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Asst. Prof. Başak Öncel

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Dear participants, distinguished researchers, and academics,

It is my great pleasure to welcome you all to the 3rd International Symposium on Traditional Foods and Sustainable Nutrition, organized by Toros University. We are honored to bring together experts and researchers from various fields to discuss the vital topics of traditional foods, sustainable nutrition, and gastronomy systems.

We are living in an era where the world is facing numerous pressing challenges, such as climate change, resource depletion, food scarcity, poverty, and global health crises. These global challenges demand urgent solutions, and as a society, it is clear that we must take responsibility to protect our ecosystems and work toward a sustainable future. Sustainability is not just an environmental concept but also a social and economic one. The protection of our limited resources and ensuring their availability for future generations are crucial.

That's why traditional foods and sustainable nutrition are more important than ever before. They not only play a key role in nourishing populations but also in preserving cultural heritage and supporting sustainable food systems. The purpose of this symposium is to explore the innovative ways in which traditional food systems can address these pressing global issues.

Over the next two days, this symposium will serve as a platform for sharing cutting-edge research findings in the areas of food, nutrition, and gastronomy, while also promoting the exchange of innovative ideas and solutions to help combat the challenges of sustainability. We aim to highlight the significance of traditional foods in creating more sustainable food systems and in promoting environmental stewardship.

Our hope is that the discussions and collaborations fostered here will lead to practical solutions that not only preserve traditional food practices but also transform them to fit the needs of a modern and sustainable world. We believe that by working together and exchanging knowledge, we can shape a more sustainable future.

I would like to extend my deepest gratitude to all of you for your participation and valuable contributions. I am confident that your insights and expertise will greatly enrich the discussions at this symposium.

Thank you, and I wish you all a productive and inspiring symposium.

Asst. Prof. Başak Öncel
Symposium Co-chair

Our dear rector, distinguished guests, dear participants, dear academic staff and students of Toros University, Welcome to toros university's 3rd international traditional foods and sustainable nutrition symposium organized by nutrition-dietetics, gastronomy and culinary arts, and cooking departments. We are living in a world that gets older and older with diminishing resources. there is a long list of scarcities that we struggle with such as inadequate food and high food prices, increasing poverty as a result of this, energy becoming more and more expensive, increasing pollution in all aspects, land, water and air pollutions, access to reliable food being more and more difficult, increasing threats to world peace through ongoing wars in various regions and so on.

Among these issues, scarcity of food, especially reliable food, is the most critical one since it is directly connected to lots of lost lives driven by global hunger. There are several interrelated factors behind the food crisis and global hunger that can be summarized as climate change, global warming, melting of glaciers, floods, reduction in the quantity of fertile lands, draught and the decrease in harvest levels, the harmful effect of greenhouse gases, increase in epidemic diseases, increasing migration from poor countries to rich ones in search of better lives, and tragic incidents experienced such as lost lives, broken families, etc.

In the face of all these negativities, there is an urgent need to see the big picture and gain insight into the relationships behind it. In a nutshell the issue that should be explored in detail to solve the problems in the big picture can be stated as follows:

How can we restore the equilibrium in a world that resources are being depleted at a rate quicker than they are replenished? Or what is the address for a peaceful world where nobody fights for food and sustainability in food is achieved in all aspects? Or how can we achieve social sustainability besides environmental sustainability?

It is my belief that answers to these vital questions will be clarified through the scientific work presented in our symposium.

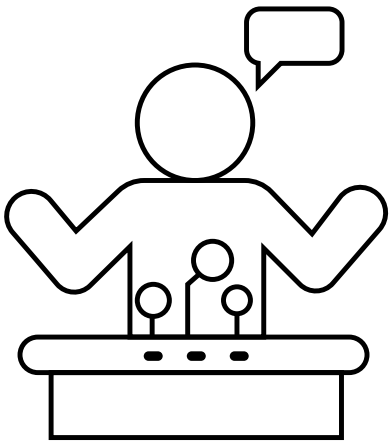
Finally, I would like to say a few words about Toros University's Journal of Food, Nutrition and Gastronomy. JFNG is a multidisciplinary journal, the product of the joint, diligent work of Toros University's three departments: Nutrition-Dietetics Department of The Faculty of Health Sciences, Gastronomy and Culinary Arts Department of The Faculty of Fine Arts, Design and Architecture and Cooking Department of The Vocational School. JFNG is in the third year of its publication and sustainable food systems is one of the popular areas of the journal.

As I conclude my speech, I thank all participants: keynote speakers, academicians who submit articles and present them, symposium committee and dear audience for their precious supports.

I wish our symposium to be a productive scientific gathering by all means and hope to be together in the 4th traditional foods and sustainable nutrition symposium next year.

Thank you all and best regards.

Prof. Dr. Bahar Taner
Editor of Toros University Journal of Food, Nutrition and Gastronomy



Keynote Speeches

Upcycling Towards New Ingredients and Sustainable Food Options

Agnieszka Piekara

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As the global demand for sustainable solutions increases, upcycling offers a promising approach to reducing food waste while creating innovative and nutritious food products. This paper explores the potential of upcycling by-products and surplus materials from food processing into new, high-value ingredients. Examples include repurposing spent grains from brewing into protein-rich flours, transforming fruit and vegetable peels into natural sweeteners or fiber-rich powders, and converting coffee grounds into baking ingredients.

Upcycling not only addresses environmental concerns by reducing waste but also enhances the nutritional profile of new food products. By leveraging the rich nutrient content found in these by-products—such as antioxidants, fiber, and plant-based proteins—manufacturers can create a diverse range of food options that cater to health-conscious consumers.

Furthermore, the paper discusses the environmental and economic benefits of upcycling, showcasing successful examples of products such as snacks, alternative flours, and plant-based alternatives currently emerging in the market. This research underscores the potential for upcycling to revolutionize the food industry by offering sustainable, healthy, and innovative solutions for the future of food production.

Keywords: Upcycling, food waste, sustainable food production, plant-based ingredients, functional foods, circular economy

The Thin Line Between Social Disparity and Obesity In Children

Ines Banjari

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Social inequality, especially poverty has the most severe consequences for children. It affects child's nutritional and overall health status, and if prolonged to adulthood reduces quality of life and life expectancy. Children from low-socioeconomic countries are more prone to malnutrition, both undernourishment and obesity, with health consequences that extend far beyond individual, they affect the whole society. The most worrying trend is that of childhood obesity which skyrocketed and projections for the near future are not bright. Still, both extremes in nutritional status of children are related to inadequate intake of nutrients, i.e. unbalanced diet. Nutrition quality directly depends on income, deteriorating greatly with limited finances or access to fresh produce, especially fruits and vegetables. Food insecurity is a worldwide problem, with more so-called food deserts in areas where people live in poverty or are at risk of poverty. The trend is especially pronounced in some of the most developed countries in the world including the US and Great Britain. Foods packed with nutrients are the first ones cut off one's diet in times of income reduction, and children are especially susceptible to lack of nutrients given their intense growth and development.

Keywords: Childhood obesity, malnutrition, poverty, social inequality, nutrition quality

Polycyclic Aromatic Hydrocarbons (PAHs) in Cooked Meat: Public Concern and Mitigation Strategies

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It is well known the effort of the worldwide scientific community to study the relationship of diet and nutrition to cancer. In the last decades, the presence of mutagenic/carcinogenic substances in foods has become a major concern for consumers. In particular, has gained great interest the study of polycyclic aromatic hydrocarbons (PAHs), well-known carcinogens, presenting different pathways of contamination among food, such as meat. These toxic substances can be generated during man-made processing involving cooking processes undergoing high-temperature, such as grilling. In the light of the health risk issue from exposure to PAHs, effective strategies to inhibit or reduce their formation, such as the use of antioxidants, has drawn the attention of the scientific community. Among antioxidants, vitamin E has been pointed out as a principal natural antioxidant. The focus of our research activities is the study of the effect of vitamin E on PAHs formation through meat model systems, by adding directly different concentrations of vitamin E to meat prior cooking and animal model system, by intramuscularly injecting it to broiler chickens in order to study how endogenous tissues levels of vitamin E could affect PAHs formation. Our findings report that although the cooking did not generate high PAHs in the meat model high PAHs amount, the effectiveness of vitamin E in inhibiting PAHs formation depends on the concentration used and on fat content and fatty acid profile of meat. Although PAH4 and BaP contents, the most appropriate indicators for occurrence and toxicity of PAHs in foods, were well below the maximum levels of 30 ng/g and 5 ng/g respectively defined by EU legislations, vitamin E significantly reduced their content in beef and chicken meat models. Intramuscular levels of vitamin E such as those found in treated chickens were not effective in reducing the formation of PAHs in grilled meat. Ongoing research is centered on the use of a higher number of samples and with a wider range of doses of vitamin E and a different timeline to better understand the pharmacokinetics of vitamin E in relation to its role on formation PAHs in grilled meat.

Keywords: Polycyclic Aromatic Hydrocarbons, meat, antioxidants, vitamin E

Gastronomy 4.0

Seden Doğan

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In the year 2045, Chef Elena runs a high-tech industrial kitchen, where robotic arms, 3D food printers, IoT appliances, and AI assistants work together to craft intricate dishes with precision. Elena oversees the kitchen like a conductor, using technology to enhance efficiency while focusing on creativity and flavor. Despite the advanced machinery, she ensures the human element remains central, adding the warmth and soul that transforms food into a memorable experience. The future of cooking blends technological mastery with the timeless art of cuisine, creating a culinary journey that is both advanced and heartfelt.

Augmented Reality (AR) technology is used in dining experiences to add a layer of interactive visual content that enhances both the presentation and storytelling aspects of a meal. For instance, AR menus allow guests to view 3D models of dishes, helping them visualize their order before making a decision. This not only makes ordering more engaging but also reduces uncertainty about the food. In immersive dining experiences, AR projections on the table can tell stories about the origin of ingredients, showcase the preparation process, or transport diners to virtual environments that match the theme of the meal, creating an emotional connection.

The rise of robotic kitchens, like Moley and Flippy, is transforming the culinary industry by automating repetitive cooking tasks and optimizing kitchen operations. Moley, the world's first fully robotic kitchen, features robotic arms capable of executing complex recipes with remarkable precision, emulating the movements of a professional chef. By handling tasks such as stirring, chopping, and even cooking entire meals, Moley reduces the need for multiple kitchen staff, thereby cutting labor costs. This allows restaurants to operate more efficiently, especially during labor shortages or peak hours.

Flippy, developed by Miso Robotics, is another example, primarily designed to operate fryers and grills in fast food settings. Flippy's ability to work consistently, without breaks or errors, ensures that every burger is cooked to perfection and fries are prepared uniformly. This consistency is crucial for chain restaurants where maintaining a standardized quality across all locations is key to customer satisfaction.

These robotic systems not only improve operational efficiency and reduce labor costs but also free up chefs for more creative tasks. By automating mundane, repetitive processes, chefs can focus on areas that require a human touch—such as recipe innovation, creative plating, and crafting new dining experiences. This shift allows for greater emphasis on culinary artistry and the development of unique flavors, rather than spending time on routine cooking duties. Additionally, robotic kitchens can enhance safety by minimizing the risk of burns and other common kitchen injuries, further supporting a more sustainable and creative kitchen environment.

AI and IoT technologies in kitchens help reduce food waste, increase efficiency, and enhance both cooking and dining experiences. AI-driven inventory management minimizes waste by predicting ingredient needs and suggesting optimal use, while smart sensors track spoilage and expiration dates. IoT integration allows connected appliances to communicate, optimizing workflows and synchronizing cooking processes. Smart kitchen appliances automate routine tasks, allowing chefs to focus on creativity and improving consistency. This efficiency leads to faster meal preparation, reduced errors, and a more personalized dining experience, ensuring higher customer satisfaction and more sustainable kitchen practices.

3D printing technology allows chefs to experiment with food presentation and texture in innovative ways, enhancing personalization and sustainability in the culinary industry. By creating intricate, customizable shapes, chefs can craft visually stunning, personalized dining experiences, meeting the growing demand for unique dishes. The precise layering capabilities also allow chefs to manipulate food textures, offering diverse sensory experiences. Additionally, 3D printing supports sustainability by minimizing ingredient waste through exact portion control and utilizing alternative ingredients creatively. This technology enables chefs to push the boundaries of gastronomy, combining aesthetics, customization, and eco-friendly practices.

Benefits

•**Competitive Edge:** Stand out by adopting cutting-edge innovations.

•**Unforgettable Experiences:** Delight customers with technologies like 3D food printing, AR/VR dining, and AI-driven personalization.

•**Sustainability:** Reduce waste and enhance energy efficiency with AI and smart kitchen systems.

Challenges & Concerns

•**High Investment Costs:** Initial tech adoption can be financially demanding.

•**Training Needs:** Workforce upskilling is essential for smooth implementation.

•**Customer Concerns:** Balancing high-tech with personal touch.

•**Data Privacy:** Protecting customer data as personalized tech becomes more prevalent.

Keywords: Gastronomy, artificial intelligence, robotics

Modern Advances in Technological Aspects of Fruit and Berry Jelly Production

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Conceptual changes in nutrition become the basis of technological developments. There is a need to create new food systems that are balanced in terms of micro and macronutrients and that can be adapted to different tastes of consumers. Food products based on gelling agents are very popular and jelly sweet dishes as a culinary product are among of them. Their popularity is growing significantly.

In the modern human nutrition, a special place is given to sweet dishes. Due to the balanced taste properties due to the content of a significant amount of sugar, fruit and berry components (fresh berries and fruits, fruit and berry juices, mashed potatoes, syrups, jam), mainly delicate consistency and attractive appearance, they are quite popular among the population. Sweet dishes complete the meal, so it should be emphasized, the expediency of their easy assimilation by the human body.

The use of fruit and berry raw materials in the recipe composition significantly reduces the energy and increases the nutritional value of the finished product due to the high content of vitamins, organic acids, biologically active substances, macro- and microelements.

It has a scientific interest to use a low-esterified amidated pectin in the technology of jelly sweet dishes not only for technological, but also for functional purposes. It is advisable to use eggshell powder as a source of calcium in the technology of jelly sweet dishes. Presented ovocalcium has a high level of digestibility and good structure-forming properties with pectin in the technology of jelly sweet dishes.

Keywords: Jelly production, gelling agents, fruit and berry components

Physicochemical Properties of Various Animal Fat Mixtures

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Abstract

The Kyrgyz Republic (Kyrgyzstan), is a mountainous country of Central Asia. Livestock husbandry is part of the history and nomadic culture of the Kyrgyz Republic. Most of the arable land is devoted to pasturage for livestock and to growing hay. Important livestock products include meat, fat and the milk of goats, sheep, and cattle. Chickens, horses, yaks and pigs are also raised in the country. Visceral fat is generally not used in the daily diet and rarely used in processing. So, by studying the chemical and physical properties of animal fats and their mixtures, it is possible to use them in various fields and comprehensively increase their use. The objective of this study was to determine the chemical, physical and rheological properties of animal fat and their mixtures. The fat samples of cow, horse, sheep, goat and pig were obtained for determining moisture, dry matter, fat and ash content. Colour characteristics (L, a, and b values) and rheological properties of the samples also were determined. The total fat content of the samples was between 87.5 – 90.5 %. Among the samples goat fat showed the highest L (lightness) parameter. Depending on the origin of animal fats, the melting temperature varied significantly. For the horse, pork, cow, sheep and goat fat, melting temperatures of 38, 43.2, 47.6, 49 and 53.4 °C were determined, respectively. From the rheological analysis, fat mix №7 was close to the physical and rheological properties of pork fat. This fat mix has the potential to be used as a substitute for pork fat in the production of the halal products. The results of the study are of interest to manufacturers and future research into finding natural raw materials to produce sustainable fatty compositions.

Keywords: Animal fats, chemical content, rheological properties, melting point.

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Enrichment Of Wheat-Acha Flour Based Cookies with Elm Oyster Mushroom (Hypsizygus Ulmarius) Flour

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ABSTRACT

Introduction and Aim

Poor utilization of food produce is one of the factors that contribute immensely to postharvest losses and thus, food insecurity and malnutrition. This study evaluated the proximate and mineral contents as well as the physical and sensory properties of cookies produced from wheat-acha composite flour enriched with Elm oyster mushroom (Hypsizygus ulmarius) flour.

Method

Cookies were produced from wheat-acha composite flour blended with sun and oven dried Elm oyster mushroom flour in the following ratios: 100:0:0, 0:100:0, 47.5:47.5:5; 45:45:10; and 42.5:42.5:15, respectively. The cookie samples were analyzed for proximate and mineral compositions as well as the physical and sensory properties. Data obtained were statistically analyzed by ANOVA and means were separated by Duncan multiple range test. There were significant differences ($p \leq 0.05$) in the proximate and mineral compositions of the cookies.

Results

Cookie samples made with 5% sundried and oven dried mushroom flours had the highest protein, ash and fibre values while the fat contents of the samples with oven dried mushroom were significantly higher ($p \leq 0.05$) than those containing sundried mushroom flour. For mineral contents, cookies with 5% sundried mushroom flour had the highest copper (2.90 mg/100g), zinc (0.76 mg/100g), calcium (0.84 mg/100g), magnesium (0.70 mg/100g), potassium (8.00 mg/100g) and iron (10.00 mg/100g) contents while cookies containing 5% oven dried mushroom had the highest sodium content amongst the samples made from the flour blends. The cookie samples with 5% oven dried mushroom flour had the highest weight (12.03 g) and thickness (9.10 mm) values while the sample with 10% oven dried mushroom flour had the highest diameter (48.1 mm). Sensory mean score values showed that the samples with up to 10 % of mushroom flour (both sun and oven dried) competed very well with those from 100 % wheat flour (control sample) and their overall acceptability values were not significantly different ($p > 0.05$) from each other.

Conclusion

Overall, the addition of mushroom flour (up to 15%) improved the proximate and mineral contents of cookie samples produced from the mushroom enriched composite flours.

Keywords: Enrichment, elm oyster mushroom, food processing, cookies, food security

1. Introduction

Composite flour is defined as a mixture of flours obtained from tubers which rich in starch such as cassava, yam, and protein-rich flour and cereals, with or without wheat flour that created to satisfy specific functional characteristics and nutrient composition (Noorfarahzila *et al.*, 2014). The composite flours utilization has several benefits for developing countries such as Nigeria in terms of food nutrient enhancement, under-exploited crops utilization, thus, disabling them from going extinct and reducing the importation of wheat flour, thereby saving of foreign exchange (Hasmadi *et al.*, 2014; Aparana, 2018). The bakery products that are made from composite flour are typically of good quality that can prevent the onset of degenerative diseases that have been associated with the current fast paced lifestyle that is now very common. Some properties of pastries that are made from composite flour are similar to wheat flour-based products, though the texture and the properties of these products may be different from those made from wheat flour, with an increased nutritional value and appearance (Hasmadi *et al.*, 2014; Chillo *et al.*, 2019).

Wheat (*Triticum spp*.) is one of the major cereal crops around the world and it is a fundamental source of calories and nutrients that is mainly milled and used for baking (Adeyanju *et al.,* 2021). It contains moisture 12.8%, protein 11.8%, fat 1.5%, minerals 1.5%, crude fibre 1.2%, carbohydrates 71.2%, energy value 346 Kcal, calcium 41 mg/100 g, phosphorus 306 mg/100 g and iron 5.3 mg/100 g respectively (Gopalan *et al.,* 2000; Rana, 2020).

Acha (*Digitaria exilis*) also known as "hungry rice" is a cereal crop of West African origin belonging to the family *Graminaea*. Two acha varieties are said to be most popular, the white acha (*Digitaria exillis*) and brown acha (*Digitaria iburua*) (Aviara *et al.*, 2017). In West Africa, acha is mainly grown and cooked as a special food for treats at weddings, and other ceremonies (Chinwe *et al.*, 2015). The study of Jideani (2012) reported that acha is a food security crop because of its potentials to serve as a food fortificant and its composition. It is also gluten-free and it contains crude protein (7%) that is high in leucine (19.8%), methionine and cysteine (7%) and valine (5.8%) (Okeme *et al.,* 2017). According to Ayo *et al.* (2018), Acha can contribute notably to the macronutrients and whole grain consumption in diets, wellness, improved food security and economic status in developing economies. However, despite its possible relevance in food application and nutritional benefits, it is still underexploited (Glew *et al.*, 2013).

Elm oyster mushroom, *Hypsizygus ulmarius* (BULL.), is a high yielding edible mushroom. It is one of the most widely consumed mushrooms (Ma *et al.*, 2017). It is a natural source of bio active compounds, including carbohydrates, peptides, dietary fibres (Shivashankar and Premkumari, 2014). Studies have shown that *Hypsizygus ulmarius* exhibits good antioxidant, anti- inflammatory, etc. (Al-Faqeeh *et al.,* 2018). For this reason, there has been great interest in incorporating *Hypsizygus ulmarius* into foods, such as baked goods, to enhance their nutritional profiles and potential health benefits (Anmut and Galana, 2022).

Cookies are one of the best-known quick snack products and they are also known as 'biscuit'. They are popularly baked products due to their low manufacturing cost, convenience and longer shelf life (Lalmuanpuia *et al.*, 2017; Culetu *et al.*, 2021). Ideally, the recipes for baking cookies mainly consist of wheat, margarine, sugar, salt, emulsifier etc., however, they can be enriched with other food materials, like mushroom, to improve their nutritional and health benefits so they can meet specific needs of the consumers (Ajibola *et al.*, 2015; Uchenna and Omalayo, 2017).

Most cookies are produced from refined wheat which results in a low nutrient density product that is devoid of health protective ingredients. Also, people from developing countries, like Nigeria, subsist mainly on starch rich diets and this has resulted in the prevalence of malnutrition, with children being the worst sufferers in form of diseases like kwashiorkor and marasmus. Due to their consistent quality, bakery products have gained an important place in the food industry and therefore, has potential to serve as a medium for nutrition improvement of populace at low cost and without much compulsion. Hence, this study aimed to evaluate the proximate and mineral composition, physical and sensory properties of cookies produced from whole wheat-acha composite flour fortified with Elm oyster mushroom (*Hypsizygus ulmarius*) flour.

2. Methods

2.1 Materials

2.1.1 Raw material collection

Fresh *Hyspizygus ulmarius* was procured from Life Plus Foundation International, Owode-Ede, Osogbo, Osun State. Acha grains were obtained from a local market in Kano State. Other ingredients for the cookies like wheat grain, sugar, eggs, sugar, margarine, baking powder, salt were purchased at Orisunmbare market Osogbo, Osun State. All materials and chemical that were used for analysis were of analytical grade.

2.2 Methods

The methods used for the preparation of acha, whole wheat and mushroom flours are represented in Figures 1a-c as described by Olapade and Aworh (2012), Peter *et al.* (2017) and Ayo *et al.*, (2018), respectively.

The method described by Peter *et al.* (2017) with some modifications was used for the production of the cookies as shown in Figure 2. The whole wheat, acha and Elm oyster mushroom flours were blended as shown in Table 1. The margarine, sugar and egg were measured to a bowl and creamed for 30 minutes using the rubbing method. In separate bowls, cream was added each flour blend and made into dough. Each dough was rolled and flattened before cutting out to shapes using a cookie-cutter. The cut-out dough pieces were baked at 150 ºC for 30 minutes in the oven. After baking the cookies were cooled to room temperature, packed in laminated aluminum foil pouches till needed for further analysis.

2.3 Sample Analyses

The cookie samples were analyzed for their proximate content, mineral composition, physical and sensory properties.

2.3.1 Proximate composition of the cookie samples

The cookie samples were analyzed for moisture, fat, protein, ash and crude fiber contents in triplicate using AOAC (2012) method. Protein content was estimated using conversion factors 6.25. Carbohydrate was estimated by difference method.

2.3.2 Mineral composition of the cookie samples

The copper, zinc, calcium, magnesium, sodium, potassium and iron contents of the samples were evaluated using AOAC (2005) method.

2.3.3 Physical properties of the cookie samples

The weight of the cookies was measured using the method described by Ayo *et al.* (2018) while their thickness and diameter were evaluated using the method described by Zucco *et al.* (2011). The hardness of the cookies samples was measured using Instron Universal Texturometer (Shimadzu AG-Xplus). Each cookie was placed on the loading cell and compressed as described by Singh *et al.*, (1993).

2.3.4 Sensory evaluation of the cookie samples

The organoleptic properties of the cookie samples, taste, colour, texture, aroma and overall acceptability, were assessed by a 20-member panel, who were instructed regarding the evaluation procedures in both written and verbal formats prior to the evaluation of the cookie samples. Each panelist was presented with the cookie samples to rank based on preference using a nine (9) point hedonic scale where 9 represented ―like extremely, 5 represented neither like nor dislike and 1 represented dislike extremely (Peter *et al.*, 2017).

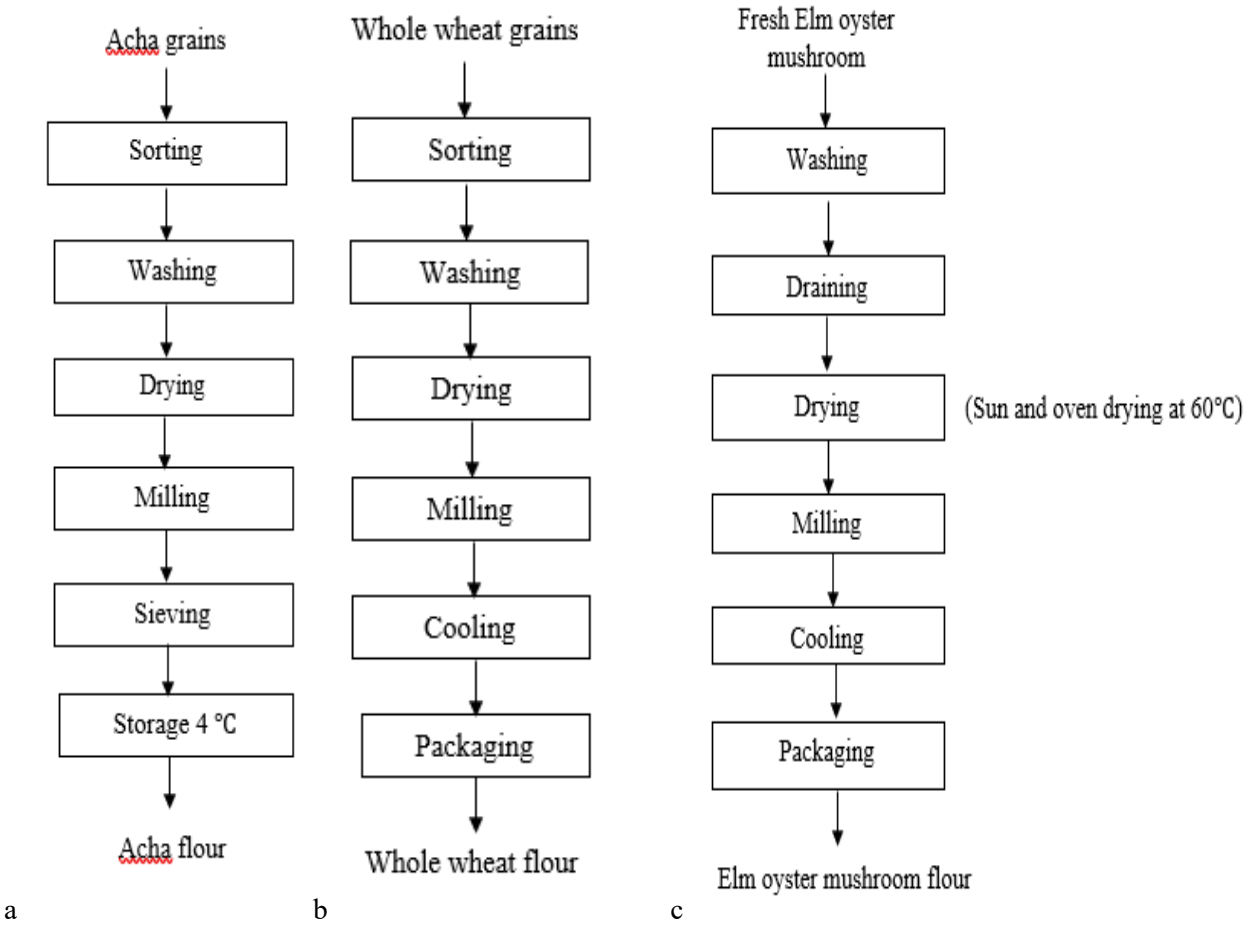

Figure 1: Flow chart for the production of: a) Acha flour (Olapade and Aworh 2012); b) Whole wheat flour (Peter et al., 2017) and c) Elm oyster mushroom flour (Ayo et al., 2018).

Table 1: Blending Ratio of Whole Wheat, Acha and Mushroom Flour for Cookies Production

Sample Code	Wheat flour (%)	Acha flour (%)	Sundried mushrooms flour (%)	Oven dried mushroom flour (%)
WTC	100	0	0	0
AHC	0	100	0	0
CSD5	47.5	47.5	5	0
CSD0	45	45	10	0
CSD1	42.5	42.5	15	0
COD5	47.5	47.5	0	5
COD1	42.5	42.5	0	15

Sample code : WTC = 100% Whole wheat flour (control); AHC = 100% Acha flour (control); CSD5 = 95% whole wheat and acha to 5% sundried mushroom flour blend, CSD0= 90% whole wheat and acha to 10% sun-dried mushroom flour blend, CSD1 = 85% whole wheat and acha – to – 15% sundried mushroom flour blend, COD5 = 95% whole wheat and acha to 5% oven dried mushroom flour blend, COD0 = 90% whole wheat and acha to 10% oven dried mushroom flour blend, COD1 = 85% whole wheat and acha to 15% oven dried mush-room flour blend.

2.3.6 Statistical Analysis

The results obtained from the sample analyses were subjected to statistical analysis. One way analysis of variance (ANOVA) was used to compare the means and the means were separated using Duncan multiple range test. Significance difference accepted at $P<0.05$ using statistical product for service solution (SPSS) version 25.

3.0 Results and discussion

3.1 Proximate composition of the cookie samples

Table 2 shows the proximate composition of cookie samples. The moisture content of the samples varied from 7.94 to 11.48%. The moisture content of the samples was significantly different ($p<0.05$) from each other except for WTC and CSD0. The samples blended with oven dried mushroom had higher moisture content than those blended with sundried mushroom. The overall highest moisture content occurred in the cookie samples produced from 100% acha flour and while COD0 had the highest (10.86%) moisture content among the samples that were blended with mushroom, CSD0 had the overall least (7.94%) moisture content. This indicated that CSD0 will likely have longer shelf life than other samples. According to Ndulaka and Obasi (2018), cookies are generally low moisture foods. Baked products with moisture content less than 13% are stable from moisture-dependent deterioration (Ayo-Omogie and Odekunle, 2015). According to the report of Awuchi (2019), cookies with moisture content below 13% can be stored at room temperature and will be less prone to fungal and microbial infection.

The fat content of the cookie samples varied from 8.43% for AHC to 16.59% for COD5. The fat content of samples with sundried mushroom flour and AHC are not significantly different ($p<0.05$) but were significantly lower than the fat content of the other samples. For the mushroom enriched cookies, COD5 and CSD0 had the highest (16.59%) and lowest (8.75%) fat content respectively. It was deduced from these results that cookies enriched with oven dried mushroom flour had high oil binding capacity than those made with sundried mushroom flour. Fat is a rich source of energy and also serves as carriers of fat-soluble vitamins A, D, E and K (Ikuomola *et al.*, 2017). It also serves as a lubricating agent that improves the mouth feel, flavour and palatability of foods (Ikuomola *et al.*, 2017). However, high level of fat in food products could lead to rancidity and development of unpleasant odour/flavor (Ufot *et al.*, 2018).

The fibre content in the cookies ranged from 0.47 % to 1.78 %. WTC has the highest fibre value (1.78 %) while COD0 has the least value (0.47 %). The fibre content of samples with sundried mushroom flour are not significantly different ($p<0.05$) from each other but they are significantly different ($p<0.05$) from other samples. The fibre contents of the mushroom enriched cookies were significantly lower ($p<0.05$) than those of the control samples (cookies from 100% whole wheat and 100% acha flour). The fibre content of WTC (1.78 %) was higher than 1.59% reported for whole wheat cookies by Peter *et al.* (2017) but lower than 2.45 % reported by Ajibola *et al.* (2015) for whole wheat cookies. The presence of high fibre in food product is essential owing to its ability to facilitate bowel movement, bulk addition to food and prevention of constipation (Satinder *et al.*, 2011). It is well known that soluble fibre generally increases transit time through the gut, slow emptying of the stomach and slow glucose absorption (Swaminathan, 2002; Chukwuma *et al.*, 2010). Also, the risk of mortality and morbidity from cardiovascular disease, stroke, diverticulitis, colon cancer and diabetes are reduced when high fibre diet is consumed (Slavin, 2005; Weickert, 2008).

The ash content of the cookies ranged from 1.18 to 2.03 % and it was significantly different ($p<0.05$) in the control samples and those enriched with oven dried mushroom flour. CSD5 had the overall highest ash content and also among the enriched cookies while COD0 had the least ash content among the enriched cookie samples. The enriched cookie samples all had higher ash content than those made from 100% acha flour and they compared relatively well with the ash content of sample made from 100% of whole wheat flour except for COD0.

Table 2: Proximate Composition of Cookies Produced from Blends of Whole wheat, Acha and Elm Oyster Mushroom (Hypsizygus ulmarius)

Sample code	Moisture (%)	Fat (%)	Fibre (%)	Ash (%	Protein (%)	Carbohydrate (%)
WTC	10.29±0.03^{c}	10.43±0.14^{c}	1.78±0.02^{a}	1.89±0.02^{b}	12.82±0.45^{a}	62.80±0.27^{c}
AHC	11.48±0.10^{a}	8.43±0.25^{d}	1.55±0.04^{b}	1.18±0.09^{d}	7.35±0.22^{e}	69.43±0.36^{a}
CSD5	8.96±0.02^{e}	8.90±0.05^{d}	0.96±0.03^{d}	2.03±0.02^{a}	11.44±0.2^{7}b	67.72±0.20^{b}
CSD0	7.94±0.11^{f}	8.75±0.15^{d}	0.89±0.06^{d}	1.87±0.04^{b}	10.57±0.45^{bc}	70.00±0.08^{a}
CSD1	8.85±0.13^{e}	8.79±0.64^{d}	0.96±0.04^{d}	1.95±0.04^{ab}	11.41±0.36^{b}	68.08±0.24^{b}
COD5	10.38±0.06^{c}	16.59±0.21^{a}	1.23±0.03^{c}	1.87±0.07^{b}	10.94±0.44^{bc}	59.00±0.66^{e}
COD0	10.86±0.03^{b}	15.37±0.04^{b}	0.47±0.02^{f}	1.22±0.01^{d}	9.07±0.4^{5}d	61.78±0.54^{d}
COD1	9.69±0.21^{d}	16.14±0.41^{a}	0.76±0.01^{e}	1.74±0.01^{c}	10.32±0.45^{c}	62.61±0.24^{cd}

Values are mean ± SD. Samples with different superscripts within the same column were significantly ($p<0.05$) different.

Sample codes: CSD5 = 95% whole wheat and acha– to – 5% sundried mushroom cookies; CSD0= 90% whole wheat and acha – to – 10% sundried mushroom cookies; CSD1 = 85% whole wheat and acha – to – 15% sundried mushroom cookies; COD5 = 95% whole wheat and acha to 5% oven dried mushroom cookies; COD0 = 90% whole wheat and acha to 10% oven dried mushroom cookies; COD1 = 85% whole wheat and acha to 15% oven dried mushroom cookies; WTC = Whole wheat cookies; and AHC = Acha cookies.

The value for WTC was 1.89% which was higher than 0.64% reported by Awan *et al.* (1995) for whole wheat biscuits but lower than 2.31 % reported by Ajibola *et al.* (2015) for whole wheat biscuits. The ash content of a food sample is an index of the mineral element of such food (Ufot *et al.*, 2018).

The protein content of WTC was the overall highest (12.82%) while the cookies from 100% acha flour (AHC) had the overall least (7.35%) protein content. This could be attributed to the fact that both acha is not a good source of protein (Ubbor *et al.*, 2022). The protein content of the enriched cookies was not significantly different ($p<0.05$) from each other except in COD0 which had the lowest (9.07%) protein content among the enriched cookies while CSD5 had the highest at 11.44%. The range of protein value obtained in cookies produced in this study was higher than values (5.39 - 6.32 %) reported for wheat-based cookies substituted with OFSP flour (Temesgen *et al.*, 2015), but lower than values (12.61 - 15.03 %) obtained in cookies from wheat, acha and pigeon pea flour blends (Adeyanju *et al.*, 2018).

Carbohydrate contents of the samples ranged from 59.00 to 70.00 %. The carbohydrate content of AHC is higher than the carbohydrate content of WTC. COD5 had the least carbohydrate content. There was no significant difference ($p<0.05$) between CSD0 and AHC but they were significantly different ($p<0.05$) in the carbohydrate content of the cookies. Similar findings were reported by Ubbor *et al.* (2022) where the cookies produced from wheat:acha:whole OFSP flour (50:50:0) had the highest carbohydrate content value (67.62 %) and this could probably be due to the significant amount of acha flour which Istifanus and Agbo (2016) has reported to be a rich source of carbohydrate. Similar finding was reported by Ufot *et al.* (2018) where whole wheat-based biscuit supplemented with 25% acha flour had the highest carbohydrate content (68.70 %) compared to 67.90 % obtained in the control (biscuit processed from 100% wheat flour). Besides carbohydrate content of food products like cookies, protein and fat contents also contribute to their energy value (Ikuomola *et al.*, 2017).

3.2 Mineral composition of the cookie samples

The results of the copper, zinc, calcium, magnesium, sodium, potassium and iron contents of the samples were presented in Figure 2. The mineral contents of the samples produced with sundried mushroom flour were generally higher than those of the cookies enriched with oven dried mushroom flour. The copper content of the cookies ranged from 1.60 to 2.90 mg/100g with AHC having the least value while CSD5 had the highest value. The copper contents of the cookies were significantly ($p<0.05$) different from each other.

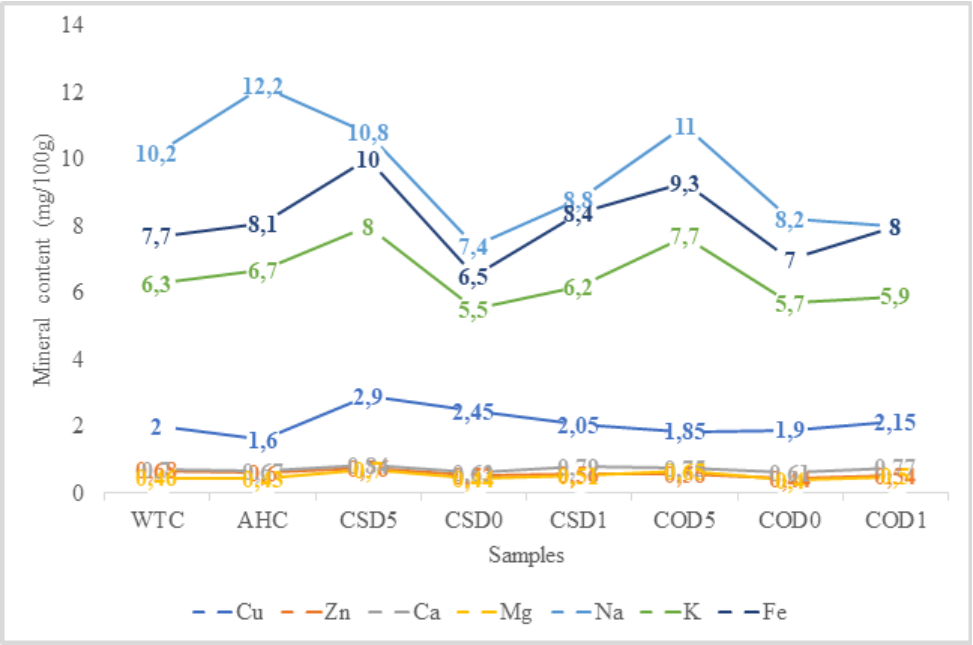

Figure 2: Mineral Composition of Cookies Produced from Blends of Whole wheat, Acha and Elm Oyster Mushroom (Hypsizygus ulmarius)

Sample codes: CSD5 = 95% whole wheat and acha– to – 5% sundried mushroom cookies; CSD0= 90% whole wheat and acha – to – 10% sundried mushroom cookies; CSD1 = 85% whole wheat and acha – to – 15% sundried mushroom cookies; COD5 = 95% whole wheat and acha to 5% oven dried mushroom cookies; COD0 = 90% whole wheat and acha to 10% oven dried mushroom cookies; COD1 = 85% whole wheat and acha to 15% oven dried mushroom cookies; WTC = Whole wheat cookies; and AHC = Acha cookies.

CSD5 had the overall highest copper content while CSD5 had the least copper content among the enriched cookie samples. The copper content decreased with increasing addition of sundried mushroom flour while a reverse trend was observed for the copper results of the cookies enriched with the oven dried mushroom flour.

The zinc and calcium contents of the cookies followed the same trend and they ranged from 0.44 to 0.76 mg/100g and 0.67 to 0.84 mg/100g, respectively. COD0 having the least zinc and calcium values while CSD5 had the highest values. For zinc, CSD1 and COD5 were not significantly different ($p<0.05$) from each other but they were significantly different ($p<0.05$) from the other samples while all the calcium contents were significantly different ($p<0.05$) from one another. Nisar *et al.* (2017), who observed high in zinc contents in cookies produced from buckwheat flour showed contrasting results to the findings of the present study. According to Abulude *et al.* (2006), calcium plays important role in blood clotting, muscle contraction, and in certain enzymes in metabolic processes. The calcium results obtained in this study were lower than the values (97.18 to 160.92 mg/100g) of calcium content reported by Emelike *et al.* (2020) for breakfast cereals formulated from acha, wheat, cashew kernel and prawn.

The magnesium content in the cookies ranged from 0.40 to 0.70 mg/100g with COD0 having the lowest value while CSD5 had the highest value. AHC and CSD0 were not significantly different ($p<0.05$) from each other but they were significantly different ($p<0.05$) from CSD1 and COD1 and also from other samples. Magnesium is important for bone health, is needed as a cofactor for numerous reactions in the body and is also essential for nerve and muscle conductivity (Grosvernor and Smolin, 2002). High amount of magnesium, potassium and calcium have been reported to reduce blood pressure in humans (Ranhotra *et al.*, 1998).

The sodium content in the cookies ranged from 7.40 to 12.20 mg/100g with CSD0 having the least value while AHC had the highest value. CSD5 and COD5 were not significantly different ($p<0.05$) from each other but they were significantly different ($p<0.05$) from COD0 and COD1 and also from other samples.

Potassium content in the cookies ranged from 5.50 to 8.00 mg/100g. CSD0 had the least value while the highest value occurred in CSD5. WTC and CSD1 were not significantly different ($p<0.05$) from each other but they were significantly different ($p<0.05$) from COD0 and COD1 and also from other samples. The values 535.67 to 695.33mg/100g reported by Nisar *et al.* (2017) were higher than the results reported for this study. Potassium intake is required in relatively large amount in the body because it functions as an important electrolyte in the nervous system and has also been shown to exert a powerful, dose-dependent inhibitory effect on sodium sensitivity (Adrogue *et al.*, 2007).

The iron content in the cookies ranged from 6.50 to 10.00 mg/100g. The overall lowest iron value was found in CSD0 while the highest occurred CSD5. WTC, AHC and COD1 were not significantly different ($p<0.05$) from each other but they were significantly different ($p<0.05$) from other samples. The end results achieved were higher than the finding of Ufot *et al.* (2018), who examined iron content of 3.89 to 5.12mg/100g from whole wheat flour supplemented with acha (fonio) and kidney bean flours. Adequate iron in the diet is essential to minimize the incidence of iron deficiency anemia, which is considered as the most common nutritional disorder worldwide (Short and Domagalski, 2013).

3.3 Physical properties of the cookie samples

The hardness results of the cookie samples ranged from 0.006 to 0.071 MPa as shown in Figure 3. Samples AHC, CSD1, COD0 and COD1 were not significantly different ($p<0.05$) from each other and samples CSD5, CSD0 and COD5 were not significantly different from each other ($p<0.05$) but they were significantly different from the rest of the sample. The hardness of the samples decreased with increasing addition of acha and mushroom flours to the blends used for the production of the cookies. Hardness of the cookies was the maximum forces that were achieved after the increase in the trigger force until the cracking of cookies into two or more pieces (Shubli *et al.*, 2020).

The physical properties of cookies produced were represented in Figure 4. The physical properties of samples produced from blends that were enriched with oven dried mushroom flour were mostly higher than those produced with sundried mushroom samples. The weight of the cookies ranged from 7.67 to 12.03 g. COD0 and COD1 with CSD5 and CSD0 were not significantly different ($p<0.05$) from each other but significantly different ($p<0.05$) from other samples. Cookies produced from all the enriched flour blends except CSD1 had higher weights than WTC (8.20 g). This could be attributed to the high carbohydrate content of the cookie samples compared to the control sample (Ubbor *et al.*, 2022). The weight range for cookies recorded in this study was lower than 18.20 -19.75g reported for cookies produced from wheat and fermented *Afzelia africana* composite flour (Igbabul *et al.*, 2018).

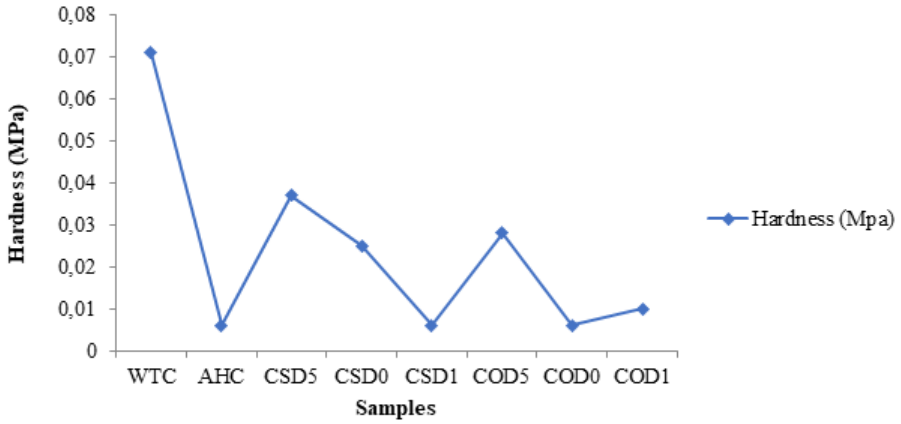

Figure 3: Hardness of Cookies Produced from Blends of Whole wheat, Acha and Elm Oyster Mushroom (Hypsizygus ulmarius)

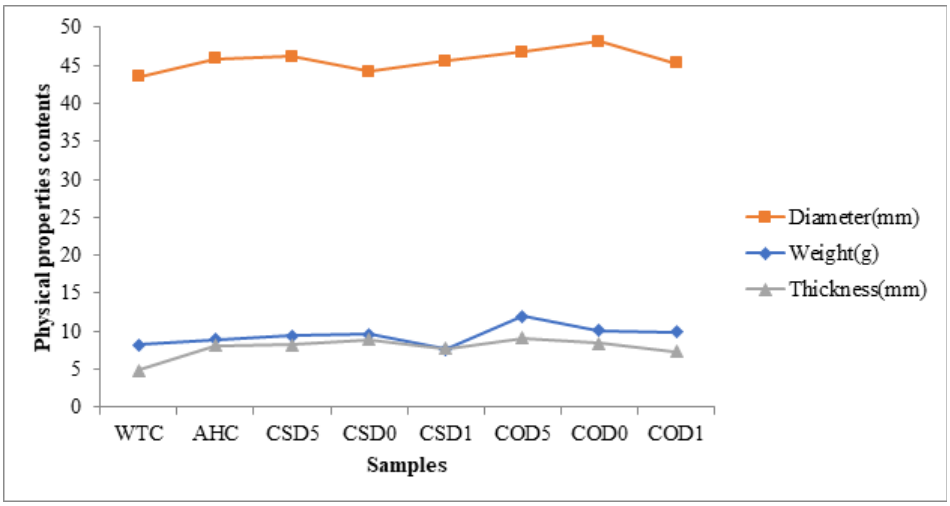

Figure 4: Physical Properties of Cookies Produced from Blends of Whole wheat, Acha and Elm Oyster Mushroom (Hypsizygus ulmarius)

Sample codes: CSD5 = 95% whole wheat and acha– to – 5% sundried mushroom cookies; CSD0= 90% whole wheat and acha – to – 10% sundried mushroom cookies; CSD1 = 85% whole wheat and acha – to – 15% sundried mushroom cookies; COD5 = 95% whole wheat and acha to 5% oven dried mushroom cookies; COD0 = 90% whole wheat and acha to 10% oven dried mushroom cookies; COD1 = 85% whole wheat and acha to 15% oven dried mushroom cookies; WTC = Whole wheat cookies; and AHC = Acha cookies.

The cookies diameter ranged from 43.50 to 48.17 mm. WTC had the lowest value while sample COD0 has the highest value. AHC and CSD5 were not significantly different ($p<0.05$) from each other but were significantly different ($p<0.05$) from other samples. It was observed that all cookie samples enriched with mushroom flour had higher values than the control (WTC). Diameter determines the quality of flour used (Bala *et al.*, 2015). According to Adejuyitan *et al.* (2009), wheat flour has a higher gluten content than its composite counterparts which forms an elastic network and results in the cookies structure contraction during baking. This explains the low diameter value recorded for WTC produced from 100 % wheat flour.

The thickness of the cookies ranged from 4.87 mm for the control (WTC) to 9.10 mm for COD5. There were significant differences ($p<0.05$) in the thickness of the cookie samples. The higher value for thickness obtained in cookies made with composite flours could be due to the higher absorption of moisture of the dough owing to the presence of water binding component (Ikuomola *et al.*, 2017). Interestingly, Peter *et al.* (2017) reported

that the higher the thickness of cookies the higher its ability to withstand stress. This implies that cookies from the enriched flour blends and AHC should have the ability to withstand stress more than the control (WTC). The range of thickness obtained in cookies produced in this study was lower than 12.50 - 13.50 mm reported for cookies produced from wheat flour and fermented *Afzelia africana* flour (Igbabul *et al.*, 2018) and values (9.49 - 10.13 mm) of thickness obtained in wheat-based cookies supplemented with OFSP flour (Temesgen *et al.*, 2015). The differences in the various cookies could also be attributed to the thickness of the dough rolled out during processing (Ubbor *et al.*, 2022).

3.4 Sensory properties of the cookie samples

The score ranking for the colour of the cookies ranged from 7.00 to 8.15. The colour value for WTC and the cookie samples enriched with sundried mushroom flour were not significantly different ($p<0.05$) from each other but they were significantly different ($p<0.05$) from those that were produced from blends enriched with oven dried mushroom flour. AHC was the most preferred in term of colour with the mean value of 8.15. COD1 had the lowest ranking among the samples with the mean value of 7.00. The value for appearance obtained in this study was higher than the range of values (6.40 to 8.00) reported by Adeyanju *et al.* (2021) for cookies produced from wheat, acha, and African yam bean composite flour. Colour is a very important parameter in judging properly baked cookies that not only reflect the suitable raw materials used for the preparation but also provides information about the formulation and quality of the product (Ikpeme *et al.*, 2010).

The values of the taste of the cookies ranged from 5.60 to 7.75. The taste results for CSD5, CSD1, COD5 and COD0 were not significantly different ($p<0.05$) from each other but they are significantly different ($p<0.05$) from other samples. WTC was the best in term of taste but COD5 compared well with it while COD1 had the lowest value. However, the values for taste obtained in this study were slightly different from the values (6.57 - 7.80) obtained in wheat-based cookies substituted with OFSP flour (Temesgen *et al.*, 2015) but lower than the values (7.44-8.36) obtained from cookies produced from composite flour of whole wheat, acha and whole orange fleshed sweet potato (Ubbor, *et al.*, 2022).

The texture of the sample ranged from 6.15 to 8.05 with COD5 having the highest ranking while COD1 had the lowest ranking. Furthermore, the texture ranking for WTC, AHC, CSD5 and COD0 are not significantly different ($p<0.05$) from each other and CSD0 and CSD1 were also not significantly different ($p<0.05$) from each other but significantly different ($p<0.05$) from the other samples. The sensory scores that were obtained in this study were averagely higher than the scores (5.90 to 8.00) from cookies produced from germinated pigeon pea, fermented sorghum, and blanched cocoyam flour blends (Okpala *et al.*, 2013), and the scores (6.20 to 7.51) reported by (Ufot *et al.*, 2018).

Another factor that determines acceptance of cookies prior to being tasted is aroma (Ubbor and Akobundu, 2009). The aroma score values of the cookies ranged from 6.05 to 7.95, with COD0 having the highest value while COD1 with the lowest. This result show that there was no significant difference ($p<0.05$) between sample WTC, AHC and COD5 also CSD5 and CSD1 were not significantly different ($p<0.05$) from each other but they were significantly different ($p<0.05$) from other samples. Eke-Ejiofor (2013) and Taiwo *et al.* (2015) also reported aroma values of biscuits made from wheat – breadfruit flour composites which agreed with the present study. However, Ojinnaka *et al.* (2016) reported aroma values which were slightly higher than those obtained for the present study.

The values of the overall acceptability ranged from 6.10 to 8.20 with WTC having the highest score, although COD5 and COD0 had scores that were very close, while COD1 had the lowest score. The cookies produced from the enriched flour were not significantly difference ($p<0.05$) from each other in terms of acceptability except COD1. Preference in terms of overall acceptability can be related to preference in terms of taste which has distinct and influential effects on food acceptability (Piqueras-Fiszman and Spence, 2015).

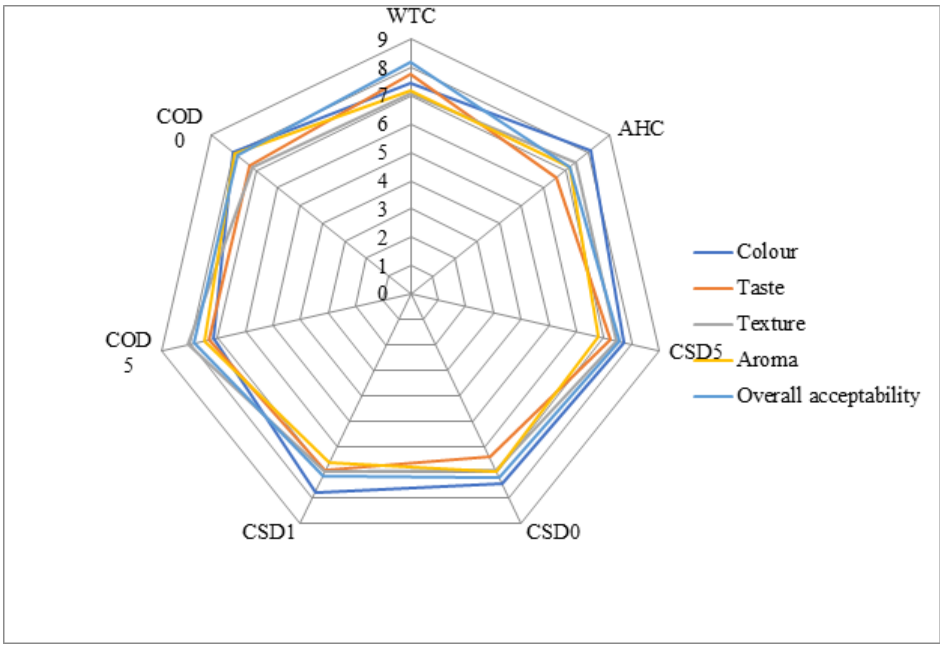

Figure 5: Sensory characteristics of cookies produced from blends of wheat, acha and Elm oyster mushroom (Hypsizygus ulmarius)

Sample codes: CSD5 = 95% whole wheat and acha– to – 5% sundried mushroom cookies; CSD0= 90% whole wheat and acha – to – 10% sundried mushroom cookies; CSD1 = 85% whole wheat and acha – to – 15% sun-dried mushroom cookies; COD5 = 95% whole wheat and acha to 5% oven dried mushroom cookies; COD0 = 90% whole wheat and acha to 10% oven dried mushroom cookies; COD1 = 85% whole wheat and acha to 15% oven dried mushroom cookies; WTC = Whole wheat cookies; and AHC = Acha cookies.

4. Conclusion

The study has shown that acceptable cookies of improved nutritional quality and increased protein can be produced from blends of whole wheat and acha flours enriched with *Hypsizygus ulmarius* (Elm Oyster Mush-room). The research work also showed that drying methods had significant effect on the mushroom flour sam-ples used for the enrichment of the cookies and in turn affected the nutritional and functional qualities of the cookies. From the sensory evaluation scores, COD0 and COD5 competed favourably with the control sample (WTC) with no significant difference ($p<0.05$) in their overall acceptability ranking. The high protein content in *Hypsizygus ulmarius* (Elm Oyster Mushroom) incorporated in the cookies could be used to alleviate the problem of protein-energy malnutrition that is still prevalent in most developing countries.

This study recommends that 15% elm oyster mushroom flour inclusion can be explored in the production of ready to eat and convenient foods such as cookies.

Figure 6: Pictures of cookies produced from blends of wheat, acha and Elm oyster mushroom (Hypsizygus ulmarius)

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Quality Improvement of Cookies Produced Using Wheat And *Abelmoscus Caillei* Flour Blends

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Abstract

Introduction and Aim: West African okra plant or *Abelmoschus caillei* is a significant tropical and subtropical vegetable crop that contains antioxidants called polyphenols and lecithin. This study investigated the nutritional, pH and sensory qualities of cookies produced using wheat and *Abelmoscus caillei* flour blends.

Method: The okra leaf was dried at room temperature and processed into flour and mixed with wheat flour at three different proportions; WOC 2 (17% okra leaf flour, 83% wheat flour), WOC 3 (33% okra leaf flour and 67% wheat flour) and WOC 4 (50% okra leaf flour, 50% wheat flour) with WOC 1 (100% wheat flour) as the control . The samples were subjected to proximate analysis, mineral analysis, pH determination and sensory evaluation using standard methods.

Results: The proximate analysis shows that WOC 4 had the highest crude fiber (2.155 ± 0.035), crude protein (14.89 ± 0.035) and ash content (4.76 ± 0.028), WOC 3 had the highest ether extract content (20.985 ± 0.0354). WOC 4 has the highest Zinc (10.20 ± 0.0012), Iron (87.00± 0.0015) and Folate (19.64 ± 0.179). WOC 2 was the most preferred cookie in terms of appearance(5.56 ± 1.97), colour (5.28 ± 2.04), texture(5.92 ± 1.79), flavour (6.00 ± 1.80), taste (5.58 ± 1.75) overall acceptability(5.68 ± 1.71) on a 9 points hedonic scale.

Conclusion: The research findings highlight that Wheat flour and okra leaf flour blend is suitable for the production of fortified cookies because of the richness in terms of nutrient contents such as Zinc, Iron and Folate.

Keywords: *Abelmoschus caillei,* nutritional, sensory, blends, composition.

1. Introduction

West Africa Okra (*Abelmoschus caillei*) is one of the species that is rich in carbohydrates, protein, antioxidants, vitamins, and minerals including folic acid essential to human consumption. West African okra plant or *Abelmoschus caillei*, is a significant tropical and subtropical vegetable crop (Kehinde 1999).

Okra is mostly used as a dietary vegetable and its seed have been used for oil production (National Research Council, 2006).

Snacks are light, fast, and ready-to-eat foods, the demand for snack foods, characterized by their convenience and ease of consumption, has increased significantly in urban areas. These foods are often consumed as a quick substitute for a full meal, due to the fast-paced nature of modern lifestyles. With changing dietary habits and increasing work demands, the popularity of snack products has risen globally (Okafor and Ugwu, 2014). Okra mucilage refers to the thick and slimy substance found in fresh as well as dried pods; mucilaginous substances are usually concentrated in the pod walls and are chemically acidic polysaccharides associated with proteins and minerals (Wolfe et al., 1977).

One of the most significant and popular vegetable crops in the tropics and subtropics is okra. It belongs to the genus *Abelmoschus* and the family *Malvaceae.* Several studies by Agbangnan et al., 2018 have established and reported that Okra provides an important input of vitamins and mineral salts, protein, and fiber. *Abelmoschus caillei* is one of the specie. In addition, wheat is the most significant stable food crop, and it provides more calories and protein to the global diet than any other cereal crop. It can be transformed into many different forms of food, is nutritive, and is simple to store and transport. Also despite the fact that environmental factors may have an impact on the nutritional makeup of wheat grains and their vital bran, vitamin, and mineral coating, wheat is still regarded as a good source of protein, minerals, B-group vitamins, and dietary fiber. Wheat is also a fantastic food for promoting good health. The essential quality that sets it apart from other temperate crops is the special qualities of dough made from wheat flour, which enables it to be processed into a variety of bread (Johnson *et al*., 1985).

Snacks are a popular way to tide people over between meals, especially for kids and adults. And wheat flour-based snacks are particularly popular in Nigeria, meanwhile, there are several other crunchy, delicious, and healthy snacks made from maize, yam, plantain, coconut, groundnut, melon, and other crops widely grown locally. The snacks produced from these crops include ‘kokoro’ and ‘aadun’ from maize; ‘ojojo’ from yam; ‘kulikuli’ and groundnut candy from groundnut or peanut; ‘donkwa’ from a mixture of maize and groundnut; ‘robo’ from a mixture of melon and groundnut; ‘ikpekere’ from plantain; ‘baba dudu’ (coconut candy) from grated coconut; ‘ofio’ from tiger nut and many others.(Karigidi *et al*., 2022)

Cookies are very popular bakery products dried to a low moisture content and have a soft chewy texture (Okaka, 2009). They are consumed extensively all over the world as a snack food and on a large scale in developing countries where protein and caloric malnutrition are prevalent (Chinma and Gernah, 2007).

According to World Health Organization, nutrition is a critical part of health and development. Better nutrition is related to improved infant, child, and maternal health, stronger immune systems, and safer pregnancy and childbirth. This study will help in introducing how to fortify daily snacks (cookies) with *Abelmoschus caillei* which may help to improve fertility in women of reproductive age and the general well-being of the body

system. Several developing countries have encouraged the initiation of a program to evaluate the feasibility of alternative locally available flour (Abdelghafor et. al., 2011). This study is important to influence the consumption of cookies produced from wheat flour and *Abelmoschus caillei,* which may be helpful in improving fertility in women of reproductive age. The composition of Vitamin C and folic acid present in the *Abelmoschus caelli* may help to improve ovulation and prevent neural tube defects in the brain and spine of the fetus between three to four weeks after conception.

Objectives of this study are to prepare cookies from wheat flour and *Abelmoschus caillei* blends, determine the sensory properties of cookies made with wheat flour and *Abelmoschus caillei.*, evaluate the nutritional composition of the modified cookies sample and to evaluate the pH of the modified cookies sample.

2. Materials and methods

2.1. Materials

Freshly harvested West Africa Okra (*Abelmoschus caillei*) leaves were obtained from Igbo-Ora, Oyo state, Nigeria. Wheat flour, baking powder, sugar, egg and flavours were gotten from a supermarket, Ile-Ife, Osun State, Nigeria. The preparation of the fortified cookies took place at Food, Nutrition and Dietetics Laboratory in the Department of Family, Nutrition and Consumer Science, Faculty of Agriculture University.

Tools and Equipment: All the tools and equipment that were used to prepare the fortified cookies include; trays, bucket, weighing scale, oven, mesh (60um and 250um size), chopping board, rolling pin and knife, cookie cutter, spoon, bowl, plate.

Preparation of West Africa okra (*Abelmoschus caillei*) flour: The West African okra (*Abelmoschus caillei*) leaves were selected and rinsed with clean running water for optimal removal of dirt and to reduce contamination, physically damaged leaves were manually removed; the leaves were gently spread out on a clean tray. The leaves were dried at room temperature for five days until total dryness was observed, the dried Okra leaves were ground with an electric blender, and the flour was sieved to obtain uniform particle size using a 0.315 mm mesh screen.

Preparation of cookies with 100% wheat flour: Ingredients used: 300g of wheat flour, 150g of margarine, 17g of baking powder, 3 tsp. of vanilla flavour, 123g of icing sugar, 17g of powdered milk, 5g of preservative, 130g of egg.

The dry ingredients such as wheat flour, okra leaf flour and icing sugar were sieved before measurements. The wheat flour (300g) and other dry ingredients were weighed accurately. The pre-weighed dry ingredients were mixed together. Margarine and icing sugar were mixed till fluffy and whisked eggs were added accurately. All the ingredients were beaten together with a wooden spoon. The dough formed was transferred to the chopping board; a small amount of flour was sprinkled on the chopping board to prevent the dough from sticking to the chopping board and rolling pin during spreading. The rolled-out dough was cut out using a circular cookie cutter. The cookies were placed on the oven tray which was greased, lined with baking paper and transferred to the oven. The cookies produced were baked at 180°C. The oven was preheated for 15 minutes before needed.

Preparation of fortified cookies with 83% wheat flour and 17% Okra flour: Ingredients used: 250g of wheat flour, 50g of Okra leaf flour,150g of margarine, 17g of baking powder, 3 tsp. of vanilla flavour, 123g of icing

sugar, 17g of powdered milk, 5g of preservative, 130g of egg.

The dry ingredients such as wheat flour, okra leaf flour and icing sugar were sieved before measurements to prevent a reduction in size. The 250g of wheat flour, 50g of okra leaf flour and other dry ingredients were weighed accurately. The pre-weighed dry ingredients were mixed together. Margarine and icing sugar were mixed till fluffy and whisked eggs were added accurately, followed by the remaining ingredients. All the ingredients were beaten together with a wooden spoon. The dough formed was transferred to the chopping board, small amount of flour was sprinkled on the chopping board to prevent the dough from sticking to the chopping board and rolling pin during spreading. The rolled-out dough was cut out using a circular cookie cutter. The cookies were placed on the oven trays lined with baking paper and transferred to the oven. The cookies produced were baked at 180°C. The oven will be preheated for 15 minutes before needed.

Preparation of fortified cookies with 67% wheat flour and 33% Okra flour: Ingredients used: 200g of wheat flour, 100g of Okra flour, 150g of margarine, 17g of baking powder, 3 tsp. of vanilla flavour, 123g of icing sugar, 17g of powdered milk, 5g of preservative, 130g of egg.

The dry ingredients such as wheat flour, okra flour and icing sugar were sieved before measurements to prevent a reduction in size. The 200g of wheat flour, 100g of okra flour and other dry ingredients were weighed accurately. The pre-weighed dry ingredients were mixed together. Margarine and icing sugar were mixed till fluffy and whisked eggs were added accurately, followed by the remaining ingredients. All the ingredients were beaten together with a wooden spoon. The dough formed was transferred to the chopping board, a small amount of flour was sprinkled on the chopping board to prevent the dough from sticking to the chopping board and rolling pin during spreading. The rolled-out dough was cut out using a circular cookie cutter. The cookies were placed on the oven trays which were lined with baking paper and transferred to the oven. The cookies produced were baked at 180°C. The oven was preheated for 15 minutes before needed.

Preparation of fortified cookies with 50% wheat flour and 50% of Okra flour: Ingredients used: 150g of wheat flour, 150g of Okra flour, 150g of margarine, 17g of baking powder, 3 tsp of vanilla flavour, 123g of icing sugar, 17g of powdered milk, 5g of preservative, 130g of egg.

The dry ingredients such as wheat flour, okra flour and icing sugar were sieved before measurements to prevent a reduction in size. The 150g of wheat flour, 150g of okra flour and other dry ingredients were weighed accurately. The pre-weighed dry ingredients were mixed together. Margarine and icing sugar were mixed till fluffy and whisked eggs were added accurately, followed by the remaining ingredients. All the ingredients were beaten together with a wooden spoon. The dough formed was transferred to the chopping board, and a small amount of flour was sprinkled on the chopping board to prevent the dough from sticking to the chopping board and rolling pin during spreading. The rolled-out dough was cut out using a circular cookie cutter. The cookies were placed on the oven trays which were lined with baking paper and transferred to the oven. The cookies produced were baked at 180°C. The oven was preheated for 15 minutes before needed.

Sample Formulation: Three flour blends from Okra leaves flour and wheat flour were used for the production of fortified cookies. The Okra leaf flour and wheat flour were mixed in the ratio 50:50, 33:67 and 17:83. For the purpose of this research, the first sample was made with 100% wheat flour and it served as the control sample (Table 1).

Table1: Mixing proportion of the flours

	WOC 1	WOC 2	WOC 3	WOC 4
Wheat	300g	250g	200g	150g
Okra leaf flour	0	50g	100	150g

Note: WOC 1: 100% Wheat Flour

WOC 2: 17% Okra leaf flour, 83% wheat flour

WOC 3: 33% Okra leaf flour, 67% wheat flour

WOC 4: 50% Okra leaf flour, 50% wheat flour

Test of Quality: This test of quality for the sample was done through sensory evaluation, nutritional, pH and statistical analysis.

Sensory Analysis Test: Sensory characteristics of all the cookie samples (WOC 1, WOC 2, WOC 3, and WOC 4) were evaluated for different sensory attributes through sensory evaluation. The evaluation consisted of one hundred (100) semi-trained panelists chosen at random from among the Obafemi Awolowo University students and staff who are women of reproductive age. Each sample was randomly coded and given to the panelists (Amerine et al., 2013). Subjective analysis (color, flavor, tastes, texture, and overall acceptability) of samples was done on a 9-point hedonic scale. The hedonic scale was in the following sequence: 9= like extremely, 8= like very much, 7= like moderately, 6= like slightly, 5= Neither like nor dislike, 4= Dislike slightly, 3=dislike moderately, 2= dislike very much and 1 dislike extremely. The sensory analysis test was conducted in Food Nutrition and Dietetics Laboratory in the Department of Family, Nutrition and Consumer Science, Faculty of Agriculture University.The panelists were instructed to rinse their mouths thoroughly with water after testing any of the samples before proceeding to the next samples from interfering with one another.

Proximate Analysis: The proximate analysis was used to determine the following contents in the cookies sample. This analysis was done at Anatomy Laboratory, Obafemi Awolowo University.

i. Moisture content determination
ii. Crude protein content determination
iii. Ash content determination
iv. Crude fiber determination
v. Fat content determination
vi. Carbohydrate content determination

Moisture content determination

The moisture content of the cookies sample was determined according to the standard method of AOAC (2006). 2g of sample was weighed into a crucible which is made up of platinum or ceramic. It was transferred into a hot-air oven and dried overnight at 70° Celsius. It was removed from the hot-air over and allowed to cool in a desiccator. The final weight of the sample was taken with the aid of a Mettler-Toledo analytical balance.

Calculation:

$$\% \text{ Moisture} = \frac{\text{Initial wright (g) - Final weight (g)}}{\text{Weight of sample taken (g)}} \times 100$$

Crude protein content determination

The protein content of the samples was determined according to the standard method of AOAC (2006). Using the Kjeldahl method, comprised three steps which are digestion, distillation and titration.

Digestion of sample: the sample (0.5g) was weighed into a kjeldahl flask. A little scoop of digestion catalyst/ mixture which consists of 50g of Anhydrous CuSO4 (blue), 500g of Anhydrous Na2SO4 and 0.5g of Mercy Oxide (HgO) was added. 20ml of concentrated H2SO4 was added carefully and the flask was transferred to the Kjeldahl digestion system (Tecator digestion system 1007 digester), the mixture was heated or digested for 2 hours. The sample was cooled and distilled water was added to make the volume up to the 50ml mark

Distillation: 50ml of 2% Boric acid was measured into a 200ml conical flask. The conical flask was placed under the receiving tube of the distillation; below the level of the Boric acid.20ml of the digested sample was measured into the Kjeldahl digestion flask. 50ml of 40% NaOH was added into the Kjeldahl flask which contains the sample without shaking the flask.

Titration: The distillate was titrated with standard HCl, a known concentrated amount of HCl e.g. 0.1N HCl, 0.097N HCl etc. until the endpoint is reached.

Calculation:

$$\textbf{\%N} = \frac{\text{Titer value} \times \text{Concentrated of Acid} \times 0.014 \times \text{Dilution factor.}}{\text{Weight of the sample taken}} \times 100$$

$$\text{Dilution factor} = \frac{\text{Volume of the digested sample.}}{\text{The volume of a sample taken}}$$

%Crude protein = %N × 6.25

Ash content determination

The ash content of the products was determined according to the standard method of AOAC (2006). 2g of the sample was weighed into a crucible which is made of platinum or ceramic. The weight of the empty crucible was noted before taking the weight of the sample. The crucible containing the sample was transferred into a

muffle furnace and Ash at 600°C for 3 hours. It was removed and allowed to cool in a desiccator. The final weight was taken with the aid of a Mettler-Toledo analytical balance.

Calculation:

$$\%Ash = \frac{[\text{weight of empty crucible + Ash (g)}] - \text{the weight of empty crucible (g)}}{\text{Weight of sample taken (g)}} \times 100$$

Crude fiber determination

The crude fiber content of the sample was determined using the standard method of AOAC (2006). 2g of the sample was weighed into a 600ml beaker made of Pyrex. 200ml of 1.25% H2SO4 was added. It was boiled and refluxed for 30 minutes. It was filtered with a sieving cloth and rinsed with hot distilled water to remove the acid; the sample was transferred into a beaker. 200mls of 1.25% NaOH will be added, the sample was boiled and refluxed on a hot plate for 30 minutes. It was transferred into the hot-air oven and dried overnight at 70°C. It was removed and allowed to cool in a desiccator. The weight of the sample was taken with the aid of a Mettler-Toledo analytical balance and transferred into a muffle furnace for ignition; it was ashed at 600°C for 3 hours. It was removed and allowed to cool in a desiccator and the final weight was taken with the aid of Mettler-Toledo analytical balance.

Calculation:

$$\%Crude\ Fiber = \frac{\text{Initial weight (g) - Final weight (g).}}{\text{Weight of sample taken (g)}} \times 100$$

Fat content determination: The crude fiber content of the sample was determined using the standard method of AOAC (2006). The fat content is also referred to as ether extract, oil or lipid. The fat content was determined by the Soxhlet extraction method. 2g of the sample was weighed into a fat-free extracting thimble, the sample was placed in the thimble in the Soxhlet extractor and 125ml of normal Hexane was added until it siphons over once. The condenser was replaced and it was placed in a water bath after ensuring all the joints are tight. The condenser was connected to the tap water for 3 hours. The control on the water bath was adjusted to 50°C so that the N-Hexane boils gently and was left to siphon over for 3 hours, when N-Hexane stops siphoning, the thimble was removed and the solvent (N-Hexane) was recorded. The flask (which contained all the oil) was detached and the exterior was cleaned and dried in the hot air oven to a constant weight at 70°C overnight. The final weight of the flask and oil was taken with the aid of a Mettler-Toledo analytical balance.

Calculation:

$$\%\text{Fat content} = \frac{\text{Weight of flask + oil (g) - weight of the flask (g).}}{\text{Weight of the sample taken}} \times 100$$

% Fat content is also referred to as % oil, and % ether extract or % lipid

Carbohydrate content determination

The carbohydrate content of the sample was determined by the difference in the percentage of moisture, Ash, protein, fat and crude fiber according to the standard method of AOAC (2006). As follows:

% Nitrogen Free Extract =100- (% moisture + % ash + % protein + % fat + % crude fiber) while,

Total Carbohydrate = (%NFE + %Crude fiber)

Determination of mineral elements: The mineral content of the sample was determined using the standard method of AOAC (2006). The sample was ashed at 550^0c. The ash was boiled with 10ml of 20% hydrochloric acid in a beaker and then filter into a 100ml standard flask. This is made up to the mark with deionized water. The minerals were determined from 5g of the resulting solution. Iron (Fe), Zinc (Zn) and Folate (B9) were determined using Atomic Absorption Spectrophotometer.

Determination of pH: 5g of each sample was blended and dissolved in 50 ml of distilled water. It was allowed to rest for 5 minutes. The pH meter was used to measure the pH of each sample.

2.2. Statistical Analysis

All experiments were conducted in triplicate. Data obtained was analyzed using the software, IBM, SPSS Statistics, version 24. The data were presented as mean standard deviation, and one-way analysis of variance (ANOVA) was used to assess for statistical significance; a p-value of < 0.05 was regarded as significant.

3. Results and discussion

Sensory Evaluation of smoothie's samples

Table 2 shows that sample WOC 2 (5.56 ± 1.30^b) was rated highest in terms of appearance, followed by sample WOC 3 (5.30 ± 1.93^c) and sample WOC 4 (4.08 ± 2.09^d) was rated lowest in term of appearance apart from the control sample WOC 1 (8.02 ± 1.12^a). Table 2 shows that sample WOC 2 (5.28 ± 2.04^b) was rated highest in terms of colour, next to the control sample WOC 1 (8.08 ± 0.90^a) followed by WOC 3 (5.18 ± 2.22^c) and WOC 4 (4.52 ± 2.21^d) was rated lowest in term of colour. Table 2 shows that WOC 2 was rated highest ($5.58 \pm .1.75^b$) in terms of taste, followed by WOC 3 (4.90 ± 1.62^c) and WOC 4 (3.38 ± 1.64^d) was rated lowest in terms of taste apart from the control sample WOC 1 (7.90 ± 1.02^a). Table 2 shows that WOC 2 was rated highest in terms of texture (5.92 ± 1.30^b), next to the control sample WOC 1 (7.52 ± 1.30^a) followed by WOC 3 (5.28 ± 1.65^c) and WOC 4 (4.45 ± 1.86^d) was rated lowest in term of texture. WOC 2 was rated highest in terms of flavour (6.00 ± 1.79^b), followed by WOC 3 (5.52 ± 1.90^c) and WOC 4 (3.58 ± 1.92^d) was rated lowest in terms of flavour apart from the control sample WOC 1 (8.04 ± 0.78^a). Sample 1 which is the control, WOC 2 was rated highest in terms of overall acceptability (5.68 ± 1.71^b) next to the control sample WOC 1 (8.10 ± 0.82^a)

followed by WOC 3 (4.98 ± 1.76^{c}) and WOC 4 (3.66 ± 1.97^{d}) was rated lowest in term of overall acceptability.

Table 2: The sensory properties of cookies produced from composite flour of okra leaf flour and wheat flour blend.

Sample	Appearance	Colour	Taste	Texture	Flavour	Overall Acceptability
WOC 1	8.02 ± 1.12^{a}	8.08 ± 0.90^{a}	7.90 ± 1.02^{a}	7.52 ± 1.30^{a}	8.04 ± 0.78^{a}	8.10 ± 0.82^{a}
WOC 2	5.56 ± 1.97^{b}	5.28 ± 2.04^{b}	5.58 ± 1.75^{b}	5.92 ± 1.79^{b}	6.00 ± 1.80^{b}	5.68 ± 1.71^{b}
WOC 3	5.30 ± 1.93^{c}	5.18 ± 2.22^{c}	4.90 ± 1.62^{c}	5.28 ± 1.65^{c}	5.52 ± 1.90^{c}	4.98 ± 1.76^{c}
WOC 4	4.08 ± 2.09^{d}	4.54 ± 2.21^{d}	3.38 ± 1.64^{d}	4.44 ± 1.89^{d}	3.58 ± 1.92^{d}	3.66 ± 1.97^{d}

The significant difference is < 0.05

WOC 1: 100% Wheat Flour (control)

WOC 2: 17% Okra leaf flour, 83% wheat flour

WOC 3: 33% Okra leaf flour, 67% wheat flour

WOC 4: 50% Okra leaf flour, 50% wheat flour

Proximate composition of cookies produced from composite flour of okra leaf flour and wheat flour blend.

According to the results in Table 3, WOC 1 (10.03 ± 0.014^{a}) was rated highest in terms of moisture content, next was WOC 4 (9.86 ± 0.028^{b}), followed by WOC 3 (9.805 ± 0.021^{c}), and WOC 2 (8.67 ± 0.042^{d}) was rated lowest in term of moisture content. Table 3 shows that WOC 4 (4.76 ± 0.028^{a}) was rated highest in terms of ash content, next was WOC 3 (3.55 ± 0.028^{b}), followed by WOC 2 (2.575 ± 0.035^{c}), and WOC 1 (1.55 ± 0.042^{d}) was rated lowest in term of Ash content. Table 3 also shows that WOC 4 (2.155 ± 0.035^{a}) was rated highest in terms of fiber content, next, was WOC 3 (1.05 ± 0.029^{b}), followed by WOC 2 (0.315 ± 0.021^{c}), and WOC 1 (0.016 ± 0.01^{d}) was rated lowest in term of crude fiber content. Table 3 shows that WOC 3 was rated highest in terms of ether content (20.99 ± 0.035^{a}), next was WOC 4 (20.88 ± 0.028^{b}), followed by WOC 2 (20.05 ± 0.021^{c}), and WOC 1 (17.90 ± 0.021^{d}) was rated lowest in term of ether extract contents. According to the results in Table 3, WOC 4 (14.89 ± 0.014^{a}) was rated highest in terms of protein content, next was WOC 3 (14.24 ± 0.021^{b}), followed by WOC 1 (13.800 ± 0.028^{c}), and WOC 2 was rated lowest (13.05 ± 0.035^{d}) in term of crude protein content. Table 3, also shows that WOC 1 (56.71 ± 0.02^{a}) was rated highest in terms of Carbohydrate content, next was WOC 2 (55.616 ± 0.03^{b}), followed by WOC 3 (50.37 ± 0.01^{c}), and WOC 4 was rated lowest (47.47 ± 0.05^{d}) in term of Carbohydrate content.

Table 3: Proximate analysis of cookies produced from okra leaf flour and wheat flour blend.

Sample	Moisture	Ash	Crude Fiber	Fat	Crude Protein	Carbohydrate
WOC 1	10.2 ± 0.02^{a}	1.55 ± 0.43^{d}	0.016 ± 0.01^{d}	17.89 ± 0.02^{d}	13.80 ± 0.028^{c}	56.71 ± 0.02^{a}
WOC 2	8.67 ± 0.04^{d}	2.58 ± 0.04^{c}	0.032 ± 0.02^{c}	20.06 ± 0.02^{c}	13.05 ± 0.021^{d}	55.616 ± 0.03^{b}
WOC 3	9.80 ± 0.02^{c}	3.55 ± 0.03^{b}	1.050 ± 0.03^{b}	20.99 ± 0.04^{a}	14.24 ± 0.029^{b}	50.37 ± 0.01^{c}
WOC 4	9.85 ± 0.03^{b}	4.76 ± 0.03^{a}	2.155 ± 0.04^{a}	20.88 ± 0.03^{b}	14.89 ± 0.035^{a}	47.47 ± 0.05^{d}

The significant difference is < 0.05

WOC 1: 100% Wheat Flour (control)

WOC 2: 17% Okra leaf flour, 83% wheat flour

WOC 3: 33% Okra leaf flour, 67% wheat flour

WOC 4: 50% Okra leaf flour, 50% wheat flour

Mineral Composition

Table 4 shows that WOC 4 (10.20 ± 0.0012^{a}) was rated highest in terms of Zinc composition, followed by WOC 2 (8.20 ± 0.001^{b}), next was WOC 3 (8.00 ± 0.001^{c}) and WOC 1 (6.40 ± 0.009^{d}) was rated the lowest. Table 4 shows that WOC 4 was rated highest (87.00 ± 0.0015^{a}) in Iron composition, followed by WOC 2 (62.00 ± 0.0012^{b}), next was WOC 3 (60.00 ± 0.0011^{c}) and WOC 1 (0.68 ± 0.058^{d}) was rated the lowest. Table 4 shows that WOC 4 (19.64 ± 0.179^{a}) was rated highest in terms of Folate composition, next was WOC 3 (13.87 ± 0.058^{b}), followed by WOC 2 (12.75 ± 0.267^{c}) and sample WOC 1 (54.00 ± 0.0010^{d}) was rated the lowest.

Table 4 : Minerals and vitamin analysis of cookies produced from okra leaf flour and wheat flour blend.

Sample	Zinc (mg/l)	Iron (mg/l)	Folate (µg/g)
WOC 1	6.40 ± 0.009^{d}	54.00± 0.0010^{d}	0.68 ± 0.058^{d}
WOC 2	8.20 ± 0.001^{b}	62.00 ±0.0012^{b}	12.75 ± 0.267^{c}
WOC 3	8.00 ± 0.0010^{c}	60.00 ±0.0011^{c}	13.87 ± 0.058^{b}
WOC 4	10.20 ± 0.0012^{a}	87.00± 0.0015^{a}	19.64 ± 0.179^{a}

The significant difference is < 0.05

WOC 1: 100% Wheat Flour (control)

WOC 2: 17% Okra leaf flour, 83% wheat flour

WOC 3: 33% Okra leaf flour, 67% wheat flour

WOC 4: 50% Okra leaf flour, 50% wheat flour

pH of cookies produced from okra leaf flour and wheat flour blend

The results in Table 5, shows that WOC 1 was rated highest (8.28 ± 0.014^{a}) in terms of alkaline content, followed by WOC 2 (8.00 ± 0.014^{b}). Sample WOC 3 (7.67 ± 0.007^{c}) and WOC 4 (7.81 ± 0.008^{d}) was rated less alkaline and almost neutral in term of pH content in the samples.

Table 5: pH of cookies produced from okra leaf flour and wheat flour blend**.**

Sample	pH
WOC 1	8.28 ± 0.014^{a}
WOC 2	8.00 ± 0.014^{b}
WOC 3	7.67 ± 0.007^{c}
WOC 4	7.81 ± 0.008^{d}

The significant difference is < 0.05

WOC 1: 100% Wheat Flour (control)

WOC 2: 17% Okra leaf flour, 83% wheat flour

WOC 3: 33% Okra leaf flour, 67% wheat flour

WOC 4: 50% Okra leaf flour, 50% wheat flour

4. Discussion

Moisture content plays a critical role in food quality and safety, it can affect a range of attributes, including shelf life, texture, flavor, and microbial growth. Food safety is achievable when the moisture content is regulated (Eskin and Labuza, 2017). The lower moisture content of (17% okra leaf flour, 83% wheat flour) results in better shelf stability. The considerable low moisture in the samples is an indication of good keeping quality of the products when properly packaged and stored since high moisture content in food has been shown to encourage microbial growth (Alawode, *et al*., 2017).

Higher folate content shows that sample 4 was preferable for women of reproductive age because it promotes access to a sufficient amount of folate. The high quantity of folate included in the okra is helpful for the fetus while pregnant (Agbangnan, et al., 2018). Foliate is a vital nutrient that increases the growth and development of the fetus's brain. The high quantity of folic acid within okra performs a huge role in the neural tube formation of the fetus through the fourth to the 12th week of pregnancy (Zaharuddin *et al*., 2014)

Higher Iron and zinc content shows that sample 4 (50% okra leaf flour, 50% wheat flour) is preferable for women of reproductive age because it promotes access to a sufficient amount of Zinc and Iron composition. Cookies contribute valuable quantities of iron, calcium, protein, calorie, fiber and some of the B vitamins to our diet and daily food requirement, cookies are important baked products in the human diet, which are usually consumed with a beverage and also used as weaning foods for infants (Morsy & Sohaimy, 2011). Potassium, Sodium, Magnesium and Calcium are the principal elements in pods, which contain about 17% seeds. The presence of Iron, Zink, Manganese and Nickel also has been reported (Moyin-Jesu, 2017)

This study reveals that cookie made from 83% wheat flour and 16% okra leaf flour is mostly preferred in term of sensory properties as it was ranked the highest in term of appearance, taste, texture, flavour and overall acceptability. Finally, it is also suitable for consumption by women of reproductive age and individuals seeking a snack rich in folate, zinc and Iron.

5. Conclusion

The study has shown that out of all the samples produced, the sample with 17% okra leaf flour, and 83% wheat flour was the most accepted product in terms of appearance, colour, taste, texture, flavour and general acceptability, apart from the control sample. The cookie also contains essential nutrients which are associated with women of reproductive age, 50% okra leaf flour, 50% wheat flour blend has the highest amount of nutrient composition followed by 17% okra leaf flour, 83% wheat flour, 33% okra leaf flour, 67% wheat flour and 100% wheat flour which are also highly accepted and they all have nutritional benefits, these cookies are produced with ingredients which are locally available and can be easily prepared.

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Impact of *Acha* Flour and Starch on the Microbiological, Physicochemical and Sensory Properties of Soymilk

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Abstract

Plant milk is becoming popular and acceptable all over the world for health, religious and economic reasons. Plant milk produced from soymilk was substituted with *acha* flour and *acha* starch to increase its energy content and also serve as nutritive thickener. The microbiological characteristics, total sugar, pH and TTA were determined during refrigerated storage at 0, 7, 14 and 21 days. Viscosity and sensory properties were also evaluated using standard methods. The TVC, LAB and fungi counts were between 2.71 – 9.17, 3.99 – 9.25 and 2.77 – 4.40 log CFU. ml^{-1} respectively. *Bacillus subtilis, Bacillus licheniformis, Leuconostoc mesenterioides, Lactobacillus plantarum*, *Saccharomyces cerevisiae* and *Candida tropicalis* were isolated during storage. Microorganisms isolated from the products during storage were not pathogenic and the microbial count was within the permissible limit from 0 – 7 days according to WHO standards. The total sugar content was in the range of 12.98 to 16.56 $mg.m^{-1}$ and the pH range was between 3.96 – 6.98. The sensory analysis shows that the addition of 10g and 20g did not have a significant effect on the colour, taste, aroma and overall acceptability of soymilk showing that *acha* flour can be added as a thickener to soymilk thereby advancing the use of *acha* in food processing.

Keywords: *Acha*, starch, soymilk, sensory, viscosity, microbial.

1. Introduction

Soybean (*Glycine max*) is a rich leguminous crop that provides adequate quantity of protein, minerals, fat, vitamins and minerals. The protein contains all essential amino acids and the fat is also rich in polyunsaturated fatty acids (Borode, 2017). Plant milk and milk products are gaining more attention, especially in the Tropics due to the high cost of animal products, vegetarianism and health purpose. Production of milk from legumes such as soybean increases the nutritional status of the world population and prevents nutritional-related diseases (Malomo and Abiose, 2020).

Soymilk is an extract of soybean which is consumed globally as an alternative source of protein for low-income earners and vegetarians. It is a cheaper and highly accessible source of protein with a lot of health benefits. It is the most popular alternative to cow milk because of its similar protein profile and lower calories (Saini and Morya, 2021). It is produced by soaking soybean, dehulling, milling, sieving and boiling the filtrate obtained. It has a cream appearance and is acceptable among all age groups because of its unique taste and its nutritional composition which is close to dairy milk (Borode, 2017; Malomo, 2023). Breakfast cereals are consumed all over the world because of their nutritional and health benefit. They are mixed with nuts, legumes and nutritious fluids such as cow milk, soymilk or their derivatives (Johnsen *et al.*, 2015; Ng and Loh, 2018).

Soymilk usually has a thin consistency which could be improved by adding a thickening agent (Wang *et al.,* 2001; Gatade *et al*, 2014). Sodium carboxymethyl cellulose (Na-CMC) is usually added to food such as ice cream and liquid foods to improve organoleptic and rheological properties that will make food more acceptable to consumers. The addition of CMC to soymilk has been reported to increase its sensory quality (Gatade, *et al*., 2014). Stabilizers are hydrocolloids used as thickening or bulking agents in food to improve and retain the rheological and organoleptic properties of food. They may naturally be obtained from plants, animals and microorganisms. They are added to food to improve the texture, and viscosity, prevent crystallization, and syneresis and maintain homogeneity (Bhattarai *et al.,* 2015; Macit and Bakirci, 2017; Rashidi and Dutta, 2020). Gelatin and carboxymethyl cellulose have been the most common thickener in yoghurt but in recent times attention has been drawn to the substitution of synthetic stabilizers with those obtained from natural sources especially from plant sources so that vegetable yoghurt will also be accessible to vegetarians (Sameen *et al*., 2017). There is an increase in the use of starch as a thickener due to its source, availability, affordability and desirable properties. Starch from cassava, rice, corn and potato (Aygemang, 2020, Eze *et al*., 2021) has been used in soy products but there is a dearth of information on the use of *acha* starch and flour as thickeners in beverages.

Acha (*Digitaria exilis*), a cereal of West African origin grown in Nigeria, Benin and Togo contain resistant starch that aids in the treatment and prevention of prediabetics and type 2 diabetes. Its starch has been reported to have a slow rate of retrogradation, low per cent crystallinity, low gelatinization temperatures and enthalpy which will make it a suitable stabilizer (Jideani and Jideani, 2011). This cereal matures within six to eight weeks after planting and compares favourably with millet, rice and maize in terms of nutrients. It is a non-glutenous cereal which lacks glutenin or gliadin proteins, a causative agent of celiac disease, making it suitable for people living with a condition known as gluten intolerance (Ayo *et al.*, 2014; Malomo *et al*., 2018).

Date fruit (*Balanites aegyptiaca*) is an energy-dense fruit rich in carbohydrates, including simple sugars such as glucose, fructose and sucrose, which make up about two-thirds of its flesh. It also contains protein, fat, minerals, vitamins especially B vitamins, tannin and fibre (Vayalil, 2012, Odeh, 2014; Amadou *et al*., 2012). The

fruit is traditionally used as a sweetener and a quick source of calories. Extract from the leaves, stem and root has been used as medicine in Asia and Africa for decades (Amadou *et al*., 2012). This research aims to study the effect of *acha* flour and starch on the microbial population and physicochemical properties of soymilk during storage at refrigeration temperature.

2. Materials and Methods

Soybean and date fruits were obtained from Oja tuntun Ile – Ife and *acha* was obtained from Zaria in Kaduna State, Nigeria. All chemicals and media used for this research were of analytical grade.

Preparation of *acha* flour and starch

Acha flour was produced by winnowing, washing, and drying in an oven at 45°C for 18 h, milling and sieving in a sieve with a mesh diameter of 0.5 mm. Starch was produced from *acha* by winnowing, washing and soaking in water at room temperature (28 °C) for 24 h. The water was drained, washed with clean water and ground into a smooth slurry in a domestic blender. The slurry obtained was suspended in distilled water and centrifuged at 4500 r.p.m for 30 mins. The supernatant was discarded and the starch obtained was thinly spread on a tray and dried at room temperature (Modified method of Shehu and Oshodi, 2016).

Preparation of soy-*acha* milk

Soybean was sorted, washed and steeped in portable water for 12 h at room temperature. The water was drained, rinsed with potable water and blanched for 20 min in water before dehulling. The dehulled beans was milled, homogenized with water, and sieved with muslin cloth and the residue was discarded. The milk obtained was divided into five portions. *Acha* flour (10g and 20g), and *acha* starch (10g and 20g) were added and the sample without treatment served as the control (Table 1). The samples were boiled separately for 30 mins, cooled to room temperature and dispensed into sterile bottles for further analysis (Malomo and Abiose, 2020).

Table 1 Sample preparation

Sample	Milk (ml)	*Acha*	Date fruit (g)
A	1000	Nill	10
B	1000	10 g of Flour	10
C	1000	20 g of Flour	10
D	1000	10 g of Starch	10
E	1000	20 g of Starch	10

Enumeration of microorganisms

Each sample (5 g) was homogenized with sterile peptone water (45 ml) in a Colworth Stomacher and serially diluted appropriately. Appropriate dilution was dispensed into a sterile petri dish using a sterile pipette, 20 ml of molten agar was poured and gently swirled to ensure an even distribution of growth. Nutrient Agar was poured for total viable count (TVC), Potato Dextrose Agar for fungi count and de Man Rogosa and Sharpe for lactic acid bacteria (LAB) count. Plates were incubated in Gallenkamp incubator at 35 ± 2 °C for 24 h, 25 ± 2 °C for 72 to 105 h and 35 ± 2 °C for 72 h respectively (Harrigan, 1998). After incubation, the colonies on the plates were counted using a colony counter and recorded in colony forming units per ml (log CFU/ml). Pure

isolate was obtained by streaking on media of primary isolation and the pure isolate was streaked on an agar slant and kept in the refrigerator at 4 ± 2 °C for identification (Malomo, 2023).

Bacteria isolates were identified using cultural characteristics, morphological characteristics and biochemical tests. Yeast isolated were identified using the colony characteristics, size of the cell, mode of reproduction, arrangement of cell, carbon and nitrate assimilation (Barnett *et al.,* 2000).

Determination of total sugar

Each sample was diluted appropriately and filtered through Whatman 1 filter paper. The filtrate (1 ml) was dispensed into a test tube, 4 ml of anthrone reagent was added, the mixture was boiled for 10 min in water bath (Gallenkomp, HH-S6, England) and rapidly cooled. Absorbance was read against reagent blank in the UV-spectrophotometer at 620nm (Spectrumlab 752S, YM1206PHB2, China) and the amount of sugar liberated was obtained from the standard curve based on known concentrations of glucose (10-100mg/l) (Malomo *et al*., 2019).

Determination of pH

The pH of soy-*acha* milk was determined using pH meter standardized with buffer 4 and 7. The pH electrode was inserted into each sample and the values displayed were recorded as the pH (AOAC, 2005).

Determination of titratable acidity

Each soy-*acha* sample (10 ml) was dispensed in a 200 ml conical flask and homogenized with 20 ml of distilled water. The mixture (10 ml) was measured into a conical flask, and three drops of phenolphthalein indicator were added and titrated against 0.1 N NaOH (AOAC, 2005).

Viscosity

The viscosity of the soy-*acha* milk samples was determined in a Brookfield viscometer (Model: LVDV-II pro) at 25°C. Spindle no. 1 was used at a speed of 100 rpm and readings were taken in duplicate (Ranganna, 2000).

Sensory evaluation

Panelists who are familiar with soymilk were presented with coded samples of soy-*acha* milk samples to evaluate for colour, mouthfeel, taste, thickness, aroma and overall acceptability using 9 -point Hedonic scale where the lowest score was 1 and the highest was 9. Samples were analyzed under the same conditions and the panelists were allowed to clean their palates with water after tasting each sample (Yangilar and Yildiz, 2017).

3. Results and Discussion

The microbiological population of soy-*acha* milk during storage

The results of TVC, LAB count and fungi count are shown in Table 2. TVC of soy-*acha* milk ranged between 2.71 and 9.17 log CFU.ml^{-1}. It was highest in the soymilk sample containing 20% starch and lowest in 100% soymilk at the beginning of storage. There was a general increase during storage due to the availability of nutrients for microbial growth and reproduction. Samples containing *acha* flour and starch generally had higher counts than 100% soybean probably due to an increase in carbohydrate content. The count was significantly

higher in soymilk plus *acha* starch than in *acha* flour at day 0. The higher the concentration of both *acha* starch and flour, the higher the TVC. This could be due to an increase in the availability of carbon for the metabolism of microorganisms. It has been reported that *acha* is rich in simple and complex carbohydrates which can be broken down into sources of carbon for microorganisms (Jideani and Jideani, 2011). The TVC was lower than the 5.294 log CFU.ml^{-1} recorded by Kohli *et al*. (2017). The total viable count was within the permissible limit in all samples from day o to day 7 according to CODEX (2011). The lactic acid bacteria count also increased with an increase in the days of storage (3.99 – 9.25 log CFU.ml^{-1}). It was also higher in samples containing *acha* and flour than 100% soymilk from 0 to 7th day of storage. The count was higher in samples containing 20% *acha* flour than 10% and the same observation was recorded in samples containing *acha* starch. Samples containing 20% starch had the highest count. This could be a result of a higher carbon source for lactic acid bacteria.

The fungi count of soy-*acha* milk was in the range of 2.61 and 4.40 log CFU/ml. It was lower in 100% soymilk (2.61 – 3.82 log CFU/ml) than in samples containing *acha* flour and starch. The higher count observed in samples containing *acha* can be attributed to the high carbohydrate content which is converted to organic acid by lactic acid bacteria thereby reducing the pH and making the environment conducive for fungi growth. Some authors also reported the symbiotic relationship between lactic acid bacteria and yeast in food where the bacteria produce acid for the yeast and yeast produces amino acid needed by the bacteria (Malomo *et al*., 2018).

Table 2 Microbiological population of soy-*acha* milk during storage (log CFU.ml^{-1})

Samples	Days of storage			
	0	7	14	21
		TVC		
A	2.71±0.11^{a}	3.25±0.02^{c}	8.53±0.09^{d}	9.08±0.14^{b}
B	3.00±0.10^{b}	3.31±0.06^{b}	8.37±0.12^{c}	7.72±0.11^{d}
C	4.04±0.03^{c}	3.45±0.11^{a}	8.80±0.10^{b}	9.17±0.21^{a}
D	3.84±0.04^{d}	3.47±0.13^{a}	8.85±0.05^{b}	7.64±0.10^{e}
E	4.85±0.02^{e}	3.47±0.20^{a}	8.98±0.16^{a}	8.00±0.05^{c}
		LAB Count		
A	3.99±0.02^{d}	4.47±0.05$_{ab}$	8.68±0.50^{d}	9.15±0.02^{b}
B	4.00±0.12^{bc}	4.41±0.07^{b}	8.73±0.21^{c}	8.11±0.04^{d}
C	4.25±0.09^{a}	4.49±0.07^{a}	8.80±0.09^{b}	9.25±0.02^{a}
D	4.08±0.05^{b}	4.41±0.04^{b}	8.88±0.20^{a}	8.20±0.09^{c}
E	3.90±0.09^{d}	4.34±0.03^{c}	8.73±0.18^{c}	8.30±0.14^{c}
		Yeast count		
A	2.61±0.02^{d}	3.27±0.05^{ab}	3.35±0.02^{c}	3.82±0.02^{c}
B	3.00±0.11^{ab}	3.31±0.07^{b}	3.41±0.06^{b}	4.00 ±0.12^{b}
C	3.15±0.10^{a}	3.29±0.07^{a}	3.52±0.10^{a}	4.21±0.05^{b}
D	3.02±0.04^{bc}	3.12±0.04^{b}	3.57±0.12^{a}	4.35±0.11^{a}
E	3.10±0.04^{b}	3.12±0.03^{c}	3.53±0.19^{a}	4.40±0.04^{a}

A: 100% soymilk; B: Soymilk and 10 g acha flour; C: soymilk and 20 g acha flour, D: Soymilk and 10 g acha starch; E: Soymilk and 20 g acha starch. Values are means of three replicates ± standard error. Means followed by different superscript in the same column are significantly different at $p < 0.05$

Microorganisms isolated from soy-*acha* milk

Microorganisms isolated from the soy-*acha* milk during storage were Bacillus species and lactic acid bacteria. *Bacillus subtilis* and *Bacillus licheniformis* were isolated from all soy-*acha* samples throughout the period of storage. These two Bacillus species are thermophilic gram-positive endospore-forming rods (Farinde *et al*., 2016). They produce proteolytic enzymes that break down protein into amino acids and ammonia. The presence of Bacillus species has been reported in food produced from leguminous crops such as soybean and locust beans (Malomo *et al*., 2020). Soymilk plus *acha* flour and starch contains *Lactobacillus plantarum* and *Leuconostoc mesenteroides* in addition to *Bacillus subtilis* and *Bacillus licheniformis*. This could be due to the higher carbohydrate content of *acha* flour and *acha* starch which were broken down into sugar making the environment favourable for lactic acid bacteria. The yeast isolated from the samples were *Saccharomyces cerevisiae* and *Candida tropicalis. Saccharomyces cerevisiae* has been isolated from many beverages by several authors (Owuzu-kwanteng, 2013). Malomo (2023) isolated Bacillus species and lactic acid bacteria from soy-tiger nut yoghurt. Farinde *et al*. (2016) isolated *Lactobacillus plantarum, Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* from soy yoghurt coagulated with maize water.

Total sugar content of soy-*acha* milk

The total sugar content of soy-*acha* milk (Figure 1) decreased with increase in the period of storage (12.98 – 16.56 mg.ml^{-1}). It was generally higher in samples containing *acha* flour (15.55 -15.57 mg/ml) than *acha* starch (15.44 – 15.50 mg.ml^{-1}) and lowest in 100% soymilk (14.88 mg.ml^{-1}) at the beginning of storage. This could be as the result of the low carbohydrate content of soymilk and high carbohydrate content of *acha* being a cereal crop. Sani *et al*. (2019) reported higher total sugar content in soy yoghurt containing tiger nuts because of the higher carbohydrate content of the nut. On day 7, the total sugar generally increased in all samples probably due to increase in the breakdown of carbohydrates into simple sugars by fermenting microorganisms and their enzymes. The total sugar content decreased in all samples from day 7 to day 21 probably because the microorganisms present in the samples utilized the sugar as carbon source (Adepoju *et al*., 2016). This decrease is in agreement with the result obtained by Malomo *et al*. (2021) in ogi produced from *acha*, maize and sorghum.

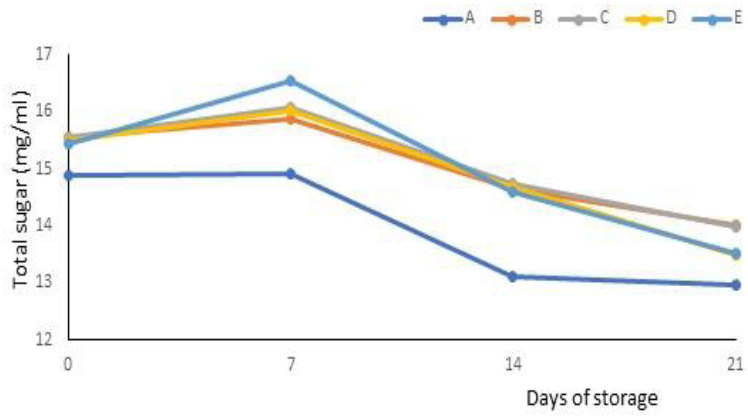

Figure 1 Total sugar content of soy-*acha* milk during storage

A: 100% soymilk; B: Soymilk and 10 g acha flour; C: soymilk and 20 g acha flour, D: Soymilk and 10 g acha starch; E: Soymilk and 20 g acha starch.

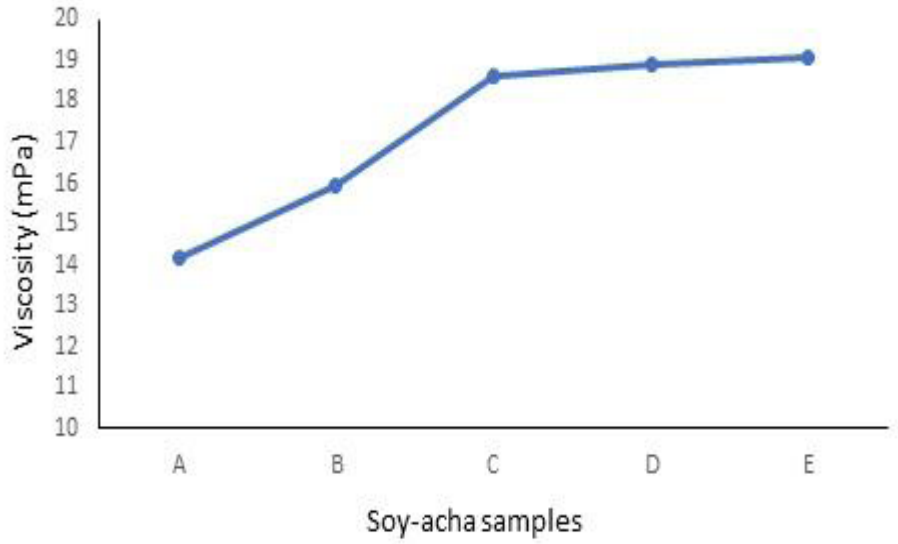

Figure 2 Viscosity of freshly prepared soy-*acha* milk.

A; 100% soymilk; B: Soymilk and 10 g acha flour; C: soymilk and 20 g acha flour, D: Soymilk and 10 g acha starch; E: Soymilk and 20 g acha starch.

The viscosity of soy-*acha* milk is presented in Figure 2. It was within the range of 14.2 to 19.09 mPa during storage. It was lowest in 100% soymilk (14.20 mPa) and highest in soymilk plus 20g of *acha* starch (19.09 mPa). It was also higher in soymilk plus *acha* flour (15.93 – 18.60 mPa) than 100% soymilk. The viscosity of soy-*acha* milk increased with an increase in the concentration of both *acha* starch and *acha* flour. Gatade *et al*. (2014) reported an increase in viscosity with increase in the addition of carboxymethylcellulose (CMC) to soymilk. Increase with the addition of *acha* starch and flour is also in agreement with Eze *et al*. (2021).

pH of soy-*acha* milk during storage

The pH was within the range of 3.96 – 6.98 during the period of storage (Figure 3). It was generally higher in 100% soymilk (6.46-6.98) than other samples. Soymilk containing *acha* flour had higher pH than *acha* starch probably because starch contain more carbohydrate that can be broken down into sugar for the production of acid by microorganisms. An increase was observed in samples containing soymilk while a decrease was observed in samples containing *acha* starch and flour. Increase in 100% soymilk could be attributed to the high protein content of soybean (35 – 40%) combined with the proteolytic activities of microorganisms associated with the samples during storage (Fakuda *et al*., 2017) while the decrease in samples containing starch and *acha* flour could be attributed to the production of organic acid which in turns reduced the pH of the samples (Adepoju *et al*., 2016). The decrease in pH observed in samples containing ach flour and starch could be due to the production of organic acids such as lactic and acetic by microorganisms (Makinde and Oyeleke, 2012).

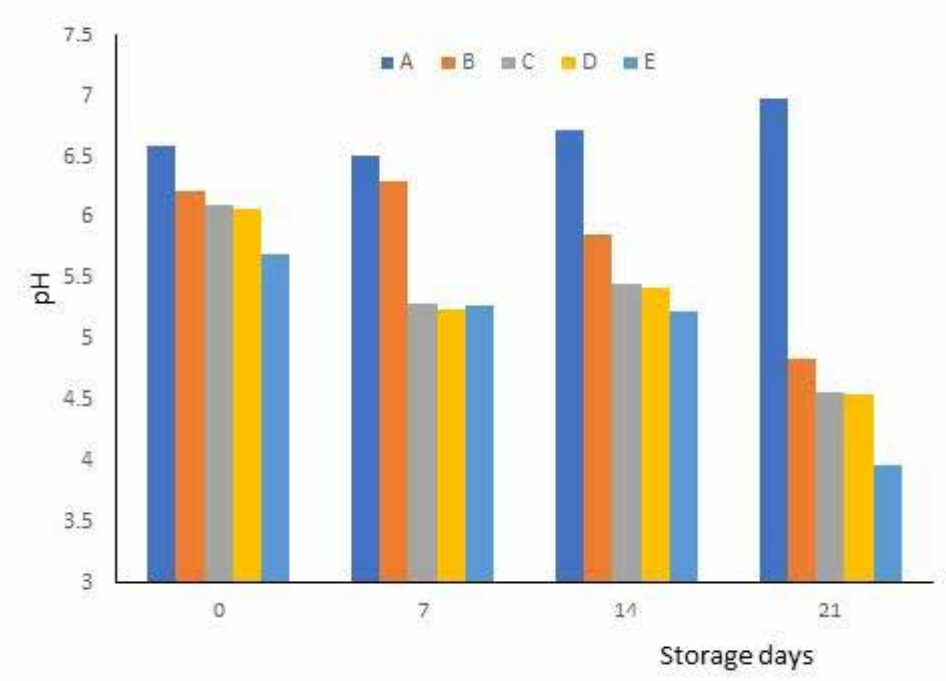

Figure 3 pH of soy-*acha* milk during storage

A; 100% soymilk; B: Soymilk and 10 g acha flour; C: soymilk and 20 g acha flour, D: Soymilk and 10 g acha starch; E: Soymilk and 20 g acha starch.

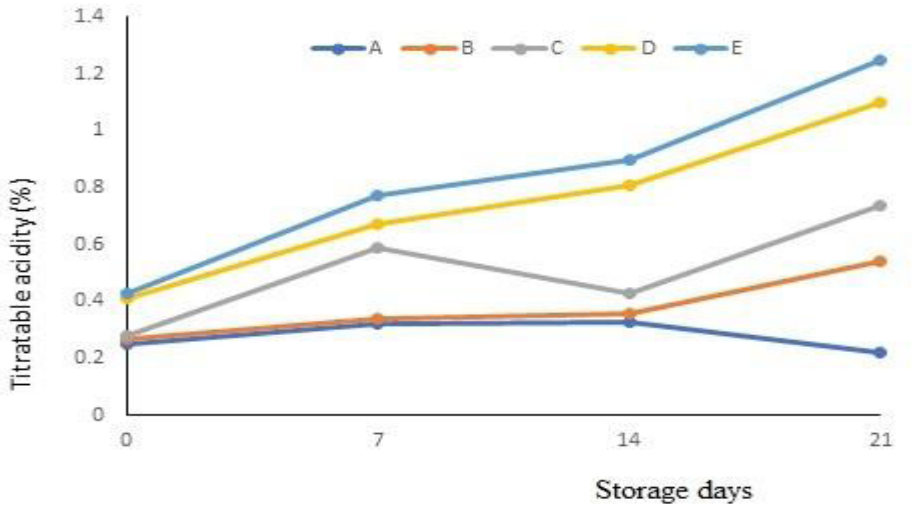

Figure 4 TTA of soy-*acha* during storage

A; 100% soymilk; B: Soymilk and 10 g acha flour; C: soymilk and 20 g acha flour, D: Soymilk and 10 g acha starch; E: Soymilk and 20 g acha starch.

TTA of soy-*acha* milk during storage

The TTA of soy-*acha* milk is presented in Figure 4. There was a general increase in the TTA of soymilk samples containing *acha* flour and *acha* starch (0.27 – 1.25%) while a decrease was observed in 100% soymilk from day 14 (0.33) to 21 days (0.22%). The TTA was also higher in samples containing *acha* starch (0.41 – 1.25%) than *acha* starch (0.27 – 0.74%) and also increased with increase in the concentration of both *acha* flour and starch. Increase in TTA could be linked to the production of acids from sugars by microorganisms associated with the samples (Adepoju *et al*., 2016). Higher levels in samples containing *acha* could be attributed to the higher carbohydrate content of *acha*. Eze *et al*. (2021) reported an increase in the TTA of soymilk and yoghurt with the addition of corn starch.

Sensory evaluation of soy-*acha* milk.

The result of the sensory evaluation (Table 3) shows that sample C containing 20 g *acha* (7.50) had the highest score for colour while 10 g (6.20) and 20% starch (6.20) had the lowest score. The scores for the taste of samples A, B and C containing 100% soymilk (6.20), soymilk plus 10 g of *acha* (6.10) and 20 g of *acha* flour (6.50) respectively had no significant difference ($p > 0.05$) but significantly different ($p < 0.05$) from D and E which are produced from soymilk plus 10 and 20 g of *acha* starch respectively. Soymilk (100%) had the highest score for aroma (7.30) while soymilk plus 10% starch had the lowest (4.60). There was no significant difference ($p > 0.05$) in the aroma and taste of samples A, B and C showing that the addition of *acha* flour did not have a negative effect on the aroma and taste of soymilk. The addition of *acha* flour increased the mouthfeel of soymilk. The score for mouthfeel was significantly higher in samples containing *acha* flour (6.40 – 6.50) than *acha* starch (4.70 - 4.80). The score for thickness was also higher in 100% soymilk than in samples containing *acha* flour (6.30 – 6.80) and starch (5.20 – 5.40). Overall acceptability shows that soymilk containing 10 g and 20 g *acha* flour had the highest score of 6.90 followed by 100% soymilk with no significant difference ($p > 0.05$) while soymilk plus 10 g and 20 g of starch had a significantly lower score ($p < 0.05$) of 3.90 and 4.40 respectively.

This shows that the quantity of starch added had a negative effect on the quality of soymilk. Increase in the addition of CMC has also been reported to have a negative effect on the taste, flavour, mouthfeel and overall acceptability of soymilk (Gatade *et al*., 2014). This shows that the addition of *acha* flour (10 – 20 g) can improve the organoleptic properties of soymilk. It has been reported that malted *acha* contains flavouring components such as aldehydes, heterocyclic compounds, aromatic compounds, terpenes, hexanal, esters and ethylacetate (Lasekan *et al*., 2001).

Table 3: Sensory properties of soy-*acha* milk during storage

Sample	Days of storage					
	Colour	Taste	Aroma	Mouthfeel	Thickness	Overall acceptability
A	7.10±1.27^{a}	6.20±1.54^{a}	7.30±1.41^{a}	6.20±1.31^{ab}	7.10±1.19^{a}	6.50±0.85^{a}
B	7.00±1.35^{a}	6.10±1.29^{a}	6.90±1.06^{a}	6.40±1.77^{a}	6.30±1.49^{abc}	6.90±1.66^{a}
C	7.50±1.05^{a}	6.50±1.65^{a}	6.70±1.10^{a}	6.50±1.07^{a}	6.80±1.31^{ab}	6.90±1.26^{a}
D	6.20±1.39^{a}	4.50±1.95^{b}	4.60±0.97^{b}	4.70±1.41^{b}	5.20±1.93^{c}	3.90±1.37^{b}
E	6.20±1.54^{a}	4.30±1.94^{b}	4.90±1.97^{b}	4.80±2.25^{b}	5.40±1.95^{bc}	4.40±2.01^{b}

A: 100% soymilk; B: Soymilk and 10 g acha flour; C: soymilk and 20 g acha flour, D: Soymilk and 10 g acha starch; E: Soymilk and 20 g acha starch. Values are means of three replicates ± standard error. Means followed by different superscripts in the same column are significantly different at $p < 0.05$

Panel analysis

Responses obtained from soy-*acha* milk assessors are presented in Figure 5. Assessor number 9 had the highest variance in colour, followed by 8 while other assessors had similar scores for colours as their response clusters at the outer eclipse. Assessor 8 had the highest variance for taste followed by assessor 5. Other assessors had similar responses as they also clustered together at the outer eclipse. Assessors 1 and 8 had different views of aroma compared to other assessors. Assessor number 9 had the highest variance for mouthfeel, followed by 8, 5 and 10 while 1, 2,3,4,6 and 7 had similar responses. Assessor 9 also had the highest variance for thickness, followed by 2 while others have similar responses. The responses for overall acceptability clustered together showing that all assessors had similar responses for overall acceptability.

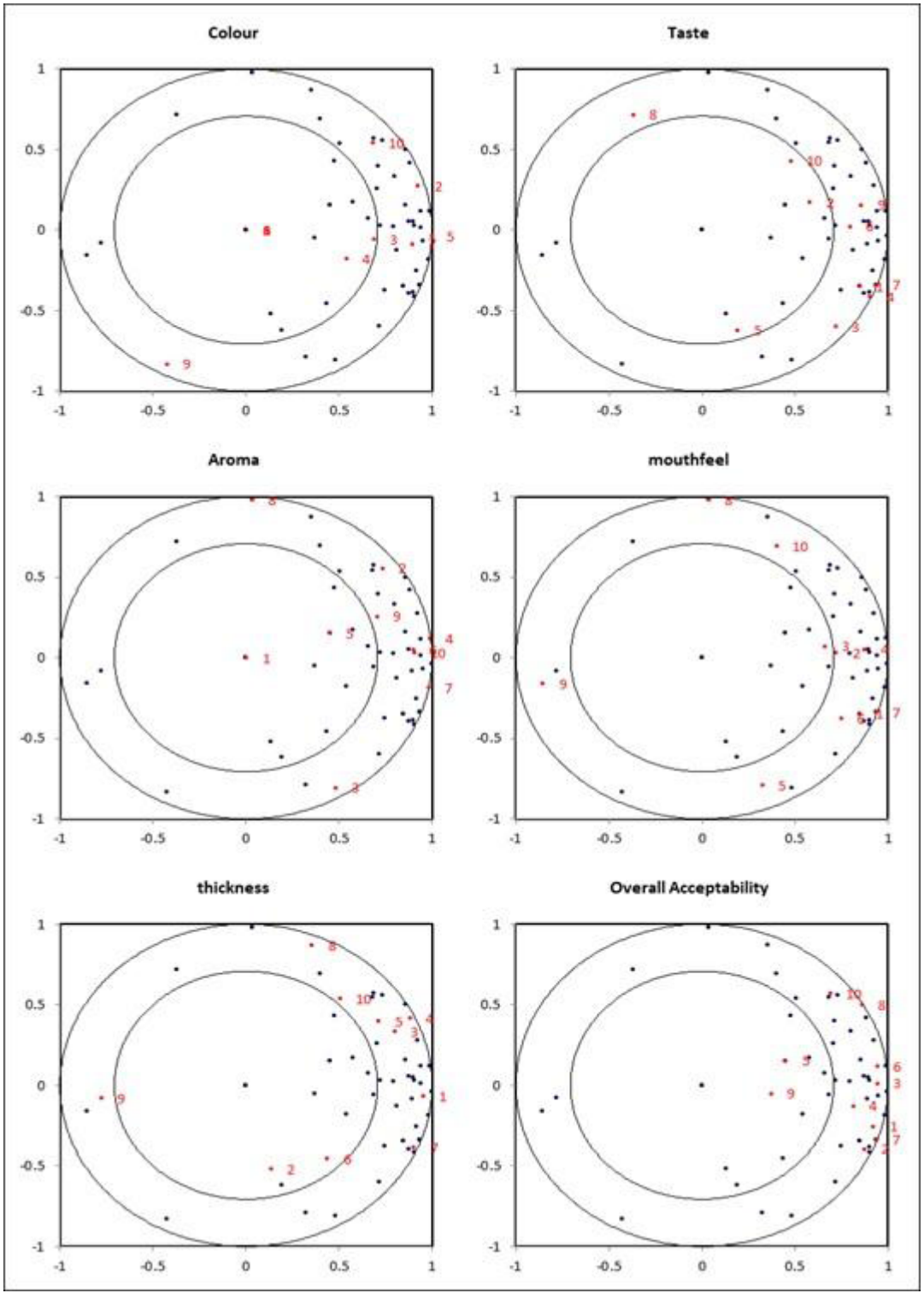

Figure 5 Panel analysis for soy-*acha* milk

4. Conclusion

Safe and hygienic protein-energy beverages can be produced from the combination of soymilk and *acha* flour or starch due to the absence of pathogenic organisms in the products during storage. The soy-*acha* samples were also acceptable to panelists and soybean containing *acha* flour compared favourably with 100% soymilk in terms of organoleptic assessment showing that *acha* is a suitable nutritive thickener in soymilk. Soymilk samples containing 10 g and 20 g of *acha* were the most preferred by the panelists.

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Conflict of Interest

Authors declare no conflict of interest.

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Comparative Assessment of Some Selected Bread Brands in Ondo State, Nigeria, For Public Acceptability

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Abstract

The study examined the level of acceptability of five different bread brands in Akure, Ondo State. The bread brands are oyato bread, omo oroki bread, Fortunate Tumbo bread. The bread samples were randomly selected and purchased. Fresh from bakery. Proximate composition and sensory evaluation of these breads were conducted. The study revealed that the ingredients used by bakers of the selected breads are common to all of them except those that are not using flavors and additives. FUTA Bread used preservatives and improver and had the highest protein content. Findings: The FUTA bread was rated best as regards crust color (x=8.25), crumb color (x=8.1), aroma (x=8.55), taste (x=7.95) and overall acceptability (x=8.35). There was no significant different in the bread samples f oyato with respect to crust, crumb, aroma, taste, texture and overall acceptability. Bread uses the best range of ingredients: flour, yeast, sugar, salt, fat, water, improper, flavor and preservatives. This is to recommended that FUTA should endeavor to set up production point in all the two local government of Akure city of the university in order to meet the bread demand of the students.

Keywords: Addictive assessment, bread, brands and crust.

1. Introduction

Dough has been a household staple food. Being an important part of our diet for thousands of years (jones et. al,, 2016). Bread celebrates the richest and simplest pleasures of daily living, for most European cultures it is the single inevitable presence at the table during all three meals of the day, for very few would contemplate a meal without read (Smallwood,2018), bread has a major component of flour, water, yeast, fat, salt and sugar. The flour for bread production is derived mainly from wheat. It is the least expensive staple food in the past and yet most important staple food in the world (Hoover,2014). The sugar in the bread improves the texture, colour (browning/caramelisation) and support yeast reaction during the production of brad apart from the sweetness it impart into the flavor (Lendel 1015 and Anderson,2019). Salt contributes to the flavour of the bread, and also assit in creating the right PH for yeast to operate. The yeast added performs a basic function of fermenting the sugar in the dough, thereby giving the bread its spongy texture, water,which is commonly used, helps dissolve other ingredients and also provides moisture for yeast activity.

Bread is now produced almost everywhere in the world based on different recipes. Also different countries have bread particular to their countries such as the Americans' corn bread, the Punjabis, the 'naan' etc (small-wood,2018). In Nigeria, different establishments now produce bread based on different recipes develop due to various factors and influences such as social influences, target market, companies' policy and more.

Federal University of Technology, Akure owns bakery and has developed its on recipe for bread production. The quality and acceptability of this bread produced i.e FUTA Bread will be assessed along with some selected bread brands produced in Akure in Ondo State ingredients used in the production of the selected breads, compare the quality with other selected breads and determine the sensory attributes of the selected breads

2. Materials and Methods

Five breads were freshly purchased from Akure, Ondo State FUTA was purposely selected and purchased fresh from FUTA bakery Technology Akure while oyato Bread and captain cook Bread were randomly selected and purchased fresh from oyato bakery and captain cook bakery in Akure, Omo oroki Bread and Fortunate Tumbo Bread were randomly selected and purchased fresh from omo oriki bakery and Fortunate Tumbo bakery in Akure Township.

Proximate analysis method as described by A.O.A.C (1990) was used to determine moisture content, nitrogen/protein content, ash content, crude Fibre content of the defatted bread and carbohydrate contents of all the bread samples and compared. Also conducted for the purpose of the study was sensory evaluation of the select-ed bread to evaluate the level of acceptability of the bread using a twenty-member panel of judges consisting of students and staff in the Federal University of Technology Akure. The criteri used include: crusts, crumb (colour), aroma, taste, texture, and overall acceptance. The judges were asked to rate all bread samples on a 9 point hedonic scale as described by Ihekoronye and Ngoddy (1985) and Rothman (1990) as follow: 9-like extremely, 8-like very much, 7-like Moderately,6-like slightly, 5-Neither like nor dislike, 4-Dislike slightly, 3-Dislike moderately, 2- Dislike very much and 1-Fislike extremely.

The data collected was subjected to inferential statistical analysis using ANOVA and Duncan multiple range test (Duncan, 2016).

3. Results and Discussion

Table i shows the ingredients used in the production of selected bread brands namely: oyato, ommo oroki, captain cook. FUTA breads. Its shows that the ingredients used in the production of the five bread samples varies slightly. However, its shows that that ingredients used include: flour, yeast, sugar, salt, fat, water. Improver, flavor and preservatives. These ingredients match up with that of Bakers' Association of Nigeria. this will contribute to the overall quality of the bread.

Table 1: Recipe Used for Production of Selected Bread Brands

Ingredient used in the production of selected bread brands	Bread brands location		Ship		
	Akure Town	Federal university of Technology			
	Bread brands		Akure		
	Oyato	Omo oroki	Captain cook	Fortunate	FUTA delight
Flour (kg)	50	50	50	50	50
Yeast (g)	250	250	250	250	250
Sugar(kg)	5	5	5	5	5
Salt(g)	600	600	600	600	600
Fat (kg)	2	2	2	2	2
Water (Litres)	25	25	25	25	25
Improver (g)	-	120	-	150	150
Flavour	-	-	-	-	150
Preservatives (g)	-	-	-	-	150

Source: Field survey,2024

Proximate composition of selected Bread Brands

The proximate composition of all the bread samples is presented in table 2. The component analysed in the samples include: moisture content, protein, fat, ash, fibre and carbohydrate. The result shows that captain cook bread has the highest moisture content of 42.19%, followed by bread (36%). Halleluyah bread (38.95). omo oroki bread (38.24%) and finally FUTA bread (36%). This shows that FUTA bread has the least moisture content. Hence it will be less vulnerable to mould attack. As compared to the standard given by Leverton (1985). Moisture content of bread should be within the range 35-36%, thus FUTA meet up with the recommended standard.

The table further shows the protein constituent of bread samples. Its shows that FUTA has the highest protein content of 11.4%, followed by bread (10.88%), Omo oroki bread (10.68%), Fortunate tumbo bread (10.22%) and Ata bread (9.80%). As regards the fat content of the sampled bread, the table shows that omo oroki bread has the highest fat content (2.43%) while FUTA bread with the lowest (1.75%) fat content

Furthermore, the Ash composition of oyato is highest (1.22%) compared to FUTA bread that is having the lowest (0.98%). The ash content is the reflection of minerals constituent that were present in the selected bread, Fibre is highest in FUTA bread (2.45%) and according to the UK'S Committee on Medical Aspects (COMA, 1994) advised that for a healthy, balanced digestive system, it is important that we eat enough fibre and a high intake of fibre may help protect our help protect our body against certain cancers and types of diabetes.

The carbohydrate content is highest for FUTA bread (47.42%) followed by Oyato bread (46.33%).

Table 2: Proximate Composition of Selected Bread Brands

Bread bands	Moisture Content	Protein	Fat	Ash	Fibre	Carbohydrate
Oyato	38.24±0.02	10.88±0.04	2.25±0.03	1.22±0.08	1.08±0.04	46.33±0.04
Omo-Oroki	38.95±0.04	10.68±0.08	2.43±0.04	1.08±0.06	1.22±0.08	45.64±0.08
Captain Cook	42.19±0.04	9.80±0.04	1.85±0.04	1.12±0.04	1.15±0.04	43.82±0.06
Fortunate Tumbo	41.36±0.00	10.21±0.02	1.93±0.04	1.06±0.04	1.15±0.04	44.28±0.04
FUTA	36.00±0.02	11.40±0.02	1.75±0.05	0.98±0.04	2.45±0.05	47.42±0.08

Sensory Attributes of Selected Bread Brands

Tables 3 shows that FUTA bread was rate best as regard crust colour (X = 8.25) followed by Omo-Oroki bread (ū=6.25), Fortunate Timbe bread (X=4.60), Captain cook bread (X = 3.95) and lastly Oyato bread (X=3.85). Also FUTA bread was rated in terms of crumb colour with (X = 8.1) while Captain cook bread was least (X = 3.75).

The results further shows that the aroma attributes of the bread is highest for FUTA (X=8.55) and followed by Omo-Oroki bread (X=6.75). The mean evaluation report of the panelist on taste is least for Omedun bread (X=3.65) and height for O.O.U Delight bread (X=7.95). Also for texture, the mean response of the panelist was highest for O.O.U Delight bread (X=7.90) and least for bread (X=4.00). The table finally shows that the overall acceptability of the selected breads to be highest for FUTA bread (X=8.35) and least for bread (X=3.45).

However, the sensory evaluation results for the bread samples were correlated for specified sensory quality attributes (crust and colour, aroma, taste, texture and overall acceptability). The result show a variation in the tested attributes between the bread samples. It shows that FUTA bread and Omo-Oroki bread are not significantly different based on crumb, aroma, taste, texture and overall acceptability, through there are slight differences in their means at 5% level LSD.

Also, there are significant differences between the means of FUTA bread and Oyato bread, Captain cook and Fortunate Tunbo breads as regards crust, crumb, aroma, taste, texture and overall acceptability. Thus, it could be concluded based on sensory evalution that Oyato and FUTA bread is rated best, followed by Omo- Oroki bread, Fortunate tumb bread, captain cook bread and lastly Oyato bread, FUTA bread.

Table 3: Mean Sensory Evaluation of Quality Attributes of Selected Bread Brands

Bread bands	Crust (X)	Crumb (X)	Aroma (X)	Taste (X)	Texture (X)	Overall acceptability (X)
Oyato	-3.85b	3.85b	3.80b	3.65b	4.00b	3.45b
Omo-Oroki	-6.45a	6.45a	6.75a	6.10a	6.40a	6.40a
Captain Cook	3.95a	3.75b	4.65b	4.40b	4.90b	4.15b
Fortunate Tum-bo	4.60b	4.65	4.85b	5.05b	5.60b	5.30b
FUTA	-8.25a	8.10a	8.55a	7.95a	7.90a	3.85a
LSD 5%	0.194	0.194	0.228	0.319	0.223	0.208

Mean (X) with the same with the same letter are not significantly different

4. Conclusion

Based on the findings of this study it could be concluded that the ingredients used by bakers of the selected breads are common to all of them except those that are not using flavours and additives. FUTA Bread uses the best range of ingredients: flour, yeast, sugar, salt, fat, water, improver, flavor and preservatives and overall acceptability (X = 8.35). Therefore, it became pertinent that a careful analysis of baked products should be carried out before they get to the general for consumption. Despite the advocate of NAFDAC in ensuring compliance of product meant for consumption to healthy and required standards it shows that little has been done in the area of bread industries in complying to the set standards it shows that little has been done in the area of bread industries in complying to the set standards.

Recommendation

- All Government established Agencies Monitoring the activities of Bakers Association should ensure total compliance with health and safety standards.
- FUTA Bread should endeavor to setup production unit in two local governments in Akure Township, This will assist in solving part of the nutritional problems of the masses.

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Quantification of Saponins by Chromatographic and Spectrophotometric Methods

Saponinlerin Kromatografik ve Spektrofotometrik Yöntemlerle Kantifikasyonu

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Özet

Birçok yararlı biyoaktif etkilere sahip olan saponinler, sabun oluşturma özelliği ile ayırt edilmektedir. Saponinler, bazı deniz hayvanlarında ve farklı bitki çeşitlerinde bulunmaktadır. Doğal kökenli olmaları ve biyolojik olarak parçalanabilmeleri sayesinde sağlık ürünleri ve hayvan yemlerinde olduğu gibi kozmetik ve ilaç sanayisinde de kullanılabilmektedir. Saponinlerin besinsel kalitesi ve miktarı, sıcaklık, ışık, su gibi eko fizyolojik etkenler tarafından etkilenirken; coğrafi konum, yetiştirme şekli ve hasat zamanı gibi faktörler saponinlerin farklı yapıda olmalarına neden olmaktadır. Farklı yapıdaki saponinler, biyolojik aktivitede düşüşe ve sınırlı klinik uygulamalarına neden olduğundan bu durumundan kaynaklanan problemlerin çözümü için bazı analitik yöntemler önerilmektedir. Bu yöntemler arasında kromatografik ve spektrofotometrik yöntemler yer almaktadır. Kromatografik ve spektrofotometrik yöntemler, saponinlerin karmaşık yapıları ve çeşitlilikleri göz önüne alındığında, yüksek doğruluk ve hassasiyet sağlamaktadır. Bu yöntemlerin uygulanmasından önce saponinlerin bitki hücrelerinden ayrılması amacıyla ekstraksiyon işlemi gerçekleşmektedir. Genellikle ekstraksiyon süresinin uzun olması ve yüksek sıcaklık uygulanması ekstraksiyon verimini arttırmaktadır. Kromatografik yöntem ile saponin bileşiklerinin kantifikasyonunda genellikle ince tabaka kromatografisi (TLC), düşük ve orta basınçlı sıvı kromatografisi (LPLC-MPLC) ve yüksek performanslı sıvı kromatografisi (HPLC) kullanılmaktadır. TLC, saponinlerin hızlı ve niteliksel analizi için kullanılmaktadır. Saponinlerin HPLC-UV detektörü ile belirlenememesi durumunda, kütle spektrometrisi (MS) veya nükleer manyetik rezonans (NMR) gibi yeni tekniklerle HPLC'nin birlikte kullanımı sonucunda yüksek hassasiyetli analizler sağlamaktadır. HPLC, saponin analizinde en yaygın kullanılan ve en güvenilir yöntem olup, diğer tekniklerle birleştirildiğinde saponinlerin yapısal çeşitliliğini ve konsantrasyonlarını doğru bir şekilde belirleyebilme kapasitesine sahiptir. Spektrofotometrik yöntemler ise basit, hızlı ve ekonomik çözümler sağlamaktadır. Saponinlerin karakterizasyonunda kütle spektrometrisinin kromatografi ile kullanımı oldukça yaygındır. Ayrıca bazı yöntemlerin kullanılarak saponinlerin tespitinde karşılaşılan zorluklar, sıvı kromatografisi ile kütle spektrometrisini birleştiren ek tekniklerin geliştirilmesini teşvik etmiştir.

Anahtar kelimeler: Saponin, saponinlerin kantifikasyonu, kromatografik yöntem, spektrofotometrik yöntem.

Abstract

Saponins, which have many beneficial bioactive effects, are distinguished by their soap-forming properties. Saponins are found in some marine animals and various plant species. Due to their natural origin and biodegradability, they are used in the cosmetic and pharmaceutical industries, as well as in health products and animal feed. The nutritional quality and quantity of saponins are influenced by ecophysiological factors such as temperature, light, and water; while geographical location, cultivation methods, and harvest time cause saponins to have different structures. Different structures of saponins result in reduced biological activity and limited clinical applications, thus some analytical methods are proposed to solve the problems arising from this situation. These methods include chromatographic and spectrophotometric techniques. Given the complex structures and diversity of saponins, chromatographic and spectrophotometric methods provide high accuracy and sensitivity. Prior to the application of these methods, an extraction process is carried out to separate the saponins from plant cells. Generally, a longer extraction time and higher temperature increase the extraction yield. In the quantification of saponin compounds by chromatographic methods, thin layer chromatography (TLC), low and medium pressure liquid chromatography (LPLC-MPLC), and high performance liquid chromatography (HPLC) are commonly used. TLC is used for the rapid and qualitative analysis of saponins. HPLC, combined with newly coupled techniques such as mass spectrometry (MS) or nuclear magnetic resonance (NMR) when saponins cannot be detected with a HPLC-UV system, provides highly sensitive analyses. HPLC is the most widely used and reliable method in saponin analysis, and when combined with other techniques, it has the capacity to accurately determine the structural diversity and concentrations of saponins. Spectrophotometric methods provide simple, fast, and economical solutions. The use of mass spectrometry with chromatography is quite common in the characterization of saponins. Additionally, the challenges encountered in the detection of saponins using certain methods have encouraged the development of additional techniques that combine liquid chromatography with mass spectrometry.

Keywords: Saponin, quantification of saponins, chromatographic method, spectrophotometric method.

1. Saponinler

Günümüzde tüketicilerin sağlık bilincinin artması ile biyoaktif bileşenlerin potansiyel faydalarına yönelik ilgi de giderek artmıştır (Nguyen ve ark., 2020). Bitkisel ve hayvansal kaynaklı olabilen bu bileşenler, tip II diyabet, osteoporoz, kardiyovasküler hastalıklar ve kanser risklerinin azaltılmasında rol oynamaktadır (Abuajah, 2017). Saponinler, birçok yararlı biyoaktif etkilere sahip olan önemli bir biyoaktif bileşendir. Sabun oluşturma özelliği ile ayırt edilen saponinler, bazı deniz hayvanlarında ve farklı bitki çeşitlerinde bulunmaktadır (Elekofehinti ve ark., 2021). Orta Asya'da yetişen bitki türleri üzerinde yapılan bir çalışmada, bu bitki türlerinden %76'sının saponin içerdiği saptanmıştır (Savarino ve ark., 2023). Bitkilerin kök, gövde, yaprak, çiçek ve tohumlarında yaygın olarak bulunan saponinler, insan beslenmesinde yer alan birçok meyve ve sebzelerin içeriğinde de tespit edilmiştir (Wang ve ark., 2022). Özellikle baklagillerde olmak üzere, ıspanak (47 g/kg KM), pancar (58 g/kg KM) ve yonca filizlerinde (87 g/kg KM) yüksek miktarlarda bulunmaktadır (Küçükkurt ve Fidan, 2008). Bazı bitki türlerinde bulunan saponin miktarları Tablo 1'de gösterilmektedir.

Tablo 1. Bazı bitki türleri ve saponin miktarları

Bitki türü	Saponin Miktarı	Bitki türü	Saponin Miktarı
Nohut (*Cicer arietinum L.*)	56 g/kg KM	Mercimek (*Lens culinaris*)	4,1 g/kg KM
Soya fasulyesi *(Glycine Max L. Merr*)	43 g/kg KM	Bakla (*Vicia faba L.*)	3,5 g/kg KM
Kuşkonmaz (*Asparagus officinalis*)	15 g/kg KM	Amarant (*Amaranthus* sp.)	0,1-0,5 mg/100g
Yeşil Fasulye (*Phaseolus vulgaris*)	13 g/kg KM	Susam tohumu (*Sesamum indicum L.)*	3 g/kg KM
Yeşil Bezelye (*Pisum sativum* ssp.)	11 g/kg KM	Sarımsak (*Allium sativum)*	2,9 g/kg KM
Kuşkonmaz (*Asparagus officinalis*)	15 g/kg KM	Yulaf (*Avena sativa*)	1 g/kg KM
Kinoa (*Chenopodium quinoa Willd.*)	470-1633,3 mg/100 g	Maş fasulyesi (*Vigna radiata*)	0,5-5,7 g/kg KM

Saponinler, şeker zincirinden oluşan ikincil metabolitlerdir (Abashev ve ark., 2021). Bir şeker zincirinden meydana gelen saponinler monodesmozid, iki şeker zincirinden oluşan bidesmozid ve üç şeker zincirinden oluşan saponinler tridesmozid olarak adlandırılmaktadır (Oleszek ve Oleszek, 2020). Bu şeker kompozisyonları arasındaki fark ve bağlanma olasılıkları, saponin yapısında çeşitliliklere neden olmaktadır. Yalnızca bir bitki türünde bile farklı yapıda saponinler bulunabilmektedir. Saponinler, yapılarındaki hidrofobik aglikon (triterpenoid, steroidal) ve bir veya daha fazla hidrofobik şeker zinciri sayesinde yüzey aktif özellikler sergilemektedir (Timilsena ve ark., 2023). Saponinlerin yapısal olarak hem hidrofilik hem de hidrofobik bileşenlere sahip olmaları, onların gıda alanında emülgatör ve stabilizatör olarak kullanılmalarına olanak sağlamaktadır. Ayrıca tat değiştirici ve tatlandırıcı olarak kullanılan saponinler, gıdalarda tekstüre ve stabiliteye katkı sağlamaktadır (Oleszek ve Oleszek, 2020). Bazı içeceklerde köpük oluşmasını sağlamak amacıyla da kullanılabilen saponinlerin kolesterol düşürücü ve antikanserojen etkileri gibi potansiyel sağlık yararları bulunmaktadır (Kafle ve ark., 2021). Saponinlerin antimikrobiyal özellikleri, onların gıda koruyucu olarak kullanılmalarını sağlamaktadır. Bu sayede gıdalarda patojenik mikroorganizmaların büyümesini engelleyerek gıdaların raf öm-

rünün uzatılmasını sağlamaktadır (Ma ve ark. 2024).

Geçmiş yıllarda yapılan çalışmalarda saponinlerin antinütrosiyonel etkileri dikkate alınarak bu bileşenlerin zararlı olduğu düşünülmüştür (Küçükkurt ve Fidan, 2008). Ancak yapılan yeni çalışmalar sonucunda saponinlerin yüksek dozlarda toksik olduğu; kullanım ve doz sıklığına göre yararlı etkilerinin bulunduğu saptanmıştır. Günümüzde saponinlerin antioksidan, antibakteriyel, antiviral ve antikarsinojenik etkileri üzerinde durulmaktadır (Desai ve ark., 2009). Saponinlerin yüksek yapısal çeşitlilikleri sayesinde kanser hücrelerini inhibe etme gibi olumlu etkileri bulunmaktadır (Elekofehinti ve ark., 2021). Bunlara ek olarak saponinler, kandaki glukoz ve lipid değerlerini düşürmeye yardımcı olmaktadır (Wang ve ark., 2022).

Saponinler, doğal kökenli olmaları ve biyolojik olarak parçalanabilmeleri sayesinde sağlık ürünleri ve hayvan yemlerinde olduğu gibi kozmetik ve ilaç sanayisinde de kullanılabilmektedir (Kafle ve ark., 2021). Saponinler, hayvan yemlerinde kullanılarak anti-besinsel faktörlerin olumsuz etkilerini azaltarak hayvan sağlığını iyileştirebilmektedir. Örneğin, bazı saponinler rumen mikroflorasını düzenleyerek metan üretimini azaltmakta ve böylece çevresel sürdürülebilirliğe katkı sağlamaktadır (Kafle ve ark., 2021). Böcek öldürücü ve fungisidal özellikleri sayesinde tarımda biyopestisit olarak kullanılabilen saponinler, tarımda yaygın olarak böcek kovucu işlevi görmektedir (Wang ve ark., 2022). Saponinlerin besinsel kalitesi ve miktarı, sıcaklık, ışık, su gibi eko fizyolojik etkenler tarafından etkilenmektedir (Szakiel ve ark., 2011). Ayrıca coğrafi konum, yetiştirme şekli ve hasat zamanı gibi faktörler saponinlerin farklı yapıda olmalarına neden olmaktadır (Wang ve ark., 2022). Farklı üreticilerden gelen farklı yapıdaki saponinler, biyolojik aktivitede düşüşe ve sınırlı klinik uygulamalarına neden olmaktadır. Araştırmacılar bu durumundan kaynaklanan problemlerin çözümü için bazı analitik yöntemleri önermektedir. Bu yöntemler arasında spektrofotometrik ve kromatografik yöntemler yer almaktadır (Fu ve ark., 2021).

1.1. Saponinlerin Ekstraksiyonu ve İzolasyonu

Saponinlerin bitki hücrelerinden ayrılması amacıyla ekstraksiyon işlemi gerçekleştirilmektedir. Basit ekstraksiyon yöntemi (maserasyon), saponinler belirli bir süre boyunca uygun bir spesifik çözücüye daldırılarak veya ıslatılarak kolayca ekstrakte edilebilmektedir. İşlem süresi, uygulanan sıcaklık, karıştırma hızı ve çözücünün polaritesi saponinlerin çözünürlüğü ile ekstraksiyon sürecinin verimliliğini etkileyen ana değişkenlerdir (El Aziz ve ark., 2019). Genellikle ekstraksiyon süresinin uzun olması ve yüksek sıcaklık uygulanması ekstraksiyon verimini arttırmaktadır.

Saponinlerin bitki kaynaklarından ekstraksiyonu ve izolasyonu, yapısal çeşitlilikleri, bitki biyokütlesinde düşük konsantrasyonlarda bulunmaları ve kimyasal olarak kararsız olmaları nedeniyle oldukça karmaşıktır. Saponinler, çeşitli teknikler kullanılarak bitki materyallerinden ekstrakte edilebilmektedir. Basit ekstraksiyon yöntemi, su, alkol ve diğer solventleri içerirken, soxhlet ve solvent ekstraksiyonunun ultrason veya mikrodalga gibi teknikler ile birleştirilmesi, daha yüksek verimlilik sağlamaktadır. Soxhlet ekstraksiyonu, basit ekstraksiyon sürecine kıyasla daha verimli bir süreç olarak kabul edilmektedir. Bunun nedeni, soxhlet ekstraksiyonu sırasında, organik çözücünün bitki dokusu ile doğrudan temas etmesinden kaynaklanmaktadır (Seidel, 2005). Ekstraksiyon işleminin tamamlanmasının ardından yoğunlaştırma işlemi gerçekleştirilmektedir. Yoğunlaştırma işlemi, çözücüye buharlaştırma veya vakum distilasyonu kullanılarak gerçekleştirilmektedir. Ancak, yoğunlaştırma işleminden sonra istenmeyen safsızlıklar görülebilmektedir. Bu nedenle saponinlerin daha fazla fraksiyonlanması veya saflaştırılması gerekmektedir (McCloud, 2010). Saflaştırma işleminde kullanılacak

olan polar çözücünün hedef saponin moleküllerine uygun olarak seçilmesi gerekmektedir.

Saponin bileşenlerini saflaştırmak için kullanılan yöntemler arasında kromatografik yöntemler yer almaktadır. Bu yöntemlerin avantajları arasında, saponinlerin oldukça yüksek saflıkta fraksiyonlarını elde etmek için özelleştirilebiliyor olması yer almaktadır.

2. Saponinlerin Kantifikasyonu İçin Kullanılan Kromatografik Ve Spektrofotometrik Yöntemler

Saponinler, bitkilerde benzer yapısal bileşikler halinde karmaşık bir şekilde bulunmaktadır (Majinda, 2012). Bu nedenle saponin bileşiklerinin izolasyonu, tanımlanması ve ayrılması zor bir işlemdir. Saponinlerin kimyasal yapılarını belirlemek amacıyla etkili bir ekstraksiyon ve izolasyon prosedürünün geliştirilmesi gerekmektedir (Mroczek, 2015). Saponinlerin ekstraksiyon işlemi için kullanılan ekstraksiyon çözücüleri arasında aseton, etanol, dietil eter gibi çözücüler bulunmaktadır.

Saponinlerin kantifikasyonu genellikle spektrofotometrik ve kromatografik yöntemler kullanılarak gerçekleştirilmektedir. Bu iki yöntem arasındaki fark, kromatografik yöntemin belirli saponin bileşiğini kantitatif olarak ölçerken, spektrofotometrik yöntemin toplam saponin değerini vermesinden kaynaklanmaktadır (Cheok ve ark., 2014).

2.1. Kromatografik yöntemler

Kromatografik yöntem ile saponin bileşiklerinin kantifikasyonunda genellikle ince tabaka kromatografisi (TLC), düşük ve orta basınçlı sıvı kromatografisi (LPLC-MPLC) ve yüksek performanslı sıvı kromatografisi (HPLC) kullanılmaktadır.

İnce tabaka kromatografisi (TLC): TLC, saponin bileşiklerinin kimliğinin ve saflığının belirlenmesinde kullanılan destekleyici bir tekniktir (Majinda, 2012). Bu teknik, saponinlerin farklı türlerini ve miktarlarını belirlemek için hassas ve güvenilir sonuçlar sunmaktadır. TLC, bazı baklagillerde saponin içeriğinin belirlenmesi için kullanılır ve çok bileşenli saponin karışımlarının ayrıştırılmasını sağlamaktadır (Oleszek ve Bialy, 2006). Saponinlerin ayrıştırılmasında TLC'nin kullanımı, bitkisel ürünlerin kalitesinin belirlenmesinde de önemli bir rol oynamaktadır. Genellikle TLC ile soya fasulyesi ve sarımsak ürünlerinde bulunan saponinlerin içeriği belirlenmektedir (Oleszek ve Bialy, 2006; Fenwick ve Oakenfull, 1983). TLC için en yaygın olarak kullanılan çözücü kloroform- glasiyel asetik asit- metanol- su (60:32:12:8) ve etil asetat- formik asit- glasiyel asetik asit- su (100:11:11:26) karışımıdır (Gómez-Caravaca ve ark., 2014).

Düşük basınçlı sıvı kromatografisi (LPLC): Saponinlerin kantifikasyonu için LPLC kullanımında sabit ve mobil fazların dikkatli bir şekilde seçilmesi gerekmektedir. Saponinlerin ayrıştırılması içini, LPLC'lerde sabit fazlar açısından hala sınırlı sayıda seçenek bulunmaktadır. Ayrıca bu yöntemle saponin ayrıştırılmasında tek kolon kullanımı verimli olmadığından normal ve ters faz ayrımlarının kombinasyonuna ihtiyaç duyulmaktadır. Bu yöntemle saf saponin elde edilmesi amaçlanmaktadır. LPLC yöntemi ile kinoada bulunan saponin bileşikleri ayrıştırılabilmektedir (Medina-Meza ve ark., 2016).

Yüksek performanslı sıvı kromatografisi (HPLC): HPLC, saponin bileşiklerinin kantifikasyonunda en etkili olanıdır. Ancak saponin bileşiklerinde belirli bir dalga boyundaki ışığı emerek renk yayan molekül olan kromofor bulunmamaktadır. Bu durum saponin bileşiklerinin ultraviyole ışık altında tespit edilmesini engelleyerek yalnızca 200-210 nm'de tespit edilmesine neden olmaktadır. Bu dalga boyu aralığında saponin dışındaki bileşenler de absorbe olabileceğinden saponin tespiti zorlaşmaktadır (Oleszek ve Bialy, 2006). HPLC kullanı-

mında bu sorunların üstesinden gelmek amacıyla bazı analitik yöntemler geliştirilmiştir. Bu yöntemlerden biri saponin tespiti için buharlaşmalı ışık saçılım dedeksiyonu (ELSD) uygulamasıdır. ELSD, örneğin buharlaşma süreci sırasında oluşan uçucu olmayan partiküllerin saçtığı ışığı ölçerek tespit edilmesini sağlamaktadır. Yapılan bir çalışmada, Çin geleneksel tıbbi bitkilerinden olan *Flos Lonicerae* örnekleri kullanılmış ve ELSD yöntemi ile saponin içerikleri belirlenmiştir (Li ve Fitzloff, 2001; Chai ve ark., 2005).

Bunun yanı sıra saponinlerin ayrılmasında, C18 ters faz kolonu kullanılan HPLC yöntemlerinin daha etkili olduğu belirtilmektedir (Negi ve ark., 2011). Yapılan bir çalışmada, beş farklı soya fasulyesi kullanılarak saponinlerin ayrıştırılması işlemi gerçekleştirilmiştir (Berhow ve ark., 2002). Bu amaçla %40 sulu asetonitril ve %0,025 triflorasetik asidi mobil faz olarak kullanılan HPLC sisteminde, C18 ters faz kolon ile çalışılmıştır. İşlem sonunda zenginleştirilmiş bir fraksiyon elde edilmiştir. HPLC için en yaygın olarak kullanılan çözücü metanol-su (MeOH- H_2O) sistemidir. Ayrıca HPLC kullanımının ppm ile ppb aralığında algılama yaparak yüksek hassasiyet gösterdiği belirtilmektedir. HPLC kullanılarak saponinlerin kantifikasyonuna ilişkin bir özet Tablo 2'de gösterilmektedir.

Tablo 2. Saponinlerin kantifikasyonu için HPLC kullanımı (Cheok ve ark., 2014).

Bitki türü	Kullanılan çözücü	Saponin verimi	Kaynak
Aesculus chinensis	Asetonid/0,1%-fosforik asit	Tohumlarda 17,3 mg/g düzeyinde aeskin saptanmıştır.	Chen ve ark., 2007
Gynostemma pentaphyllum	%0,1 formik asit çözeltisi/asetonitril	Toplam 17 tip saponin tespit edilmiştir (1278 µg/ml).	Cheng ve ark., 2011
Vaccaria hispanica	Asetonitril-su-formik asit/asetonitril-formik asit	En yüksek saponin verimi %80 metanol kullanılarak 42,2 g/100g olarak elde edilmiştir.	Güçlü-Üstündağ ve ark., 2007
Medicago truncatula	Su-asetonitril	Toplam saponin değerleri kökte 5924 ng/mg/dw, yaprakta 1064 ng/mg/dw ve tohumda 991 ng/mg/dw olarak elde edilmiştir.	Huhman ve ark., 2005

Kütle spektrometrisi (MS): Saponinlerin karakterizasyonunda kütle spektrometrisinin kromatografi ile kullanımı oldukça yaygındır. Buna örnek olarak yapılan bir çalışmada saponin açısından zengin kökleri olan chubak (*Acanthophyllum Glandulosum)* bitkisi kullanılmıştır (Dabestani ve ark., 2021). Bu amaçla bitki ekstraktından saflaştırılmış saponinlerin karakterizasyonu işlemi için kütle spektrometresinin ultra-performans sıvı kromatografisi (UPLC) ile kullanıldığı belirtilmektedir.

Gaz kromatografisi-kütle spektrometresi (GC-MS): Bu yöntem, saponinlerin kapsamlı bir şekilde saflaştırılmasını gerektirmektedir. Ayrıca bu yöntemde, saponinlerin ilgili sapogeninlerine dönüştürülmesi ve ardından uçucu türevlerin hazırlanması gibi işlemler yer almaktadır. Kapsamlı saflaştırma süreci ve işlem basamaklarının fazla olması, bu yöntemin bir dizi zaman alıcı ve istenmeyen bozulmalara neden olması ile bilinmektedir (Muir ve ark., 2000).

Sıvı kromatografisi-elektro sprey iyonizasyon kütle spektrometrisi (LC/MS): Bazı yöntemlerin kullanılarak saponinlerin tespitinde karşılaşılan zorluklar, sıvı kromatografisi ile kütle spektrometrisini birleştiren ek tekniklerin geliştirilmesini teşvik etmiştir. MS, bitki ekstraktlarındaki saponin miktarını belirlemek ve saponinleri karakterize etmek için yaygın olarak kullanılmaktadır. Soya fasulyesi örneklerinde LS/MS yöntemiyle analiz yapılmadan önce saponinlerin karmaşıklığını azaltmak ve stabilitelerini arttırmak amacıyla bazı işlemler yapılmaktadır. Bu işlemler, asetil ve 2,3-dihidro-2,5-dihidroksi-6-metil-4-piron (DDMP) gruplarını elimine eden alkali bozunmayı içermektedir (Oleszek ve Oleszek, 2020). Bu şekilde bu gruplar dışında olan saponinler, seçici iyonlar sayesinde kolaylıkla seçilebilmektedir.

Yonca bitki türlerinden *Medicago sativa* ve *Medicago truncatula* üzerinde yapılan bir çalışmada, LC/MS tekniği kullanılarak saponin profilleri karşılaştırılmıştır. Elde edilen sonuçlara göre *Medicago truncatula* köklerinin, *Medicago sativa* köklerine göre daha zengin saponin kaynağına sahip olduğu belirtilmektedir (Oleszek ve Bialy, 2006).

2.2. Spektrofotometrik yöntemler

Saponinlerin kantifikasyonunda spektrofotometrik yöntemlerin yaygın olarak kullanılması bu yöntemin basit, hızlı ve ekonomik olmasından kaynaklanmaktadır.

Vanilin-sülfürik asit testi: Saponin kantifikasyonunda yaygın olarak kullanılan spektrofotometrik yöntemler arasında yer almaktadır. Ancak bu spektrofotometrik yöntemin kullanılabilmesi için standart seçimi, dalga boyu ve diğer faktörlerin dikkate alınması gerekmektedir. Toplam saponin değerinin belirlenmesi için başlangıçta dikkate alınması gereken standart seçimi hakkındaki bilgiler literatürde oldukça az bulunmaktadır (Cheok ve ark., 2014). Uygun dalga boyunun seçimi konusunda önceki çalışmalarda bir açıklama yer almamakla birlikte, çoğu araştırmacının 544 nm dalga boyunu seçtiği gözlemlenmiştir.

Vanilin-sülfürik asit testinin temel prensibi, oksitlenmiş triterpen saponinlerin vanilin ile reaksiyona girmesi olarak belirtilmektedir (Li ve ark., 2010). Oksidan olarak sülfirik asit ve perklorik asit kullanılabilen bu reaksiyonun belirgin rengi mordur (Chen ve ark., 2007; Hiai ve ark., 1976).

3. Kromatografik ve Spektrofotometrik Yöntemler ile Saponinlerin Kantifikasyonunu İçeren Bazı Çalışmalar

Kinoa ile yapılan bir çalışmada, ilk kez fenolik bileşikler ve saponinlerin eş zamanlı tayini için geliştirilmiş yeni bir sıvı kromatografisi metodolojisi, diyot dizinli dedektör ve uçuş zamanlı kütle spektrometresi ile kullanılmıştır. Analiz sırasında C18 kolon kullanılmış ve analiz 27 dakikadan kısa sürede gerçekleştirilmiştir. Elde edilen sonuçlara göre kinoada saponin miktarının, %5,6 ile %7,5 arasında değişen bir konsantrasyonda olduğu bulunmuştur (Gómez-Caravaca ve ark., 2011).

Bir başka çalışmada ise *Panax notoginseng*'de 11 adet saponin çeşidinin belirlenmesi için hızlı ultra performans sıvı kromatografisi foto diyot dizinli dedektör yöntemi (UPLC-PDA) kullanılmıştır. Birçok çalışmada olduğu gibi bu çalışmada da C18 kolondan faydalanılmıştır. Kullanılan yöntem, *P. notoginseng*'deki saponinlerin analizi için başarıyla kullanılmış olup, analitler için genel geri kazanım %93–101,6 olarak bulunmuştur. Sonuçlar, UPLC'nin Çin ilaçlarındaki bileşenlerin analizi için güçlü bir araç olduğunu göstermektedir (Guan ve ark., 2007).

Astuti ve arkadaşları (2011) tarafından gerçekleştirilen bir çalışmada tıbbi bitkilerden biri olan Binahong bitkisindeki saponin bileşiklerinin tanımı yapılmaktadır ve bu bitkinin çeşitli hastalıkların tedavisindeki etkisine

katkıda bulunabileceği düşünülmektedir. Yapılan çalışmalar sonucunda Binahong bitkisinin her kısmında saponin bileşiklerinin bulunduğu tespit edilmiştir. Ayrıca, Binahong yapraklarında (28,14±0,22 mg/g), saplarında (3,65±0,11 mg/g) ve yumrularında (43,15±0,10 mg/g) olmak üzere saponinler bulunmuştur (Astuti ve ark., 2011).

Farklı bir çalışmada ise 14 farklı tahin helvası örneği kullanılarak bu örneklerin saponin içerikleri belirlenmiştir. Saponin içeriğinin belirlenmesi amacıyla HPLC yöntemi kullanılmıştır. Sonuçta tahin helvasının saponin miktarının 32-172 mg/kg olduğu tespit edilmiştir. Tahin helvasının ana bileşenlerinden biri çöven otunun sıvı ekstresidir. Çöven otu ekstresinin aktif maddesi olan saponin, tahin helvasının rengini ve kıvamını olumlu yönde etkilemektedir. Aynı zamanla helva ürünlerinde yağın sızmasını önleyerek emülsifikatör görevi görmektedir (Sezgin ve Artik, 2010).

Kromatografik yöntem ile farklı gıdaların saponin içeriğinin belirlenmesine yönelik bazı çalışmalar Tablo 3'te verilmiştir.

Tablo 3. Saponin içeriğinin kromatografik yöntem ile belirlenmesine yönelik bazı araştırmalar

Yapılan çalışma	Sonuç	Kaynak
Kinoa tohumlarının saponin içeriğinin belirlenmesi için GC-MS yöntemi kullanılmıştır.	Kinoa örneklerinde yüksek miktarda saponin içeriği (50,88 mg/100g) tespit edilmiştir.	Gómez-Caravaca ve ark., 2014
Kolon kromatografisi kullanılarak Yuka bitkisinde saponin belirlenmesi için yapılan bir çalışmada saponin miktarı, renk reaksiyonlarına dayanarak 430 nm'de absorbans ölçülerek belirlenmiştir.	Sonuçta gıda katkı maddesi olarak kullanılan ticari yuka özütlerinin %5,6 ile %6,4 (w/w) arasında saponin içermekte olduğu tespit edilmiştir.	Uematsu ve ark., 2000
Geleneksel Çin otu olarak bilinen *Gynostemma pentaphyllum* bitkisindeki saponin içeriğinin belirlenmesi için uygun bir ekstraksiyon, saflaştırma ve HPLC-MS yöntemi geliştirilmiştir.	Bu çalışmada LC-MS ile 18 saponin tanımlanmıştır.	Kao ve ark., 2008
Kinoaların saponin içeriklerinin belirlenmesi amacıyla GC-MS yöntemi kullanılmıştır.	Toplam saponin içeriği çeşitler arasında 3,81 ile 27,1 mg/g arasında değişim gösterirken; en baskın saponin çeşidi toplam saponin içeriğinin yaklaşık %70›ini oluşturan fitolakkajenik asit olarak belirlenmiştir (16,72 mg/g).	Medina-Meza ve ark., 2016

4. Sonuç

Saponinler, oldukça önemli farmasötik özellikleri nedeniyle çeşitli bitki kaynaklarından türetilen önemli ikincil metabolitlerdir. Bu derleme, saponin bileşiklerinin kantifikasyonu için kullanılan kromatografik ve spektrofotometrik yöntemlere odaklanmaktadır. Saponinlerin kromatografik ve spektrofotometrik yöntemlerle kantifikasyonu, çeşitli bitki materyallerinden bu önemli bileşiklerin tanımlanması ve miktarlarının belirlenmesi için etkili bir yaklaşımdır. Kromatografik ve spektrofotometrik yöntemler, saponinlerin karmaşık yapıları ve çeşitlilikleri göz önüne alındığında, yüksek doğruluk ve hassasiyet sağlamaktadır. TLC, saponinlerin hızlı ve niteliksel analizi için kullanılırken, HPLC özellikle ters faz kolonları kullanılarak saponinlerin kantitatif analizi için en yaygın kullanılan yöntemdir. HPLC, saponin analizinde en yaygın kullanılan ve en güvenilir yöntem olup, diğer tekniklerle birleştirildiğinde saponinlerin yapısal çeşitliliğini ve konsantrasyonlarını doğru bir şekilde belirleyebilme kapasitesine sahiptir. HPLC-UV sisteminin saponinlerin belirlenmesinde yetersiz kaldığı durumda HPLC, MS veya nükleer manyetik rezonans (NMR) gibi tekniklerle birleştirilmektedir. Böylece yüksek hassasiyetli analizler dedeksiyonlar yapılabilmektedir. GC, saponinlerin büyük moleküller ve uçucu olmayan bileşikler olmaları nedeniyle sınırlı uygulamaya sahiptir. Sonuç olarak, saponinlerin kromatografik ve spektrofotometrik yöntemlerle ayrıştırılması, bitki materyallerinden bu bileşiklerin güvenilir bir şekilde tanımlanması ve miktarlarının belirlenmesi için kritik öneme sahiptir.

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Functional Properties, Vitamin Content and Sensory Attributes of Instant Swallow Meal from Pre-Gelatinized Composite Flours

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Abstract

Introduction and Aim: Swallow meals are traditional foods made from popular staple foods often hand moulded and generally served with Nigerian soups. This study was carried out to produce instant swallow meal from whole wheat, yellow cassava, acha and soy protein isolate, which will reduce the rigors and time of preparing swallow meals.

Method: The raw materials were processed into flours, packaged in polyethylene bags and kept at room temperature. The flours were blended in the following ratios, whole wheat :yellow root cassava flours (90:10) (95:5), acha :yellow root cassava (90:10) (95:5). Blends were enriched with 5g of soy protein to boost protein content of the product they were pre-gelatinized by mixing 400g of each blend with 250ml of water, molded and tied with polyethylene bag and preheated at 100°c for 1hr, oven dried at 60°c for 48hrs. The pre-gelatinized blends were subjected to functional properties analysis, selected vitamin content and sensory evaluation using standard methods.

Results: Functional properties recorded the following ranges water absorption capacity (96.7-130.61%), Oil absorption (69.53-84.44%), solubility (3.26-5.12%), bulk density (0.71-0.87g/ml), swelling capacity (60.98% to 83.45%) and gelatinization temperature (82.0-86.6). Vitamin E ranged from 0.81 to 0.93 mg/100g, vitamin B3 ranged from 2.27 to 3.45mg/100g and Pro-vitamin A ranged from 0.19 to 0.25 mg/100g Pre-gelatinization significantly ($p>0.05$) increased the water absorption capacity solubility and swelling capacity of the flour blends. The most acceptable instant swallow meal was WCY2 which contains whole wheat flour and yellow root cassava (90:10).

Conclusion: Acceptable instant swallow meal was achieved which reduced the rigors and time involved in the preparation of swallow meals.

Keywords: Swallow, meal, pre-gelatinized, instant, flour

1. Introduction

Swallow meals are traditional thick, elastic, easily malleable dough-like food made from popular staples that are consumed by moulding and dipping sizable dough into soup /sauces or stews of fish, meat and vegetables (Esnhemokha, 2021). Swallow meals are popular in Nigeria. It is so described because rather than chewing, it is just swallowed after dipping in a soup. Swallow meals vary from place to place, cassava products (Garri, fufu), pounded yam are indigenous to the eastern part of Nigeria, amala to the western part of Nigeria, Tuwo Shinkafa is majorly consumed in the northern part of Nigeria (Quora, 2017). Other such foods include whole wheat swallow (semolina), starch, oatmeal swallow, millet swallow among several others.

Pre-gelatinization is a hydrothermal process that consists of boiling and drying (Du, *et al.,* 2014). Pre-gelatinization process is one of the commonly used methods of physical modification of starch and widely used in the food industry to improves the functional characteristics of starches (Zia-ud, et al*.,* 2017,Ulfa et al., 2020). Pre-gelatinization could improve the flow ability of some cereals starch with increase of swelling power and water-binding capacities of starches (Liu et al., 2017) which favours the making of instant products (Butt et al., 2018).

Acha (*Digitaria exilis*) is a small annually herbaceous plant (forest seed/grain) commonly found in Nigeria, Sierraleon, Ghana, Guinea Bissau, Togo, Mali, Benin republic, and Ivory Coast (Jideani and Jideani, 2011). The protein content of acha grain is rich in methionine and cysteine which are vital for human health and lacking in most cereals (Sarwar et al., 2013). Scientist has reported the crude protein content of the grain to be similar to that of maize and complementary to legumes in methionine and cysteine contents (Glew et al., 2013). The English name of the seed grain "Hungry Rice" was believed to have been named by European's which was considered misleading by some authors (Ukim et al., 2013). In West Africa, the common species cultivated are *Digitaria exilis* (white acha), and *Digitaria iburua* (black acha) (Dansi et al., 2010). Acha is now gradually being used as feed due to it nutritional value and high yield during cultivation (Li et al., 2018).

Wheat (*Triticum aestivum L.*) belongs to the family of poaceae, which is cultivated worldwide as a staple food. it is an excellent source of dietary fibre, antioxidants (black wheat), free and esterified phenolic acids, vitamins and minerals (magnesium, phosphorous, and selenium)

which play important roles in maintaining and regulating various body metabolic functions (Xu et al., 2019). The nutritional value of wheat is extremely important as it takes an important place among the few crop species being extensively grown as staple food sources. The importance of whole wheat is mainly due to the fact that the seed can be ground into flour as seen in semolina production or refined and thus form the basic ingredients of bread and other bakery products, as well as pastas, thereby presenting the main source of nutrients to most of the world population.

Yellow cassava (*Manihot esculenta Crantz*) is an essential root crop for safeguarding the future of more than 800 million people throughout the world (Da Silva et al., 2015). It is widely cultivated in many parts of Sub Saharan Africa (Parkes et al., 2013). Yellow cassava is used as a stabilizer in the instant swallow meal due to the binding properties of the flour. Yellow cassava is rich in pro vitamin A carotenoid (Ilona et al., 2017). These bios fortified cassava varieties have yellow flesh due to their provitamin A carotenoid content are more bioavailable than in other crops (Berni. et al., 2014).

Soybean (*Glycine max L. Merrill*), is considered among ancient cultivated crops. It was domesticated in the 11th century BC around northeast of China. It is one of the grown leguminous crops in the world. Soy protein isolate (SPI), as a prime source of high-quality protein it has been extensively used in beverage, baking, and dairy alternatives due to its high nutritional value (Cui et al., 2013). Compared with other plant proteins, soy protein isolate (SPI) is rich in essential amino acids needed by human body, especially for the amount of lysine, which is normally lacking in other cereals (Astawan and Prayudan, 2020).

Cooking of most swallow foods from raw materials can be multitasking requiring many laborious unit operations and time consuming as well, as experienced in making of swallow from maize flour. Also, cooking of the fermented cassava paste to fufu which involves series of cookings and poundings.

The purpose of this study was to produce nutrient dense instant swallow meal that will save preparation time as well as circumvent the rigorous cooking processes associated with many swallow foods and still maintain good organoleptic properties.

2. Methods

2.1 Procurement of raw materials

Eight kilograms (8kg) of acha was bought from Terminus market, Jos, Plateau state, Nigeria. Seven kilograms (7kg) of whole wheat, five kilograms (5kg) of soybean and fifteen kilograms (15kg) of yellow cassava tubers, as well as other materials used for this work were obtained from Ogige market in Nsukka, Enugu State, Nigeria. The entire work was conducted in the laboratories of the Department of Food Science and Technology. University of Nigeria, Nsukka.

2.2 Preparation of samples

The raw materials were processed into flour as described below:

2.2.1 Production of whole wheat flour

The whole wheat grains were processed into flour according to the modified method of Ndife *et al,* (2011). The grains were cleaned to remove stones and other extraneous materials, washed in excess water, drained, oven dried 60°C for 3hrs., milled using attrition mill to obtain the flour and was packaged using polyethylene bags. Stored inside a covered bucket at room temperature.

2.2.2 Production of acha flour

The method of Ayo *et al,* (2007) was adopted. The grains were cleaned to remove stones and other extraneous materials, washed, drained, oven dried at 60°C for 2hrs. Milled using the attrition mill, packaged using polyethylene bags. Stored inside a covered bucket at room temperature.

2.2.3 Production of yellow cassava flour

The yellow cassava tubers were peeled, washed, grated to reduce into mesh and pulp, the pulp was poured inside a bag and heaped with a heavy stone to dewater the mesh, it was dried and milled using the attrition mill and then sieved using 0.02mm sieve and was finally packaged using polyethylene bag Shittu and Adedokun (2010).

2.2.4 Production of soybean protein isolate

Soybeans were processed into soy protein isolate according to the method of Kondjoyan et al., (2015). The cleaned and sorted soybean was cracked using an attrition mill, The cracked beans were then winnowed to remove the hulls, milled using attrition mill. The flour was then defatted in a soxhlet extractor for 6 h. using n hexane with boiling point of 50–55°C, desolventized by leaving to dry at room temperature for about 24 h. The defatted soy flour was extracted in 200 ml water at pH 8.9. The pH of the distilled water was adjusted using 1 N NaOH. The slurry was stirred continuously for 30 min. The mixture was then centrifuged, the extract was mixed with the first extract while the residue was discarded. The pH of the combined extracts was then adjusted to pH 4.5. The supernatant was discarded while the residue, that is the precipitate was given two 50 ml washes with distilled water and re-centrifuged. The precipitate (ASPI) was then neutralized to pH 7 using NaOH and then spread thinly in a crucible and dried in an air-draught oven at 40°C for 12 h.

2.3 Composite flour formulation for pre-gelatinization

Six different formulations were obtained from the flour blends as shown in Table 1 below. Acha flour (AF) and wheat flour WF) blended with yellow cassava flour (YCF) and enriched with soy protein isolate (SPI) each, two formulations were acting as controls, one was from 100% Acha flour and another one was from 100% whole wheat flour.

Sample	Yellow cassava	Acha		Wheat		Soy protein isolate
		Pregelatinized	Not pre-gelatinized	Pregelatinized	Not pre-gelatinized	
AC	-	-	100%	-	-	-
WC	-	-	-	-	100%	-
ACY1	5%	95%	-	-	-	5g
ACY2	10%	90%	-	-	-	5g
WCY1	5%	-	-	95%	-	5g
WCY2	10%	-	-	90%	-	5g

Table 1: Formulation of samples for pre-gelatinization

2.4 Pre–gelatinization process

The flours were pre-gelatinized according to the method of Obinna-Echem (2017). 400g of flours were manually mixed with 250 ml of water each to get a sticky dough. The dough was tied using polyethylene bag and precooked for 1 hr at 100°C. They were oven dried at 60°C for 48 hrs, [Fulton, model NYC -101] cooled. Milled using the attrition mill. The samples were packed using a polyethylene bags and stored for further analysis.

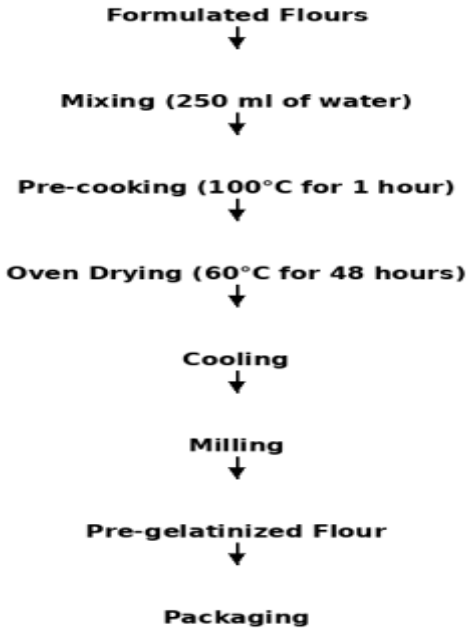

Figure :1 Pre-gelatinization of flours, Obinna-Echem (2017).

2.5 Determination of functional properties

2.5.1 Water absorption capacity (WAC)

Water absorption capacity was determined using the method described by Adeleke and Odedeji (2010), where 15 ml of distilled water were added to 1 g of the flour in a weighed 25 ml centrifuge tube. The tube was agitated on a mixer for 2 min. It was centrifuged (Sarvalle ®-rotor GSA, Newton, USA) at 4000 rpm for 20 min. The clear supernatant was decanted and discarded. The adhering drops of water were removed and then reweighed. Water absorption capacity is expressed as the weight of water bound by 100 g dried flour.

2.5.2 Oil absorption capacity (OAC)

Oil absorption capacity was determined using the method described by Adeleke and Odedeji (2010). Ten milliliters (10 ml) refined corn oil were added to one gram (1 g) of the flour in a weighed 25- or 80-ml centrifuge tube. The tube was agitated on a mixer for 2 min. It was centrifuged (Sarvalle ®-rotor GSA, Newton, USA) at 4000 rpm for 20 min. The volume of free oil was recorded and decanted. Fat absorption capacity was expressed as milliliter of oil bound by 100 g dried flour.

2.5.3 Solubility Determination

The cold-water extraction method, as described by Udensi and Onuora (1992), was adopted. Flour dispersion (10% w/v, db.) was prepared with each of the flour samples by dispersing one (1) gram (dry basis) of sample in 5 ml distilled water and making it up to 10ml. It was left for 60 mins. while it was stirred every 10 mins. Then it was allowed to settle for 15 mins. after which 2ml of the supernatant was weighed in a dry Petri dish, evaporated to dryness and re-weighed. The difference in mass is the total soluble solids. Solubility was calculated as follows:

Solubility = TSS (%) ((VsMe-Md) x 100

Where;

Vs = Total supernatant/ filtrate

Md = Mass of empty, dry Petri dish0

Me = Mass of Petri dish plus residual solid after evaporative drying

Ms = mass of flour sample used in the preparation of the dispersion

2.5.4 Swelling capacity

This is the ratio of the expanded volume of a unit weight of flour to its initial volume. We followed the steps Onwuka (2018) had outlined. One (1) gramme of the flour sample was weighed into a pristine, dry measuring cylinder. After measuring the sample's height (beginning height), 5cm of distilled water was added. Having been allowed to stand for an hour without being disturbed, this was observed and captured once more (final height). After then, the swelling index was calculated by dividing the final height by the starting height.

Bulk density

Bulk density was determined using the method described by Adeleke and Odedeji (2010). Fifty grams (50 g) flour sample was put into a 100 ml measuring cylinder. The cylinder was tapped several times on a laboratory bench to a constant volume. The volume of sample was recorded.

$$\text{Bulk density } \left(^{g}/_{cm^3}\right) = \frac{\text{Weight of sample}}{\text{Volume of sample after tapping}}$$

2.5.5 Gelatinization temperature

The method of Onwuka (2018) was used. Determination of Gelatinization Temperature A beaker containing one gramme (1g) of sample was filled with 10 ml of distilled water, which was then agitated in order to create a homogeneous mixture. The sample was heated and continually whirled in a beaker filled with boiling water until it gelled. A thermometer was used to measure the temperature 30 seconds after gelation (Onwuka, 2018).

2.5.6 Apparent viscosity

Apparent Viscosity of flour dispersions was determined using Brookfield DV-II viscometer (Brookfield Eng., Middleboro, Mass., USA) by preparing dispersions of 5, 10, 15, and 20 g flour in 100 mL of distilled water in 250-mL beaker. The apparent viscosity was measured using spindle number 2 at shear rate of 100 rpm at 23 ± 1ºC. The readings were recorded after 30 secs. shearing time. All viscosity measurements were done in triplicate and results are reported in mPa.s

2.6 Vitamin analysis

2.6.1 Determination of pro-vitamin A

Pro-vitamin A was determined by the calorimetric method of Kirk and Sawyer (1991). One gram (1 g) of the sample and standard were mixed with 30 ml of absolute alcohol and 3 ml of 5% KOH solution was added to it and was boiled for 30 min under reflux. After washing with distilled water, vitamin A was extracted with 150ml of diethyl ether. The extract was evaporated to dryness at low temperature and then dissolved in 10 ml of isopropyl alcohol. Exactly 1 ml of standard Vitamin A solution was prepared and that of the dissolved extract were transferred to separate cuvettes and their respective absorbance were read in a spectrophotometer at 325 nm with a reagent blank at zero.

Conc. of pro-vitamin A in Sample = x conc. of Standard

2.6.2 Determination of Vitamin E

Vitamin E was determined using the method described by Kirk and Sawyer (1991). One gram of sample was weighed into 100 ml flask, 10ml of absolute alcohol and 20 ml of M alcoholic tetraoxosulphate VI acid (H_2SO_4) was added. Ten milliliters of clear solution were pipetted into a test tube and heated in a water bath at 90 °C for 3 minutes. This was allowed to cool. The absorbance was read in a spectrophotometer at 470 nm wavelength.

$$\text{Vitamin E in } {}^{\text{Mg}}/_{100\text{ g}} = \frac{(a-b)}{(s-b)} \times \frac{c}{w}$$

Where, a= Absorbance of test sample

b= Absorbance of blank

s= Absorbance of standard solution

c= Concentration of standard in mg/100 g

w= Weight of the sample used

2.6.3 Determination of vitamin B3

Vitamin B3 (Niacin) was determined by using AOAC (2010) procedure. Five grams of each sample was treated with 50 ml of 1 N sulphuric acid for 30 minutes. 0.5 ml ammonia solution was added to it and then it was filtered. To 10 ml of the filtrate, 5 ml of 0.5 % potassium cyanide was added. This was further acidified with 5 ml of 0.02 N sulphuric acid. The absorbance of the resultant solution was recorded at 420 nm. The absorbance obtained from the sample extract was converted to niacin concentration by means of a calibration curve generated using different standard concentrations.

2.7 Preparation of the pre-gelatinized composite flours for swallow meals

Two hundred millilitres (200mls) of water were boiled at 100°C, poured into a bowl and 350g of pre-gelatinized flour were added and turned until smooth, shaped and wrapped ready to be served with soup.

2.7.1 Sensory Analysis

Sensory attributes of pre-gelatinized instant swallow was assessed based on appearance, taste, aroma, mould-ability, texture and overall acceptability. Twenty panelists were selected from Department of Food Science and Technology, University of Nigeria, Nsukka based on the fact that they are conversant with swallow meals. The quality indices were assessed on a 9-point Hedonic scale (from 1= extremely dislike to 9= extremely like) as described by Singh et al. (2012).

2.8 Experimental Design and Statistical Analysis

The experiment was laid out on a completely randomized design (CRD). Data Obtained were subjected to Analysis of Variance (ANOVA) using Statistical Product for Service Solution (SPSS) software version 23.0. Duncan's New Multiple Range Test (DNMRT) was used to determine the difference between means of the tested parameters. Statistical significance was accepted at ($p < 0.05$) (Steel and Torrie, 1980).

3. Results

Table 2: Functional properties of pre-gelatinized composite flours

Samples	Water absorption Capacity (%)	Oil absorption Capacity (%)	Solubility	Swelling Capacity (%)	Bulk density (g/cm3)	Gelatinization Temperature (°C)
WCY1	$121.13^{b}\pm1.93$	$69.53^{e}\pm0.49$	$5.12^{ab}\pm4.65$	$71.95^{c}\pm0.06$	$0.85^{b}\pm0$	$83.5^{ab}\pm0.71$
ACY2	$112.64^{c}\pm1.09$	$84.44^{a}\pm4.2$	$4.83^{a}\pm7.87$	$76.09^{b}\pm0.007$	$0.72^{d}\pm0$	$86.5^{a}\pm0.71$
WCY2	$130.61^{a}\pm1.92$	$71.84^{de}\pm1.1$	$4.86^{b}\pm0.01$	$.83.45^{a}\pm064$	$0.87^{ab}\pm0$	$82.0^{b}\pm1.41$
ACY1	$106.52^{d}\pm0.04$	$80.6^{ab}\pm0.17$	$4.31^{b}\pm0.76$	$69.60^{d}\pm0.01$	$0.75^{c}\pm0.01$	$85.5^{ab}\pm0.71$
AC	$107.81^{d}\pm2.62$	$76.56^{bc}\pm0.8$	$3.27^{d}\pm0.62$	$63.78^{f}\pm0.02$	$0.72^{d}\pm0$	$85.0^{ab}\pm0.00$
WC	$96.7^{e}\pm2.5$	$75.15^{cd}\pm0.66$	$3.26^{e}\pm3.25$	$60.98^{e}\pm0.72$	$0.71^{e}\pm0.01$	$83.0^{ab}\pm2.83$

Values are mean ±SD of duplicate determinations. Values within the same column with different superscripts are significantly ($p<0.05$).

Key: Sample WC - Wheat flour (100%) Sample AC - Acha Flour (100%) Sample WCY1 – Wheat flour + yellow root cassava flour (95:5%) Sample WCY2 – Wheat flour + yellow root cassava flour (90:10%) Sample ACY1 - Acha flour + yellow root cassava flour (95:5%) Sample ACY2 - Acha flour + yellow root cassava flour (90:10%).

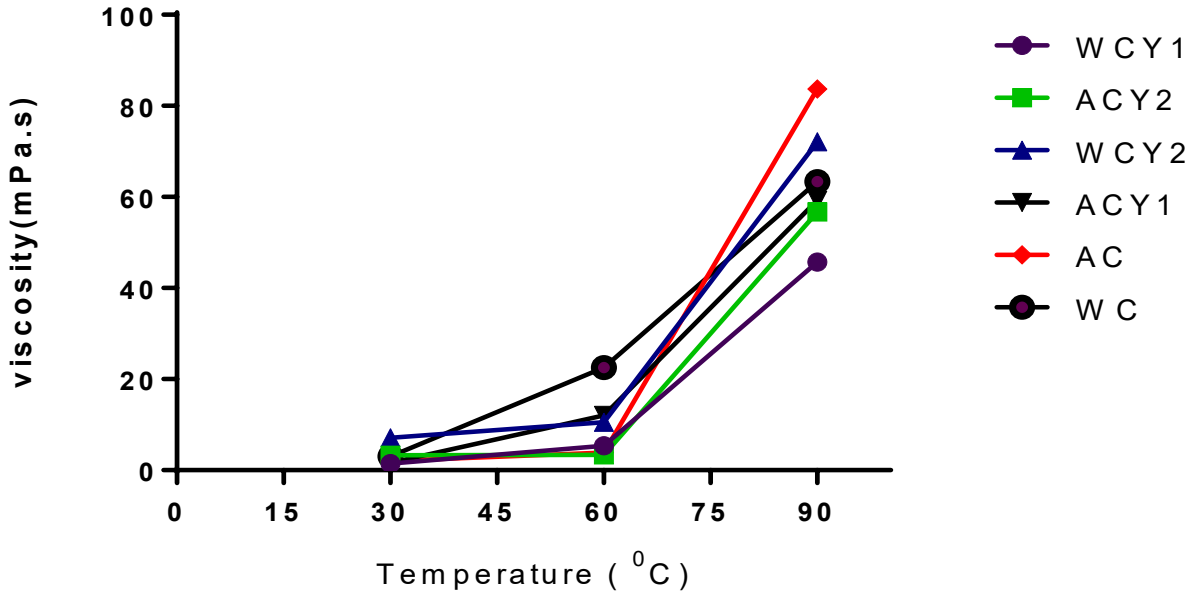

Figure 1: Change in viscosity of the composite flours with temperature

Key: Sample WC - Wheat flour (100%) Sample AC - Acha Flour (100%) Sample WCY1 – Wheat flour + yellow root cassava flour (95:5%) Sample WCY2 – Wheat flour + yellow root cassava flour (90:10%) Sample ACY1 – Acha flour + yellow root cassava flour (95:5%) Sample ACY2 - Acha flour + yellow root cassava flour (90:10%).

Table 3: Vitamin composition of pre-gelatinized composite flours and the controls (mg/100g)

Sample	Vitamin E	Vitamin B3	Pro-vitamin A
WCY1	$0.93^{bc}\pm0.10$	$3.33^{a}\pm0.42$	$0.21^{c}\pm0.01$
ACY2	$0.81^{c}\pm0.05$	$2.79^{a}\pm0.78$	$0.25^{b}\pm0.01$
WCY2	$0.82^{c}\pm0.04$	$3.45^{b}\pm0.25$	$0.22^{bc}\pm0.01$
ACY1	$0.93^{bc}\pm0.06$	$2.27^{a}\pm0.03$	$0.19^{c}\pm0.01$
AC	$0.99^{c}\pm0.00$	$3.31^{a}\pm0.32$	$0.21^{c}\pm0.01$
WC	$1.37^{a}\pm0.05$	$3.72^{a}\pm0.95$	$0.32^{a}\pm0.01$

Values are mean ±SD of duplicate determinations. Values within the same column with different superscripts are significantly ($p<0.05$).

Key: Sample WC - Wheat flour (100%) Sample AC - Acha Flour (100%) Sample WCY1 – Wheat flour + yellow root cassava flour (95:5%) Sample WCY2 – Wheat flour + yellow root cassava flour (90:10%) Sample ACY1 - Acha flour + yellow root cassava flour (95:5%) Sample ACY2 - Acha flour + yellow root cassava flour (90:10%).

Table 4: Sensory scores of the instant swallow meal

Sample	Appear-ance	Taste	Texture	Aroma	Mouldability	General Accept-ability
WC	5.26^{b}±1.52	6.0^{b}±1.03	6.85^{a}±0.67	5.53^{c}±1.22	6.6^{c}±0.75	6.04^{b}±0.72
AC	3.63^{d}±1.51	4.20^{c}±1.15	2.50^{c}±1.59	6.16^{b}±1.08	2.43^{c}±1.30	3.78^{c}±1.32
WCY1	7.05^{a}±1.1	6.65^{ab}±1.04	7.15^{a}±0.81	6.35^{a}±1.09	7.85^{a}±0.93	6.9^{ab}±0.79
ACY1	5.23^{c}±1.77	6.05^{b}±0.69	5.83^{bc}±1.78	6.2^{a}±1.06	6.96^{a}±1.29	6.1^{b}±0.62
WCY2	7.3^{a}±0.66	6.45^{ab}±0.76	7.15^{a}±0.93	6.4^{a}±1.14	8.00^{a}±0.73	7.35^{a}±0.59
ACY2	7.5^{a}±0.69	6.4^{ab}±0.75	7.3^{a}±0.86	6.45^{a}±1.1	7.2^{b}±0.52	7.25^{ab}±0.64

Values are mean ±SD of duplicate determinations. Values in the same column with different superscript are significantly ($p>0.05$) different.

Key: Sample WC - Wheat flour (100%) Sample AC - Acha Flour (100%) Sample WCY1 – Wheat flour + yellow root cassava flour (95:5%) Sample WCY2 – Wheat flour + yellow root cassava flour (90:10%) Sample ACY1 - Acha flour + yellow root cassava flour (95:5%) Sample ACY2 - Acha flour + yellow root cassava flour (90:10%).

4. Discussion

Functional properties are the basic physico-chemical characteristics that show the intricate relationship between the molecular conformation, content, structure, and physico-chemical characteristics of food constituents as well as the type of environment in which these are related and quantified. They are necessary to assess and maybe assist in anticipating the potential behaviour of food components in certain food systems (Suresh and Samsher, 2013). The functional properties such as bulk density, water and oil absorption capacity, gelation temperature**,** swelling capacity and solubility of the of the pre-gelatinized composite flour were evaluated and result presented in Table 2. Functional properties can be used to predict and precisely evaluate how new proteins, fat, carbohydrates (starch and sugars), and fibre may behave in specific food systems as well as demonstrate whether such can be used to stimulate or replace conventional protein, (Suresh and Samsher, 2013), fat, carbohydrates (starch and sugars), and fibre.

Water absorption capacity (WAC) is the amount of water (moisture) taken up by food/flour to achieve the desirable consistency and create quality food product. Very low or excessive water absorption can negatively affect the quality of food products. Water absorption capacity of a flour is influenced by starch, pentosans, proteins contents, among others. In this study, the values for WAC of the instant swallow composite flours ranged from 96.7%-130.61% which were higher than the controls (the un pregelatinized composite flours). Significant ($p<0.05$) differences existed among samples in water absorption capacity. Composite samples containing whole wheat flour recorded higher WAC in the present work, this is contrary to the report of Suresh et al. (2015) where WAC increased with a decrease in wheat flour incorporation. These high WAC value can therefore be attributed to many hydrophilic components contained in these composite flours, such as carbohydrate

(especially polysaccharides), proteins, especially polar amino acid residues, which have high affinity for water molecules (Sreerama et al., 2012). Increase in the WAC can also be associated with increase in the amylose leaching and solubility, and loss of starch crystalline structure. High WAC of composite flours suggests that the flours can be used in formulation of foods in dough forms, and bakery products.

Oil Absorption Capacity (OAC) is the binding of fat by the non-polar side chain of proteins. The OAC of the composite flours ranged from 71.84 to 84. 44%. Significant ($p<0.05$) differences existed among samples in oil absorption capacity. Composite flours containing acha flour recorded higher oil absorption capacity as compared to samples containing whole wheat. This can be traced to the fact that the acha flour also recorded a higher value when compared to whole wheat flour. This is because rate of oil absorption is very high in foods with high protein content. The oil and water binding capacity of protein in food depend on the intrinsic factors such as protein conformation, amino acid composition, and surface polarity or hydrophobicity (Suresh and Samsher, 2013). The ability of flours to bind with oil makes them useful in food applications. Oil absorption capacity is an essential functional property that contributes to enhancing mouth feel while retaining the food products' flavour (Iwe et al., 2016). The major chemical component affecting OAC is protein which is composed of both hydrophilic and hydrophobic parts. Non-polar amino acid side chains can form hydrophobic interaction with hy drocarbon chains of lipids (Jitngarmkusol et al., 2008).

Solubility The ability of solid, liquid, or gaseous food (chemical) substances known as solutes to dissolve in liquid, gaseous, or solid solvent is known as solubility in the food system. The solubility of a material is primarily determined by the solute's and solvent's physical and chemical characteristics, as well as temperature, pressure, pH, and the presence of other substances in the solution. The extent of the solubility of a food (flour) substance in a specific solvent is commonly measured as the saturation concentration, in which addition of more solute does not increase concentration of the solution(Awuchi et al., 2019). Flour solubility is the amount of the flour that dissolves into solution, usually with water as solvent. Flour solubility is one of the functional properties usually determined during the development and testing of a new flour or flour composite. The smaller the size of a food particle is, the quicker it dissolves (Awuchi et al., 2019). Solubility of the controls which is 100% whole wheat and 100% acha flours were seen to increase after pre-gelatinization. This is an indication that pre-gelatinization increased solubility. This could be attributed to change in the crystalline structure of the flours after pre-gelatinization. Solubility ranged from 4.31 to 5.12 with composite flours containing whole wheat recording higher solubility. Significant ($p<0.05$) differences existed among the samples in solubility.

Swelling capacity Swelling capacity (SC), is the volume in milliliter taken up by the swelling of one gram (1 g)of food material under specific conditions, usually based on the addition of water. It is the measure of the ability of starch to absorb water and swell, it reflects the extent of associative forces in the starch granules. Swelling capacity is considered a quality measure in some food products such as bakery products. It is an indication of the non-covalent bonding between the molecules of starch granules and also one of the factors of the αamylose and amylopectin ratios (Iwe et al., 2016). Swelling capacity is influenced by factors such as particle size, species or variety and method of processing or unit operations (Suresh and Samsher, 2013) Swelling capacity ranged from 60.98% to 83.45%, with the pre-gelatinized flours recording higher values. Significant ($p<0.05$) differences existed among the samples in swelling capacity. The swelling capacity of the blends increased due to pre-gelatinization which increase swelling properties of the flours. High starch content increases swelling capacity of foods and flours, especially in starch with higher amount of the branched amylopectin. Starch is made up of amylose (linear chain) and amylopectin (branched chains), both are chains of

glucose units. Starch is found in very small packets known as granules. The amount and proportion of amylose and amylopectin found in starch vary according to the plant source. This explains why different flours from different (plant) sources and species have different swelling capacities (Awuchi et al., 2019).

Bulk density Bulk density is a measure of the heaviness of a flour sample. It is used to determine packaging requirements. Bulk density can as well be referred to as the density measured without the influence of any compression. Bulk density ranged from 0.72 g/cm^3 to 0.87 g/cm^3 .Composite flours containing whole wheat recorded higher levels in bulk density. There were Significant ($p<0.05$) differences in bulk densities of the composite flours. The high bulk density of flour suggests their suitability for use in food preparations. On the contrast, low bulk density would be an advantage in the formulation of complementary foods (Akapata and Akubor 1999). The higher the starch content the more likely the increase in bulk density. Also, bulk density depends on factors such as geometry, method of measurement, particle size, surface properties, and solid density of the materials and can be improved when the particles are smaller, properly tapped/vibrated, compactible, and with a suitable packaging material (Iwe et al., 2016). Therefore, in the present study the highest bulk density of composite flour (WCY2) suggests its suitability to be used as thickener in food products and for use in food preparation since it will be able to thicken and readily form a dough..

Gelatinization temperature Gelatinization is a process of breaking down intermolecular bonds of starch molecules in the presence of heat and water, allowing the hydrogen bonding sites (hydroxyl hydrogen and oxygen) to absorb more water. This causes the starch granule to irreversibly dissolve in the water. As soon as starch granules are heated in liquid like water, they absorb the liquid, then swell and rupture, leading to increase in viscosity (stickiness) of the starch. Finally, the mixture thickens. Gelatinization temperature is the temperature at which the gelatinization of starch takes place. The gelatinization temperature of starch depends on the plant type and amount of water present, pH, salt concentration and types, sugar, protein, and fat in the recipe, as well as the technology of starch derivatization employed. Some type of unmodified native starches begin swelling at 55 °C, some other types at 85 °C (Hans-Dieter et al., 2004). Also, gelatinization temperature depends on the amount of the damaged starch granules, these will swell faster. Damaged starch granules can be produced, for instance, during milling process. The gelation temperatures of the composite flours ranged from 82 to 86.5 ^{0}C. Slight decrease in gelation temperature was observed with the sample (WCY2).

Factors influencing gelatinization are types of starch, stirring, water, temperature, presence of other ingredients, such as protein, sugar, acids, fat among others. Starch from different sources differs in the thickening and water-binding capability. Starch with more amylopectin often has higher thickening ability. The root starches are generally more effective than cereal starches, thus necessitated the addition of yellow fleshed cassava in the present study. The hydrophobic and waterproof fat molecules coating the flour components such as starch granules decrease their interaction with water, reduce absorption of water and decrease gelatinization (Awuchi et al., 2019).

Effect of temperature on the viscosity of the pre-gelatinized composite flours

The effect of temperature on the viscosity of the composite flours is illustrated in figure 1. The viscosities of composite flours were observed to increase slightly from 30 to 60^{0}C. However, a sharp increase in viscosity was observed between 60 to 90^{0}C, which could be attributed to gelation thus suggesting that the gelation temperatures of the composite flours are between 60 to 90^{0}C.

Vitamin composition of pre-gelatinized composite flours and the controls

Table 3, shows selected vitamin content of pre-gelatinized composite flours. Pro-vitamin A are found in plant based foods, the most common carotenoid in foods and dietary supplements is beta carotene. It isa pale yellow fat soluble vitamin. Pro-vitamin A content of the formulated instant swallow composite flours varied slightly among the samples. Pro-vitamin A content ranged from 0.19 to 0.25 mg/100g for the pre-gelatinized composite flours. A value of 0.32 mg/100g was observed in whole wheat as a single flour that was not pre-gelatinized (control) this can be compared to 4 mg/kg reported by Hussain *et al*. (2015) for carotene content in organically produced wheat. Pro-vitamin A content of composite flours containing 10 % yellow cassava was slightly higher than samples containing 5 % inclusion. Significant ($p<0.05$) differences was observed by 10% increase in yellow cassava flour addition. Pro-vitamin A is unstable to light and solutions that are slightly alkaline. It is destroyed when heated in the presence of oxygen and thus the process of pre- gelatinization caused a depletion in the initial content of pro-vitamin A. Carotene are very important in the food, pharmaceutical and cosmetic industries due to the coloring properties. In addition, they are also used in food fortification because of their possible activity as pro-vitamin A and their beneficial biological functions in health, such as vision, its deficiency results in retarded growth, night blindness or defective sight when the light is deem. It has the ability to strengthen the immune system, reducing the risk of degenerative diseases. It also possesses antioxidant properties, and ant-obesity/hypolipidemic activities (Mezzomo *et al.,* 2015).

Vitamin E or tocopherol is a fat-soluble vitamin that is stored in the liver and released in small doses whenever necessary. Therefore, it is not necessary to take it on a regular basis, such as through foods. It is a nutrient that is important to vision, reproduction, and the health of the blood, brain and skin. Vitamin E also has antioxidant properties. Antioxidants are substances that might protect your cells against the effects of free radicals. Free radicals might play a role in heart disease, cancer and other diseases. The vitamin E content of the pre-gelatinized composite flours ranged from 0.81mg/100 to 0.93 mg/100g. The control samples of 100% whole wheat flour and 100 % acha flour recorded higher values than the pre-gelatinized composite flours. Significant ($p<0.05$) differences existed among the samples in vitamin E content. This can be explained by the fact that the raw materials are not known sources of vitamin E, which is also sensitive to heat and light, so it tends to disperse in the presence of high temperatures thus the depletion in the pre-gelatinized samples. A higher amount of vitamin E (2.60 mg/100g) was reported by James and Nwabueze (2013) for African breadfruit-soybean-acha flour. Vitamin E deficiency can cause nerve pain (neuropathy).

Vitamin B3 is also known as niacin. It is one of the water-soluble B vitamins. It is essential for the health of the nervous system, digestive system, and skin. Sources of niacin includes legumes, and many cereals. In higher amounts, niacin may be recommended as a dietary supplement to improve cholesterol levels, slow the progression of certain types of heart disease, and even help prevent memory loss and dementia (Galloway et al., 2024).

Niacin content ranged from 2.27 to 3.45 mg/100g in the pre-gelatinized composite flours. Significant ($p<0.05$) differences was observed among the pre-gelatinized samples.

Among the control samples 100 % whole wheat flour and 100 % acha flour, it was observed that whole wheat flour contained more niacin than acha, this explains why the pre-gelatinized composite flours containing whole wheat recorded higher levels of niacin than samples containing acha. A lower amount (0.22–0.01 µg/ml) was reported by (Ayo et al., 2016) for quality characterization of Acha-mushroom flour blends. Niacin deficien-

cy could develop a serious skin condition called pellagra.

Sensory scores of the instant swallow meal

Table 4: Shows the sensory scores of the instant swallow meal from the different composite flours. Generally, there is an indication that blending of the composite flours with yellow cassava flour as a binding agent and pre-gelatinization process improved the swallow meals. in appearance, sample ACY2, recorded the highest score (7.50) among the pre-gelatinized composite flours although not significantly ($p>0.05$) different from other samples except sample ACY1 which recorded the least score (5.23). Samples that were pre-gelatinized were preferred in appearance. The swallow meals were brown in colour. The brown colour is a result of Maillard reaction between the carbohydrate and protein content of the raw materials during heating (Olapade et al., 2014). However, it is still acceptable because other swallow meals like amala, (a popular swallow meal in Nigeria, popularized by the Yoruba ethnic group of the Southestern Nigeria) also appear brown in colour.

In terms of taste, sensory scores ranged from 6.05 for sample ACY1 to 6.65 for sample WCY1, significant ($p<0.05$) difference were observed among the pregelatinized samples which were all preferred to the un pre-gelatinized composite flours.

The score for texture ranged from 5.83 for sample ACY1 to 7.30 for sample ACY2. There were no significant ($p>0.05$) differences among the pre-gelatinized composite flours except for sample ACY1 which scored the least. The texture and mouldability, of any swallow meal gives an overview of the elasticity and ease of shaping the swallow meal. Mouldability scores ranged from 6.96 (ACY1) to 8.00 (WCY2). It was observed that in mouldability there was no significant ($p>0.05$) difference among the pre-gelatinized composite flours. Sample WCY2 was the most preferred in terms mouldability.

Panelists scored sample ACY2 highest in aroma, although there were no significant ($p>0.05$) differences among the pre-gelatinized composite flours. The values for the overall acceptability of the instant swallow meal ranged from 6.10 (ACY1) to 7.35 (WCY2) for the pre-gelatinized composite flours. Significant ($p<0.05$) differences existed among the pre-gelatinized composite flours in general acceptability. However, it was generally observed that 10 % incorporation of yellow cassava flour significantly improved acha containing instant swallow meal, as sample ACY2 received the highest scores in most of the sensory parameters evaluated. Although sensory the scores generally are as a result of individual preferences, which could be influenced by many factors inherent in the individual. Texture and moudability are the most important parameters in assessing swallow meal because they show the ease with which the swallow meals can be moulded and swallowed and sample ACY2 was rated highest in both parameters.

5. Conclusion

This study has shown that pre-gelatinization and blending were able to improve the functional properties and vitamin contents of the swallow meals. Pre-gelatinization significantly increased the water absorption capacity, solubility and swelling capacity which are important factors considered in flours for food products that requires the formation of dough. Sample WCY2 containing whole wheat: yellow cassava (90:10) was the best sample in most of the important functional properties determined. Pre-gelatinization was also instrumental in reducing preparation time for the products and also eliminate the rigors of producing swallow meals. The sensory scores of the swallow meals revealed higher acceptability of sample ACY2 containing acha : yellow cassava (90:10). Thus incorporation of 10 % yellow cassava improved the products greatly.

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Search On Puffed Innovative Product Production From Red Lentils

Kırmızı Mercimekten Patlatılmış Yenilikçi Ürün Üretimi Üzerine Araştırma

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Özet

Baklagiller zengin bir protein, enerji, vitamin ve mineral kaynağıdır. Yüksek besin değerlerine rağmen baklagillerin küresel tüketimi azalmaktadır. Tüketimdeki bu azalma, zaman alan hazırlama ve pişirme süreçlerine, sindirim sisteminde gaz oluşumuna ve bazı beslenme kaygılarına bağlanabilmektedir. Ancak son zamanlarda baklagillere ve doğal pişirme yöntemlerine olan ilgi, özellikle tüketicilerin daha sağlıklı gıdaları ve işleme tekniklerini tercih etmesiyle yeniden canlanmaya başlamıştır.

Kırmızı mercimek, besin değeri yüksek olan bir baklagildir ve protein, lif, vitamin ve mineral bakımından zengindir. Aynı zamanda düşük yağ oranı ile kalp sağlığına faydalıdır. Mercimek, çeşitli yemeklerde kullanılabilen ve dünya genelinde yaygın olarak tüketilen bir gıdadır.

Bu çalışma, yüksek sıcaklığa maruz bırakılarak ani basınç değişimi ile kırmızı mercimeğin şişirilmesi sürecini incelemekte ve aynı zamanda optimum dokusal özellikleri belirlenerek istenilen dokusal özelliklere sahip şişirilmesi amaçlanmaktadır. Farklı basınç (6, 7 ve 8 bar) ve su miktarları (25, 50 ve 75 mL) kullanılarak kırmızı mercimek patlatılmıştır. Ancak, elde edilen sonuçlar, hem basıncın hem de su miktarının mercimeklerin şişme performansı üzerinde anlamlı bir etkisi olmadığını göstermiştir. Mercimeklerde şişme gerçekleşmemiş, dolayısıyla kütle yoğunluğu ve dokusal kalite de değişmemiştir. Sadece renkte bazı değişiklikler gözlemlenmiş, fakat bu değişiklikler tutarlı değildir. Bu çalışmada gerçekleştirilen analizler, son üründe nem içeriği, yığın yoğunluğu, boyut, renk, duyusal özellikler, mikro yapı ve doku profili parametrelerini kapsamaktadır.

Anahtar Kelimeler: Patlatma prosesi, şişirme, bakliyat, kırmızı mercimek, patlatma.

Abstract

Legumes are a rich source of protein, energy, vitamins and minerals. Despite their high nutritional value, global consumption of legumes is declining. This decline in consumption can be attributed to time-consuming preparation and cooking processes, gas formation in the digestive tract and some nutritional concerns. However, recently there has been a resurgence of interest in legumes and natural cooking methods, especially as consumers prefer healthier foods and processing techniques.

Red lentils are a legume with high nutritional value and are rich in protein, fiber, vitamins and minerals. They are also beneficial for heart health with their low fat content. Lentils can be used in a variety of dishes and are a widely consumed food around the world.

This study examined the puffing process of red lentils under high temperature and sudden pressure changes, aiming to achieve optimal textural properties. Various pressures (6, 7, and 8 bar) and water amounts (25, 50, and 75 mL) were used to attempt puffing. However, the results showed that neither pressure nor water significantly affected the puffing performance, as the lentils did not puff. Consequently, there were no changes in bulk density or textural quality. Only some inconsistent changes in color were observed. Analyses included moisture content, bulk density, size, color, sensory properties, microstructure, and texture profile.

Keywords: Puffing process, legumes, red lentils, puffing gun, puffing.

1. Introduction

Legumes are seeds or fruits from plants belonging to the Leguminosae or Fabaceae family. The term 'legume' is derived from the Latin word 'Legumen', referring to the harvested seeds of beans in their pod. Varieties such as red lentils, cowpeas and soybeans are pivotal sources of protein, carbohydrates, vitamins and minerals, widely embraced in diets. Regarded as a sustainable and cost-effective meat alternative globally, legumes hold a significant place in human nutrition. (1)

The significant importance of legumes in human nutrition was recognized in 2016, declared as the "International Year of Pulses". This declaration was adopted at the 146th FAO Council and later acknowledged by the 68th General Assembly of the United Nations. Species within the legume family represent a crucial source of vegetable protein globally. These products are integral components of general food baskets within initiatives such as the "World Food Program" and other food aid endeavors. (2)

Simply put, all pulses are legumes, but not all legumes are pulses (Figure 1.1). According to the FAO, pulses represent an important component of healthy diets. In addition to their significant contribution to human nutrition and food security, pulses are associated with reduced risk factors for chronic disease, foster sustainable agriculture, and support climate change mitigation. Pulses have also been identified as having important potential roles in sustainable and healthy food systems. (3)

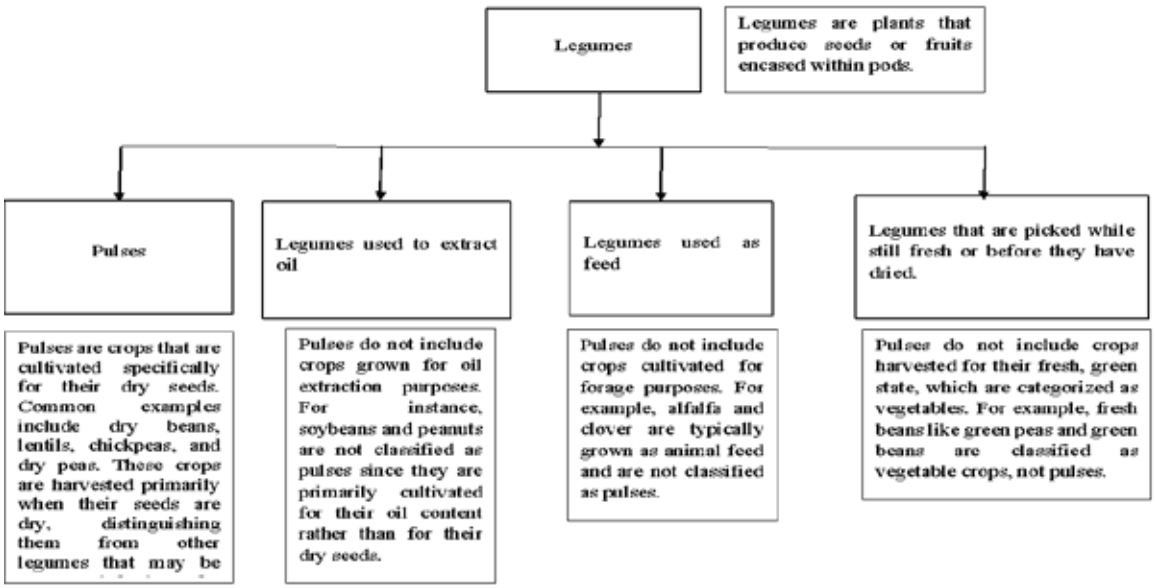

Figure 1. Summary of various types of legumes

The macronutrient contents for several common pulses along with soybeans are shown in Table 1. Pulses can contain various components in smaller amounts which should also be considered. These include protease inhibitors, lectins, phytates, phenolic compounds, saponins, oligosaccharides, phytoestrogens and non-protein amino acids (4). These are important as many are considered to be antinutrients, although some have also been considered to have health benefits (5).

Table 1. Macronutrient content of pulses and soybeans (g/100 g dry matter) (6)

	Protein	Fat	Carbohydrate	Ash	Dietary fibre
Kidney bean	17-27	1-5	63-74	3.2-5.2	18-30
Navy bean	19-27	2	67-75	4-4.9	14-25
Chickpea	19-27	1-3	52-71	1.8-3.5	6-15
Lentil	23-31	1-3	42-72	2.1-3.2	7-23
Pea	14-31	1-4	55-72	2.3-3.7	3-20
Lupin	32-55.3	5-15	4.5-47	2.6-5.09	14-55
Soybeans	32-43.6	8.1-24.7	31.7-35	4.5-6.4	19.7-31.9

The primary global production and trade of lentils predominantly focuses on red lentils (7). The majority of red lentils are consumed in split or whole cotyledon forms, commonly known as the 'football,' after removing the seed coat through dehulling, enhancing the cooking quality of red lentils. (8).

In this study, the effects of water and pressure on the puffing performances of red lentils were investigated. The aims of this study were;

- the effects of the types of legumes as a raw material (red lentils) on the swelling performance,
- the effect of puffing process on the dimensional size of red lentils,
- the bulk density and moisture content of the puffed products,
- relationship between starch content, ash content, protein content and color value analysis of the raw materials and the puffed red lentils,
- the quantitative and qualitative characteristics of the puffed products.

2. Materials and Methods

Materials

Materials used in the pulse puffing process; red lentils (*Lemnaceae*) (Grano Turco Tarım İşletmeleri Sanayi ve Ticaret A.Ş./Turkey) was used in this study. Moisture content device (Pfeuffer HE 50-5i, Germany) and colour device (colorflex, USA) were used. The puffing machine (Puritan Co. 1939, U.S.A.), (Figure 2) was used in this study (9).

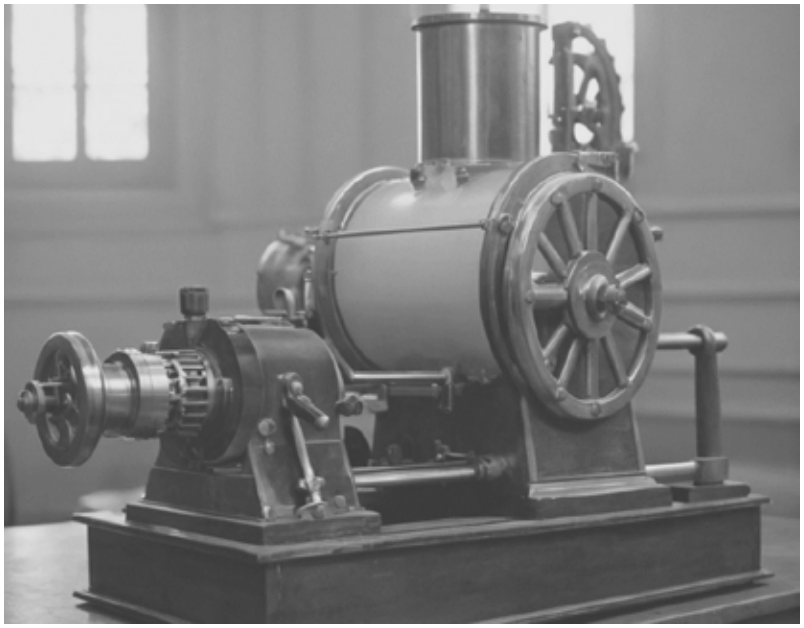

Figure 2. Puritan puffing machine

Preparation of the Samples

Five kilograms of red lentils were individually mixed with water at different levels (25, 50 and 75 mL) and then they were puffed at different pressures (6, 7 and 8 bar). During the puffing operation, initial (IT, °C) and final (FT, °C) temperatures of puffing systems and puffing time were determined. Bulk density, color values (L*, a*, b* values) and moisture content were measured for puffed products.

Experimental Set-ups

The experimental set-up for raw materials is given in Figure 3.

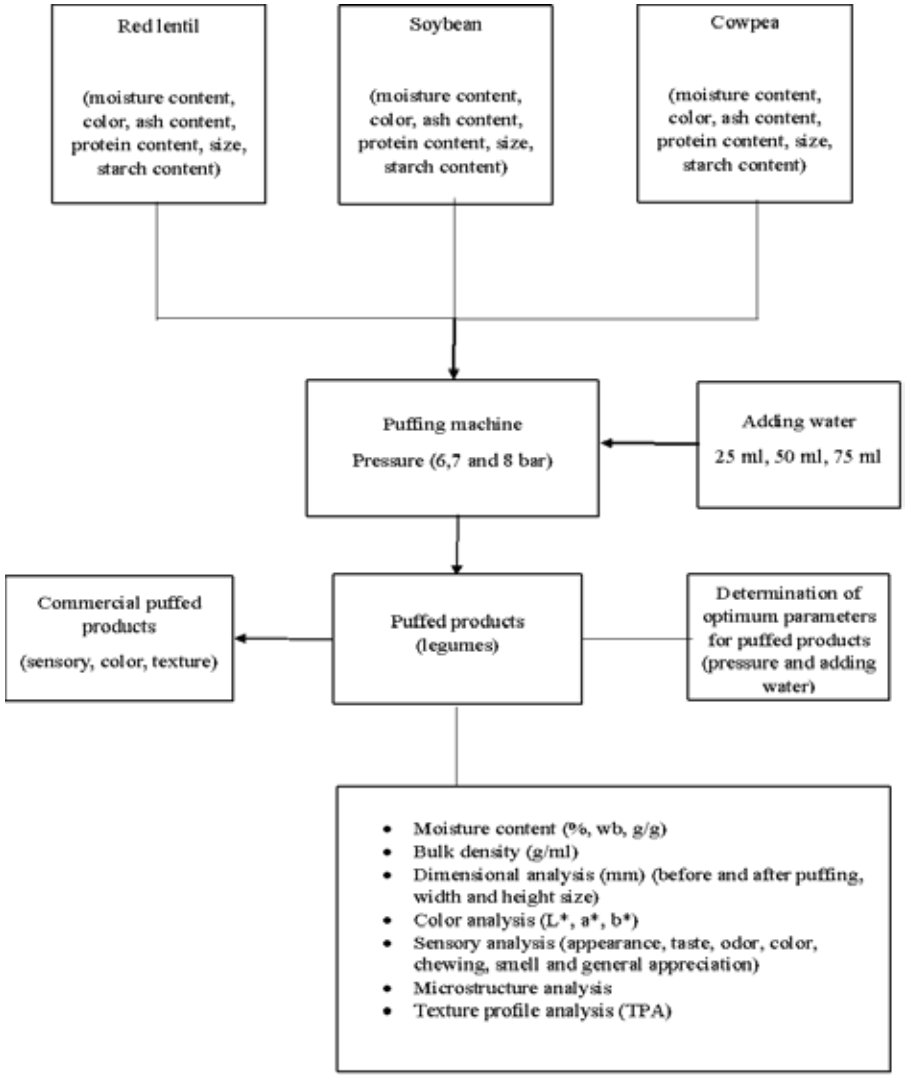

Figure 3. Experimental set-up for raw materials and puffed samples.

3. Result and Discussion

In this study, red lentils were puffed by adding 25, 50 and 75 mL of water at 6, 7 and 8 bar in the puffing machine. Initial and final temperatures of the puffing operation were measured and given in Table 2.

Table 2. Controlled parameters (amount of water and pressure) of red lentils puffing

Sample number	Controlled parameters		Measured variables	
	Added water (mL)	Pressure (bar)	Initial temperature (°C)	Final temperature (°C)
Red lentils 1	25	6	132	196
Red lentils 2	25	6	140	170
Red lentils 3	50	6	150	197
Red lentils 4	50	6	155	200
Red lentils 5	75	6	160	202
Red lentils 6	75	6	166	205
Red lentils 7	25	7	166	210
Red lentils 8	25	7	170	214
Red lentils 9	50	7	172	211
Red lentils 10	50	7	174	213
Red lentils 11	75	7	174	208
Red lentils 12	75	7	182	219
Red lentils 13	25	8	127	154
Red lentils 14	25	8	141	171
Red lentils 15	50	8	145	190
Red lentils 16	50	8	148	166
Red lentils 17	75	8	154	170
Red lentils 18	75	8	156	184

In this study, gun puffing technology was used. Gun puffing is a process in which the milled grains are introduced into the gun or high-pressure chamber after preheating, which is introduced to the closed rotating chamber (10). Red lentils were used as raw materials in the study. In this study, the effects of water and pressure on the puffing performances of red lentils were investigated.

Physical, chemical, sensory and statistical analyzes were performed. Raw material analysis; Moisture content, color value, protein value, starch content and dimensional size were determined. Moisture content, bulk density, color value, dimensional size, microstructure and texture of puffed red lentils were analyzed.

4. Conclusion

In this study Initial and final temperatures were determined in the inflation process. As the pressure increased, the final temperature in the inflation process also increased. According to the literature, an increase in temperature has a positive effect on puffing (11). However, the increase in temperature did not provide puffing on red lentils.

In this study, the swelling did not change as the pressure increased, while the moisture content decreased. When the water turned into vapor and escaped from the grains due to the high pressure and temperature during the explosion, the moisture content of the puffed grains decreased significantly (12).

The moisture value of the puffed red lentils was analyzed as 6.85% maximum, while the maximum moisture value was measured as 2.53% after baking. In the values measured throughout the study, the grain width and

height of the grain did not change as the pressure and the amount of water added increased. The density did not change as the amount of water added to the study increased.

Based on the results of this research, the following recommendations can be made such as:

- By increasing and decreasing the amount of water and pressure applied in the study, its effect on inflation performance can be observed.
- In addition to this study, the effect of increasing the moisture content of the raw material on the swelling performance and texture of the final product can be determined.
- Inspired by this study, it would be possible to observe the optimum operating parameters of legumes and grains during this swelling process. For example, bread wheat, durum wheat, buckwheat, rice, oats, barley, quinoa and amaranth etc.

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Physicochemical Composition And Microbiological Studies of Stored Kunu-Zaki, Produced From Millet (*Pennisetum Glaucum*)/Acha (*Digitaria Exilis*) And (*Sesame Sesamum Indicum L.*) Blends

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Abstract

Kunu-zaki, is an energizing beverage typically made from grain. Depending on the locality, the beverage is produced from millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor)*, acha (*Digitaria exilis*), maize (*Zea mays*), or wheat (*Triticum typhoidum*) cereals, among others, it could be utilized in non-composite ratios. This studywas carried out to determine the physicochemical and microbial status of kunun-zaki produced from millet/acha-sesame blends. A sample of 300g of the grains were cleaned, weighed, before steeping in water, for about 6-8h it was then washed thoroughly with clean water to remove dirt. Ginger (*Zingiber officinales*), alligator pepper (*Afromonium melegueta*), red pepper (*Capsicum species*), black pepper (*Piper guineense*) and cloves (*Syzygiumaromaticum*) were added to each of the sample to improve flavor and taste while, sliced sweet potatoes were also added to act as a sweetener. These all blended together to form a thick paste. The mixture was transferred into a bow, and about 1/3 portion of each sample was set aside, and for the 2/3 portion of the sample, boiling water was poured into each mixture in the bow and stirred until it became thick then the 1/3 portion of the uncooked, was then poured into the larger portion and stirred. Then, it's allowed to stand overnight for about 12-15 hours for fermentation and flavour development. The fermented samples were sieved to give the different kunu-zaki samples which were used for subsequent analysis. Results obtained revealed the following ranges: Moisture (84.97–93.20), protein (3.30-4.08%), fat (1.04-2.75%), ash (0.04-1.05%), carbohydrate (0.54-9.01%), phytate (0.15-2.24mg/100ml), tannin (0.10-3.09mg/100ml), andoxalate (0.27-1.75mg/100ml). Other nutrients were vitamin C(0.40-3.88mg/100ml), vitamin B_1(0.30-1.65mg/100ml), and vitaminB_2 (0.15-0.92mg/100ml) while minerals: Potassium (37.00-132.18mg/100ml), calcium (5.83-14.97mg/100ml), iron (2.92-3.88mg/100ml), and zinc (0.15-2.27 mg/100 ml) were obtained. Microbial load for total bacteria counts (TBC) ranged from 1.0-3.0 × 10^5cfu/ml in day one (1), 1.2- 9.3 × 10^5 cfu/ml in day two, 1.1 × 10^5 - 1.15 × 10^6cfu/m in day three, 1.01- 1.35 × 10^6 cfu/ml in day four, in day five, it range from 1.10 × 10^6 to an uncountable value (TNC). The microbial load for total fungal counts (TFC) ranged from 2.0 × 10^5 -1.30 × 10^6 cfu/ml in day one and experienced exponential growth in day two and three respectively, while the values showed too numerous counts in day four and five. The probable microorganisms characterized, belong to *Bacillus* spp*, Lactobacillus* spp, *Staphylococcus aureus, Streptococcus* spp, *Aspergillus flavus, Aspergillus niger and Trichoderma.* The sensory score scores showed that the panelists preferred sample AS1 with an overall acceptability of 7.20. This could be attributed to aroma and taste which had the values of 7.35 and 7.60 respectively. From the study, it was deduced that sample AS6 had the highest nutritional composition. It was also observed that aroma, taste and overall acceptability in sample AS1 was the most preferred by the panelists. Some samples showed slow growth of microorganisms. In conclusion, this research shows that kunu-zaki could be produced from millet (*Pennisetum glaucum)*/acha (*Digitaria exilis)* and sesame (*Sesamum indicum L.*) blends.

Keywords: Kunu-zaki, millet, acha, sesame, physicochemical and microbial.

1. Introduction

The production of soft drinks in Nigeria has relied primarily on imported raw ingredients for many years. Emphasis is now placed on the manufacturing of locally produced beverages, and the nation's focus is beginning to shift toward the use of locally sourced raw materials for economic development, such as millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor)*, Acha (*Digitaria exilis*), maize (*Zea mays*), or wheat (*Triticum typhoidum*) according to (33). In Nigeria, especially in the North, kunu-zaki, an energizing beverage typically made from grain, is highly popular. Depending on the locality, the beverage is produced from millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor)*, acha (*Digitaria exilis*), maize (*Zea mays*), or wheat (*Triticum typhoidum*) cereals, among others, could be utilized in non-composite ratios.

Kunu-zaki is frequently flavored with a mixture of spices known as kayan-yaji, which includes ginger (*Zingiber officinale*), black pepper (*Piper guinese),* and tamarind to enhance flavour and aroma as well as act as a purgative and treat conditions associated with flatulence. Kunu-zaki could quench thirst, as a result, even though consumption may be seen throughout the year, it is frequently consumed during the dry season. Because of the components used in manufacture, it is a relatively inexpensive drinking drink, which makes it easily accessible (4, 28). The procedures involved in producing kunu-zaki include wet milling the grain, wet sieving, mild fermentation, adding sugar, and bottling. The slurry is then partially gelatinized. For all people, cereal grains are a significant source of nutritional content, but particularly for those living in poor nations (43). Nevertheless, owing to lower protein content, a lack of some essential amino acids, lower protein and starch availability, the presence of some anti-nutrients, and the coarse texture of the grains, cereal grains' nutritional quality and the sensory qualities of their products are lacking (26).

In Nigeria, kunu-zaki is frequently used to wean infants off of solid foods and beverages. However, because the beverage is mostly made from cereals, it may not be as nutrient-dense as it could be, especially in terms of protein, vitamins, and minerals. As a result, supplementation with more nutrient-dense sources may be necessary (6). Research efforts are needed to ensure this beverage's nutritional quality is improved due to its low nutritional value. Exploiting sesame seeds (*Sesamum indicum*), might be a useful strategy, which is known to be rich in some nutritional values. A 100-gram serving (3.5 oz) of dried whole sesame seeds contains 573 calories, comprising 5 % water, 23 % carbohydrates (with 12 % being dietary fiber), 50 % fat, and 18 % protein.

These seeds are especially rich in essential minerals, including iron, magnesium, calcium, phosphorus, and zinc, offering 20 % or more of the Daily Value per serving. These nutritional attributes make sesame seeds a valuable addition to address some of the nutrient gaps found in kunu-zaki, a cereal-based beverage (31). The raw material, depending on the grain or legume differs from each other. Such as in millet, they are warm-weather, annual, small-grained cereals from the grass family. These are extremely resistant to drought and other adverse weather,

and they contain comparable amounts of nutrients to other important grains (19). When comparing it to other cereals, millets are a significant source of food in many traditional cuisines around the world as well as in arid and semiarid areas (21).

Acha is another grain that has been underutilized worldwide. Fonio or acha, as it is fondly called, is an erect, annual herbaceous plant with a height range of 30 to 80 centimeters. The ears are made up of two to five long, narrow parts that can be up to 15 cm wide. A fertile flower and a sterile flower make up each spikelet, and it is the fertile flower that develops into the fonio grain. Caryopsis, the grain, is still encased in glumes and husks. Only 1.5 mm in size, it has about 2000 seeds per gram. White, yellow, and purple are some of the available colours. All other grains mature more slowly than fonio. Some types can be picked 42 to 56 days after planting. Some mature more slowly, typically taking 165–180 days (16).

The sesame plant, an annual species, features opposite leaves that are 4 to 14 cm (1.6 to 5.5 inches) in length with smooth edges. The plant typically raises to a height between 50 and 100 cm (1.6 to 3.3 feet). Near the base, the leaves are broadly lance-shaped, reaching up to 5 cm (2 inches) in width, while those on the flowering stem narrow to just 1 cm (0.4 inches). The tubular flowers, which are 3 to 5 cm (1.2 to 2.0 inches) long, have a four-lobed opening and can be white, blue, or purple, depending on the variety. Sesame seeds themselves vary in color based on the cultivar, with the most common being off-white. Other prevalent seed colours comprise “buff, tan, gold, brown, reddish, gray, and black”, and these colors typically match the fruit's hull. The fruit of the sesame plant is a capsule, usually pubescent, with a short, triangular beak. It is rectangular in section and grooved, measuring 0.5 to 2.0 cm in width and 2 to 8 cm in length. Depending on the variety, the fruit capsule may either split open (dehisce) through two apical pores or along the septa from top to bottom to release the seeds. This degree of dehiscence is significant for breeding varieties suitable for mechanized harvesting, similar to the height of the initial capsule. Sesame seeds are tiny, typically measuring 3 to 4 mm in length, 2 mm in width, and 1 mm in thickness. The seeds are ovate, slightly flattened, and narrower at the hilum than at the opposite end, with 100 seeds weighing approximately 0.203 grams (46).

2. Methods

Sample collection and preparation

Millet (*Pennisetum americanum*), hungry rice (locally referred to as fonio or acha, *Digitaria exilis*), and sesame seeds (*Sesamum indicum*) were all sourced from Garki market in Abuja, Nigeria. A 300-gram sample of each grain was cleaned, weighed, and then steeped in water for partial fermentation, which lasted for about 6-8 hours. After steeping, the grains were thoroughly washed with clean water to remove any remaining dirt. To enhance the flavour and taste of each sample, “ginger (*Zingiber officinales*), alligator pepper (*Afromonium melegueta*), red pepper (*Capsicum species*), black pepper (*Piper guineense*), and cloves (*Syzygium aromaticum*) were added”. Additionally, sliced sweet potatoes were included as a natural sweetener. These all blended together to

form a thick paste. Approximately one-third (1/3) of each sample was set aside after the mixture had been placed into a bow, and for the 2/3 portion of the sample, each mixture in the bow was filled with boiling water, which was then stirred until it thickened. then the 1/3 portion of the uncooked, was then poured into the larger portion and stirred. Then, it is left to stand for 12 to 15 hours at night to allow for flavor development and fermentation. To create the various kunu-zaki samples utilized for the subsequent analysis, the fermented samples were sieved through muslin fabric (figure 1). The composite grains were used in different proportions and coded as shown in table 1.

Analysis of samples

The proximate analysis of the kunu zaki samples was conducted following the methods outlined by the (10). The carbohydrate content was determined by difference.

Table 1: Formulation of the composite flour for kunu-zaki

Sample code	Acha Grain	Millets Grain	Sesame seeds
MS1	–	100	0
MS2	–	90	10
MS3	–	80	20
MS4	–	70	30
MS5	–	60	40
MS6	–	50	50
AS1	100	–	0
AS2	90	–	10
AS3	80	–	20
AS4	70	–	30
AS5	60	–	40
AS6	50	–	50

Key → M = Millets grains; A = Acha grains; S = Sesame seeds

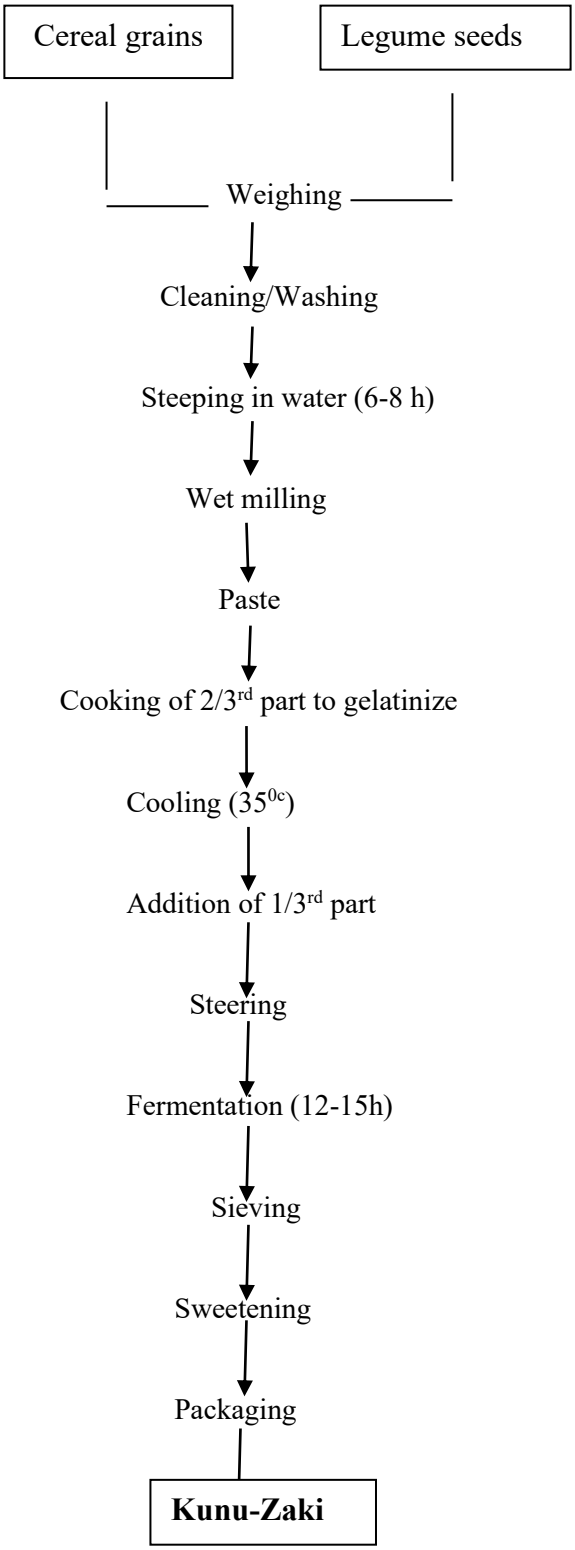

Fig 1: Kunu-zaki production, from millet/acha blends fortified with sesame seeds Source; (44

Proximate Analysis of the Samples

Determination of moisture content

The moisture content was examined by calculating the weight difference before and after drying the samples. To achieve a stable initial weight (W1), stainless steel oven dishes were first cleaned and then dried in an oven at 100 °C for an hour. The dishes were dried, then cooled in a desiccator until they were ready to weigh. Then, 5 ml of each sample was placed into the dishes (W_2), and they were dried at 100 °C until a constant weight was obtained. In a desiccator, the dishes and the sample were chilled, and then weighed (W_3).

$$\% \text{ Moisture content} = \frac{W2-W3}{W2-W1} \times \frac{100}{1}$$

Where; W_1 = Weight of dish; W_2 = weight of dish + sample before drying; W_3 = weight of dish + sample after drying.

Determination of protein content

Calculating the crude protein content was done using the Kjeldahl technique. Afterwards, 5ml of the Kunu beverages were poured into the digestive tube. A clear solution was achieved by adding two Kjeldahl tablets and concentrated sulfuric acid (tetraoxosulphate (VI) acid) to the sample, then placing the tube in a preheated digester at 400 °C for approximately 45 minutes. Here's a paraphrased version: Following digestion, the tube was taken out of the digester, left to cool, and then water was distilled through it. The digested and diluted sample was then transferred into the distillation unit. A conical flask containing 25 ml of 2 % boric acid was positioned under the condenser outflow to collect the distillate. After adding 25 ml of 40 % sodium hydroxide, 4 minutes are spent distilling. Hydrochloric acid 0.01 M was added to the ammonium borate solution to titrate it to a purple-grey end point.

Calculation:

$$\% \text{ Total Nitrogen} = \frac{(Titre-blank) \times Normality\ of\ the\ acid \times N2}{Weight\ of\ sample}$$

Nitrogen factor = 6.25

Crude protein = % Total N x 6.25

Determination of crude fat

The fat content was examined using the Soxhlet extraction technique as recommended by the (10). To begin, 300 ml of petroleum ether was placed in a 500 ml round-bottom flask, which was then connected to the Soxhlet extractor. A labeled thimble containing 5 ml of the sample was placed inside the extractor and sealed with cotton wool. After that, the apparatus was heated and refluxed for six hours. After the extraction process, the thimble was carefully removed, and the petroleum ether was recovered for reuse. The flask, now free of the solvent, was placed in an oven at 105 °C for one hour, then cooled in a desiccator and weighed to determine the fat content.

$$\% \text{ Fat} = \frac{weight\ of\ fat}{weight\ of\ sample} \text{ x } \frac{100}{1}$$

Determination of ash content

The ash content was examined using the (10) technique. The kunu sample was weighed into a crucible that had been pre-ignited, cooled, and weighed beforehand. To remove any gases that could accumulate in the furnace, the sample was pre-ashed on a hot plate. After pre-ashing, the crucible was placed in a muffle furnace and heated at 550 °C for four hours. Once the process was complete, the crucible was removed from the furnace, allowed to cool, and then reweighed to determine the ash content.

The ash content was calculated as;

$$\%\text{Ash} = \frac{w3-w1}{w2-w1} \text{ x } \frac{100}{1}$$

Where; W_1 = Weight of dish; W_2 = Weight of dish + sample before ashing; W_3 = Weight of dish + sample after ashing.

Determination of crude fibre

The crude fiber content was examined using the (10) technique. Initially, 5 ml of each sample was weighed into a 500 ml beaker, and 150 ml of pre-warmed sulfuric acid (H_2SO_4) was added. The mixture was then boiled for 30 minutes over a Bunsen burner, followed by filtration using suction, and thoroughly washed with hot distilled water to neutralize the acidity. The residue was transferred to a clean beaker and boiled in 150 ml of pre-warmed potassium hydroxide (0.223 %). After boiling, the mixture was filtered, and the remaining residue was washed multiple times with hot water to remove any residual alkalinity.

The residue was then placed into a pre-weighed and labeled crucible (W_2). These crucibles were dried in a hot air oven at 110 °C for two hours, Here's a paraphrased version: The apparatus was then cooled in a desiccator, weighed (W_2), and subsequently ashed at 550 °C for four hours in a muffle furnace. After ashing, the samples were cooled in a desiccator once more and weighed (W3). The crude fiber content in the samples was calculated using the following formula: (%) Crude fibre = $\frac{w2-w3}{w1}$

Where ; W_1= weight of original sample ; W_2 = Weight of the crucible + the residue; W_3 = Weight of the crucible + the ash

Determination of carbohydrate content

The carbohydrate content of the kunun-zaki samples was determined using the nitrogen-free extraction method as outlined by (10). This was calculated by subtracting the combined percentages of protein, crude fat, moisture, and crude fiber from 100. The resulting value represents the carbohydrate content of the samples.

Percentage (%) carbohydrate = 100 — (protein + crude fat + moisture + crude fibre + ash) %

Micronutriments analysis

Mineral content

The potassium (K) content of the samples was analyzed using the flame photometry technique according to (10). For the other elemental contents, such as calcium (Ca), iron (Fe), and zinc (Zn), the wet digestion method as described by (10) was employed. Each sample (0.5 g) was weighed into a clean ceramic crucible. A blank crucible, containing no sample, was also prepared. The crucibles were placed in a cool muffle furnace and heated to 500°C for two hours. After this initial heating, they were baked at 500 °C for an additional two hours before being allowed to cool in the oven. Once cooled, the sample was transferred into a labeled 50 ml centrifuge tube. The crucible was rinsed with 5 ml of distilled water and 5 ml of aqua regia, and this rinse was added to the centrifuge tube. The crucible was further cleaned twice with 5 ml of aqua regia each time, bringing the total volume to 20 ml. The mixture in the centrifuge tube was vortexed to ensure proper mixing. The contents were then centrifuged for ten minutes, and the resulting solution was transferred to clean vials. The mineral elements were then quantified using "an atomic absorption spectrophotometer (model AA-7000 Shimadzu, Japan ROM version 1.01, S/N A30664700709)". Before analyzing the samples, a blank sample was used to calibrate and reset the device, ensuring accurate measurements for the subsequent samples.

Vitamin content

Determination of vitamin C (Ascorbic acid)

The ascorbic acid content was measured according to the (10) technique. A 2 ml sample was homogenized and extracted using an acetic acid solution. To prepare the vitamin C standard solution, a 50 mg standard ascorbic acid tablet was dissolved in 100 ml of distilled water. After dissolution, the solution was filtered to obtain a clear filtrate. From this, 10 ml of the filtrate was added to a conical flask containing 2.5 ml of acetone. The mixture was then titrated with an indophenol dye solution (2,6-dichlorophenol indophenol) for 15 seconds to determine the ascorbic acid content.

Determination of vitamin B_1 (Thiamin)

The thiamine content was examined using the (10) technique. In this process, 2 ml of the sample was placed in a conical flask along with 75 ml of 0.2 N HCl, and the mixture was incubated overnight at 37 °C. After incubation, the solution was filtered through a silicate column to purify the filtrate. A portion of the filtrate, 25 ml, was then mixed in a conical flask with 5 ml of acidic potassium chloride (KCl) eluate, 3 ml of alkaline ferricyanide solution, and 15 ml of isobutanol. The mixture was shaken vigorously for 3 minutes to ensure thorough mixing. Once the solution had separated, the alcohol layer was carefully collected. To this alcohol layer, 3 grams of anhydrous sodium sulfate were added. For the standard thiamine solution, instead of using alkaline ferricyanide, 3 ml of 15 % NaOH was added to the flask to create the normal 5 ml of thiamine solution.

Determination of vitamin B_2 (Riboflavin)

The riboflavin content was examined using the (10) technique. Initially, 2 ml of the sample was rinsed and

placed in a conical flask, followed by the addition of 50 ml of 0.2 N HCl. The mixture was boiled for one hour and then allowed to cool. Sodium hydroxide was added to raise the pH of the solution to 6.0. Subsequently, the pH was lowered to 4.5 by adding 10 ml of HCl. The solution was then brought up to a predetermined volume and labeled as solutions 1 and 2 before being filtered into a 100 ml volumetric flask. Tube 1 received approximately 10 ml of water, while test tube 2 was given an additional 10 ml of filtrate along with 1 ml of riboflavin standard. Each tube then received 1 ml of glacial acetic acid, and the contents were mixed. Subsequently, 0.5 ml of a 3% potassium permanganate ($KmnO_4$) solution was added to each tube. After allowing the mixtures to stand for 2 minutes, 0.5 ml of 5 % sulfuric acid (H_2SO_4) was added, and the mixtures were thoroughly blended. The fluorimeter was calibrated at an excitation wavelength of 525 nm. It was set to 100 using tube 2 (standard) as the reference and zero deflection was set against 0.1 N H_2SO_4. The fluorescence of tube 1 was then measured within 10 seconds, and the readings were recorded for analysis.

Determination of anti-nutrients

Tannin content

The tannin content was examined using the technique stated by (38). Each sample was diluted by adding 2 milliliters of the sample to 10 milliliters of distilled water, stirred, and then left to stand at room temperature for thirty minutes. After this period, the mixtures were centrifuged, and 2.5% of the supernatants were distributed into six 50 ml volumetric flasks. Each flask was then supplemented with 2.5 ml of a standard tannic acid solution. Following this, 1 milliliter of Folin-Denis reagent and 2.5 milliliters of saturated sodium carbonate (Na_2CO_3) were added to each flask, and the flasks were filled to the 50 ml mark. The mixtures were diluted and then incubated at room temperature for 90 minutes. The absorbance of each sample, along with the standard and a blank reagent, was measured using a spectrophotometer (single beam UV/VIS spectrometer by Lab Aids India) at a wavelength of 250 nm. The absorbance readings were recorded and used to determine the tannin content in the samples. Readings was taken with the reagent blank and the tannin content was calculated as expressed:

$$\% \text{ Tannin} = \frac{An \times C \times 100 \times 5}{As \times W}$$

Where A_n = Absorbance of test sample; As = absorbance of standard solution;

W = weight of the sample and C = concentration of standard solution.

Oxalate content

The titration technique recommended by (7) was employed for the analysis. Initially, 50 milliliters of distilled water were boiled with 2 milliliters of the sample. After boiling, the heated sample was treated with 0.3 M hydrochloric acid (HCl). The mixture was then heated to 100°C, following the addition of 3 drops of methyl red indicator and ammonium hydroxide (NH_4OH) solution to the cooled filtrate. Once the mixture reached 100°C, it was allowed to cool. The cooled filtrate was reheated, and 10 cm^3 of 10% calcium chloride ($CaCl_2$) solution was

added. The mixture was then left to stand overnight. After standing, the mixture was filtered using Whatman No. 1 filter paper. The precipitate remaining on the filter paper was washed to remove any residual calcium ions (Ca^{2+}), then dissolved in a sulfuric acid (H_2SO_4) solution. To complete the analysis, the solution was brought to a boil and maintained at that temperature for at least 30 seconds while being titrated with a 0.05 M potassium permanganate ($KMnO_4$) solution. The titration was conducted while the solution was warm to ensure accurate results. Then, using the following calculation, the amount of oxalate in 1 ml of 0.05 m $KMnO_4$ (Potassium permanganate) was determined to be 2.2 mg:

$$O = Ts \times Md \times Mo \times 100/Ws$$

Where; O = Oxalate concentration in mg/100 ml; Ts = Volume of Potassium permanganate used for sample, Md = number of moles of potassium permanganate reacted, Mo = number of moles of oxalate reacted; Ws = sample weight

Phytate content

The levels of phytic acid in the samples were examined using the technique outlined by (7). For each sample, 2 ml was weighed into a 125 ml Erlenmeyer flask. The phytic acid was extracted by adding 50 ml of 3 % Trichloroacetic acid (TCA) and allowing the mixture to sit for 30 minutes, with periodic hand spinning for 45 minutes. After this extraction period, the suspension was centrifuged, and a 10 ml aliquot of the supernatant was transferred into a 50 ml conical flask.

Next, 4 ml of $FeCl_2$ solution was quickly added to the aliquot using a pipette. The mixture was then heated in a boiling water bath for 45 minutes. After 30 minutes of heating, two drops of 3% sodium sulfate were added to the TCA extract, and the heating continued. The supernatant was decanted after centrifugation for 15 minutes. The precipitate was rinsed by thoroughly dispersing it in 20 to 25 ml of 3% TCA, then it was boiled in a water bath for 10 minutes and centrifuged. This washing step was repeated using water. The precipitate was then dissolved in a mixture of 27 ml of water and 3 ml of 1.5 N sodium hydroxide with thorough mixing. The solution was diluted with water to approximately 30 ml and then boiled in a water bath for 30 minutes. The precipitate was filtered using Whatman No. 2 filter paper, which has medium retention. The filtrate was discarded, and the precipitate was removed from the filter paper using 40 ml of 3.2 N nitric acid (HNO_3) into a 100 ml volumetric flask. The filter paper was thoroughly rinsed with water in several portions, ensuring that the rinsed water was collected in the same flask without exceeding the 100 ml mark. After cooling the solution to room temperature, water was added to bring the volume up to 100 ml. A 5 ml aliquot of this solution was transferred to another 100 ml volumetric flask and diluted to approximately 70 ml. Then, 20 ml of 1.5 M potassium thiocyanate (KSCN) was added, and the mixture was diluted to the final volume. The color development was measured at 480 nm within one minute using a spectrophotometer. A reagent blank was run alongside each set of samples to ensure accuracy. The following formula was utilized to ascertain the phytate

content of the sample:

$$\text{Phytate content in } \mu g/100 \text{ ml sample} = C \times \frac{E}{S} \times Av \times 100$$

Where; C = phytate concentration from standard graph, E = total extraction volume, S = analytical sample taken, and Av = analytical volume.

Microbial analysis

Determination of total viable Count

Total bacteria count (TBC)

Formula for calculation: CFU/ml- (Number of colonies × dilution factor/Volume of cultured plate

The TBC analysis used conventional microbiological techniques. For each sample, 10 ml of sterile normal saline was aseptically added to 90 ml of the solution before being well mixed with additional dilution to a concentration of 10^{-6}. The freshly prepared media was then inoculated with 0.1 ml of the diluted sample using a spread-plate technique, and the incubation period was then extended to 36 hours at a temperature of 37 °C. A computerized colony counter was used to count the colonies, according to (25).

Fungi count (FC)

It was decided to use the approach outlined by (25). For plating on Sabor and dextrose agar, the pour plate method was utilized. The kunu sample was diluted by adding 9 ml of water to 1 ml of the sample. Following that, triplicates of 0.1 ml were gently spined onto molten sabor and dextrose agar plates. The content was given time to harden before being incubated for 72 hours at 28 °C.

Cultivation of microorganism

The cultivation process followed the method described by(29). Initially, bottles containing the previously macerated plant material were prepared. The suspected organism was then cultured by transferring it from the sample bottles into a freshly prepared general-purpose medium, specifically nutrient agar. The nutrient agar was prepared and poured into sterile plates, which were allowed to solidify and cool. Once set, the medium was streaked with the culture and incubated at 37°C for 24 hours to allow for organism growth.

Isolation of microorganism

The nutrient agar was sterilized in an autoclave at 121°C and 15 lb pressure for 15 minutes. After sterilization, the agar was poured into glass plates, which had been previously sterilized in a hot air oven at 160°C for one hour and then dried for 30 minutes at 60°C. Individual samples from preparations diluted to a factor of 10^{-3} or 10^{-5} were then streaked onto the agar plates using an inoculating loop that had been sterilized by flaming over a Bunsen burner. The plates were incubated at 37°C for 24 hours to allow for bacterial growth. The isolates were tentatively identified based on colony morphology, spore production, Gram reaction, and various biochemical assays. Following this preliminary identification, the isolates were sub-cultured on agar slants and incubated

again at 37°C for 24 hours. Additional morphological and biochemical tests were then performed to further characterize the organisms, following the procedures outlined by (39).

Colonial morphology

After the isolation and purification of the isolates, their colonial morphology was observed and documented. This included characteristics such as form, size, chromogenesis, opacity, elevation, surface texture, edge definition, consistency, emulsifiability, and odor of the isolates as they grew on MacConkey agar. These observations were conducted following the methodology described by (29).

Microscopy

Following colonial morphology, gram reaction in organisms was monitored.

Gram's staining

The Gram staining procedure followed the method outlined by (29). An 18- to 24-hour culture grown in nutrient agar broth was used to prepare a heat-fixed smear on a microscope slide. The smear was then stained with crystal violet for 1-2 minutes, followed by rinsing with water. The slide was subsequently incubated in Gram's iodine solution for 1 minute. After incubation, the iodine was poured off, and the slide was dried using a cloth. Next, the slide was washed with 95% ethanol (or industrial methylated spirit) until no more stain was visible, which typically took about 5 to 15 seconds for well-prepared smears. After decolorization, the slide was stained with a diluted carbol fuchsin solution for 20 seconds, followed by a thorough rinse under running water. The slide was then dried completely. Finally, the slide was examined under a microscope using oil immersion to observe the stained bacteria.

Biochemical characterization of microorganisms

In general, additional tests are necessary for identifying bacteria in addition to staining reactions. Extensive biochemical and other assays are crucial for bacterial identification because some bacterial species have identical morphological, culture, or staining characteristics. According to (24), these biochemical assays include the catalase test, citrate test, and sugar fermentation test among others.

Characterization and identification of the isolate

Standard inocula were prepared using the cultured sample and were aseptically inoculated onto sterile nutrient agar plates. Following the method described by (15), these plates were incubated at 37 °C for 36 hours. After incubation, the bacterial colonies were characterized using various tests, including "Gram staining responses, motility test, indole test, ornithine decarboxylase test, oxidase test, catalase test, triple sugar iron (TSI) agar test, citrate test, and coagulase test".

Sugar fermentation test

This procedure was carried out using compounded peptone water sugar. The indicator solution for the base medium was arranged by dissolving 0.1 g of bromothymol blue in 2.5 ml of 0.1 mole per liter NaOH, followed

by the addition of 47.5 ml of sterile distilled water with thorough mixing. To prepare 1000 ml of the basal medium, 100 g of peptone, 12.5 ml of the indicator solution, and 5 g of sodium chloride were added, and the pH was adjusted to 7.4. The mixture was then divided into 200 ml portions, which were poured into stoppered conical flasks and autoclaved at 121°C and 15 lb pressure for 15 minutes. In addition, a 0.5% peptone water sugar solution was prepared by dissolving 5 g of various sugars in 50 ml of distilled water. This sugar solution was then dispensed into Bijou bottles in 10 ml ampoules for further use (29).

Citrate test

The procedure was carried out using Simmons' Citrate medium. A 23 g portion of the dehydrated medium was suspended in one liter of distilled water and thoroughly homogenized. Then, 8 ml of the medium was dispensed into test tubes, which were laid on their sides and plugged with cotton wool. After sterilization, pure isolates were inoculated into the sterile medium, and the tubes were incubated at 37°C for 24-48 hours. A positive test result was indicated by the growth of the organism and a change in the medium's color from green to blue, signifying the utilization of citrate as the sole carbon source. A negative result was indicated by no growth and no change in the color of the medium, which remained green (29).

Catalase test

The catalase test was conducted following the technique stated by (29). First, 2-3 ml of hydrogen peroxide (H_2O_2) solution was transferred into a test tube using a clean glass rod or wooden stick. A loopful of the test organism, exhibiting good growth, was then removed and immersed in the H_2O_2 solution. The presence of catalase in the test culture was determined by observing the reaction. If immediate bubbling (effervescence) occurred, it indicated the release of free oxygen gas, signifying a positive test for catalase production. According to the reaction equation, active bubbling confirmed the presence of catalase, whereas the absence of bubbles indicated a negative test, meaning no catalase was produced.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

Hydrogenperoxide Water Oxygen

Oxidase test

This test was carried out according to (15). The test involved picking the suspicious organism with a sterile wire loop and mixing it with 2–5 drops of newly prepared oxidase (P-aminodimethylamine) reagent in a filter paper. If the colour changes from its natural state to a deep purple, the result is positive (+), whereas if it does not, it is negative (-).

Coagulate test

According to (15), this test was executed. Then, 2-3 drops of normal saline was added to the suspected organism, along with 1-2 drops of plasma and rock, to distinguish between *Staphylococcus aureus* and other staphylococcus species. Agglutination Indicates a positive (+) result, while its absence indicates a negative (-)

result.

Indole test

According to the method described by (34), the indole test was used to identify E. coli and other members of the Enterobacteriaceae family that are indole positive (+). The test involved inoculating the test organism into a test tube containing 3 ml of sterile tryptone water. The tube was then incubated for 24 hours at 37°C. 0.5 ml of Kovac's reagent was added after incubation, to the tube, then gently shaken (the mixture). The presence of indole was determined by observing the color change on the surface layer of the solution within 10 minutes. A red color on the layer's surface indicated a positive (+) test result, signifying the production of indole, while the absence of color change indicated a negative (-) test result.

Motility test

This test was designed to identify the majority of motile members of the Vibrionaceae and Enterobacteriaceae families. The test organism was inoculated into a motility medium by stabbing five times with a needle to a depth of 1-2 cm from the bottom of the tube. The tube was then incubated for 24 hours at 37°C. After the incubation period, the medium was examined for cloudiness along the line of inoculation. The presence of cloudiness indicated that the organism was motile, as it had moved away from the stab line, while the absence of cloudiness indicated non-motility (15).

Sensory evaluation

The sensory qualities of appearance, viscosity, aroma, taste, and acceptability of the kunun-zaki samples were assessed. Scores were assigned to the traits based on a 9-point Hedonic scale, ranging from 1 (extremely dislike) to 9 (extremely like), and a semi-trained 20-member panel was used (24).

Storage studies

The freshly prepared samples were stored at ambient temperature for 120 hours to undergo further analysis. The analysis was conducted every 24 hours over a five-day period to monitor any changes or developments in the samples.

Experimental design and data analysis

All data obtained from this study were subjected to statistical analysis using analysis of variance (ANOVA) with the Statistical Package for the Social Sciences (SPSS) Version 26.0 software. Duncan's New Multiple Range Test (DNMRT) was employed to separate the means obtained from triplicate analyses, following the method described by (17). A significance level of $p < 0.05$ was applied to assess statistical significance.

3. Results and Discussion

Formulated Kunu- zaki Prepared from Millets and Sesame blend

Plates 1- 5: Shows the formulated kunu - zaki from millets and sesame blends.

Plate 1: Packaged samples

Plate 2: MS2

Plate 3: MS1

Plate 4: MS4

Plate 5: MS6

Key:

MS1- Millet/sesame (100/0) %

MS2- Millet/sesame (90/10) %

MS4- Millet/Sesame (70/30) %

MS6- Millet/Sesame (50/50) %

Formulated Kunu zaki prepared from Acha and Sesame blend

Plates 6-10: Show the formulated kunu zaki from acha and sesame blends.

Plates 6: Sieving of Kunu zaki

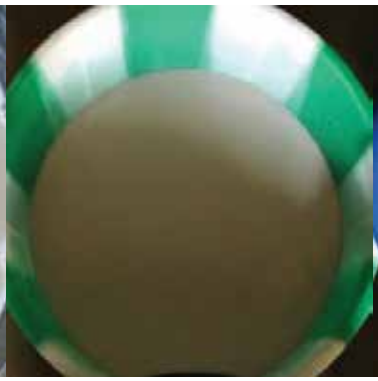

Plate 7: AS2

Plate 8: AS3

Plate 9: AS4

Plate10: AS6

Key:

AS2- Acha/Sesame (90/10) %
AS3-Acha/Sesame (80/20) %
AS4- Acha/Sesame (70/30 %
AS6- Acha/Sesame (50/50) %

Sensory Scores of kunu-zaki produced from millet/acha – sesame blends

The sensory scores of kunu – zaki produced from millet/acha – sesame blends are presented in Table 2. Based on the preliminary research, two samples (AS1 and MS1) were used as control (100 % cereal) and blended with sesame at 10, 20, 30, 40 and 50 % respectively and stored at cold temperature (refrigerated at 5±2 °C). The average mean score of appearance slightly increased for both millet and acha sample, with an increasing percentage of added sesame in appearance for the kunu-zaki sample made from millet and sesame if ranged from 6.95 – 7.25 and that of acha and sesame ranged from 6.85 – 7.25. The slight change in appearance with

increasing concentration of sesame could be due to the golden yellow colors of added sesame which is quite different from the appearance of locally produced kunu–zaki (11). Overall, there was no significant difference ($p < 0.05$) in appearance across all samples. However, the aroma scores revealed a significant difference ($p < 0.05$) among the samples.

The aroma scores ranged from 6.10 to 7.35 across the different samples. However, the highest scores were recorded in the content samples (AS1, AS2 and MS1) which were 7.35, 7.35 and 7.25 respectively. This also showed that the panelists prepared the aroma of their three samples (AS1, AS2 and MS1). The aroma of the kunu-zaki samples decreased from 7.35 to 6.10 as the level of added sesame increased in both millet and acha-based preparations. This finding aligns with the sensory quality research conducted by (11), which also reported that the addition of sesame reduced the aroma of kunu-zaki.

Regarding taste, the scores revealed a significant difference ($p < 0.05$) between the samples, with taste scores ranging from 4.70 to 7.60. The addition of sesame at various levels in both millet and acha resulted in a decrease in taste scores, with values dropping from 7.60 to 4.70. This was also in accordance with (11) that as the concentration of benne seed increased; the average means score for taste decreased. The highest score (7.60) was recorded in one of the content (AS1), which is modified that the panelists preferred A1 followed by MS1 and AS5. The lowest scores were recorded in AS6, followed by MS6 and AS5. This is probably due to the bitter taste in sesame which may be due to the high concentration seen in the Kunu-Zaki sample (11, 28).

The average means score for after taste ranged from 7.10 – 3.85 as the concentration of the sesame increases, in both kunu – zaki sample made from acha and millet. The aftertaste score decreased from 6.40 – 4.80 for kunu – zaki made from millets sesame blend and 7.10 – 3.85 for kunu – zaki made from acha – sesame blend. There was significant ($p < 0.05$) difference in the score for after taste. Most panelists preferred sample AS1 (control) which have the3 highest score 7.10 followed by MS1 (6.40) and AS2 (6.35).

The mouth feel of the different samples ranged from 6.80 – 4.30. But sample AS1 (control 2) has the highest score (6.80) followed by MS1 (control) and AS2 which have 6.35 and 6.05 respectively. There was significant ($p < 0.05$) difference amongst the samples. Nevertheless, the score for mouth feels in millet based kunu – zaki decreased from 6.35 – 4.00 and that of acha – sesame based kunu – zaki decreased from 6.80 – 4.30 with the increased addition of sesame at different proportion (0 – 50%). This could be as the result of the texture of sesame which is not as fine as that of millet and acha based kunu – zaki. This outcome is in agreement with (23). That sesame is rich in fibre (4 – 5%).

The average mean score for consistency ranged from 6.85 – 4.45. There was significant ($p < 0.05$) difference among the samples, it was recorded that panelists desired sample MS1 (control) which had 6.70 and 6.40 respectively. The decrease in scores for consistency across the sample which occurred as a result of increased concentration of sesame in the Kunu – Zaki may be explained by the fact that sesame contains high amount of

oil (22) which was not appreciated by the panelists.

The overall acceptability of the different samples ranged from 7.20 to 4.45. Sample AS1 (control 2) received the highest score of 7.20, followed by MS1 (control 1) and AS2, which scored 6.80 and 6.65, respectively. A significant difference ($p < 0.05$) was observed between the samples. This difference might be attributed to the fact that the panelists preferred the flavor of sample AS1 (control 2) over the others.

Proximate composition (%) of kunu-zaki produced from millet/acha – sesame blends

The proximate composition (%) of kunu-zaki produced from millet/acha-sesame blends is presented in Table 2. The moisture content of the control samples, MS1 and AS1, was 91.84% and 90.37%, respectively. For the other samples, which included both millet and acha-based kunu-zaki with sesame blends, the moisture content ranged from 84.97% to 93.75%. There was also an increase in the mixture content of the sample with the exception of few (AS3, AS2 and AS5) which had 84.97 %, 86.95 % and 89.75 %. This was most likely caused by the product's total water content during formulation. “A measure of stability and vulnerability to microbial contamination, the moisture content of any food is an indicator of its water activity” (29). This implies that the kunu – zaki sample due to high moisture content, can have a limited shelf life.

Table 2: Sensory scores for kunun zaki produced from millets/acha – sesame blends

Samples	Appearance	Aroma	Taste	After taste	Mouth feel	Consistency	Overall acceptability
MS1	$7.05^{a} \pm 1.468$	$7.25^{c} \pm 0.933$	$6.95^{ef} \pm 0.887$	$6.40^{de} \pm 1.309$	$6.35^{ef} \pm 1.309$	$6.85^{e} \pm 0.813$	$6.80^{cd} \pm 1.056$
MS2	$6.95^{a} \pm 1.395$	$6.85^{abc} \pm 0.988$	$6.30^{de} \pm 1.302$	$5.80^{cd} \pm 1.281$	$5.60^{cde} \pm 1.143$	$5.95^{cde} \pm 1.234$	$6.20^{bc} \pm 1.473$
MS3	$7.25^{a} \pm 0.910$	$6.75^{abc} \pm 0.910$	$6.05^{cde} \pm 1.317$	$5.35^{c} \pm 1.461$	$5.50^{bcde} \pm 1.504$	$5.55^{bcd} \pm 1.050$	$5.90^{bc} \pm 1.410$
MS4	$7.20^{a} \pm 1.005$	$7.05^{bc} \pm 0.887$	$5.80^{bcd} \pm 1.542$	$5.00^{bc} \pm 1.686$	$5.25^{abcd} \pm 1.293$	$5.35^{abc} \pm 1.725$	$5.35^{ab} \pm 1.814$
MS5	$7.25^{a} \pm 1.020$	$6.55^{ab} \pm 1.234$	$5.25^{abc} \pm 1.552$	$4.25^{ab} \pm 1.293$	$4.55^{ab} \pm 1.395$	$5.30^{abc} \pm 1.261$	$5.45^{ab} \pm 1.234$
MS6	$7.25^{a} \pm 1.118$	$6.40^{ab} \pm 1.231$	$4.80^{a} \pm 1.735$	$4.00^{a} \pm 1.622$	$4.35^{a} \pm 1.531$	$4.45^{a} \pm 1.605$	$4.65^{a} \pm 1.872$
AS1	$6.85^{a} \pm 2.495$	$7.35^{c} \pm 1.137$	$7.60^{f} \pm 0.940$	$7.10^{e} \pm 0.968$	$6.80^{f} \pm 1.322$	$6.70^{e} \pm 1.418$	$7.20^{d} \pm 1.005$
AS2	$7.15^{a} \pm 1.872$	$7.35^{c} \pm 0.933$	$6.90^{ef} \pm 1.483$	$6.35^{de} \pm 0.988$	$6.05^{def} \pm 1.234$	$6.40^{de} \pm 0.940$	$6.65^{cd} \pm 1.137$
AS3	$7.05^{a} \pm 1.504$	$6.95^{bc} \pm 1.146$	$6.30^{de} \pm 1.380$	$5.50^{cd} \pm 1.762$	$5.75^{cde} \pm 1.773$	$6.00^{cde} \pm 1.806$	$5.96^{bc} \pm 1.504$
AS4	$6.95^{a} \pm 1.395$	$6.45^{ab} \pm 1.276$	$5.25^{abc} \pm 1.743$	$4.25^{ab} \pm 1.585$	$4.85^{abc} \pm 1.137$	$5.15^{abc} \pm 1.424$	$4.85^{a} \pm 1.424$
AS5	$6.95^{a} \pm 1.317$	$6.15^{a} \pm 1.268$	$4.95^{ab} \pm 1.356$	$3.85^{a} \pm 1.531$	$4.30^{a} \pm 1.261$	$4.95^{ab} \pm 1.432$	$4.70^{a} \pm 1.455$
AS6	$7.25^{a} \pm 1.293$	$6.10^{a} \pm 1.119$	$4.70^{a} \pm 1.174$	$3.95^{a} \pm 1.638$	$4.80^{abc} \pm 1.542$	$4.80^{ab} \pm 1.436$	$4.45^{a} \pm 1.468$

Values are means ± standard deviation of 20 panelists. Values bearing different superscripts within the same column are significantly ($p<0.05$) different.

Key: AS1- Acha/Sesame (100/0; AS2- Acha/Sesame (90/10); AS3-Acha/Sesame (80/20); AS4- Acha/Sesame (70/30); AS5 – Acha/Sesame (60/40); AS6- Acha/Sesame (50/50); MS1- Millet/sesame (100/0); MS2- Millet/sesame (90/10); MS3- Millet/Sesame (80/20); MS4- Millet/Sesame (70/30); MS5-Millet/Sesame (60/40); MS6- Millet/Sesame (50/50)

Table 3: Proximate composition (%) for kunun zaki produced from millets/acha – sesame blends

Samples	Moisture	Protein	Fat	Fibre	Ash	Carbohydrate	Energy
M1	$91.84^{ef} \pm 0.39$	$3.30^{a} \pm 0.02$	$1.04^{a} \pm 0.03$	$0.26^{d} \pm 0.01$	$0.24^{e} \pm 0.01$	$3.32^{bc} \pm 0.40$	$35.84^{b} \pm 2.76$
MS2	$93.20^{g} \pm 0.45$	$3.36^{ab} \pm 0.06$	$1.05^{a} \pm 0.04$	$0.31^{e} \pm 0.01$	$0.12^{b} \pm 0.01$	$1.96^{ab} \pm 0.30$	$30.73^{a} \pm 0.33$
MS3	$90.60^{cd} \pm 0.40$	$3.45^{ab} \pm 0.00$	$1.40^{c} \pm 0.02$	$0.36^{f} \pm 0.01$	$0.26^{f} \pm 0.00$	$3.93^{c} \pm 1.41$	$42.12^{b} \pm 1.58$
MS4	$92.84^{fg} \pm 0.48$	$3.55^{b} \pm 0.00$	$1.68^{d} \pm 0.00$	$0.54^{h} \pm 0.01$	$0.22^{d} \pm 0.00$	$1.17^{a} \pm 0.33$	$34.00^{a} \pm 0.96$
MS5	$92.55^{efg} \pm 0.02$	$3.77^{c} \pm 0.00$	$1.80^{e} \pm 0.01$	$0.71^{i} \pm 0.00$	$0.34^{h} \pm 0.12$	$0.83^{a} \pm 0.42$	$34.60^{a} \pm 2.01$
MS6	$92.38^{efg} \pm 0.25$	$3.90^{cd} \pm 0.00$	$1.91^{f} \pm 0.04$	$1.05^{j} \pm 0.02$	$0.22^{d} \pm 0.00$	$0.54^{a} \pm 0.08$	$34.95^{a} \pm 1.63$
A1	$90.37^{cd} \pm 0.35$	$3.41^{ab} \pm 0.06$	$1.15^{b} \pm 0.05$	$0.04^{a} \pm 0.01$	$0.04^{a} \pm 0.02$	$4.99^{d} \pm 0.35$	$43.95^{b} \pm 1.29$
AS2	$86.93^{b} \pm 0.42$	$3.46^{ab} \pm 0.06$	$1.68^{d} \pm 0.07$	$0.11^{b} \pm 0.11$	$0.18^{c} \pm 0.10$	$7.64^{e} \pm 0.53$	$59.52^{c} \pm 1.45$
AS3	$84.97^{a} \pm 0.06$	$3.74^{c} \pm 0.13$	$1.83^{ef} \pm 0.03$	$0.15^{c} \pm 0.04$	$0.30^{g} \pm 0.00$	$9.01^{f} \pm 0.19$	$67.47^{d} \pm 0.01$
AS4	$91.49^{dc} \pm 0.04$	$3.80^{c} \pm 0.00$	$2.46^{g} \pm 0.00$	$0.16^{c} \pm 0.01$	$0.18^{c} \pm 0.00$	$1.91^{ab} \pm 0.04$	$44.98^{b} \pm 0.33$
AS5	$89.75^{c} \pm 1.44$	$3.81^{c} \pm 0.00$	$2.69^{h} \pm 0.04$	$0.31^{e} \pm 0.01$	$0.26^{f} \pm 0.05$	$3.18^{ab} \pm 0.74$	$52.17^{c} \pm 6.01$
AS6	$91.82^{ef} \pm 0.13$	$4.08^{d} \pm 0.24$	$2.75^{h} \pm 0.08$	$0.54^{h} \pm 0.01$	$0.24^{e} \pm 0.00$	$0.57^{a} \pm 0.44$	$43.35^{b} \pm 0.14$

Values are means ± standard deviation of triplicate determinations. Values bearing different superscripts within the same column are significantly ($p<0.05$) different.

Key: AS1- Acha/Sesame (100/0; AS2- Acha/Sesame (90/10); AS3-Acha/Sesame (80/20); AS4- Acha/Sesame (70/30); AS5 – Acha/Sesame (60/40); AS6- Acha/Sesame (50/50); MS1- Millet/sesame (100/0); MS2- Millet/sesame (90/10); MS3- Millet/Sesame (80/20); MS4- Millet/Sesame (70/30); MS5- Millet/Sesame (60/40); MS6- Millet/Sesame (50/50).

Hence, it is recommended that adequate preservative measures (such as bottling and storing under refrigeration temperature (4 – 7)) are taken to decrease instant spoilage of the products. There was a significant difference ($p < 0.05$) among the samples, with the exception of MS5 and MS6. The protein content of the control samples, MS1 and AS1, were 3.30% and 3.41%, respectively. For the other samples, the protein content increased from 3.36% to 4.08% as the addition of sesame increased. The difference between the samples was statistically significant ($p < 0.05$). The highest protein content (4.08 %) was observed in sample A6, which contained 50% sesame, while the lowest protein content (3.30 %) was found in MS1, which was 100% millet. This higher protein content aligns with the discoveries of (11), which noted that sesame is rich in protein and could be used to fortify kunu-zaki. Additionally, it was observed that the acha-sesame based samples generally had higher protein content compared to the millet-sesame blends. This is likely because acha has higher protein content than millet, as reported by the (47), with acha having around 10 % protein.

The fat content of the kunu-zaki samples ranged from 1.04% to 2.75%. The lowest fat content was in MS1 (control 1) at 1.04 %, while the highest fat content was in sample AS6 at 2.75%. There was a significant difference ($p < 0.05$) between the samples. Furthermore, the fat content of the kunu-zaki increased with the addition of sesame (ranging from 10% to 50%) to both the millet and acha-based kunu-zaki.

The fat content of the control (MS1 and AS1) are 1.04 and 1.15 % which is close to the finding that kunu – zaki has a fat content of 1 %. (Sopade and Kassum, 1992). Similarly, the increased fat content of the samples as the result of added sesame (10 – 50 %) is in accordance with (22), which reported that sesame seed has a fat content of 49.7 %.

The crude fibre content of kunu – zaki produced, ranged from 0.04 - 1.05%. The control (MS1 and AS1) had a fibre content of 0.26 and 0.04 %. However, there was significant increase of crude fiber in the samples with increased addition of sesame seed (10 – 50 %). This outcome supports the conclusion of (23), that sesame seed is rich in fibre (4.8 %).

The kunu – zaki, ash content of the sample ranged from 0.04 - 0.34 %. The least value (0.04 %) was recorded AS1 (control 2) while the highest value (0.34 %) was recorded in sample MS5 which have about 40 % sesame. There was rise in the ash content with added proportion of sesame (10 – 50 %) across the samples, except for sample MS2, MS4 and MS6 which had 0.12, 0.22 and 0.22 lower than sample MS1 (control 1)

The carbohydrate content of the samples was 3.32 and 4.99 % for the control (MS1 and AS1). But if generally ranged from 0.54 % - 9.01 %. it was noted that the control (MS1 and AS1) which had 0 % sesame, had high carbohydrate content except for sample MS3, AS2 and AS3 which had 3.93, 7.64 and 9.01 % respectively. These changes could have been because of the drop in moisture (90.60, 86.93, 84.97

and 89.75 %). However, all the samples showed a decrease in carbohydrate with addition of sesame (10 – 50 %). This indicates that the nutrified material (sesame) which has fewer carbohydrates may have had an impact on the samples' carbohydrate content by reducing it content and simultaneously increasing the protein of the kunu – zaki samples.

The energy value of the kunu-zaki sample ranged from 30.73-67.47 kcal. The control samples (MSI and ASI) which were 100 % cereals had 35.84 and 43.95 kcal. Conversely, there was a significant ($p<0.05$) increase in the energy value. Most notably in sample AS2 and AS3 which had a value of 59.52 and 67.47 kcal as a result of increase concentration of sesame which has high energy value (573 kcal) as against the cereals (acha and millet) which are lower (21). This might not have been because of the increased concentration of sesame alone, but also as a result of the high carbohydrate content (7.64 and 9.01%).

Mineral composition (mg/100ml) of kunu-zaki produced from millet/acha – sesame blends

The mineral composition of different kunu-zaki samples is detailed in Table 4. The potassium content ranged from 37.00 to 132.18 mg/100 ml. The control samples, AS1 and MS1, had mean values of 37.00 mg/100 ml and 41.08 mg/100 ml, respectively. The lowest potassium content was recorded in MS1 (37.00 mg/100 ml), while the highest was in AS6 (132.18 mg/100 ml), which contained 50% sesame. There was a significant increase in potassium content with the addition of sesame (10-50%), likely due to sesame's high potassium content (47). Additionally, kunu-zaki samples made from acha-sesame blends had higher potassium content than those from millet-sesame blends. This may be attributed to acha's higher potassium content (26.53 mg/100 ml) compared to pearl millet (24.13 mg/100 ml) as reported by (42). Potassium is crucial for the body, playing a key role in amino acid and protein synthesis, as well as regulating fluid balance within cells (28). (41) And (27) further stress that inadequate fluid balance can result in dehydration, which impacts the heart and kidneys.

The iron content in the kunu-zaki samples ranged from 2.92 to 3.91 mg/100 ml, with MS1 (control 1) showing the lowest value at 2.92 mg/100 ml, which aligns with the known low iron content in millet (47). This finding is consistent with (28), who reported that the iron content in sorghum-based kunu-zaki fortified with benne seeds ranged from 2.70 to 4.20 mg/100 g. An increase in iron content was observed with higher sesame concentrations (10-50%). Iron is a vital dietary component, particularly for pregnant women, lactating mothers, newborns with convulsive disorders, and the elderly, as it helps prevent anemia and related conditions (36). (20) also noted that the mineral content of cereals can increase significantly during fermentation due to the activity of fermenting microorganisms.

The zinc content in the kunu-zaki samples ranged from 0.15 to 2.27 mg/100 ml. The lowest zinc content (0.15 mg/100 ml) was recorded in AS1 (control 2), while the highest was in MS6. Similar to iron, zinc content increased with the addition of sesame at various proportions. "Zinc is crucial for immune function, wound healing, blood clotting, thyroid function, and vision, and it may also possess antiviral

properties (2)."

The calcium content in the kunu-zaki samples ranged from 5.83 to 14.97 mg/100 ml. The control sample MS1 had the lowest calcium content (5.83 mg/100 ml). The increase in calcium content with higher levels of sesame substitution can be attributed to sesame's high calcium content. The highest calcium content (14.97 mg/100 ml) was recorded in sample AS6. Calcium is important for regulating muscle contractions, transmitting nerve impulses, and supporting teeth development (14). This finding is consistent with the results reported by (28).

Vitamin composition (mg/100 ml) of kunu-zaki produced from millet/acha-sesame blends

The vitamin composition (mg/100 ml) of kunu-zaki produced from millet/acha-sesame blends is presented in Table 5. The Vitamin C content in the control samples (AS1 and MS1) was 3.88 and 4.89 mg/100 ml, respectively. Overall, the Vitamin C content in the samples ranged from 0.40 to 4.89 mg/100 ml, with the lowest value observed in AS6 (50% sesame) and the highest in MS1 (control 1). There was a significant ($p < 0.05$) decrease in Vitamin C content as the concentration of sesame increased. This decrease may be attributed to the low Vitamin C content in sesame, as reported by the (22), which indicates that sesame contains 0 mg/100 g of Vitamin C. Additionally, the reduction in Vitamin C could also be due to oxidation during processing (29).

An increase in thiamine (B1) and riboflavin (B2) content was recorded in the kunu-zaki samples containing sesame (10-50%), which might influence consumer food intake. The control samples (MS1 and AS1) had the lowest values of 0.30 and 0.35 mg/100 ml for thiamine (B1) and 0.29 and 0.15 mg/100 ml for riboflavin (B2), respectively. The sample with 50% sesame (AS6) had the highest values of 1.65 mg/100 ml for thiamine and 0.92 mg/100 ml for riboflavin. Similar results (1.05 and 0.56 mg/100 ml) were reported by (37), who found that a 30% incorporation of tiger nut milk in kunu-zaki increased the content of vitamins B1 and B2. The increase in these vitamins in the sesame-containing samples could be due to the contribution of sesame, particularly in boosting the levels of vitamins B1 and B2 (12).

Antinutrient composition (mg/100ml) of kunu-zaki produced from millet/acha-sesame blends

The result of the anti – nutrient content of the kunu – zaki sample as presented in Table 6. The tannin content of the control samples (AS1 and MS1) were 0.11 – 0.10 mg/100ml. Generally, the tanin content ranged from 0.10 – 3.09 mg/100ml. The least value 0.10 mg/100ml was recorded in sample MS1 (control 1) while the highest value (3.09 mg/100ml) was recorded in MS6. It was noted that the kunu – zaki prepared from 100 % acha and millet had lower values of tannin compared to the products with added sesame (10 – 50 %). This could be due to both the cereal and sesame seeds containing tannin. However, the tannin content in these samples is below the acceptable levels (<76-90 g/kg dry matter) as reported by (8), “who highlighted that tannin contents as high as 76-90 g/kg dry matter might be harmful when

consumed".

The total phytate and oxalate content ranged from 0.15 to 2.27 mg/100 ml and 0.27 to 1.75 mg/100 ml, respectively. The lowest phytate content (0.15 mg/100 ml) was recorded in AS1 (control 2), and similarly, the lowest oxalate content (0.27 mg/100 ml) was also found in AS1. Conversely, the highest values for phytate (2.27 mg/100 ml) and oxalate (1.75 mg/100 ml) were recorded in samples AS6 and MS6, respectively, both of which contained 50% sesame. Both phytate and oxalate content increased in the kunu-zaki samples with the increased concentration of sesame. This rise could be attributed to the high phytate concentration (5.36 g/100 g) in sesame, as reported by (1).

Table 4: Mineral composition (mg/100ml) of kunu-zaki produced from millet/acha-sesame blends

Sample codes	Potassium	Iron	Zinc	Calcium
AS1	$41.08^{b} \pm 0.60$	$3.04^{a} \pm 0.05$	$0.15^{a} \pm 0.03$	$10.11^{a} \pm 1.14$
AS2	$82.96^{e} \pm 1.27$	$3.46^{b} \pm 0.03$	$0.21^{ab} \pm 0.02$	$10.47^{a} \pm 1.52$
AS3	$102.11^{g} \pm 2.03$	$3.65^{cd} \pm 0.04$	$0.49^{b} \pm 0.02$	$11.41^{a} \pm 1.94$
AS4	$120.81^{h} \pm 0.00$	$3.77^{de} \pm 0.06$	$0.35^{ab} \pm 0.04$	$12.56^{a} \pm 1.88$
AS5	$120.99^{h} \pm 0.10$	$3.86^{e} \pm 0.03$	$1.31^{d} \pm 0.04$	$12.61^{a} \pm 1.12$
AS6	$132.18^{j} \pm 0.00$	$3.91^{e} \pm 0.07$	$2.00^{fg} \pm 0.04$	$14.97^{a} \pm 1.42$
MS1	$37.00^{a} \pm 4.07$	$2.92^{a} \pm 0.07$	$0.48^{b} \pm 0.13$	$5.83^{a} \pm 0.18$
MS2	$45.48^{c} \pm 0.00$	$3.44^{b} \pm 0.08$	$0.86^{c} \pm 0.05$	$5.92^{a} \pm 0.15$
MS3	$77.78^{d} \pm 2.34$	$3.50^{bc} \pm 0.00$	$1.53^{de} \pm 0.04$	$6.72^{a} \pm 0.25$
MS4	$89.54^{f} \pm 0.00$	$3.58^{bc} \pm 0.00$	$1.63^{e} \pm 0.08$	$8.91^{a} \pm 0.00$
MS5	$125.02^{i} \pm 0.21$	$3.67^{cd} \pm 0.13$	$1.76^{ef} \pm 0.02$	$8.98^{a} \pm 0.01$
MS6	$125.07^{i} \pm 0.00$	$3.88^{e} \pm 0.15$	$2.27^{g} \pm 0.44$	$9.26^{a} \pm 0.65$

Values are means ± standard deviation of triplicate determinations. Values bearing different superscripts within the same column are significantly ($p<0.05$) different.

Key: AS1- Acha/Sesame (100/0; AS2- Acha/Sesame (90/10); AS3-Acha/Sesame (80/20); AS4-Acha/Sesame (70/30); AS5 – Acha/Sesame (60/40); AS6- Acha/Sesame (50/50); MS1- Millet/sesame (100/0); MS2- Millet/sesame (90/10); MS3- Millet/Sesame (80/20); MS4- Millet/Sesame (70/30); MS5-Millet/Sesame (60/40); MS6- Millet/Sesame (50/50)

Table 5: Vitamin composition(mg/100ml) of kunun-zaki produced from millet/acha-sesame blends

Sample codes	Vitamin C	Vitamin B1	Vitamin B2
AS1	$3.88^{f} \pm 0.05$	$0.35^{b} \pm 0.00$	$0.15^{a} \pm 0.05$
AS2	$2.69^{e} \pm 0.05$	$0.39^{bc} \pm 0.01$	$0.19^{b} \pm 0.01$
AS3	$1.61^{d} \pm 0.00$	$0.41^{bc} \pm 0.06$	$0.23^{bc} \pm 0.01$
AS4	$0.53^{abc} \pm 0.00$	$0.54^{c} \pm 0.01$	$0.27^{cd} \pm 0.02$
AS5	$0.46^{ab} \pm 0.03$	$1.88^{e} \pm 0.01$	$0.32^{de} \pm 0.18$
AS6	$0.40^{a} \pm 0.02$	$2.59^{f} \pm 0.02$	$0.36^{ef} \pm 0.01$
MS1	$4.89^{g} \pm 0.00$	$0.30^{a} \pm 0.04$	$0.29^{d} \pm 0.07$
MS2	$2.63^{e} \pm 0.00$	$0.37^{bc} \pm 0.48$	$0.38^{e} \pm 0.61$
MS3	$1.54^{d} \pm 0.03$	$0.39^{bc} \pm 0.11$	$0.44^{f} \pm 0.03$
MS4	$1.46^{d} \pm 0.04$	$0.46^{bc} \pm 0.01$	$0.47^{fg} \pm 0.02$
MS5	$0.77^{c} \pm 0.12$	$1.32^{d} \pm 0.22$	$0.50^{g} \pm 0.12$
MS6	$0.76^{c} \pm 0.21$	$1.65^{de} \pm 0.10$	$0.92^{h} \pm 0.44$

Values are means ± standard deviation of triplicate determinations. Values bearing different superscripts within the same column are significantly ($p<0.05$) different.

Key: AS1- Acha/Sesame (100/0; AS2- Acha/Sesame (90/10); AS3-Acha/Sesame (80/20); AS4- Acha/Sesame (70/30); AS5 – Acha/Sesame (60/40); AS6- Acha/Sesame (50/50); MS1- Millet/sesame (100/0); MS2- Millet/sesame (90/10); MS3- Millet/Sesame (80/20); MS4- Millet/Sesame (70/30); MS5- Millet/Sesame (60/40); MS6- Millet/Sesame (50/50)

The levels of phytate in the products were as "So low that it may have a negative effect on the body, as it falls significantly below the required daily intake (150–1400 mg) needed by the body (40)". Whereas, the oxalate content of the kunu zaki samples was also below the safety thresholds of 2-5g/kg as reported by (32).

Microbiological count

Microbiological count of kunun-zaki produced from millet/acha – sesame blends

Colony counts of bacteria isolates from fresh kunu samples are presented in Table 7. The bacteria count ranged from 1.0×10^5 - 3.4×10^5 cfu/ml after 24 hours, but showed significant growth in day 2 (48 hours), days 3 (72 hours), days 4 (96 hours) and day 5 (120 hours) respectively. However, one of the control sample MSI showed slow growth, which resulted in no count for day 1 (24 hours) and day 2 (48 hours). This could be as a result of insufficient nutrient found in pearl millet, as compared to fonio or acha (47). In general, most foods might have limited amount of one or a few nutrients for rapid growth of some gram (+) bacteria, especially some fastidious *Lactobacillus* species (13). But samples AS2, AS4, AS5 and MS3 were too numerous to count.

Fungal count (cfu/ml) of kunun-zaki produced from millet/acha – sesame blends

Colony counts of fungi isolate from fresh kunu samples are presented in Table 8. After 24 hours, colonies were counted and it range between 2.0 x 10^5- 2.35 x 10^6 cfu/ml. But showed massive growth in day 3 (72 hours), day 4 (96 hours) and day 5 (120 hours) which were too numerous to count in day 3 for both MS5 and MS6. However, despite the massive growth, AS5 was still visible for counting in day 4 (96 hours).

Microbiological status of kunu- zaki produced from millet/acha – sesame blends

The pH range of all kunu samples was between 5.5 and 5.8, making them all mildly acidic. This has been explained by various studies as being caused by "the presence of fermentative microorganisms in kunu zaki, which spoil the beverage by fermenting its carbohydrate circuits and causing undesirable changes in them". This also gives them their flavor and aroma, rendering them unappealing for human consumption. According to (3).

Six microbial isolates including for (4) species of bacteria and two (2) species of fungi were isolated and identified from the kunu-zaki samples. The bacterial isolates include; *Staphylococcus* spp, *Streptococcus* sp, *Bacillus* spp, and *Lactobacillus* spp. (Table 9) while the fungal isolates were the specie of *Aspergillus* and *Trichorderma* (Table 10). Following preliminary microscopy and biochemical investigations, these microorganism species were identified (Table 9).

Table 6: Antinutrient composition (mg/100ml) of kunu-zaki produced from millet/acha-sesame blends

Sample codes	Tanin	Oxalate	Phytate
AS1	$0.11^{a} \pm 0.01$	$0.27^{a} \pm 0.11$	$0.15^{a} \pm 0.08$
AS2	$0.16^{a} \pm 0.00$	$1.08^{bc} \pm 0.05$	$0.41^{ab} \pm 0.26$
AS3	$0.21^{a} \pm 0.01$	$1.44^{cd} \pm 0.18$	$0.69^{abc} \pm 0.02$
AS4	$0.25^{a} \pm 0.01$	$1.58^{cd} \pm 0.40$	$0.58^{ab} \pm 0.55$
AS5	$0.31^{a} \pm 0.01$	$1.59^{d} \pm 0.39$	$1.37^{cd} \pm 0.41$
AS6	$0.36^{a} \pm 0.01$	$1.66^{d} \pm 0.32$	$2.27^{f} \pm 0.55$
MS1	$0.10^{a} \pm 0.01$	$0.89^{b} \pm 0.10$	$0.56^{ab} \pm 0.04$
MS2	$0.14^{a} \pm 0.01$	$1.39^{cd} \pm 0.14$	$0.90^{abcd} \pm 0.06$
MS3	$0.95^{b} \pm 0.05$	$1.43^{cd} \pm 0.16$	$1.08^{bcd} \pm 0.04$
MS4	$1.89^{c} \pm 0.18$	$1.69^{d} \pm 0.02$	$1.51^{de} \pm 0.09$
MS5	$2.58^{d} \pm 0.19$	$1.71^{d} \pm 0.03$	$2.16^{ef} \pm 0.27$
MS6	$3.09^{e} \pm 0.39$	$1.75^{d} \pm 0.06$	$2.24^{f} \pm 0.48$

Values are means ± standard deviation of triplicate determinations

Key: AS1- Acha/Sesame (100/0; AS2- Acha/Sesame (90/10); AS3-Acha/Sesame (80/20); AS4-Acha/Sesame (70/30); AS5 – Acha/Sesame (60/40); AS6- Acha/Sesame (50/50); MS1- Millet/sesame (100/0); MS2- Millet/sesame (90/10); MS3- Millet/Sesame (80/20); MS4- Millet/Sesame (70/30); MS5-Millet/Sesame (60/40); MS6- Millet/Sesame (50/50)

Table 7: Total bacteria count (cfu/ml) of kunun zaki produced from millet/acha – sesame blends

Sample code	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
AS1	3.0×10^5	5.6×10^5	5.9×10^5	6.9×10^5	9.8×10^5
AS2	3.0×10^5	7.9×10^5	8.0×10^5	1.00×10^6	TNC
AS3	1.0×10^5	4.6×10^5	6.0×10^5	7.0×10^5	9.0×10^5
AS4	2.0×10^5	8.4×10^5	9.3×10^5	1.01×10^6	TNC
AS5	3.4×10^5	5.8×10^5	1.15×10^6	1.35×10^6	TNC
AS6	1.5×10^5	4.0×10^5	7.1×10^5	8.0×10^5	1.27×10^6
MS1	2.0×10^4	1.2×10^5	1.9×10^5	2.5×10^5	4.7×10^5
MS2	1.4×10^4	5.0×10^4	1.1×10^5	2.8×10^5	5.0×10^5
MS3	2.5×10^5	9.3×10^5	8.0×10^5	1.20×10^6	TNC
MS4	2.7×10^5	4.7×10^5	5.1×10^5	8.7×10^5	1.28×10^6
MS6	1.5×10^5	4.0×10^5	5.2×10^5	8.5×10^5	1.50×10^6

Values are means ± standard deviation of triplicate determinations

Key: AS1- Acha/Sesame (100/0; AS2- Acha/Sesame (90/10); AS3-Acha/Sesame (80/20); AS4-Acha/Sesame (70/30); AS5 – Acha/Sesame (60/40); AS6- Acha/Sesame (50/50); MS1- Millet/sesame (100/0); MS2- Millet/sesame (90/10); MS3- Millet/Sesame (80/20); MS4- Millet/Sesame (70/30); MS5-Millet/Sesame (60/40); MS6- Millet/Sesame (50/50)
TNC – Too numerous to count

The scope of this investigation did not, however, include the molecular analysis of the strains and the pathogenicity test, which reveal the growth of the microorganisms inoculated on the fresh sample and indicate their spoiling activities. These analyses are used to determine the genetic makeup of the microorganisms and validate their identity. It has been shown that isolated lactic acid bacteria such *Lactobacillus, Streptococcus*, and *Bacillus spp.* have the capacity to ferment carbohydrates and generate lactic acid (18). It is also known that *Lactobacillus* has been isolated from other native non-alcoholic beverages including zobo, which lowers pH (18). Humans have long known about and used the lactic acid fermentation caused by bacteria to produce food products (29). During lactic acid fermentation, lactic acid bacteria (LAB) create a variety of substances like organic acids, deacetyl hydrogen peroxide, or bactericidal proteins, according to Mbaeyi-Nwaoha and Muotolu (2016).

All of the kunu-zaki samples' elevated bacteria counts might be linked to poor hygiene habits and potential contamination from the water and utensils used to prepare the drink.

S. aureus is a typical component of the flora of the skin, nose, throat, palm hairs, and other areas. It is a pervasive microbe that can enter food from a variety of sources, including handless with acute pyogenic illnesses and healthy carriers who keep the microbe in their noses or throats.It frequently bears blame for tainted water and food. S. aureus can cause a variety of diseases, most notably foodborne poisoning,

making its diagnosis crucial for maintaining good health.In facts Staphylococcus spp. levels of 10^8 ml^{-1} have been reported to be potentially dangerous to consumers (5).

It is not surprising that *Lactobacillus sp*. was found in some samples because it has been shown to flourish in environments with plenty of fermentable substrates like sugars, which frequently result in the generation of acids following fermentation. Their fermenters' presence and activities may be to blame for the typical souring of flavor noticed if not consumed within 6 to 8 hours of processing (35). In humans, *Lactobacillus sp*. is a non-intestinal flora that is typically not harmful. It is said to have advantageous characteristics. These can counteract the actions of some food spoilage pathogenic bacteria, like "*S. aureus* and *E. coli,* and include colon cancer prevention, immune system improvement, and allergy reduction" (Aboh and Oladusu, 2014). The spores of *Bacillus spp*., on the other hand, can withstand high temperatures and are therefore present in the kunu-zaki samples that have undergone heat treatment during processing. According to (9), some of these related microorganisms have been linked to food poisoning. *Bacillus* and *Lactobacillus species* were also discovered to be easily detected in foods with low acid content, such as juice and beverages, where they create organic acid (30). Additionally, *Aspergillus species* have been linked to food rotting, especially when there is a carbohydrate substrate. These are the cereals' storage microorganisms. Mycotoxins, which are important for both public health and the economy, could be produced and accumulated as a result of thr proliferation (3).

4. Conclusion and Recommendation

This research showed that kunu-zaki could be produced from millet (*Pennisetum glaucum)*/acha (*Digitaria exilis)* and sesame (*Sesamum indicum L.*) blends. It was observed that, the aroma and taste in sample AS1 and AS2 were most chosen by the panelist, and had the highest ratings, respectively. Addition of sesame to acha or millets in kunu-zaki production increased the tannin, phytate and oxalate, minerals (potassium, zinc, iron and calcium), Vitamins (Vitamin B1 and B2), protein, fats and fibre contents, as well as a reduction in Vitamin C and carbohydrate as across the samples, though there were no much difference in moisture, however, sample MS2 had the highest moisture content. There were no spoilage microorganisms in some samples. However, the probable microorganisms characterized were: *Bacillus* spp, *Lactobacillus* spp, *Staphylococcus aureus, Streptococcus* spp*, Aspergillus flavus, Aspergillus niger and Trichoderma* were found in minimal amount in both the control and the blended samples, "which might not be detrimental to the human body"

Increased protein, fat and fibre in supplementation of the cereals with sesame showed a significant increase in the nutritional properties of the kunu-zaki, which is a step towards making the beverage a more complete drink. The maintaining the health of the nervous system, and stimulating growth are all made possible by vitamin B complexes. However, this contradicted the sensory attributes as the panelists preferred sample AS1 which had the highest value in overall acceptability.

Based on the obtained results, it would be advisable to implement roasting or germination of sesame seeds as these processes can reduce the levels of anti-nutrients in the drink, which have shown an increase when sesame is added to either acha or millet. Anti-nutrients are known to interfere with the absorption and utilization of micronutrients, so reducing their levels is essential for improving the nutritional quality of the drink. Additionally, processing under aseptic conditions should be adopted to lower the microbial load in the products, thereby preventing contamination and reducing the risk of infection or intoxication when consumed.

Furthermore, conducting molecular studies to characterize the isolated organisms is recommended. This might provide valuable data for food processors, nutritionists, industrialists, and consumers, aiding in the selection of the best blend for optimal nutritional benefits and safety.

Table 8: Total fungal count (cfu/ml) of kunun-zaki produced from millet/acha – sesame blends

Sample code	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
AS1	4.0×10^5	8.0×10^5	1.45×10^6	TNC	TNC
AS2	3.5×10^5	7.0×10^5	1.80×10^6	TNC	TNC
AS3	5.6×10^5	8.5×10^5	1.87×10^6	TNC	TNC
AS4	2.0×10^5	5.6×10^5	1.02×10^6	TNC	TNC
AS5	4.4×10^5	6.5×10^5	1.16×10^6	1.39×10^6	TNC
AS6	7.5×10^5	1.00×10^6	1.93×10^6	TNC	TNC
MS1	7.8×10^5	1.20×10^6	2.01×10^6	TNC	TNC
MS2	1.20×10^6	1.80×10^6	2.15×10^6	TNC	TNC
MS3	7.5×10^5	1.05×10^6	1.75×10^6	TNC	TNC
MS4	1.30×10^6	1.80×10^6	2.35×10^6	TNC	TNC
MS5	7.8×10^5	1.88×10^6	TNC	TNC	TNC
MS6	9.5×10^5	2.10×10^6	TNC	TNC	TNC

Values are means ± standard deviation of triplicate determinations.

Key: AS1- Acha/Sesame (100/0; AS2- Acha/Sesame (90/10); AS3-Acha/Sesame (80/20); AS4-Acha/Sesame (70/30); AS5 – Acha/Sesame (60/40); AS6- Acha/Sesame (50/50); MS1- Millet/sesame (100/0); MS2- Millet/sesame (90/10); MS3- Millet/Sesame (80/20); MS4- Millet/Sesame (70/30); MS5-Millet/Sesame (60/40); MS6- Millet/Sesame (50/50)
TNC – Too Numerous count

Table 9: Morphological and biochemical characterization of bacterial isolates of kunun-zaki produced from millet/acha – sesame blends

Code	pH	Gram Staining	Shape	Catalyst	Coagulase	Citrate	Indole	Oxidase	Glucose	Sucrose	Lactose	Moltility	Probable organism
ASI	5.5	+	Coci	+	−	+	−	−	+	+	+	−	*Staphylococcus aureus*
AS6	5.85	+	Coci	+	+	+	−	−	+	+	+	−	*Streptococcus* spp
MS3	5.80	+	Rod	+	−	+	−	−	+	−	−	−	*Bacillus* spp
MS4	5.85	−	Rod	−	−	−	−	−	+	+	+	+	*Lactobacillus* spp

Values are means ± standard deviation of triplicate determinations

Key: AS1- Acha/Sesame (100/0), AS6- Acha/Sesame (50/50), MS3- Millet/Sesame (80/20), MS4- Millet/Sesame (70/30)

Table 10: Morphological and cultural characteristics of fungi isolates of kunun-zaki produced from millet/acha – sesame blends

Isolates	Cultural Characteristics	Morphological feature	Back of plate	Microscopy	Identity
AS1	Gray/White	Smooth and flat	Redish Gold	Presence of conidial head and conidia sphores	*Aspergillus flavus*
AS6	Black/ White	Rough	Gold	Black spore with smooth and colorless conidia sphores	*Aspergillus niger*
MS6	Greenish	Hairy/ Rough	Greenish	Tree like structure with branches	*Trichoderma*

Values are means ± standard deviation of triplicate determinations

Key: AS1- Acha/Sesame (100/0), AS6- Acha/Sesame (50/50), MS6- Millet/Sesame (50/50)

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Proximate Composition and Functional Properties of Wheat (*Triticum aestivum*) and Fermented Bambara Groundnut (*Vigna subterranean Verdc*) Flour Blends for Production of Acceptable Cookies

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Abstract

The study was undertaken to investigate the proximate composition and functional properties of wheat and fermented Bambara groundnut flour blends for production of acceptable cookies. The flour blending was based on Mixture I-optimal design using design expert software version 13.0. Experimental constraints were: $0.5 \leq A \leq 0.85$; $0.1 \leq B \leq 0.45$; $0.05 \leq C \leq 0.16$ which represented wheat flour, fermented Bambara groundnut flour and water respectively. Functional properties of the flour blends and proximate composition of the flour blends and cookies were analysed. Means were separated using Duncan multiple range test and significant difference accepted at $p<0.05$. Proximate composition of the flour blends showed that the moisture content ranged from (8.50-11.44 %), protein (21.97- 26.73 %), fat (5.83-11.22 %), ash (0.10-0.30 %), fibre (0.10-0.30 %), carbohydrate (50.81- 60.99 %). The bulk density decreased as the oil absorption capacity increases and the addition of fermented Bambara groundnut flour significantly increased the water and oil absorption capacities. Results from this study indicates that the blend ratios significantly impacted the nutritional profile of the cookies. Results from this work have shown that fermented Bambara groundnut flour could be used for substituting wheat flour up to 26% level in the production of acceptable cookies without adversely affecting the sensory attributes of the cookies. It also serves as a good cut on the cost of wheat importation in communities with supply challenges.

Keywords: Proximate composition, functional properties, wheat flour, fermented bambara groundnut, cookies.

1. Introduction

There is a global demand for healthier and more nutritionally balanced snack foods and this has led to increased interest in the development of food products with enhanced nutritional profiles. Cookies, as widely consumed snack items, present a significant opportunity for nutritional improvement and fortification [13]. Traditional cookies are predominantly made from wheat flour, which is valued for its functional properties such as gluten formation, contributing to the desired texture and structure of baked goods [12].

[34] reported that wheat flour consists of approximately 72% carbohydrates, with its composition also including 8% to 13% protein, 12% to 13% moisture, 2.5% sugar, 1.5% fat, 1.0% soluble protein, and 0.5% mineral salts. However, the reliance on wheat flour alone limits the nutritional diversity of such products, particularly in terms of protein content and micronutrient availability.

Bambara groundnut (*Vigna subterranea*) is highly nutritive but an underutilized legume that has shown great potential as an alternative flour source due to its high protein content, rich amino acid profile, and presence of dietary fibre [2]. Fermentation, a traditional processing method, can further enhance the behavioural properties of flour made from Bambara groundnut by improving its protein digestibility, increasing the bioavailability of essential nutrients and decreasing antinutritional factors.

The functional properties of flour blends are critical for predicting their behaviour in cookie production. The impact of fermentation on the functional properties of Bambara groundnut flour and the final product quality are not well documented.

The aim of this research is to investigate proximate composition and functional properties of wheat and fermented Bambara groundnut flour blends and evaluate their suitability for producing acceptable cookies. The combination of these flours could, provide a functional composite flour blend that not only meets consumer expectations for taste and texture but also develop biscuits that offers enhanced nutritional benefits. This aligns with the growing consumer demand for snack products that combine health benefits with enjoyable eating experiences [20].

2. Materials and Methods

Material Procurement

The ingredients, including wheat flour, Bambara groundnut seeds, sugar, eggs, nutmeg, baking powder, salt, butter, milk, and vanilla extract, were bought from the Ogige market in Nsukka town. The chemicals used were of high purity and were acquired from the Department of Food Science and Technology at the University of Nigeria in Nsukka, Enugu State, Nigeria.

Sample preparation

At first, stones, weevil seeds, and foreign objects were removed by carefully separating and cleaning the grains of wheat and Bambara groundnut seeds to make flour from fermented Bambara groundnut seeds, the seeds were first cleaned and soaked in water.

Processing of wheat grains into flour

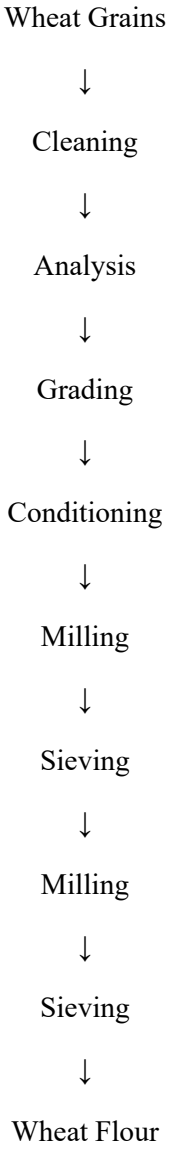

Figure 1: Flow diagram for processing wheat grains into flour, Posner and Hibbs (2005)

The outer hulls were removed and cooked before being rinsed. The cooked seeds were mixed with water and left to ferment for three days at room temperature. After fermentation, the water was removed, and the seeds were dried. The dried seeds were then ground into a fine powder using a milling machine and sifted. The resulting flour was stored in plastic containers in a refrigerator for future use. Figure 2 depicts the flow diagram for the processing of Bambara groundnut flour.

Bambara Groundnut Seeds

↓

Sorting/Cleaning

↓

Washing

↓

Soaking (24 hours at 28°C)

↓

Dehulling

↓

Cooking (20 minutes)

↓

Cooling

↓

Fermentation (72 hours)

↓

Oven Drying (60°C, 14 hours)

↓

Milling

↓

Sieving (using 1mm sieve)

↓

Fermented Bambara Groundnut Flour

Figure 2. Flow Chart for production of fermented Bambara groundnut flour, Fadahunsi (2009)

Production of Cookie

With minor modifications, cookies were made using the flour blends according to the formula in Table 2, following the procedure outlined by [3]. Baking was done at 180 °C for 15 minutes. The biscuits were collected and stored in closed high-density polyethylene nylon until further analysis.

Weighing of Ingredients

↓

Mixing

↓

Kneading

↓

Sheeting/Cutting

↓

Baking (180°C, 15 minutes)

↓

Cookies

Figure 3: Production of cookies from composite flour blends, Aliyu and Sani (2009)

Analyses

The proximate of the flour blends and cookies were determined using [5].

Proximate analyses

Moisture Content

Two grams of each flour sample and cookies was weighed into a crucible and then dried at 105 ± 2 °C for 3 hours until a constant weight was obtained. The crucible was then cooled in a desiccator and thereafter reweighed. Percentage moisture content of the sample was determined using the formula:

Moisture content (%) = (sample weight + crucible) – (crucible weight) × 100

Sample weight

Crude Protein Content

One gram of each flour sample was weighed into a 30 ml Kjeldahl flask. Fifteen (15 ml) of concentrated sulphuric acid (H_2SO_4) and 2 tablets of copper sulphate (kjeldahl catalyst mixture) were then added into the

Kjeldahl flask and then placed in the protein digestor in a fume cupboard to be heated until a clear solution was obtained. The digest was allowed to cool cautiously for 45 minutes while adding 250 ml of distilled water to the digest to avoid solidification. Ten milliliters of the diluted digest were then made alkaline with 45 % Sodium hydroxide solution (NaOH) and the mixture distilled into a receiving flask containing 5 ml of 5 % boric acid which acted as an indicator. During titration, 0.01 N hydrochloric acid (HCl) was used to treat the distillate. A blank titration was also carried out with the use of distilled water. The percentage nitrogen was determined using the formula:

$$\text{Nitrogen content (\%)} = \frac{14 \times (V_1 - V_2) \times \text{Normality of NaOH} \times \text{Dilution factor (50)} \times 100}{1000 \times \text{weight of sample}}$$

$$\text{Crude protein (\%)} = \text{Nitrogen content (\%)} \times 6.25$$

Where:

V_1 = Titre value

V_2 = Blank titre value

Determination of crude fat

A Soxhlet extractor was mounted, along with a reflux condenser and a 500 ml round bottom flask. A labeled thimble was used to measure two grams of the sample. The round bottom flask was filled with petroleum ether (300 ml), and the extractor thimble was plugged with cotton wool. After allowing the Soxhlet apparatus to reflux for about 6 hours, the thimble was removed. Petroleum ether was collected for re-use. The flask was dried for 1 hour at 105 °C in an oven, then cooled in a desiccator before being weighed. The formula used to determine the fat percentage was:

$$\text{Fat (\%)} = \frac{\text{(Weight of extract + crucible) – (Weight of flask)}}{\text{Original weight of sample}} \times \frac{100}{1}$$

Determination of total Ash

Two grams of each of the flour sample and biscuit was weighed into a porcelain crucible and then ashed in a muffle furnace at 60 °C for 2 hours. The crucible with its contents were removed, cooled in a desiccator and reweighed. The percentage weight of the ash was calculated using the formula:

$$\text{\% Ash} = \frac{\text{(Weight of sample + crucible) - (Weight of crucible)}}{\text{Original weight of sample}} \times \frac{100}{1}$$

Determination of Crude Fiber

About 2 g of flour samples were weighed into a 600 mL long beaker. About 200 mL of hot 1.25% H_2SO_4 was

added. Beaker was placed on digestion apparatus with preheated plates, boiled, refluxed for 30 mins and filtered through Whiteman GF/A paper by gravity. The beaker was rinsed with distilled water. The residue was washed on the paper with distilled water until the filtrate was neutral. The residue was transferred from the paper back to the beaker containing 200 mL of hot 1.25% NaOH. Steps 4 and 5 were repeated. The paper with residue was transferred into a crucible, dried at 100˚C overnight, cooled in a desiccator and reweighed (weight A). The samples were put in furnace at 600˚C for 6 hrs, cooled in a desiccator and reweighed (weight B). The loss in weight during incineration represents the weight of crude fibre.

% crucible fibre = (weight A) − (weight B) ×100

Determination of carbohydrate content (by difference)

The percentage carbohydrate content was obtained by difference. That is, by subtracting the amount of moisture, protein, fat, crude fibre and ash from 100 %

% Carbohydrate = 100 − % (moisture + protein + ash + crude fibre)

Table 1: Design Expert's experimental data for proportion for the blending of wheat and fermented Bambara groundnut flours

Run	A. Wheat flour (g)	B. Bambara g/nut flour (g)	C. Water (ml)
1	0.66	0.26	0.08
2	0.62	0.22	0.16
3	0.73	0.22	0.05
4	0.57	0.32	0.11
5	0.62	0.33	0.05
6	0.78	0.10	0.12
7	0.72	0.17	0.11
8	0.50	0.38	0.12
9	0.85	0.10	0.01
10	0.58	0.26	0.16
11	0.50	0.45	0.05

Table 2: Recipe for biscuit production

Components	Quantity (g)
Flour	500.00
Fat	190.00
Sugar	150.00
Salt	5.00
Baking powder	2.50
Water	125.00ml

[28]

Determination of Functional Properties

Determination of Bulk Density

The bulk density was determined as described by [5]. Ten millitre (10 ml) graduated measuring cylinder was weighed and filled with the sample. The bottom of the cylinder was then gently tapped at the laboratory bench severally until there was no more diminution of the sample. The bulk density was expressed as the ratio of the weight of the sample to the volume of the sample after tapping.

$$BD\ (g/cm^3) = \frac{W_2 - W_1}{V}$$

BD = bulk density in g/cm^3

W_1 = weight of empty cylinder (g)

W_2 = weight of cylinder + sample (g)

V = volume of cylinder occupied by the sample (cm^3)

Determination of water absorption capacity

The water absorption capacity of the samples was determined by the method of [4]. One gram (1 g) of the sample was weighed into a conical graduated centrifuge tube. Ten millitre (10 ml) of distilled water was then added to the sample and mixed for 30 minutes using a whirl mixer. The sample was then allowed to stand for 30 minutes at room temperature and thereafter centrifuged at 5000 rpm for 30 minutes. The free water was then read directly from the graduated centrifuge tube. The water absorption capacity was expressed as:

$$\text{Water absorption capacity} = \frac{\text{Total volume of water} - \text{free water}}{\text{Total volume of water}} \times \frac{100}{1}$$

Determination of Swelling Index

The method used to determine the swelling index was as described by [33]. One (1) gram of the sample was transferred into a clean dry graduated 50 ml cylinder. The samples were gently levelled and their volumes recorded. Ten (10) ml of distilled water was added to the sample. The cylinder and its content were swirled and allowed to stand for 60 minutes. The swelling index of the sample was calculated as a multiple of the original volume. The percentage swelling index was calculated as;

$$\% \text{ Swelling index} = \frac{\text{Final volume}}{\text{Initial volume}} \times \frac{100}{1}$$

Determination of Emulsion Capacity

Emulsion capacity was determined by the method of [27]. Two (2) gram of flour sample was blended with 25 ml of distilled water in a warring blender at room temperature for 30 seconds at 1600 rpm. After dispersion, 25 ml vegetable oil was added and blending continued for another 30 seconds. The mixture was transferred into a centrifuge and centrifuged at 1600 rpm for 5 minutes. The volume of oil separated directly from the tube was read. The emulsion capacity was calculated as;

$$\text{Emulsion capacity} = \frac{\text{Height of emulsified layer}}{\text{Height of whole solution in centrifuge tube}} \times \frac{100}{1}$$

Experimental design and data analysis

Data analysis and experimental design were completed in compliance with the [26]. technique. A wholly randomized design was used to create the experiment (CRD). The data was analyzed using one-way analysis of variance (ANOVA) and the Duncan multiple range test (p=0.05) to separate the means using the Statistical Product for Service Solution (SPSS) version 23.0.

3. Results and Discussion

Plates 1 - 2 depicts the single flours from wheat, and fermented Bambara groundnut. The formulated cookies are presented in plates 3 - 12.

Plate 1: Wheat flour

Plate 2: Fermented Bambara groundnut flour

Plate 3: 100% wheat made biscuit

Plate 4: S1 (66:26:08)

Plate 5: S2 (62:22:16)

Plate 6: S3 (73:22:05)

Plate 7: S4 (57:32:11)

Plate 8: S5 (62:33:05)

Plate 9: S6 (78:10:12)

Plate 10: S7 (72:17:11)

Plate 11: S8 (50:38:12)

Plate 12: S9 (85:10:01)

Plate 13: S10 (58:26:16)

Plate 14: S11 (50:45:05)

Key:

S1 = (66:26:08) Wheat flour: Bambara groundnut flour: water,

S2 = (62:22:16) Wheat flour: Bambara groundnut flour: water,

S3 = (73:22:05) Wheat flour: Bambara groundnut flour: water,

S4 = (57:32:11) Wheat flour: Bambara groundnut flour: water,

S5 = (62:33:05) Wheat flour: Bambara groundnut flour: water,

S6 = (78:10:12) Wheat flour: Bambara groundnut flour: water,

S7 = (72:17:11) Wheat flour: Bambara groundnut flour: water,

S8 = (50:38:12) Wheat flour: Bambara groundnut flour: water,

S9 = (85:10:05) Wheat flour: Bambara groundnut flour: water,

S10 = (58:26:16) Wheat flour: Bambara groundnut flour: water,

S11= (50:45:05) Wheat flour: Bambara groundnut flour: water.

Results for proximate composition of wheat and fermented Bambara groundnut flour are presented in Table 3.

Moisture content

The findings indicated that wheat flour had a higher moisture content. (11.00%) than that of fermented Bambara groundnut flour (9.61%). This is similar with 11.95% for wheat flour and 6.77% for Bambara groundnut flour reported by [12]. The results suggest that Bambara groundnut flour can keep better than wheat flour although it is within acceptable limit (10-16%) expected for long term storage of flour. It has been reported that lower moisture content of food enhances its keeping quality and shelf life ([8].

Protein content

The results showed protein content of wheat flour was 11.85% while that of Bambara groundnut flour was 18.03%. Fermented Bambara groundnut flour was higher in protein than wheat flour. The high protein content of food legumes generally constitutes the natural protein supplements to staple diet [18]. This also agrees with 17.70% and 12.90% protein reported for Bambara groundnut flour and wheat flour, respectively [12]. This result is also similar with [16] who reported that Bambara groundnut had relatively high protein content which makes it a complete food. The result of the product reveals that protein increased due to fermentation process [23].

Fat content

The results showed that fat content of wheat flour was 3.18% while that of fermented Bambara groundnut flour was 6.41%. Crude fat plays a vital role in the diet and previous work show that increased fat content causes slow rate of digestion and absorption leading to lower glycemic index.

Crude Fiber

The results showed that fibre content of Bambara groundnut flour was 3.48% while that of wheat flour was 1.03%. It's possible that sieving the flours during processing contributed to the wheat's low fibre level while fermentation process may be responsible for the fibre content of Bambara groundnut flour.

Ash content

The results showed that ash content of fermented Bambara groundnut flour was 3.50% while that of wheat flour was 1.35%. It has been shown that Bambara groundnut flour had 4.0% ash content [29]. Ash is the inorganic residue remaining, after the water and organic matter have been removed by heating in the presence of oxidizing agent. Bambara groundnut flour could be an important source of minerals than wheat flour samples used for this work.

Carbohydrate content

Fermented Bambara groundnut flour had carbohydrate content of 58.97% while wheat flour had the score of 71.59%. The high carbohydrate implies that wheat flour could serve as a source of energy needed for body metabolism than fermented Bambara groundnut flour.

Table 3: Proximate composition (%) of wheat and fermented Bambara groundnut flours

Sample	Moisture	Protein	Fat	Fibre	Ash	Carbohydrate
WF	11.00±0.00	11.85±0.00	3.18±0.28	1.03±0.10	1.35±0.88	71.59±1.73
BGF	9.61± 0.06	18.03±0.06	6.41±0.01	3.48±0.14	3.50±0.14	58.97±0.04

The values represent the means ± standard deviation of findings made in triplicate.

WF- Wheat flour BGF - Bambara Groundnut Flour

Bulk density was seen to be higher in wheat flour than in Bambara groundnut flour. The density of flours is important as it affects mixing, packaging, and transportation. Higher bulk density is advantageous since it helps to reduce the paste thickness which is an essential factor in child feeding [17]. Wheat flour is advantaged over Bambara groundnut flour as higher bulk density is desirable because it offers greater packaging advantage as greater quantity of flour can be packed within a constant volume. Nutritionally, low bulk density is advantageous because it engenders consumption of more quantity of the lighter food item and this will translate into more nutrients for the consumer [19].

The water absorption capacity was higher in Bambara groundnut flour than in wheat flour. Water absorption capacity is the ability of flour to absorb water and swell for improved consistency in food [10]. Increase in water absorption capacity is useful in baking products which requires hydration to improve dough handling [14].

Oil absorption capacity of Bambara groundnut flour was higher (129.34 %) than that of wheat flour (91.54%). This is an indication that Bambara groundnut flour could be a good retainer of flavor and could also give a better mouth feel when used in the snack bar preparation.

Swelling index of fermented Bambara groundnut flour was higher than that of wheat flour while the emulsion capacity of wheat flour was higher than that of Bambara groundnut flour. Emulsifiers are incorporated into cookie formulation to improve dough handling and the products' overall quality.

Table 4: Functional properties of wheat and fermented Bambara groundnut flour.

Sample	BD (g/ml)	WAC (%)	OAC (%)	SI (g/g)	EC (%)
WF	0.88±0.08	119.00±0.03	91.54±0.01	6.73±0.01	27.00±0.04
BGF	0.79±0.01	162.33±0.01	129.34±0.63	10.97±0.13	14.05±3.87

The values represent the means ± standard deviation of findings made in triplicate.

WF – Wheat flour BGF- Bambara groundnut flour

BD- Bulk density WAC- Water absorption capacity

OAC- Oil absorption capacity SI- Swelling index

EC- Emulsion capacity

Moisture content

The findings showed that the blends of wheat and Bambara groundnut flour had moisture contents ranging from 8.50 to 11.44%. S5 (62% wheat flour and 33% fermented Bambara groundnut flour) and S10 (58% wheat flour and 26% fermented Bambara groundnut flour) had the least and highest moisture content scores respectively. Even though there were significant ($p<0.05$) differences among moisture content scores of the flour blends, moisture contents in all the blends except S10 and S11 were within the acceptable level. The results suggest that these flour blends can keep for a long period especially if stored in airtight packaging material of low air and moisture permeability.

Protein content

Results showed that the protein content of wheat-Bambara groundnut flour blends ranged from 21.97 to 26.73%. S9 (85%wheat flour and 10% fermented Bambara groundnut flour) had the least protein content while S11 (50% wheat flour and 45% fermented Bambara groundnut flour) had the highest score for protein. The protein content was similar to the report on wheat-Bambara groundnut flour (22.36%) by previous studies. It could be seen that protein content increased with increasing substitution with fermented Bambara groundnut flour. Cereal-legume composite flours have been shown to have high protein content [7]. This is because the inner layers of the legume grains are largely comprised of cotyledon tissues, which contain large proportion of the grain protein. Therefore, the observed increase in protein content upon increasing substitution of fermented Bambara groundnut may be due to an increase in components of the grain inner layers [1]. The protein levels of the wheat and fermented Bambara groundnut flour blends differed significantly ($p<0.05$), according to the mean separation from ANOVA findings.

Fat content

Results indicated that fat content of wheat-fermented Bambara groundnut flour blends ranged from 5.83 to 11.22%. S1 (66% wheat flour and 26% fermented Bambara groundnut flour) and S11 (50% wheat flour and 45% fermented Bambara groundnut flour) had the least and highest moisture content scores respectively. The low amount of fat present in the flour blends could help to prolong the shelf-life of the flour as the rate of rancidity which could lead to the production of off flavours and odours will be reduced drastically. The results of mean separation from ANOVA indicated that there were significant ($p<0.05$) differences among fat values of wheat and fermented Bambara groundnut flour blends.

Fiber content

The findings showed that fibre content of wheat- fermented Bambara groundnut mixed flours ranged from 0.1 to 0.3%. The results of mean separation from ANOVA indicated that there were no significant ($p<0.05$) differences among fibre values of wheat-Bambara groundnut flour blends.

Ash content

Results showed that ash content of wheat-Bambara groundnut flour blends ranged from 0.1 to 0.3%. There were no significant ($p<0.05$) differences found in the ash levels of the wheat-Bambara groundnut flour blends,

according to the mean separation from ANOVA findings.

Carbohydrate content

S1 (66% wheat flour and 26% fermented Bambara groundnut flour) recorded the highest score while S11 (50% wheat flour and 40% fermented Bambara groundnut flour) recorded the least score. Dietary carbohydrate supplies the bulk of calories of an average diet. The wheat and fermented Bambara groundnut flour blends' carbohydrate levels varied significantly ($p<0.05$), according to the mean separation from ANOVA findings.

Table 5: Proximate composition (%) of fermented Bambara groundnut and wheat flour blends

Sample	Moisture	Protein	Fat	Carbohydrate	Fiber	Ash
S1	9.61^{abcd}±0.11	23.17^{ab}±0.62	5.83^{a}±0.91	60.99^{abc}±0.20	0.20±0.00	0.20±0.00
S2	9.97^{abcd}±0.30	23.23^{ab}±0.25	7.83^{bcd}±0.36	58.77^{abc}±0.47	0.10±0.00	0.10±0.00
S3	10.67^{bcd}±0.43	23.40^{ab}±0.50	9.18^{de}±1.00	56.35^{bc}±6.23	0.20±0.00	0.20±0.00
S4	9.06^{ab}±0.01	23.91^{ab}±0.98	6.39^{ab}±0.01	60.44^{a}±0.43	0.10±0.00	0.10±0.00
S5	8.50^{a}±2.16	23.15^{ab}±1.48	6.94^{abc}±0.91	60.81^{abc}±0.62	0.30±0.00	0.30±0.00
S6	10.51^{bcd}±0.07	24.23^{ab}±2.46	7.13^{abc}±0.50	57.73^{bc}±0.60	0.20±0.00	0.20±0.00
S7	10.28^{abcd}±0.23	23.17^{ab}±0.37	8.43^{cde}±0.35	57.72^{ab}±0.15	0.20±0.00	0.20±0.00
S8	9.77^{abcd}±0.16	25.30^{bc}±0.74	9.64^{ef}±0.37	54.69^{a}±0.24	0.30±0.00	0.30±0.00
S9	9.53^{abc}±0.93	21.97^{a}±0.25	7.33^{abc}±0.06	60.97^{abc}±0.96	0.10±0.00	0.10±0.00
S10	11.44^{d}±0.12	24.08^{ab}±0.25	9.68^{ef}±1.27	54.60^{c}±2.77	0.10±0.00	0.10±0.00
S11	11.04^{cd}±0.56	26.73^{c}±0.25	11.22^{f}±1.05	50.81^{ab}±0.06	0.10±0.00	0.10±0.00

The values represent the means ± standard deviation of findings made in triplicate. There is a substantial ($p<0.05$) difference between values in the same column with different superscripts.
S1 (66:26) Wheat flour: Bambara groundnut flour: S2 (62:22) Wheat flour: Bambara groundnut flour: S3 (73:22) Wheat flour: Bambara groundnut flour; S4 (57:32) Wheat flour: Bambara groundnut flour: S5 (62:33) Wheat flour: Bambara groundnut flour; S6 (78:10) Wheat flour: Bambara groundnut flour; S7 (72:17) Wheat flour: Bambara groundnut flour; S8 (50:38) Wheat flour: Bambara groundnut flour; S9 (85:10) Wheat flour: Bambara groundnut flour; S10 (58:26) Wheat flour: Bambara groundnut flour; S11 (50:45) Wheat flour: Bambara groundnut flour.

Table 6: Proximate composition (%) of cookies made from fermented Bambara groundnut wheat composite flour

Sample	Moisture	Protein	Fat	Fiber	Ash	Carbohydrate
S1	$2.24^{c}\pm0.48$	$16.51^{bc}\pm0.12$	$13.98^{ab}\pm0.79$	$0.20^{a}\pm0.00$	$0.98^{a}\pm0.00$	$66.09^{abc}\pm0.20$
S2	$1.29^{ab}\pm0.01$	$15.68^{abc}\pm0.25$	$15.58^{ab}\pm0.34$	$0.10^{a}\pm0.00$	$0.60^{a}\pm0.00$	$66.75^{abc}\pm0.58$
S3	$1.39^{ab}\pm0.28$	$16.33^{bc}\pm0.13$	$11.52^{a}\pm5.83$	$0.20^{a}\pm0.00$	$1.19^{a}\pm0.00$	$69.37^{bc}\pm6.23$
S4	$1.25^{ab}\pm0.77$	$15.18^{ab}\pm0.00$	$18.17^{b}\pm0.34$	$0.10^{a}\pm0.00$	$1.20^{a}\pm0.00$	$64.11^{a}\pm0.43$
S5	$0.65^{a}\pm0.21$	$17.05^{cd}\pm0.62$	$14.17^{ab}\pm0.21$	$0.30^{a}\pm0.00$	$1.20^{a}\pm0.00$	$66.54^{abc}\pm0.62$
S6	$1.15^{ab}\pm0.21$	$15.04^{ab}\pm0.62$	$13.79^{ab}\pm0.20$	$0.20^{a}\pm0.00$	$0.30^{a}\pm0.00$	$69.53^{bc}\pm0.60$
S7	$0.6^{a}\pm0.14$	$16.74^{cd}\pm0.25$	$15.72^{ab}\pm0.25$	$0.30^{a}\pm0.00$	$1.99^{a}\pm0.00$	$64.76^{ab}\pm0.15$
S8	$1.45^{ab}\pm0.48$	$17.98^{de}\pm0.12$	$14.93^{ab}\pm0.23$	$0.10^{a}\pm0.00$	$1.20^{a}\pm0.00$	$64.14^{a}\pm0.24$
S9	$1.84^{bc}\pm0.21$	$15.16^{ab}\pm1.73$	$13.57^{ab}\pm2.47$	$0.10^{a}\pm0.00$	$0.30^{a}\pm0.00$	$69.03^{abc}\pm0.96$
S10	$0.99^{ab}\pm0.01$	$14.31^{a}\pm0.00$	$13.09^{ab}\pm2.76$	$0.10^{a}\pm0.00$	$0.90^{a}\pm0.00$	$70.61^{c}\pm2.77$
S11	$1.24^{ab}\pm0.06$	$18.98^{e}\pm0.50$	$14.57^{ab}\pm0.33$	$0.10^{a}\pm0.00$	$0.50^{a}\pm0.00$	$64.61^{ab}\pm0.57$

The values represent the means ± standard deviation of findings made in triplicate. Values in the same column with different superscripts are significantly ($p<0.05$) different.
S1 (66:26:08) Wheat flour: Bambara groundnut flour: water; S2 (62:22:16) Wheat flour: Bambara groundnut flour: water; S3 (73:22:05) Wheat flour: Bambara groundnut flour: water; S4 (57:32:11) Wheat flour: Bambara groundnut flour: water; S5 (62:33:05) Wheat flour: Bambara groundnut flour: water; S6 (78:10:12) Wheat flour: Bambara groundnut flour: water; S7 (72:17:11) Wheat flour: Bambara groundnut flour: water; S8 (50:38:12) Wheat flour: Bambara groundnut flour: water; S9 (85:10:05) Wheat flour: Bambara groundnut flour: water; S10 (58:26:16) Wheat flour: Bambara groundnut flour: water; S11 (50:45:05) Wheat flour: Bambara groundnut flour: water

Moisture content

The moisture contents of the cookies ranged from 0.6 to 2.24%. The products that had the least and the highest moisture contents were S7 (72% Wheat flour; 17% fermented Bambara groundnut flour: 11% water) and S1 (66% wheat flour; 26% fermented Bambara groundnut flour and 8% water) respectively. However, all cookies fell below 10% maximum recommended by Standard Organization of Nigeria [31]. The moisture contents of cookies were low due to high temperature of baking and it is advantageous as it will not allow the growth of spoilage microorganisms hence improving the keeping qualities [9].

Protein content

The protein content of cookies ranged from 14.31 to 18.98%. S11 (50% wheat flour; 45% fermented Bambara groundnut flour and 5% water) had the highest protein content while S10 (58% wheat flour; 26% fermented Bambara groundnut flour and 16% water) had the least protein content which followed similar trend as in the flour blends. Studies have also reported high protein content of composite flour and baked products with the addition of legumes such as Bambara groundnut and soybean [7] [8].

Crude Fat content

The fat content of cookies ranged from 11.52 to 18.17%. S3 (73% wheat flour; 22% fermented Bambara groundnut flour and 5% water) and S4 (57% wheat flour; 32% fermented Bambara groundnut flour and 11% water) had the least and highest fat content values respectively. The increase in crude fat content of cookies may be due to the addition of margarine to the flour during production. The variations observed in the fat contents of the biscuit samples, despite the same quantity of fat used in the recipe, may be due to variations in their moisture contents. Similar values (15.1%–18.1%) were reported by [32] for cookies prepared from composite flours containing different proportions of wheat, and tiger nut.

Crude Fiber content

The fibre content of cookies ranged from 0.1 to 0.3%. The fibre content of both the flour blends and cookies were within the Recommended Daily Allowance (< 5g/100g dry matter). The results of mean separation from ANOVA indicated that there were no significant ($p<0.05$) differences among fibre values of cookies produced.

Ash content

The ash content of cookies ranged from 0.3 to 1.97%. The formulation that resulted into lowest and highest amount of ash content were S6 and S7 respectively. S6 (78% wheat flour; 10% fermented Bambara groundnut flour and 12% water) and S7 (72% wheat flour; 17% fermented Bambara groundnut flour and 11% water) and respectively.

Carbohydrate content

Carbohydrate contents ranged from 64.11 to 70.61% with S4 (57% wheat flour; 32% fermented Bambara groundnut flour and 11%water) and S10 (58% wheat flour; 26% fermented Bambara groundnut flour and 16% water) having the least and highest scores respectively. The high carbohydrate implies that the cookies could serve as a source of energy needed for body metabolism.

Table 7: Functional Properties wheat and Fermented Bambara Groundnut Flour Blends

Sample	BD (g/ml)	WAC (%)	OAC (%)	SI (g/g)	EC (%)
S1	0.55^{c} ±0.01	110.85^{a}±5.73	113.49^{b}±0.15	14.90^{e}±0.17	26.99^{d}±0.08
S2	0.50^{b}±0.03	122.85^{ab}±1.34	115.77^{cd}±1.34	12.75^{cd}±0.81	25.00^{bc}±0.01
S3	0.58^{d}±0.00	113.80^{a}±0.85	110.73^{a}±0.00	13.01^{d}±0.04	38.41^{f}±0.59
S4	0.61^{e}±0.00	117.95^{ab}±1.34	111.49^{a}±0.01	13.05^{d}±0.06	27.88^{d}±0.01
S5	0.56^{cd}±0.00	142.35^{b}±36.27	116.37^{d}±0.14	13.08^{d}±0.36	21.91^{a}±0.17
S6	0.56^{cd}±0.00	115.55^{ab}±2.19	113.31^{b}±0.07	15.54^{e}±0.64	41.51^{g}±0.71
S7	0.56^{cd}±0.00	114.45^{a}±0.78	114.85^{c}±0.18	12.89^{cd}±0.16	36.34^{e}±0.57
S8	0.44^{a}±0.00	120.90^{ab}±1.56	115.48^{cd}±0.02	12.09^{c}±0.16	25.41^{c}±0.57
S9	0.56^{cd}±0.00	114.65^{a}±2.47	111.46^{a}±0.00	14.90^{e}±0.13	43.45^{h}±0.19
S10	0.58^{d}±0.00	116.00^{ab}±0.28	118.31^{c}±0.30	11.19^{b}±0.26	24.26^{b}±0.99
S11	0.58^{d}±0.00	120.20^{ab}±0.71	119.65^{f}±0.02	9.79^{a}±0.13	20.96^{a}±0.07

Values are means ± standard deviation of triplicate determinations. Values with the same superscripts under the same column showed no significant ($p< 0.05$) difference. BD = Bulk Density; WAC = Water Absorption Capacity; OAC = Oil Absorption Capacity; SI = Swelling Index; EC = Emulsion Capacity.

S1 (66:26) Wheat flour: Bambara groundnut flour: S2 (62:22) Wheat flour: Bambara groundnut flour: S3 (73:22) Wheat flour: Bambara groundnut flour; S4 (57:32) Wheat flour: Bambara groundnut flour: S5 (62:33) Wheat flour: Bambara groundnut flour; S6 (78:10) Wheat flour: Bambara groundnut flour; S7 (72:17) Wheat flour: Bambara groundnut flour; S8 (50:38) Wheat flour: Bambara groundnut flour; S9 (85:10) Wheat flour: Bambara groundnut flour; S10 (58:26) Wheat flour: Bambara groundnut flour; S11 (50:45) Wheat flour: Bambara groundnut flour.

Bulk density

S8 (50:38 wheat to Bambara groundnut flour) showed the least bulk density while S4 (57:32 wheat to Bambara groundnut flour) showed the highest density. The bulk densities correlate negatively with oil absorption capacities (OAC). The samples with least bulk density had higher oil absorption capacity and vice versa. The result is in agreement with 0.62-0.72g/ml reported by [25]. The bulk densities suggest that flour blends may require different package space and material. The more packaging space is required the less the bulk density.

Water absorption capacity

The water absorption capacities of blends of wheat and fermented Bambara groundnut flour were in the range of 110.85 to 142.35%. S1 (66:26 wheat to Bambara groundnut flour) had the least water absorption capacity while S5 (62:33 wheat to Bambara groundnut flour) showed the highest water absorption capacity. The results show that addition of Bambara groundnut to wheat flour significantly ($P<0.05$) increased the water absorption

capacity (WAC) of the composite flours. This observation suggests that the hydrophilic constituents like carbohydrates which are less in Bambara groundnut than whole wheat did not contribute very much to higher water absorption capacity in the blends. High water absorption capacities of blends S2, S5, S8, S11 makes them suitable for energy bar production since high water absorption capacity improves yield and consistency and also gives body to the food products. It also indicates its usefulness in bakery products as they could prevent staling by reducing moisture loss [22] and helps to maintain freshness of bread, cakes and sausages. Similar increase in water absorption capacity was reported for maize Bambara groundnut blend for kpekele production. High water absorption capacity can be attributed to lose structure of starch polymers while low value indicates the compactness of the structure since Bambara groundnut is proteineous [35].

Oil absorption capacity

The sample S3 (73:22 wheat to fermented Bambara groundnut flour) and S11 (50:45 wheat to fermented Bambara groundnut flour) had the least and highest oil absorption capacities, respectively. The high oil absorption capacities of blends with higher proportion of fermented Bambara groundnut flour compare to blends with high proportion of wheat flour could also be an indication of the higher polar amino acid residues of protein having affinity for oil molecules. Oil absorption capacity is useful in flour retention and to improve palatability of bakery products [24]. The high oil absorption capacities of the flour blends also make them suitable in facilitating enhancement in flour and mouthfeel when used in food preparation [35].

Swelling Index

S11 (50:45 wheat to Bambara groundnut flour) and S6 (78:10 wheat to Bambara groundnut flour) had the least and highest swelling index respectively. Swelling index of flour is an indication of the extent of associative forces within the granules and it is also related to the water absorption index of the starch-based flour during heating. The values obtained from this study were higher than the report (5.87 to 13.48%) of cassava flour samples [6].

Emulsion capacity

Emulsion capacities of wheat- fermented Bambara groundnut flour blends ranged from 20.96 to 43.45% with S11 (50:45 wheat to Bambara groundnut flour) and S9 (85:10 wheat to Bambara groundnut flour) having the least and highest emulsion capacities respectively. High emulsion capacity is indications that flour could be an excellent emulsifier. Emulsifiers are incorporated into cookie formulation to improve dough handling and the products' overall quality [10]. Hence S9, S5, S3 and S7 would be excellent emulsifier.

Appearance

The appearance of cookies produced ranged from 6.40 to 7.70 as presented in Table 8. Samples produced with 58% wheat flour; 26% Bambara groundnut flour and 16% water ranked higher in appearance. Sample 1 and 2 showed similar appearance while all other samples appeared almost the same. The appearances of the cookies were generally accepted since the least value was 6.40. Wheat flour and fermented Bambara groundnut flour had positive effect on appearance of cookies while water showed an antagonistic effect.

Colour

The S10 produced with 58% wheat flour; 26% Bambara groundnut flour and 16% water ranked higher in appearance while S6 produces with 78% wheat flour; 10% Bambara groundnut flour and 12% water ranked lower in appearance. Colour is an important attribute because it can arouse individual's appetite. Wheat flour, fermented Bambara groundnut flour and water all had positive effect on the colour of cookies.

Taste

The S10 produced with 58% wheat flour; 26% Bambara groundnut flour and 16% water ranked higher in appearance while S6 produced with 78% wheat flour; 10% Bambara groundnut flour and 12% water ranked lower in taste. It was noted that these two samples have consistently ranked higher and lower respectively for three sensory parameters. Wheat flour, fermented Bambara groundnut flour and water all had positive effect on the taste of cookies.

After-taste

The S4 produced with 57% wheat flour; 32% Bambara groundnut flour and 11% water ranked higher in after-taste while S11 produced with 50% wheat flour; 45% Bambara groundnut flour and 5% water ranked least in after-taste. Wheat flour and fermented Bambara groundnut flour had positive effect while water had a negative effect on the after-taste of cookies.

Crispiness

The S10 produced with 58% wheat flour; 26% Bambara groundnut flour and 16% water ranked higher in appearance while S4 produced with 57% wheat flour; 32% Bambara groundnut flour and 11% water ranked lower in crispiness. Wheat flour and fermented Bambara groundnut flour had an antagonistic effect on the crispiness of cookies whereas water had a synergic effect. The interactions between wheat and Bambara groundnut flours showed a positive effect although the interactions between wheat flour and water showed a negative effect on the crispiness of cookies.

Texture

The S10 produced with 58% wheat flour; 26% Bambara groundnut flour and 16% water ranked higher in appearance while S6 produced with 78% wheat flour; 10% Bambara groundnut flour and 12% water ranked lower in texture. Wheat flour and fermented Bambara groundnut flour had synergic effect on the texture of cookies while water had an antagonistic effect. The interactions between wheat and Bambara groundnut flours and between Bambara groundnut flour and water showed a positive effect.

Table 8: Sensory scores of cookies made from Bambara groundnut wheat composite flours

Sample	Appearance	Colour	Taste	After-Taste	Crispiness	Texture	Flavour	Overall Acceptability
S1	$6.90^{abc}\pm1.02$	$7.10^{bc}\pm1.02$	$7.30^{ab}\pm1.13$	$6.85^{a}\pm1.35$	$7.20^{a}\pm0.97$	$6.70^{a}\pm0.86$	$7.10^{ab}\pm0.85$	$7.40^{ab}\pm0.82$
S2	$6.95^{abc}\pm0.83$	$6.95^{abc}\pm0.99$	$7.05^{ab}\pm1.32$	$6.80^{a}\pm1.36$	$6.95^{a}\pm0.99$	$6.70^{a}\pm1.22$	$6.90^{ab}\pm1.02$	$7.30^{ab}\pm1.21$
S3	$6.60^{bc}\pm1.14$	$6.50^{ab}\pm0.89$	$7.10^{ab}\pm1.07$	$7.30^{a}\pm1.03$	$6.95^{a}\pm1.23$	$6.95^{a}\pm1.32$	$6.80^{ab}\pm1.11$	$7.20^{ab}\pm1.05$
S4	$7.25^{bcd}\pm0.97$	$7.50^{c}\pm1.19$	$7.40^{ab}\pm0.75$	$7.35^{a}\pm1.09$	$6.65^{a}\pm1.49$	$7.05^{a}\pm0.99$	$7.00^{ab}\pm1.12$	$7.65^{ab}\pm1.27$
S5	$7.20^{bcd}\pm0.89$	$7.05^{bc}\pm1.09$	$7.25^{ab}\pm0.8$	$7.20^{a}\pm1.01$	$7.05^{a}\pm1.15$	$7.20^{a}\pm1.15$	$6.90^{ab}\pm0.72$	$7.35^{ab}\pm1.14$
S6	$6.40^{a}\pm1.27$	$6.25^{a}\pm1.21$	$6.75^{a}\pm1.29$	$6.95^{a}\pm0.99$	$6.90^{a}\pm1.33$	$6.65^{a}\pm1.35$	$6.70^{ab}\pm0.92$	$6.85^{a}\pm1.23$
S7	$7.35^{bcd}\pm1.23$	$6.90^{abc}\pm1.25$	$6.75^{a}\pm1.16$	$6.80^{a}\pm1.15$	$6.70^{a}\pm1.08$	$7.05^{a}\pm1.19$	$6.55^{a}\pm1.09$	$7.10^{ab}\pm1.41$
S8	$7.15^{bcd}\pm1.35$	$7.35^{c}\pm1.04$	$6.85^{ab}\pm1.66$	$6.70^{a}\pm1.75$	$7.30^{a}\pm0.92$	$7.05^{a}\pm1.05$	$6.90^{ab}\pm1.52$	$7.20^{ab}\pm1.47$
S9	$7.50^{cd}\pm0.89$	$7.30^{c}\pm0.73$	$7.10^{ab}\pm1.02$	$7.25^{a}\pm0.97$	$7.45^{a}\pm1.15$	$7.10^{a}\pm0.85$	$7.20^{ab}\pm1.01$	$7.60^{ab}\pm0.94$
S10	$7.70^{d}\pm0.98$	$7.45^{c}\pm0.69$	$7.65^{b}\pm0.93$	$7.35^{a}\pm.99$	$7.70^{a}\pm0.73$	$7.55^{a}\pm0.69$	$7.40^{ab}\pm0.94$	$7.95^{ab}\pm0.99$
S11	$7.15^{bcd}\pm0.75$	$7.05^{bc}\pm1.09$	$6.95^{ab}\pm1.32$	$6.45^{a}\pm1.61$	$6.85^{a}\pm1.23$	$7.15^{a}\pm1.14$	$6.55^{a}\pm1.09$	$7.35^{ab}\pm1.19$

Values are means ± standard deviation of scores of 20 panelists and values with the same superscripts showed no significant ($p< 0.05$) difference under the same column.

1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely. S1 (66:26:08) Wheat flour: Bambara groundnut flour: water; S2 (62:22:16) Wheat flour: Bambara groundnut flour: water; S3 (73:22:05) Wheat flour: Bambara groundnut flour: water; S4 (57:32:11) Wheat flour: Bambara groundnut flour: water; S5 (62:33:05) Wheat flour: Bambara groundnut flour: water; S6 (78:10:12) Wheat flour: Bambara groundnut flour: water; S7 (72:17:11) Wheat flour: Bambara groundnut flour: water; S8 (50:38:12) Wheat flour: Bambara groundnut flour: water; S9 (85:10:05) Wheat flour: Bambara groundnut flour: water; S10 (58:26:16) Wheat flour: Bambara groundnut flour: water; S11 (50:45:05) Wheat flour: Bambara groundnut flour: water.

Flavour

The S10 produced with 58% wheat flour; 26% Bambara groundnut flour and 16% water ranked higher in appearance while S11 produced with 50% wheat flour; 45% Bambara groundnut flour and 5% water ranked lower in flavour. Wheat flour, fermented Bambara groundnut flour and water all had synergic effect on the flavour of cookies. However, the interaction between wheat flour and Bambara groundnut flour had an antagonistic effect. The interactions between wheat flour and water also showed an antagonistic effect.

Overall acceptability

The S10 produced with 58% wheat flour; 26% Bambara groundnut flour and 16% water ranked higher in appearance while S6 produced with 78% wheat flour; 10% Bambara groundnut flour and 12% water ranked lower in overall acceptability. S10 showed the highest overall acceptability which implies that cookies made from substitution of wheat flour with Bambara groundnut flour up to 26% would be generally accepted. All the variables had synergic effect on the overall acceptability of cookies. The interactions among wheat flour, Bambara groundnut flours and water showed a positive effect. The mean separation also showed straight significant ($p<0.05$) differences among the means.

4. Conclusion

This research showed how fermented Bambara groundnut flour was produced and used to formulate blends with wheat flour based on mixture I-optimal design. The study found that the proximate composition of the flour blends and cookies varied significantly across different formulations, with all moisture levels being below the recommended maximum, thus enhancing shelf life. The protein content increased with higher proportions of fermented Bambara groundnut flour, while the carbohydrate content was highest in blends with more wheat flour, indicating that the blend ratios significantly impacted the nutritional profile of the cookies. This study showed that fermentation improved the protein and overall properties of the flour blends and cookies. The S10 produced with 58% wheat flour; 26% Bambara groundnut flour and 16% water showed the highest overall acceptability. Results from this work have shown that fermented Bambara groundnut flour could be used for substituting wheat flour up to 26% level in the production of acceptable cookies without adversely affecting the sensory attributes of the cookies.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper

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Green Extraction Techniques & Characterization for Recovery of Bioactives from Fruit & Vegetable Industry Wastes

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Abstract

The rising amount of waste coming from fruit and vegetable processing facilities presents environmental concerns by adding to greenhouse gas emissions and causing food security problems. Each year sees five billion tons of discarded fruit and vegetable matter being generated that holds beneficial bioactive substances with possible health promoting properties like antioxidants and anti-inflammatory effects. In response, to the repercussions of conventional extraction techniques It is urgent to implement eco-friendly extraction technologies. Methods like using microwaves for extraction (MAE) ultrasound assistance for extraction (UAE) and supercritical fluid extraction (SFE) provide eco options that are sustainable in nature. These techniques not increase the production of beneficial compounds but also reduce the amount of solvent and time needed for extraction process which helps in lowering the environmental impact of the procedure. By implementing these friendly extraction approaches to make use of fruit and vegetable leftovers can result in obtaining valuable bioactive components that have applications across multiple sectors, like food industry cosmetics business and pharmaceutical sector. Furthermore advancements in delivery methods such as nano emulsions can enhance the effectiveness and longevity of these substances while enhancing their properties This analysis intends to offer perspectives on the different eco-friendly extraction approaches used to retrieve valuable compounds from leftover materials in the fruit and vegetable sector and ways to identify these substances This knowledge is essential for food science experts researchers and professionals, in the industry looking for eco conscious approaches to handle waste and extract beneficial compounds.

Keywords: Waste valorization, extraction methods, bioactive compounds.

1. Introduction

Food processing sectors generate large volumes of waste, primarily consisting of biodegradable materials (Di Maria, 2017). The food processing industries can be classified into three primary categories of waste, collectively referred to as Food Industry Waste (FIW): (a) waste generated during food and drink production, (b) waste generated by grocery stores and restaurants, and (c) waste generated by consumers and households.

Food waste occurs throughout the entire food life cycle, including industrial processing, agricultural production, and distribution. Household activities account for up to 42% of food waste, with losses in the food process industry contributing 39%, and food management sectors (such as food preparation, catering, and cafeterias) responsible for 14%, while 5% is lost during distribution due to lack of appropriate transport vehicles (Mirabella et al., 2014). On an average, 33% of food production is globally wasted throughout the food supply chain, from farmers till it reaches consumers (Sagar et al., 2018).

The combined effect of increased production and population growth, coupled with inadequate infrastructure and handling methods, has directed to significant losses and wastage of these valuable food items, as well as their by-products and residues. Losses occur at every phase of the supply and handling chain which includes harvesting, sorting, grading, processing, storage, transportation to packaging, marketing and even during household preparation. Food items that are most commonly wasted include fruits and vegetables, followed by other perishable foods such as dairy products, eggs, meat, and fish. The rates of wastage for different food types vary greatly among developing countries across the globe (Sagar et al., 2018; Schieber et al., 2001).

2. Fruit and Vegetable Industry Wastes

As a result of changing dietary preferences and the expanding global population, there has been a significant increase in the mandate for fruits and vegetables which is due to their crucial role in our nutrition and overall well-being (Schieber et al., 2001). In certain countries, fruit and vegetable industry wastes (FVW) makes up a substantial portion of household waste, ranging from 20% to 50%. It is estimated that 1.6 billion tonnes of food are wasted annually, out of which 1.3 billion tonnes can be consumed in the form of value-added products. The waste derived from the processing of raw fruits vegetables poses a chief concern for the food sector. The solid waste from fruits and vegetables predominantly consists of soluble sugars, bioactive compounds, hydrolysable materials, and fibers.

The alteration of fruit and vegetable process waste (FVPW) into value-added products offers a feasible and cost-effective solution for improving energy, reducing environmental pollution, and increasing resource efficiency, and minimizing greenhouse gas emissions. Hence, the current review is focused on exploring the possible utilization of fruit as well vegetable processing wastes to extract valuable bioactive compounds by means of green technology.

3. Green Extraction

In the context of eco-friendly extraction of bioactive compounds from fruit and vegetable industry wastes, generally regarded as green chemistry which can be further developed as “green extraction technology”. Green extraction technology emphases on developing procedures that need less energy, utilize reuseable natural resources and alternative to chemical solvents, and produce extracts that are harmless, of high value, economically valuable and commercially feasible. This approach aims to plan and validate green extraction techniques

on both laboratory as well as industrial scales, with the goal of optimizing the utilization of raw materials, chemical solvents, and energy. Three main approaches have been identified as follows:

- Improving and optimizing existing processes
- Utilizing dedicated equipment
- Innovating processes and procedures (Chemat et al., 2012).

Green extraction technology, also known as unconventional technology, has grown attention in current years due to its capability to achieve high yields, shorten process times, produce high-quality products, and reduce waste generation (Saini et al., 2019). The extraction of bioactive compounds can be accomplished using traditional or novel means, each with its own advantages and disadvantages.

Several eco-friendly technologies have been investigated for the extraction of bioactive compounds. These technologies comprise ultrasound-assisted extraction, supercritical fluid extraction, enzyme-assisted extraction, microwave-assisted extraction, pulsed electric field-assisted extraction, and pressurized liquid extraction which are discoursed in the following sections (Azmir et al., 2013).

3.1 Ultrasound Assisted Extraction (UAE)

Ultrasound-assisted extraction (UAE) is conducted by an ultrasound device operating at a frequency of 20 kHz, featuring a submerged sonotrode with a determined power of 130 W. The probe is equipped with a 2 cm diameter flat tip. Subsequent to the completion of the extraction process, the extracted material can be subjected to further analysis based on the method variables, namely the pH, solid-liquid ratio, temperature, and extraction time. The use of ultrasound technology has revolutionized the food processing industry, finding wide applications in various processes such as extraction, pre-treatments, structural modification etc. Ultrasound waves are generated at higher frequencies that are beyond the range of human hearing, while lower frequencies have a higher attenuation coefficient. Ultrasound at complex frequencies and lower powers exhibits acceptable sensitivity and does not have any detrimental properties on the mechanical or chemical properties of materials; it only induces vibrations in the molecules (Mohammadi et al., 2014).

Therefore, it is possible to view ultrasound-assisted extraction as a viable technique that is both easier to use and more successful than traditional extraction methods to extract bioactive substances from waste and natural byproducts. It improves mass transfer by increasing the dispersion of solvent substances into cellular materials and breaks down cell barriers to release bioactive components more easily (Kumar et al., 2017).

3.2 Enzyme Assisted Extraction (EAE)

The utilization of enzymes to catalyze reactions in aqueous solutions under mild processing conditions makes enzyme-assisted extraction a promising substitute for traditional solvent-based extraction procedures (Gardossi et al., 2010). Enzyme-assisted extraction (EAE) is the extremely well-known and advanced method for removing the majority of bioactive compounds (BCs) from biological materials followed by the recovery of bioactive substances from the waste generated from fruit and vegetables (Rosenthal et al., 1996). Some BCs are dispersed within the cytoplasm, while others are destined by hydrogen or hydrophobic bonds, remaining intact within the polysaccharide-lignin network, rendering them inaccessible to solvents during a typical extraction process. To recover these free and intact BCs from biological waste especially fruit and vegetable industry wastes from the processing industry. In addressing the issue of recovery from this waste, an enzymatic

pretreatment procedure has been developed and considered as a green extraction technology which results in higher yields of bioactive compounds (Vyas & Braganza, 2019; Zulkifli et al., 2012).

3.3 Supercritical Fluid Extraction (SFE)

SFE utilizes solvents that are in close proximity to their supercritical zone (Baiano, 2014). Under these conditions, the raised temperature enhances solubility and accelerates the dispersion of solutes in the solvent, while the high pressure retains the solvent below its boiling point and facilitates deeper penetration into the sample tissues and cells. Consequently, SFE requires smaller amounts of solvent (15–40 mL) and shorter extraction periods (15–20 min) (Delazar et al., 2012).

Supercritical fluid extraction involves the parting of the extractant from the matrix, typically a solid but sometimes a liquid, using supercritical fluids. The most commonly used supercritical fluid is carbon dioxide (CO2), which may be combined with co-solvents such as ethanol to modify its porosity. CO2 is preferred for its slight critical conditions (31.1 °C and 73.8 MPa), non-hazardous, and chemical stability. The returns of supercritical fluid extraction include high solute diffusivities, reduced viscosities, lower surface tension, solvating properties comparable to liquid organic solvents, and the ability to adjust solvating power by optimizing pressure or temperature. Furthermore, the partition of solutes from the fluid phase is relatively straightforward (Delazar et al., 2012). Supercritical fluid extraction can be an excellent method for recovery of bioactive from existing fruit and vegetable industry waste. However, the high initial investment required for this technology remains a significant barrier to its widespread commercial implementations (Delazar et al., 2012).

3.4 Microwave Assisted Extraction (MAE)

Microwave-assisted extraction (MAE) is a novel extraction method that associates solvent extraction with microwave extraction. It is considered to be a more advantageous method compared to traditional extraction methods due to its shorter process time, higher rate of extraction, reduced solvent prerequisite, and lower economical process (Afoakwah et al., 2023).

One of the main benefits of MAE over Soxhlet extraction and ultrasonic-aided extraction is its ability to extract plant metabolites which contain the bioactive compounds in a shorter period of time (Bandar & Lambert, 2013). With its enhanced extraction efficiency, shorter extraction times, reduced labor requirements, and excellent extraction selectivity, the microwave-assisted approach offers numerous benefits over other extraction techniques, making it the preferred method for obtaining bioactive substances from the fruits and vegetable processing waste (Vanga et al., 2021).

3.5 Pulsed Electric Field Extraction (PEFE)

The extraction method involves disrupting the cell membrane to release the bioactive compounds. Initially, a suspension of cells is exposed to an electric field, causing an electrical potential to flow crosswise the cell membrane and separating the molecules based on their charges. This technique, known as Pulsed Electric Field Extraction (PEFE), is a non-thermal method. At the weakest points of the membrane, the charged molecules form pores that increase membrane permeability and cause electroporation once the transmembrane potential hits 1 V (Sagar et al., 2018; Azmir et al., 2013).

PEFE offers advantages such as rapid extraction, high efficiency, and low energy requirements for the ex-

traction of bioactive substances from the fruit and vegetable industry wastes. Compared to Soxhlet extraction, PEFE yields a higher final output and has a reduced environmental impact which acts as a well-known green extraction technology. However, precise control parameters and extensive maintenance are notable limitations of pulsed electric field extraction. Phytosterols and polyphenols from bioactive compounds of fruit waste can be extracted using PEFE (Barbosa-Pereira et al., 2018). Several factors, including plant matrix, energy input, pulse count, field intensity, and number of pulses, influence the bioactive compound extraction yield. To maximize the final yield, PEFE is often used as a pre-extraction treatment and can be combined with other extraction methods (Azmir et al., 2013). Generally, PEFE operates at normal temperature for less than one second (Soquettaet al., 2018). Nevertheless, in certain cases, applying electric fields at ambient temperature is insufficient, requiring the use of pulsed ohmic heating to raise the temperature by utilizing ionic movements in the series (Herrero et al., 2006).

3.6 Subcritical Water Extraction (SWE)

Subcritical water extraction is an increasingly popular substitute method for extracting phenolic contents from various food sources. Subcritical water denotes to water that is maintained at a temperature ranging from 100 to 374 °C, along with a pressure that is sufficient to keep the substance in its fluid state (below the critical pressure of 22 MPa). The pros of Subcritical Water Extraction (SCW) over traditional extraction methods include shorter extraction time, reduced solvent costs, improved extraction quality, and increased environmental awareness among ecologists (Zakaria & Kamal, 2016). SCW is currently considered the most promising engineering with a greener approach for obtaining bioactive compounds from waste generated from plants, fruit and vegetables processing industries (Gil-Chávez et al., 2013).

As a result, SCW extraction presents a more sustainable alternative to the conventional use of organic solvents for recovering phenolic compounds from agricultural waste. This extraction method offers a range of benefits compared to conventional methods, including superior extract quality, lower prices of extraction agents, shorter extraction times, and an eco-friendly approach (Shinwari & Rao, 2018).

3.7 High Hydrostatic Pressure Extraction (HHPE)

Mass transport is accelerated by high hydrostatic pressure extraction. This method can be used for both polar and nonpolar molecules. Foods with weak structural integrity may undergo various structural changes when subjected to high hydrostatic pressure, such as protein denaturation, cell membrane degradation, and cell deformation. High hydrostatic pressure extraction (HHPE) is considered a revolutionary approach for extracting bioactive components from natural biomaterials, food waste, fruit and vegetable processing waste. It is currently employed in the high-pressure processing of food. The main advantage of high hydrostatic pressure extraction is that it does not require heating and has a high extraction yield with minimal energy consumption. However, this extraction method has the disadvantage of requiring expensive equipment and being challenging to maintain due to the high level of pressure it requires. One example of bioactive substances that can be obtained using high hydrostatic pressure extraction is polyphenols (Shouqin et al., 2005; Garavand, & Madadlou, 2014).

3.8 Emulsion Liquid Membrane Extraction (ELME)

ELME offers the opportunity for easy scalability with minimal use of harmful solvents, as well as the recyclability of its components, thereby requiring minimal energy consumption. This act of ELM expresses it as one of the best methods of green extraction technology. However, it is important to consider the drawbacks associated with emulsion instability, leakage, and swelling. It should be noted that various bioactive substances, such as phenolics, flavonoids, and other phytochemicals, can be successfully extracted using this method especially from the fruit and vegetable processing waste (Papaioannou et al., 2022).

In terms of cost-effectiveness, the implementation of ELM can result in a reduction of up to 40% compared to traditional solvent extraction methods. The ELM process typically comprises three stages. The first stage involves the preparation of the emulsion, where the internal and external phases, such as water-in-oil (W/O), are combined, followed by the addition of water as minute droplets or particles within the oil. In order to facilitate the solute's transport across the membrane phase from the feeding phase to the receiving phase, the second stage concentrates on the interaction between the emulsion and a continuous phase made of metal waste. Lastly, after the emulsion and outer phases have settled, the third stage involves demulsification to recover the membrane phase (Katsinas et al., 2021). The utilization of an emulsion liquid membrane (ELM) presents numerous advantages for the extraction process, including its efficiency and selectivity.

3.9 Pressurised Liquid Extraction (PLE)

The fundamental configuration for pressurized liquid extraction (PLE), occasionally stated to as accelerated solvent extraction (ASE), pressurized solvent extraction (PSE), or enhanced solvent extraction (Srivastava et al., 2021). The pressurized solvent extraction (PSE) was developed to address the necessity for an added step in separating undesirable material from the liquid extract. This technology allows for the removal of solid and semisolid materials weighing between 1 and 100 g at high temperatures and pressures. Up to 24 samples can be extracted simultaneously and automatically using this technique (Tiwari, 2015).

Micro sized particles can be successfully extracted from various samples using PLE technology in a quick amount of time and with less solvent linked to other methods this makes it easier for obtaining bioactive compounds from the fruits as well vegetable industry waste. The PLE process is inclined by a small number of constraints, making optimization relatively easy. The extraction temperature and the choice of solvent are the primary factors to consider.

PLE syndicates accurate recoveries and sufficient precision with a quick and somewhat discerning extraction process, while also reducing the time required for sample processing related to traditional methods. The main disadvantages of this technique are its higher investments, practical challenges linked with achieving homogeneous and reproducible packing of heterogeneous samples in smaller PLE extraction cells, and its limited capability to selectively extract organic compounds from fruit and vegetable industry wastes (Srivastava et al., 2021).

4. Hybrid Technologies for Extracting Bioactive Compounds

The effectiveness and efficiency of the chosen extraction methods play a significant role in extracting bioactive substances from fruit as well vegetable processing waste (Azmir et al., 2013). Several studies have found that integrating innovative extraction techniques can result in quick and efficient extraction procedures (Aires,

2018; Chemat et al., 2017; Dash et al., 2021). The most commonly described and widely used novel extraction techniques for bioactive compound extraction include Ultrasound-assisted enzymatic extraction (UAEE), Microwave-assisted enzymatic extraction (MAEE), Ultrasonic microwave-assisted extraction (UMAE).

In the food industry, reducing waste generation and increasing waste valorization are primary concerns, which align with the growing preference for eco-friendly which comes under green extraction technology. While no single technique can be employed to develop the optimal extraction process, there are strategies to poise product quality, manufacturing charges, and solvent usage. Novel technologies such as ultrasound, microwave and enzyme-assisted extraction are employed as influential tools to achieve higher yields and healthier products. Combining these technologies could be one way to enhance this equilibrium (Cheng et al., 2015).

4.1 Ultrasound-Assisted Enzymatic Extraction (UAEE)

Ultrasound-assisted enzymatic extraction (UAEE) combines ultrasound and enzymes for the extraction of bioactive compounds from industry waste. UAEE is a blend of two extraction methods, which offers synergistic recompenses over other methods of extraction. The incorporation of enzymes in the enzymatic extraction (EAE) process helps break down cell walls and membranes, aiding in the recovery of target substances. The application of ultrasound in UAEE enhances the efficiency of EAE by inducing cavitation, which physically disrupts the matrix, facilitating the enzymatic response and subsequent release of the desired chemicals (Cheng et al., 2015).

4.2 Microwave-Assisted Enzymatic Extraction (MAEE)

MAEE is a hybrid technique that associates microwave irradiation and enzymolysis, and has shown the ability to modify the structure of cell walls and enhance their permeability. Consequently, the desired compounds present within the cellular matrix can be more effectively transferred into the solvent (Sun et al., 2019). Due to their significant advantages viz. extraction efficiency, ease of handling, reduced solvent usage, and energy consumption, both EAE and MAE have recently gained recognition as capable methodologies for Obtaining phyto-bioactive substances from fruit as well vegetable industry wastes in a greener way.

4.3 Ultrasonic Microwave-Assisted Extraction (UMAE)

The combination of Ultrasonic extraction (UAE) and Microwave Assisted extraction (MAE) is known as Ultrasonic-assisted Microwave Extraction (UMAE), and it is one of the most researched and promising methods for combined extraction. The UMAE method enables rapid sample research and accelerates the bioactive extraction method, resulting in a highly efficient extraction technique characterized by a short extraction period and a high extraction yield (Rodsamran & Sothornvit, 2019). Ultrasound, by disrupting the cell walls and improving mass transfer, enhances the dissemination of the solvent into the sample matrix, facilitates the solvation of soluble materials, and raises the contact surface area. Similarly, microwave radiation rapidly heats the sample, thus increasing the solubility of the solute and the rate of mass transfer. This expedites the desorption process of the expected bioactive compounds from the sample matrix (fruit and vegetable industry wastes), leading to improved extraction efficiency (Yin & Wang, 2018; Regalagadda, S., & Challa, 2018). Table 1 enlists Green Extraction Techniques along with some of the extracted bio-active compounds from various sources of fruit and vegetable industry wastes.

Table 1. Green Extraction techniques used for various wastes

Green Extraction Technique Used	Source of Waste	Extracted Bioactive Compounds	Reference
Ultrasound Assisted Extraction	Carrot pomace, Tomato seeds	Carotenoids, Flavonoids	Kumar et al., (2017)
Enzyme Assisted Extraction	Apple pomace	Essential oils and Flavonoids	Vyas & Braganza, (2019)
Supercritical Fluid Extraction	Tomato pomace	Lycopene and beta-carotene	Delazar et al., (2012)
Microwave Assisted Extraction	Artichoke tuber waste	Phenolic compounds	Afoakwah et al., (2023)
Pulsed Electric Field Extraction	Cocoa Bean shell	Polyphenols, Caffeine	Soquetta et al., (2018)
Subcritical Water Extraction	Orange peel, Fennel seed waste, Onion skins, Potato Peel	Essential Oils, Flavonoids, phenols	Zakaria & Kamal, (2016)
High Hydrostatic Pressure Extraction	Propolis	Flavonoids	Shouqin & Changzheng, (2005)
Emulsion Liquid Membrane Extraction	Grape Pomace, Olive Pomace	Essentials oils and Polyphenols	Garavand & Madadlou, (2014)
Pressurised Liquid Extraction	Olive pomace	Phenols	Katsinas et al., (2021).
Ultrasound-assisted enzymatic extraction	Radish peel, orange peel	Phytochemicals, Citrinin	Dash et al., (2021)
Microwave-assisted enzymatic extraction	Five flavor berry waste	Bioactive polysaccharides	Sun et al., (2019)
Ultrasonic microwave-assisted extraction	Lime peels	Limonin, Phenols, Essential oils	Rodsamran & Sothornvit, (2019).

5. Characterization & Purity Estimation of Extracted Bioactive Compounds

For estimating the level of purity of extracted bioactive compounds from fruit and vegetable industry wastes, a variety of techniques are used, including chromatographic methods such as High-Speed Countercurrent Chromatography (HSCC), Flash Chromatography, Thin-layer chromatography (TLC), Column chromatography, and High-Pressure Liquid Chromatography (HPLC) (Mahato et al., 2019).

5.1 High-Speed Countercurrent Chromatography (HSCC)

This chromatography method utilizes two-phase solvent systems that flow concurrently in the reverse direction to extract and purify bioactive compounds. Since no solid support matrix is used in this liquid-liquid partition method, sample loss due to adsorption to the solid matrix is eliminated. Moreover, by employing different gradient elution processes, this method can enhance the removal of bioactive compounds from both the crude extract and the final product. Occasionally, pure phyto-bioactive compounds are isolated from the crude extract in a single step without the necessity for sample pretreatment (Chen et al., 2003). As a support-free liquid

chromatography technique, High-speed Counter-current Chromatography (HSCC) is considered as an appropriate alternative for the parting of bioactive compounds from fruit and vegetable industry wastes because it eliminates the issues such as irreversible adsorption onto the solid support and tailing of the solute peaks. This technique is valuable for isolating, identifying, and purifying the bioactive components of natural food waste i.e. obtained from fruits and vegetables (Jayaprakasha et al., 2013).

5.2 Flash Chromatography (FC)

The chromatographic technique known as flash chromatography (FC) is used to separate coarsely purified fractions or plant crude extracts. Through the use of flash chromatography, active BCs are separated between stationary and mobile phases. By using nitrogen or compressed air, the stationary phase is a firmly closed glass column which is forced into the mobile phase. Mild pressure is generated into the column for the separation of substances in pre-packed cartridges. In contrast to column chromatography, this technique for the separation and purification of chemicals is also discussed to as medium pressure liquid chromatography. Large sample volumes of chemicals can be separated with a controlled claim of medium pressure to the column, producing highly pure substances. With the use of robotic fraction collectors, online detection units, and fully automated flash chromatography equipment, the efficiency of identifying, isolating, and purifying the constituent compounds in a complicated mixture of crude extract has been improved (Bele & Khale, 2011).

5.3 Thin-Layer Chromatography (TLC)

The separation method known as thin-layer chromatography (TLC) involves the distribution of two or more chemicals or ions between two stationary and moving phases. Most frequently, these two phases are solid-liquid, liquid-liquid, or gas-liquid. Among these, TLC is an effective, simple, dependable, and repeatable process.39 Thin-layer chromatography (TLC) is a chromatography method used to disperse mixtures. It is achieved on a sheet of glass, plastic, or aluminum foil that has been closed with a thin layer of an adsorbent substance; typically, silica gel, aluminum oxide, or cellulose (blotter paper). The adsorbent layer serves as the stationary phase. After the sample is placed on the plate, a solvent or solvent combination (referred to as the mobile phase) is pinched up the plate by capillary action. Because various analytes ascend the TLC plate at different rates, separation is obtained. Thin-layer chromatography is used to determine the purity of a substance, recognize the chemicals present in a particular sample, and track the development of a reaction (Regenstein, 2012).

5.4 Column Chromatography (CC)

Column chromatography is the easiest and most extensively used method of separation and purification. Both liquid and solid materials can be separated and purified using it. Column chromatography adsorbs and separates the chemicals moving through columns by using a fixed solid phase and a liquid mobile phase. Elution is predicated on the differential adsorption of a material by the adsorbent, and compounds are adsorbed according to their chemical composition. Depending on the kinds of chemicals that need to be isolated and separated, column chromatography uses a variety of stationary phases, such as silica, alumina, calcium phosphate, calcium carbonate, starch, and magnesia, along with varying solvent compositions (Srivastava et al., 2021).

5.5 High-Pressure Liquid Chromatography (HPLC)

For the separation of composite mixtures, high-pressure liquid chromatography (HPLC) has established itself

as a standard tool. However, the detector system used in HPLC imposes limitations on obtaining structural information about chemicals separated via HPLC. HPLC has been used to address a variety of analytical issues, including:

- The direct characterization of endogenous and xenobiotic metabolites from a biological matrix.
- Characterization of in-vitro study metabolites.
- Investigation of reactive metabolites in motion.
- Identification of natural products derived from complex mixtures (Shockcor, 2011)

6. Applications

The commercialization process for valuable components derived from food waste typically involves four stages:

i. Laboratory investigation of the recovery procedure and characterization regarding the functional properties of the final product.
ii. Acquisition of patent rights.
iii. Semi-industrial (pilot plant) and industrial-level development of the process.
iv. Exploitation of applications in the food sector and confirmation of the product in the market (Baiano, 2014).

Most fruit seeds contain pectins, which can be extracted, purified, and used as gelling agents in a wide range of culinary commodities, such as jellies, fillings, and jams etc. Pomace contains other food additives like cellulose, edible fibers, lactic acid, colors, vinegar, and natural sweeteners. Some tropical fruits, such as papaya and pineapple, contain protein-degrading enzymes called papain and bromelain, which can be utilized for meat tenderization, production of washing detergents, or even the making of beer (Zheng, & Shetty, 1998). Fruit as well vegetable industry wastes is a significant cause of various bioproducts, including those used for creating tastes and smells. Many potential products, including ethanol, enzymes, methane, citric acid, lactic acid, and numerous culinary components, have been produced from fruit and vegetable industry wastes by the conversion technique known as solid-state fermentation (SSF) (Baiano, 2014). Figure 1 illustrates some of the major applications of bioactive compounds extracted from the fruits and vegetables wastes.

As environmental consciousness continues to grow there is an increasing demand for adopting eco-friendly methods and to minimize the ecological footprint. The approach of utilizing this green extraction and characterization techniques not only addresses the issue of waste disposal but also contributes in the development of economically viable and eco-friendly strategies for obtaining bio active compounds the use of these techniques and characterization tools may be helpful for sustainable future and the validation of fruit and vegetable industry ways which will offer economic and environmental benefits.

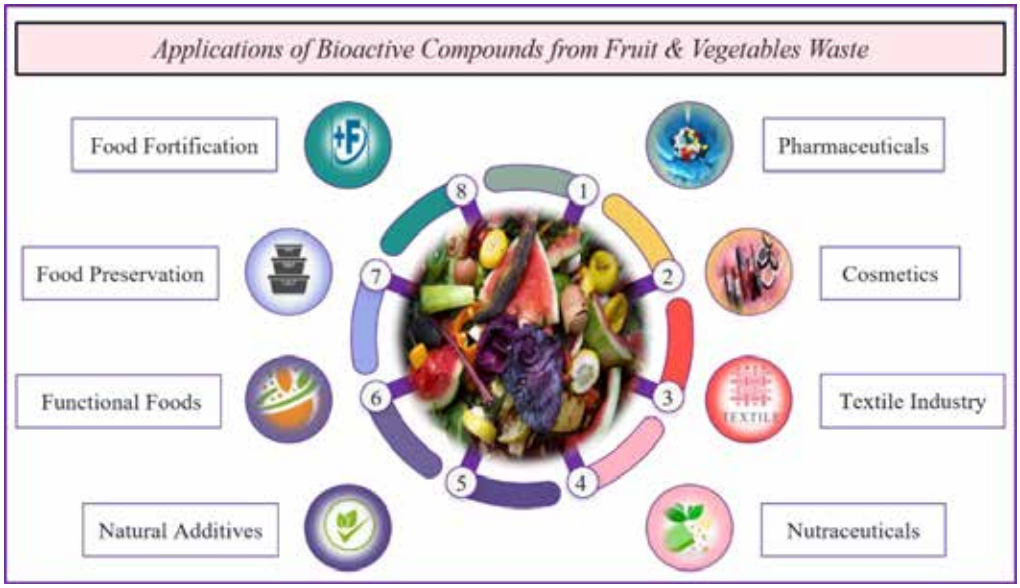

Figure 1. Application Extracted Bioactive Compounds from Fruit and vegetable Industry Wastes

7. Conclusion

The global demand for food, changing dietary habits, and population growth contribute to a significant amount of food waste, particularly in the form of biodegradable waste from fruits and vegetables. It is imperative to address this cause urgently and develop innovative resolutions to reduce waste and convert it into valuable products. Green extraction technologies, such as ultrasound-assisted and enzyme extraction, offer sustainable and efficient methods for utilizing food waste. These methods present promising alternatives to traditional extraction processes, as they are both energy-saving and environmentally friendly. The integration of emerging techniques further enhances the efficiency of extracting bioactive compounds, resulting in faster extraction, higher yields, and improved product quality. The effectiveness of these procedures is influenced by factors such as the characteristics of the source apex, its chemical composition, and process variables such as pressure, time, solvent, and temperature. Additionally, purification techniques ensure the production of high-quality, pure bioactive compounds that can be utilized in various applications, thereby contributive to sustainability and the practice of a globular economy.

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Traditional Bulgur and Flour of Develi Gaceri

Geleneksel Develi Gaceri Bulguru ve Unu

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Özet

Eski populasyonlardan kaplıca buğday grubuna giren ve yörede "GACER" olarak adlandırılan buğday Kayseri ilinin Develi ilçesi başta olmak üzere ülkemizin diğer bölgelerinde sayılı çiftçi tarafından hala kullanılmaktadır. Bu ürüne, 2022 yılı 22 Haziran tarihinde Develi Gaceri adıyla coğrafi işareti alınmıştır. Gacer harmanlanıp kavuzları uzaklaştırıldıktan sonra işlenmiş bulgura ve pirince ve öğütülmüş unu da diğer buğday unlarına alternatif olabilecek kullanım alanına sahiptir. Hasat sonrası temizlenmiş ve kavuzlarından ayrılmış buğday tohumu bulgur yapım prosesine ihtiyaç duyulmadan doğrudan bulgur olarak kullanılmaktadır. Bu temizleme esnasında eleklerin altında kalan daha küçük ya da kırılmış tanelere de düü adını almakta ve bu ürün de günümüzdeki küçük taneli bulgur (setik) gibi değerlendirilerek; başta çorba, dolma /sarma içi, içli köfte dış hamuru ve hatta çiğ köfte yapımında bile kullanılmaktadır. Diğer yandan Gacer taneleri kavuzları uzaklaştırıldıktan ve temizlendikten sonra öğütülerek unu elde edilmekte ve başta Gacer ekmeği olmak üzere, simit, poğaça, kek yapımında da diğer unlar gibi kullanılabilmektedir. Pirinç ve işlenmiş bulgura ve tüm buğday unlarına alternatif kullanımı olan bol lifli, yüksek kalitede protein ve nişastaya sahip olan Gacer ürünlerinin kullanımının yörede tekrar yaygınlaştırılması ve bu lezzetin Anadolu mutfaklarına yeniden inebilmesi için Gacer tarımına gereken önem verilmeli ve insanlık bu ata tohumu ürünlerinin gerçek lezzetinden mahrum edilmemelidir.

Anahtar Kelimeler: Kaplıca buğdayı, Develi Gaceri, Gacer bulguru, Gacer unu.

Abstract

Wheat, which is included in the emmer wheat group from the old populations and called "GACER" in the region, is still used by a few farmers in other regions of Turkey, especially in Develi district of Kayseri province. On June 22, 2022, this wheat species and its products received geographical mark under the name Develi Gaceri. After removing the husks, it can be used as an alternative to processed bulgur, rice and all wheat flours. Wheat seeds, which are cleaned and separated from their husks after harvest, are used directly as bulgur without the need for a bulgur making process. During this cleaning, the smaller or broken grains under the sieves are also called dü, and this product is evaluated like today's small grain bulgur (setik). It is mainly used in making soups, stuffed/wrapped vegetables, stuffed meatballs and even raw meatballs. On the other hand, Gacer grains are ground after the husks are removed and cleaned, and flour is obtained and can be used like other flours in making bagels, pastries and cakes, especially Gacer bread. In order to popularize the Gacer wheat and its products regionally nationally, the necessary importance should be given to Gacer wheat cultivation and people should not be deprived of the true taste of these ancestral wheat variety.

Keywords: Hulled wheat, Gacer of Develi, Gacer bulgur, Gacer flour.

1. Giriş

Anadolu'nun temel gıda maddelerinden birisi olan bulgur, Dünya'da ilk işlenen gıda maddelerinden bir tanesidir. Sözlük anlamı; kaynatılıp kurutulan kısmen kabuğu çıkarılıp kırılmış buğdaydır. Gacer bulguru ise hiçbir işleme gerek kalmadan direk tencereye girebilme özelliğine sahip olup bu hem üretim maliyeti hem de besin ve lif yoğunluğunun barındığı kabuğun alınmamasından dolayı daha besleyicidir. Tek dezavantajı ata formu buğday olması ve doğal yapısı hiç bozulmadığı için yabanilikten gelen kavuzluluğunun devam etmesidir. Tanesinin hasattan sonra kavuzlu olması kullanımını zorlaştırsa da, kavuzlarından arınmış tanesi başta pilav, dolma/sarma içi olarak, temizleme esnasında elek altında kalan ufak parçacıklar ise süt çorbası katkısı olarak ya da içli köftenin dış hamurunun yapımında doğrudan kullanılmaktadır. Sofralık bulgurun ve pirincin son ürün haline gelmesi için birçok fabrikasyon işleminden geçmesi gerekirken, Gacer tohumları sadece kavuzlarından hasat esnasında ya da sonrasında ayrılarak direk kullanılabilmektedir.

Un günlük beslenmemizde temel gıdamız olan ekmeğin yapımında kullanılan maddedir. Modern değirmenciliğin gelişimine bağlı olarak unlar çok farklı inceliğe dönüştürülüp kabuk (kepek) tan ayrılsa da beyaz unun günümüzde gastrointestinal sistem hastalıkları (sindirim sistemi) başta olmak üzere pek çok hastalığı tetikleyebilmektedir. Sağlıklı beslenmenin öneminin her geçen gün arttığı bu günlerde diyetisyen ve doktorlar esmer undan (tam buğday unu) yapılan tahıl ürünlerinin tercih edilmesi gerektiğini tavsiye etmektedirler. Bu yönüyle ele alındığında Develi Gaceri ata buğdayının tam tanesinin öğütülmesi ile elde edilen Gacer Ununun başta ekmek ve diğer unlu mamul yapımına çok uygun olduğunu söyleyebiliriz. Sofralık bulgurun ve pirincin son ürün haline gelmesi için birçok fabrikasyon işleminden geçmesi gerekirken, Gacer tohumları sadece kavuzlarından hasat esnasında ya da sonrasında ayrılarak direk kullanılabilmektedir. Pirinç ve işlenmiş bulgura alternatif kullanımı olan yüksek kalitede protein ve nişastaya sahip olan Gacerin kullanımının yörede tekrar yaygınlaştırılması ve bu lezzetin Anadolu mutfaklarına yeniden inebilmesi ve tüketicilerle tekrar buluşturulması gerekmektedir.

Bu denli önemli olan ilimizde Develi yöresinde yetişen Gacer ata buğdayı, Develi Belediyesi ve Kayseri Üniversitesi ortaklığında Türk patent enstitüsüne yapılmış olan başvuru sonrasında "Develi Gaceri" adı altında tescil edilmiştir.

Resim 1. Yerel pazarda Develi Gaceri satış merkezi ve Tescil belgesi

2. Develi Gacer Tanesi, Bulguru ve Ununun Özellikleri Ve Üretimi

1.1. Tane Elde Edilmesi ve Kaynağı

Gacer ekmeği üretiminde kullanılacak buğday unu, kesinlikle sadece organik tarım kurallara göre yetiştirilmiş gacer buğday tanelerinden el edilmelidir. Başlangıçta hasat ürünü buğday taneleri kavuzlu olduğu için öncelikle kavuzlarının taneden ayrılma işlemi yapılmalıdır. Daha sonra elde edilen tane un yapımında kullanılabilir.**Resim 2.** Gacer ve gacerin kavuzdan ayrılma işleminden kareler

Kavuzlu ve kavuzu ayrılmış Gacer

Gacerin kavuzlarından ayrılma işlemi

2.2 Gacer Bulgurunun Özellikleri ve Besin Değeri

Tarih boyunca farklı kültürler tarafından değişik isimlerle adlandırılan bulgurun (Romalılar "cerealis", İsrailoğulları "dagan", Ortadoğu halkları "arisah") menşei ile ilgili kesin bir bilgi olmamakla birlikte, bazı araştırıcılar "arisah" adi altında Tevratta bahsedildiğini bildirmektedir. Günümüzde de "bulgur" (Kuzey Amerika), "burghul" (Ortadoğu ve Kuzey Afrika) ve "burgul" (Arap ülkeleri) gibi farklı isimlerle bilinmektedir (Bayram ve Öner, 2002). Klasik bilinen bulgur genellikle durum buğdayından yapılır. Durum buğdayı bulunamadığı takdirde ekmeklik buğdaylarda bulgur yapımında kullanılabilir. Klasik bulgur temizlenmiş buğdayın 2-3 misli su ile pişirilerek kurutulması ve taneye yaklaşık %3 su verilerek 10 dakikalık bir tavlama ile kabuğunun kısmen soyulması, kırılarak iriliğine göre pilavlık, köftelik veya çorbalık olarak tasnif edilmesiyle üretilmektedir (Certel ve Ertugay, 1992; Bayram, 2000; Anonim, 2005; Diekmann, 2009; Sfayhi Terras et al., 2019; Anonim, 2024). Bulgur pirinç makarna gibi gıdalara göre daha iyi posa ve B vitamini içeren ekonomik ve dayanıklı bir besindir. Türklerin yüzyıllardan beri diyetlerinde olduğu kadar türkü, atasözü gibi sözel kültürlerinde de yerini almıştır. Günümüzde yüzlerce çeşit bulgurla yapılan yemek mevcut olmasına rağmen bulgurun tüketiminin yerini günden güne pirinç ve makarnanın aldığı görülmektedir. Türklerin çok fazla şekilde yemeklerinde kullandığı bulgur birçok yemeğe lezzet katan besinsel üstünlükleri ile sağlıklı bir yaşam sürdürülmesine katkı sağlamaktadır. Sadece bulgurdan veya bulgur katılarak yapılan yemeklerin diyette yeterince yer alması sayesinde bulgur yemekleri hem gelecek kuşaklara aktarılmış hem de bulgurla ilgili birçok Türk yemeği Türk mutfak kültürü ile birlikte tanıtılmış olacaktır (Türker, 2012). Zengin bir kültür sürecinde süzülerek gelen Türk mutfak kültüründe yer alan tüm değerlere sahip çıkılması gerekmektedir (Şeren Karakuş ve ark., 2007). Gacer tanesi ise bol lifli, yüksek proteinli fakat düşük gluten içeriğinden dolayı bulgur yapımına daha uygundur (Bulut, 2016; Bulut, 2022; Bulut, 2023). Slow Food taraftarı çiftçi ve tüketiciler bu buğdayı yaşatmaya çalışmaktadır. Tüm bulgurun üstünlük özellikleri ile birlikte Gacer buğdayından elde edilen bulgura gelince; bu ürünün en üstün özelliği klasik bulgur üretim aşamalarının hiç birine ihtiyaç duymadan (haşlama, kurutma, tavlama, değirmende kabuk soyma ve kırma vb.) doğrudan tencereye girme özelliğinin bulunmasıdır (Bulut, 2016; Bulut, 2022; Bulut, 2023). Gacer bulgurundan yapılmış bazı yemeklere ait resimler aşağıda sunulmuştur.

Resim 3. Kavuzlu buğday Gacerden elde edilmiş ürünler ve yemekler

2.3. Gacer Unu Öğütme Aşaması

Gacer tanesinin öğütülmesi ve işlenmesi esnasında, başka ürünlerle karışma ya da bulaşma riski ortadan kaldırılmalıdır. Gacer buğday tanelerinin öğütülmesinde doğal taş değirmenler tavsiye edilir. Ya da, **öğütmede** koridon madeninden yapılmış silindirlerde kullanılabilir. Her bir taş değirmen grubu hareketli dönen taş ve buna eşdeğer büyüklükte olan sabit 1 metrelik taş yatağı içermektedir. Her bir taş; yiv veya oluklarla merkezden dışarıya doğru bölümlere ayrılmıştır ve bu sayede tanenin tamamen ezilmesini sağlayacak taş yüzeye sürüklenmesi sağlanmış olur. Taneler düz taşın merkezinde bulunan göz içine beslenir ve dışardan soğuk hava

girişi olurken oluklar (yivler) sayesinde taneler taş yüzeyine eşit bir şekilde dağıtılır. Değirmen taşlarının asla birbirine dokunmaması ve yüzeydeki yivler ile konumu un kalitesi için oldukça önemlidir (Salman, 2010).

Resim 4. Gacer, Gacer bulguru, Gacer unu, Gacer nişastası

Taş değirmencilik metodunda tane ezilirken un daha az ısınır ve unun her tarafına tanedeki doğal yağ, vitamin ve mineraller eşit bir şekilde dağıtılır. Diğer değirmenlere kıyasla taş değirmenler bu nedenle öğütmede daha fazla avantaj sağlamaktadır. Bu şekilde öğütmede tanenin tamamı öğütülmüş olur. Valsli değirmende öğütme sırasında, taş değirmende öğütmeye kıyasla un daha fazla ısınmakta ve bu ısıdan besinsel değeri fazla olan tane bileşenleri olumsuz yönde etkilenmektedir. Valsli değirmende ticari şartlarda öğütme sırasında, ısıya duyarlı B vitaminleri, E vitamini, enzimlerde büyük kayıplar meydana gelmektedir. Özellikle, öğütme zamanı ve vals hızı arttıkça ısı daha fazla artmakta besin maddeleri ve gluten bundan olumsuz etkilenmektedir. 112 – 115 ^{o}C'de ısıya duyarlı vitaminlerin yapısı bozulmaya başlar ve gluten kalitesi bozulur. Dolayısıyla bu sıcaklık dereceleri güvenli öğütme için üst sınırı oluşturmaktadır. 122 ^{o}C'den sonra unun kalitesi tamamen bozulmaktadır (Arduzlar, 2010). Un ambalajının üzerinde öğütme tarihi, kullanılan öğütme metodu, kullanılan silindirlerin yapısı mutlaka belirtilmelidir.

Resim 5. Küçük işletme tipi Gacer öğütme değirmeni

Taş değirmende öğütme esnasında tüm buğday özü taş ve toprak yüzeyler arasında ezilir. Böylece endosperm, embryo ve kepek kısmı birlikte öğütülmüş olur. Taş değirmenlerde öğütülen unun ekstraksiyon oranı %100 olup kaba materyalin ayrıştırılması için elekler kullanılmaz. Silindir değirmenlerde öğütülen buğday tanesi kırılır, özü açılır ve kepeğin çoğu endospermden embriyo ile birlikte uzaklaştırılır (Kihlberg, 2004).

Buğday tanesinin kepek kısmında B vitamini, öz kısmında ise E vitamini ve doymamış yağ asitleri bulunur. Öğütme esnasında koruyucu hücre tabakası zedelenir ve doymamış yağ asitleri ve vitaminler oksidasyona maruz kalır. Un depolanmasında bu durum unun sararmasına ransid (kokmuş) tadın oluşmasına ve besin değerinde kayıplara neden olabilmektedir. Bu nedenle uzun süre depolanabilen un veya beyaz un elde edebilmek için buğday özünün uzaklaştırılması gerekir. Eğer un hemen kullanılacaksa (bir haftadan daha az bir sürede) buğday ruşeymi una katılabilir. Taş değirmende öğütülmüş, organik ekmek üretiminde kullanılacak %100 randımanlı un hemen kullanılmayacaksa uygun depolama teknikleri kullanılarak depolanmalıdır (Brandt et al., 2005).

2.4. Develi Gaceri Bulgur ve Ununu Depolamada Dikkat Edilecek Hususlar

Gacer unu; tohumdan un elde edilinceye kadarki tüm safhaları kayıt altına alınmış olan, gerekli denetimleri yapılan ve çok sıkı kontrollerden geçerek yetiştirilen ve nihayetinde sözleşmeli gacer üretimi yapan çiftçilerin üretim yaptığı gacer buğday tohumlarından elde edilen unlardır.
Gacer ekmeği üretiminde katkı maddesi kullanılmadığı için, ununun elde edildiği buğday ve un kalitesi çok önemlidir. Gacer ununun ambalajlanmasında uygun özelliklerde bez ya da kâğıt torbaların kullanılması uygundur. Un kompozisyonu, yaklaşık ekstraksiyon oranı ve öğütme metodu gibi hususların ambalaj üzerinde mutlaka belirtilmesi gerekmektedir.

Gacer ürünleri ya da unu depolama alanlarında diğer buğday ürünleri ve ununa karışmayacak şekilde ayrı bir yerde ve ambalajlarda depolanmalıdır. Ayrı olarak depolamanın mümkün olmadığı durumlarda gacer ürünleri ile diğer buğday ürünlerinin karışmasını engelleyecek tedbirler alınmalıdır.

2. Develi Gaceri Bulgur ve Ununun Üstün Özellikleri/Faydaları

• Bulgur, kandaki yağları düşürücü yönü olduğu bilinen posa/lif bakımından oldukça zengin bir gıdadır. Bu yüzden bağırsakların çalışmasında önemli bir role sahiptir. Ayrıca bulgurun glisemik endeksinin düşük olması, kana yavaş karışması ve liflerin tok tutucu özelliğe sahip olması, bulgura kilo kontrolünde de önemli bir rol yüklemiştir (Tacer, 2008).

• Bulgurda bulunan B1 vitaminleri, sinir ve sindirim sisteminde önemli rol oynamaktadır. Beriberi hastalığının önlenmesinde bu vitaminin düzenli tüketilmesi gerekmektedir.

• İçerdiği folik asitten dolayı, çocuk ve hamile kadınlar için çok önemli bir gıda maddesidir. Çocukların zekâ gelişimine katkı sağlar.

• Doymamış yağa sahiptir ve toplam yağ oranı düşük olduğu için sağlıklı bir besin maddesidir.

• Kolesterol içermez. Bu yönüyle kalp ve damar hastalıkları riskini azaltır.

• Gacer bulguru elde edilirken normal bulgur yapım aşamalarına ihtiyaç olmadığından (pişirme ve kurutma) dolayı hububat ürünlerinin en büyük dezavantajı olan fitik asit bu bulgurda bulunmaz.

• Yüksek mineral ve selülozdan dolayı besin emilimini hızlandırır, kabızlığı engeller ve bağırsak kanserini önler (Lyons et al., 2004).

• Radyasyonu emmez ve radyasyona karşı dayanıklıdır. Bu nedenle ülkelerde nükleer savaşlara karşı, askeri ve sivil amaçlar için stokta tutulan ürünlerdendir.

• Tam tane ürünü olduğundan besin değeri diğer ürünlerden (ekmek, makarna) daha yüksektir.

• Birçok farklı yemek ve salata yapımında kullanılabilmesi, kullanım alanı açısından çeşitlilik sunmaktadır.

• Develi Gaceri de başta E vitamini, B1 (tiamin), B2 (riboflavin), B3 (niasin), B6 (pridoksin) ve B9 (folat) gibi antioksidan işlevler gören maddeler yönünden zengindir. E vitamini veya tokoferol yağda çözünen önemli bir antioksidandır ve özellikle hücre zarları ve lipoproteinlerde hasar yapıcı molekülleri (serbest radikal) temizlemede önemli bir işlevi vardır (Capocchi et al., 2005; Tacer, 2008; Arduzlar, 2010; Bulut, 2016; Bulut, 2022; Bulut, 2023).

3. Sonuç

Gacer bulgurunun en üstün özelliği klasik bulgur üretim aşamalarının hiçbirine ihtiyaç duymadan (haşlama, kurutma, tavlama, değirmende kabuk soyma ve kırma vb.) doğrudan tencereye girme özelliğinin olmasıdır. Yüksek miktarda lif içermesi Gacer bulguru ve ununu bağırsak çalışmasında önemli bir pozisyona getirmektedir. Bulguru kaynatma ve kabuk soyma işlemi geçirmediğinden unu ise tüm tanenin öğütülmesi ile elde edildiğinden içlerinde barındırdıkları liflerin tok tutucu özelliğe sahip olmaları nedeniyle Gacer bulgur ve unu kilo kontrolünde de önemli bir rol üstlenebilir. Dünya Sağlık Örgütünün belirttiği üzere günde 25-30 gr. civarında lif ihtiyacımız bulunmaktadır. Bu ihtiyacımızı da 1 tabak bulgurla ya da iki dilim tam buğday ununda yapılmış ekmekle çok rahat şekilde karşılayabiliriz. Yapılarındaki B1 vitaminleri; sinir ve sindirim sistemimizin güçlenmesinde önemli rol oynamaktadır. Bulgurun bütün bunların dışında yapısında içerdiği folik asit sayesinde hamile annelerin bebeklerinin zekâ seviyesini ilerletme de oldukça gerekli olduğu uzmanlar tarafından belirtilmektedir. Gacer bulguru ve ununun glisemik endeklerinin düşük olması sebebiyle uzun süre tok tutarlar, kana yavaş karıştıkları içinde diyetlerde kullanılabilecek ürünlerdir. Ayrıca salatalarda, sıcak ve soğuk yemeklerde kullanılan bir malzeme olması nedeniyle çeşitlilik sunan bir yiyecektir. Tüm bu özellikleri ön plana çıkan Gacer bulguru ve ununun günümüzde kullanılan modern tekniklerle elde edilen bulgur ve unlara göre kolay elde edilen, daha besleyici, faydalı ve sağlığa daha az zararlı oldukları söylenebilir.

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Traditional Develi Gacer Bread

Geleneksel Develi Gaceri Ekmeği

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Özet

Buğday asırlardır insan beslenmesinde vazgeçilmez kaynaklardan biridir. Buğdaydan elde edilen ürünler başta ekmek gibi insanların temel gıda maddelerini oluşturmakta ve insan beslenmesinde gerekli olan besin öğelerinin önemli bir kısmı karşılamaktadır. Günümüzde meydana gelen teknolojik gelişmeler bitki genetiği konusunda da ilerlemelere yol açmıştır. Ancak bu gelişmelerle buğday ata formundan uzaklaşmış, modern yolla elde edilen gıdalar birçok sağlık sorununu da beraberinde getirmiştir (Kalkan ve Özarık, 2017). Bu durum insanların güvenli gıdaya ulaşmaları açısından yeni arayışlara yönelmesine sebep olmuştur. İnsanlar organik ya da üretim prosesinin güvenli olduğunu düşündükleri gıdalara yönelmeye başlamışlardır. Tam da bu noktada, organik tarıma uygunluğu ve insan beslenmesinde ihtiyaç duyulan besin maddelerini büyük oranda içeriğinde barındırması sebebiyle yöremizde coğrafi işareti alınan ve Develi Gaceri olarak bilinen emmer buğdayı (Triticum turgidum L. var. dicoccum) çeşidi güvenli gıda noktasında dikkatleri üzerine çekmektedir. Diğer çalışmamızda belirtilen özelliklere sahip Gacer unuyla geleneksel yöntemlerle ekmek üretilmektedir. Çok fazla kabarmasa da lezzet, koku ve aroması yönüyle kıyaslandığında günümüz modern teknikleri ile öğütülen unlardan yapılan ekmeklerden çok daha besleyici olduğu ve bizleri çocukluğumuza götürdüğü söylenebilir.

Anahtar Kelimeler: Kavuzlu buğday, Gacer, Gacer ekmeği, Kayseri- Develi.

Abstract

Wheat has been one of the indispensable sources of human nutrition for centuries. Products obtained from wheat constitute the basic foodstuffs of people, especially bread, and meet a significant part of the nutrients necessary in human nutrition. Today's technological developments have also led to advances in plant genetics. However, with these developments, wheat has moved away from its ancestral form, and the foods obtained in the modern way have brought many health problems. This situation has caused people to turn to new searches in terms of accessing safe food. People have started to turn to foods that they think are organic or safe. At this point, the emmer wheat (Triticum turgidum L. var. dicoccum) variety, known as Develi Gaceri, which is geographically marked in our region due to its suitability for organic agriculture and its content of nutrients needed in human nutrition, draws attention to the point of safe food. In our other study, bread is produced by traditional methods with Gacer flour, which has the properties mentioned. Although it does not rise much, it can be said that it is much more nutritious than bread made from flour milled with today's modern techniques and takes us back to our childhood when compared to its taste, smell and aroma.

Keywords: Hulled wheat, Gacer, Gacer bread, Kayseri- Develi.

1. Giriş

Dünya nüfusunun hızla artması ve beraberindeki teknolojik gelişmeler insanları tarımsal üretimde birim alanda en kısa sürede mümkün olan en fazla verimi elde etmeye yöneltmiştir. Bitkileri ve hayvanları hızla büyütmek, zararlı böcekleri öldürmek ve hastalıkları önlemek için sentetik kimyasal ilaçlar bol miktarda ve her sene daha fazla arttırılarak kullanılmaya başlanmıştır. Ayrıca, genleri değiştirilen bitkilerin sağlığımıza zararları olup olmadığı ve uzun vadeli etkileri, çevreye ne derece zarar verebilecekleri henüz kesin olarak bilinmemektedir. Dolayısıyla, daha sağlıklı ve daha güvenli bir yaşam için kimyasal madde kullanılmadan üretilmiş ürünlere olan ilgi gün geçtikçe artmakta ve organik ürün pazarı da giderek büyümektedir.

Temel gıda maddelerinin başında buğday yer almaktadır. Develi Gaceri Anadolu'da asırlardır yetişen ve nesli tükenmekte olan antik bir buğday cinsidir. Buğday araştırmalarının yetersiz olduğu ilimizde son yıllarda sayıları oldukça artan buğday çeşitlerinden yöreye uygun yüksek verim ve kaliteli olanların belirlenmesi ve buğday tarımının geliştirilmesi gerekmektedir. Yeni çeşitlerin yanı sıra yörede bulunan eski yerel populasyonlarda koruma altına alınarak gerek gen kaynağı olarak gerekse ekonomik önemi olanların yeniden üretime kazandırılması şeklinde değerlendirilmesi gerekmektedir. Bu eski populasyonlardan kaplıca buğday grubuna giren ve yörede "GACER" olarak adlandırılan buğday Kayseri ilinin Develi ilçesi başta olmak üzere ülkemizin diğer bölgelerinde sayılı çiftçi tarafından hala kullanılmaktadır. Gacer harmanlanıp kavuzları uzaklaştırıldıktan sonra işlenmiş bulgura ve pirince alternatif olabilecek kullanım alanına sahiptir. Temizlenmiş hasat ürünü buğday tohumu, ekmek, pilav, dolma içi olarak, temizleme esnasında elek altında kalan ufak parçacıklar ise süt çorbası katkısı olarak ya da içli köftenin dış hamurunun yapımında doğrudan kullanılmaktadır. Diğer yandan beyaz undan elde edilen unlu mamuller aşırı yağlanma ve kilo aldırması ve pirincin sindirim sistemimizde yüksek emilim oranına sahip olmasından dolayı obezite, kolesterol vb. sağlık sorunlarını da beraberinde getirmektedir (Ergüven ve ark., 2008; Altuncu ve ark., 2011). Gacerin bol lifli olması ve yüksek oranda antioksidan içermesinden dolayı son zamanlarda diyetisyenlerin tavsiye ettiği tüm özellikleri taşıyan sağlıklı bir gıda olarak ayrı bir üstünlüğü bulunmaktadır. Gacer buğdayı üretiminin yaygınlaşmasına en büyük engel tanelerin kavuzlardan hasatta ayrılmaması sonucu tanelerin kavuzlu kalması ve bu kavuzların ayıklanma işleminin ise meşakkatli işlemlere dayanmasıdır (Bulut, 2016; Bulut, 2022; Bulut, 2023). Çalışmalarda hedeflenen amaçlara ulaşıldığında yüksek besin değeri ve lezzetinden dolayı yerel ve ulusal pazarlarda çok rahat satılan Gacer buğday çeşidinin başta bulgur, un ve türevleri olmak üzere çok sayıda gıda üretiminde kullanılmak amacıyla uygun ekolojilerde yüksek miktarda üretiminin yapılmasına katkı sağlanacaktır.

2. Develi Gaceri Ekmeği Üretimi

1.1. Tahılların Muhafazası ve Taşınması

Organik orijinli ürünlerin depolanmasında ürünün mevcut kalitesinin korunması istenir. Diğer (konvensiyonel) ürünlerden ayrı depolanmalıdır. Temizleme ve dezenfeksiyonda yönetmelik dahilinde izin verilmiş maddeler kullanılmalıdır. Depolamada, muhafaza ve koruma işlemi havalandırma, soğutma ve kontrollü atmosfer gibi fiziksel yollarla yapılmalıdır.

Üretici, organik ürünleri toptancı ve perakendeciler de dahil olmak üzere diğer birimlere taşıma sırasında içeriğinde herhangi bir karışmaya neden olmayacak biçimde uygun paket, konteynır veya kapalı araçlarda, izlenebilirliğini sağlayacak şekilde taşınmasını sağlamakla yükümlüdür.
Konvensiyonel tahılların yetiştiriciliği, hasadı, taşınması ve depolanmasında kullanılan ekipmanların organik

tahıllar içinde kullanılması, ekipmanda arta kalan konvensiyonel tahıllarla organik tahıllar arasında fiziksel bir karışmaya neden olup yüksek risk oluşturmaktadır.

Genetik değiştirilmiş organizmaların geliştirilmesinden sonra organik tarımdaki riskler artmıştır. Bu durum hasat, taşıma, depolama veya yakın tarlalardan GDO polenlerinin organik tahılları kontaminasyona uğraması riskini oluşturmuştur. Organik standartlar, üretim ve işlemede GDO'ların kullanımına engel olmaktadır (Born, 2005).

2.2. Develi Gaceri Ekmeği Yapımı ve Özellikleri

Organik ekmek organik esaslarına göre yetiştirilmiş tahılların, taş değirmende %100 randımanlı olarak öğütüldükten sonra elde edilen un ile ekşi maya, tuz ve su dışında herhangi bir katkı kullanılmadan uygun işleme ve pişirme metotları uygulanarak üretilen ekmektir. Bu sektörde artan bir tüketici talebi mevcuttur. Tüketici artık, sertifikalı organik tarım ürünlerini marketlerde görmek istemektedir. Bu nedenle, önümüzdeki dönemde organik ürünler üreten fırınların artacağı ve süper marketlerin de kendi markalı ekmekçilik ürünlerini üretmeye ağırlık vermeye başlamalarından dolayı, özelikle organik un ve tahılların öne çıkacağı düşünülmektedir (Bulut, 2023).

Organik ekmek üretiminde, her şeyden önce organik tarım esaslarına göre elde edilen tahıl ve ondan elde edilen un çok önemlidir. Organik, ekolojik veya biyolojik tarım; sağlıklı gıdalar üretmek ve doğanın dengesini bozmamak amacıyla bitkisel ve hayvansal üretimin uygun ekolojilerde, kültürel tedbirler, biyolojik mücadele ve doğal gübreleme yoluyla gerçekleştirilen tarım şeklinde tanımlanmaktadır.

2.3. Geleneksel Develi Gaceri Ekmeğinin Üstün Özellikleri

2.3.1. Tat: Konvensiyonel tarım ile üretilen tahıl organik tahıllara kıyasla daha hızlı gelişme gösterdiği için organik tahıllarda tat unsurlarının oluşumunun daha üstün olduğu ve doğal tat ve aromanın daha iyi korunduğu söylenebilir. Tat, tam buğday unuyla elde edilen ekmekte daha iyidir. Beyaz unla yapılan ekmekte tadın çoğu fermentasyonla üretilmektedir. Tam unda ise kepek ve ruşeym en fazla tat ve aroma maddeleri içeren kısımlar olduğundan ekmeğin tat ve aroması un bileşenlerinden kaynaklanmakta ve ekmekte daha fazla tercih edilen aromaya sahip olmaktadır (Özberk ve ark., 2016).

2.3.2. GDO'lu işlemden geçmemiş olması: Genetik değiştirilmiş organizmaların ve genetiği değiştirilmiş ürünlerden elde edilen katkıların organik ekmekte kullanılması yasaklanmıştır. Dolayısıyla; tüm insanlığın ortak gıdası olan ekmek açısından düşünüldüğünde, bu prosedürler hakkındaki etik kaygıların veya güvenlikle ilgili korkuların sofralardan uzaklaştırılması sağlanmış olmaktadır.

2.3.3. Kalıntı içermemesi: Dünya genelinde, organik olmayan ürünlerde sürekli kullanılan yüzlerce pestisit vardır. Organik ekmek tüketimi ile diğer ekmeklerde bulunan pestisit kalıntılarının günlük vücuda alımı engellenmekte ve böylece kanserojenik etkiye sahip olabilecek unsurlardan korunma sağlanmaktadır (Lyons et al., 2004). Yapılan analizlerde organik olmayan farklı ekmeklerde yüksek miktarda pestisit kalıntıların varlığı ortaya çıkarılmıştır (Bourn and Prescott, 2002; Forman and Silverstein, 2012; Goetzke et al., 2014).

2.3.4. Katkı Maddesi İçermemesi: Günümüzde gıda üretiminde çok çeşitli katkı maddeleri kullanılmaktadır. Bu katkı maddelerinin yeterli bilgiye sahip olmayan kişiler tarafından kullanılmasına izin verilmesi tüketicilerde birtakım ciddi sağlık problemlerine neden olmaktadır (Kalkan ve Özarık, 2017). Gıda katkıları ileri yaşlarda ortaya çıkan osteoporosis, kalp problemleri ve migren gibi hastalıklarla çocuklarda hiperaktifliğe neden ola-

bilmektedir. Problem teşkil eden katkıların organik sertifikaya sahip ürünlerde kullanılması yasaklandığı için gıda katkıları organik ekmekte denetim altına alınmıştır (Brandt et al., 2005). Tam buğday ekmeğinin elde edilmesinde maya yerine ekşi hamur kullanması, asitliği daha fazla artırdığı için ekmekte mikrobiyolojik kaliteyi iyileştirmektedir.

2.3.5. Çevreye Zarar Vermemesi: Çevre kirliliğine neden olan herbisit, insektisit, fungusitler gibi kimyasalların organik yetiştiricilikte kullanımına izin verilmediği için, organik ekmek üretim yöntemi bakımından çevre kirliliğinin oluşmasına neden olmamakta ve doğal yaşamı korumaya katkıda bulunmaktadır. Organik tarımla toprak ve su kaynakları ile havayı kirletmeden, çevre, bitki, hayvan ve insan sağlığını korumak mümkün olabilmektedir. Buğday üretiminde karşılaşılan yabani otları elle koparma, kürekle müdahale etme, toprak işleme ve diğer mekanik metotların dönüşümlü olarak uygulanmasıyla kontrol altına alınır. Konvensiyonel tarım gibi makine ve ilaca değil de insan gücüne daha fazla yer verildiği için işsizlik içinde bir istihdam sağlamış olacaktır (Brandt et al., 2005).

2.3.6. Sağlığa Yararlı olması: Organik ekmeklerde kullanılan tam buğday unları, vitamin ve mineral açısından daha zengindir. Mineral maddeler en fazla tanenin dış kısmında bulunur ve tam buğday ekmeği üretiminde bu değer kaybolmaz. Ayrıca tam buğday ekmeği lif içeriği bakımından da zengindir. Organik ekmeğin doyurucu özelliği diğer ekmeklere göre daha fazladır. Sentetik kimyasal maddeler yüzünden vücudumuzda zamanla fazla miktarda yağ depolanmaktadır. Buna karşın organik ekmeğin düzenli olarak tüketilmesi durumunda şişmanlık problemlerini kolaylıkla aşmada yardımcı olabileceği ileri sürülmektedir (Kihlberg, 2004).

2.4. Gacer Ekmeği Üretim Aşamaları

2.4.1. İçindekiler (i**ngredientler):** Organik ekmekte kullanılan temel içerikler organik tahıl unları, su, doğal tuz ve ekşi mayadır. Tüm içerikler organik asıllı olmalıdır. Organik ekmeklerde ticari maya tatlandırıcı ve yağlar kullanılmamalıdır. Organik ekmeklerde kullanılan tam buğday unları vitamin ve mineral açısından zengindir. Dolayısıyla, doyurucu özelliği diğer ekmeklere göre daha fazladır. Bazı uygulamalarda birtakım organik bileşenler kullanılabilir. Bu sayede ekmek yapısının daha iyi gelişmesi sağlanabilmektedir.

Organik ekmek satın alan tüketiciler ekmeğin çok daha iyi tat ve tekstüre sahip olmasını isterler. Bunu sağlamak için konvensiyonel ekmek yapımındaki olgunlaşma süresinden daha fazla bir süre ve her bir ekmek için daha fazla un kullanmak gereklidir. Bunun uygulanabilir hale gelmesi için ayrılan değer payının ve fiyatlarının yükselmesi kaçınılmazdır (Brandt et al., 2005; Karaoğlu, 2007).

2.4.2. Mayalanması: Organik ekmek üretiminde mayalama ekşi hamurla sağlanmaktadır. Ekşi hamur kullanıldığı için üretim süresi uzamaktadır. Maya kullanılmayan ekmeklerin daha çeşnili, besleyici, uzun süre muhafaza edilebilir, daha iyi gelişme gösteren ve kolay sindirilebilir nitelikte olduğu bildirilmektedir (Decock and Cappelle, 2005; Karaoğlu, 2007).

Basitçe, ekşi hamur önceki pişirmeden alınan hamur parçasıdır. Ticari fırın mayası çok asitli bir ortama dayanamaz iken doğal maya böyle bir faaliyet gösterebilmektedir. Çünkü ekşi hamur kültüründeki laktobasili bolca laktik ve asetik asit üretmektedir. Bu da ekşi hamur ekmeğine kendine has tat ve lezzet vermektedir. Asitlerin oluşturduğu ortam, ticari maya için (Saccharomyces cerevisiae) fazla asidik olduğundan sadece doğal mayalar yaşayabilmektedir. Ticari mayada olduğu gibi, ekşi hamurdaki mikroorganizmalar da undaki basit şekerleri parçalayarak etanol ve karbondioksit üretmektedirler. Buna ek olarak ürettikleri laktik ve asetik asitlerin oluş-

turduğu aroma ekmeğe zengin kendine has bir tat sağlamaktadır (Hansen and Schieberle, 2005; Karaoğlu, 2007).

2.4.3. Yapımında Kullanılan Katkılar: Organik ekmek üretiminde genelde katkı maddesi kullanımına izin verilmemektedir. Katkı maddesi kullanılmadığı için ve ekmekçilik kalitesi de düşük olduğu için, organik unla en iyi kalitedeki ekmek elde edilmesi diğer bir deyişle fırıncılığın kalite isteklerine ulaşılması zordur. Yani, organik ekmekte tüm katkı maddeleri kullanımı yasaklandığı için ekmek kalite özellikleri düşmektedir. Bu nedenle organik ekmek tekstürü daha kaba, yapısı daha sert ve daha az kabaran bir ekmek özelliği göstermektedir.

Hamur özelliklerini geliştirmek için organik üretimde özel askorbik asit gibi az sayıda katkı maddesi kullanımına izin verilir. Bu durum özellikle yüksek kaliteli buğday elde edilemediği zaman önem kazanır. Ancak bilindiği şekilde organik ekmek tüketmek isteyen tüketiciler maya ve tuz dışındaki katkılar kullanılmadan yapılan ekmeği tercih etmektedirler. Bazı durumlarda askorbik asit yerine barbados kirazı tozu (aserola) gibi organik katkılar kullanılabilir (Brandt et al., 2005; Karaoğlu, 2007).

2.4.4. Yoğurulması: Yoğurucu hızı ve hamur sıcaklığı önemlidir. Yoğurucu hızı dakikada 50 deviri geçmemelidir. Büyüme safhasında bitki kalıntıları ve diğer organik gübreler besinlerini toprağa yavaşça serbest bırakırlar. Böylece organik buğday az miktarda (besleyiciliği daha az olan) protein içeriğine sahip olmaya yönelmektedir. Bununla birlikte protein içeriği dışındaki diğer faktörler de pişirme kalitesi için önemlidir. Ve organik buğday aynı protein içeriğine sahip olan konvensiyonel buğdaydan daha fazla olgunlaştırma kabiliyetine sahiptir. Bu yüzden karıştırma yoğurma ve olgunlaşma sırasındaki hamur davranışları konvensiyonel una dayalı standart uygulamalardan farklılık gösterebilmektedir (Brandt et al., 2005; Karaoğlu, 2007).

2.4.5. Pişirme: Ekmek pişirme de pasa ve tavalarda kullanılan unlar da kesinlikle organik kaynaklı taneden gelmelidir. Ve pişirme işleminde; ısıtma indirekt (müsaade edilen herhangi bir yakıt için) ya da odunla pişirilecekse direkt olabilir. Fakat odun kesinlikle yeni ve işlenmemiş olmalıdır.

Organik ekmekte koruyucu (antimikrobiyal) katkı maddesi kullanılmadığından, ekmekte mikrobiyolojik kalite ya da güvenlik açısından fırında pişirme sıcaklığı süresine azami ihtimam göstermek gerekmektedir. Ekmekte en yaygın mikrobiyolojik faaliyet küflenme ve Bacillus suptilis bozulmasıdır. Bacillus suptilis pişirme sıcaklığında canlı kalabilen sporlar üretmekte ve emekte rop hastalığına neden olmaktadır. Bu hastalık ekmekte kaliteyi düşürürken gıda güvenliği açısından önemli bir tehlike oluşturmamaktadır. Ekşi hamurla oluşan fermentasyon (laktik asit bakterisiyle) rope (sünme) yapan bakteriyi kontrol altına alabilmekte ve besinlerin biyolojik yararlılığını geliştirebilmektedir (Brandt et al., 2005; Karaoğlu, 2007).

2.4.6. Gacer Ekmeğinin Ambalajlanması: Ekmek yalnızca doğal ambalajlarla ambalajlanmalıdır. Kullanılmasının zorunlu olduğu durumlarda ambalaj malzemesi olarak selofan'a izin verilebilmektedir. Organik ekmekler genelde kraft ambalajlarla satışa sunulmaktadır. Ambalajlama materyali ekolojik görüşlere uygun seçilmiş olmalıdır. Paket üzerinde üretici ve işlemeciye ait tüm kademeleri ve isimleri belirtilmelidir (Avcı, 2004). Bileşik ürün ise (pasta, kek, peynir vb.) organik ürün ve olmayan ürün oranları belirtilmelidir. Organik tahıl ve fırın ürünleri, hammadde, yarı mamul veya mamul madde halinde ambalajlanırken, depolamama süresince organik ürün niteliği bozulmamalıdır. Yönetmelik hükümlerine göre üretilmeyen ürün etiketinde, yönetmeliğe uygun üretildiği, hazırlandığı, işlendiği, ambalajlandığı, depolandığı ima ve beyan edilememelidir. Organik olmayan ürünler etiket ve ambalaj dizaynıyla, organik ürün etiket ve ambalaj dizaynını çağrıştıracak

nitelikte ve benzerlikte olamamalıdır. Böyle ürünler için organik tarımsal ürün olarak marka, patent ve tescil alınamamaktadır. Organik olmayan ürünler için, tüketicide organik ürün izlenimi oluşturacak, haksız rekabete neden olacak, bio, biyo, eco, eko, org ön ekleri kullanılmamalıdır.

2. Develi Gacer Ekmeği Bazı Resimler

Develi Gacer ekmeği Develi'de geleneksel yöntemlerle yapılmakta, pişirilmekte ve tüketilmektedir. Kayseri'de ise Develi Gacer Ekmeği markası ile 2020 yılı aralık ayından itibaren Büyükşehir Belediyesi Kent Ekmek Fabrikası tarafından daha fazla tüketiciye ulaştırılmak üzere üretilmekte ve şehrin farklı noktalarında bulunan 27 büfe tarafından satışı yapılmaktadır. Ayrıca daha nitelikli ve katma değeri yüksek ürün geliştirme kapsamında farklı şehirlerde de Develi Gaceri ekmeği yaptırılmış ve başta e ticaret kanalları olmak üzere tüketicilere ulaştırma çalışmaları devam etmektedir. Bu sayede Ülke geneline ürünlerinin yayılması sağlanarak ülke ekonomisine doğrudan ya da dolaylı katkı sağlanmış olacaktır. Tüm bu üretimlere ait bazı resimler aşağıda sunulmuştur.

Resim 1. Çeşitli Gacer ekmeği ve diğer ürünler

4. Sonuç

Develi ilçesiyle adı özdeşleşmiş Gacer buğdayından yapılan ekmek, zengin protein içeriği, kepekli öğütülmesi ve tam organik olması ile diğer ekmeklerden ayrılmaktadır. Yüksek lif ve kepek içeriği, düşük glisemik indeksi ile hazmı kolaylaştıran ekmek, kilo problemi yaşayanlar için de faydalı olacaktır. Modern ekmeklik buğdaylara göre daha düşük gluten içermesinden dolayı dikkat çeken Gacer buğdayı ürünleri, posa içeriğinin yüksekliği ve protein içeriğinin kaliteli (düşük glüten) olmasından dolayı rahatlıkla tercih edilebilir. Develi Gaceri diğer tahıllardan farklı olarak harmandan sonra hiçbir işlem görmeden tüketilebilme özelliğinden dolayı besin kaybına da uğramamaktadır. Gacer Buğdayı ve ürünlerinin üstün özelliklerine rağmen; Develi Gacerinin üretiminin yaygınlaşmasında en büyük engel tanelerin hasat sonrasında kavuzlarından ayrılmaması ve kavuzların ayıklanma işleminin ise elle ya da yarı makinalı meşakkatli işlemlere dayalı olmasıdır. Bundan sonraki yapılacak çalışmalarla ilk yapılması gereken yüksek kapasiteli ürün işleme tesisi kurulmasıdır. Daha sonra da çeşit ıslahı, organik tarıma uygunluk, mekanizasyona uygunluk, yüksek kaliteli lokal ürün geliştirmeye yönelik araştırma ve projeler yapılmalıdır.

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Quality Evaluation of Herbal Yoghurt Produced Using Utazi (*Gongronemalatifolium*) and Uziza (*Piper guineense*) Leaf Extract

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Abstract

Introduction and Aim: Yoghurt is a diary product produced by bacterial fermentation of milk (addition of a starter of active yoghurt containing a mixed culture). Most flavorings used for yoghurt production are exotic. Underutilized indigenous herbs could be used in place of these exotic fruits and plants. Yoghurt could be flavoured with utazi and uziza leaves to provide natural antioxidant, anti-microbial and medicinal properties, while improving the sensory qualities and acting as bio-preservative. The aim of the research was to produce herbal yoghurt using utazi and uziza aqueous extract and to evaluate the proximate, micronutrient, microbial, sensory and physicochemical composition.

Methods: The utazi/uziza leaves were destalked, washed using clean water. Fifty grams of leaves was blended with 200ml of water and the juices were extracted using a muslin cloth. Utazi extract was added at different level of 4 and 8% while uziza extract was between 4 and 12% to the produced yoghurt. Nutritional, phytochemical, microbial and sensory properties were carried out using standard procedures.

Results: The proximate composition ranged; thus, moisture 59.88 to 67.03, protein 0.11 to 0.18, fat 3.43 to 5.50%, ash 0.01 to 0.04%. Flavonoid, tannin and glycosides ranged from 4.23 to 5.01, 0.02 to 0.24 and 13.02 to 18.48%, respectively. There was no mold growth detected in any of the samples, while the total viable count ranged from 1.34 x 10^3 to 9.40 x 10^2cfu/ml and the LAB was the most viable microorganism which ranged from 1.12 x 10^3 to 9.20 x 10^2cfu/ml. The control without the herb was most preferred followed by samples formulated with 4% aqueous extract of utazi and uziza.

Conclusion: Extracts of utazi leaf and uziza leaf in herbal yoghurt production improved the nutritional properties and antioxidant properties of the yoghurt.

Keywords: Yoghurt, herbs, uziza leaf, utazi leaf, antioxidant properties.

1. Introduction

Yoghurt is a diary product produced by bacterial fermentation of milk (addition of a starter of active yoghurt containing a mixed culture). The bacteria used to make yoghurt are known as yoghurt cultures. These cultures include *Streptococcus salivarius* subspecies thermophilus and *Lactobacillus delbrueckii* subspecies *bulgaricus*. Fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give yoghurt its texture and characteristic tangy taste (Xue Han *et al.,* 2016). According to the Code of Federal Regulations of the United States Food and Drug Administration (FDA), yoghurt can be defined as a food produced by culturing one or more of the optional dairy ingredients namely, full cream milk, partially skimmed milk, and skim milk, used alone or in combination with a characteristic bacterial culture that contains lactic acid producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* Yoghurt should contain at least 3.25% of milk fat and 8.25% of Milk Solids Non Fat (MSNF) with a titratable acidity of not less than 0.9 percent, expressed as lactic acid according to Weerathilake *et al* (2014). Yoghurt is considered as healthy food due to its high digestibility and bioavailability of nutrients and also could be recommended to people with lactose intolerance, gastrointestinal disorders such as inflammatory bowel disease and irritable bowel disease, and aids in immune function and weight control according toYadav *et al* (2015). Yoghurt, apart from being a probiotic carrier, is a rich source of protein, calcium, milk fat, potassium, magnesium, and vitamins B_2, B_6, and B_{12}. The fact that most of the lactose in milk precursor is being converted to lactic acid by the bacteria culture during fermentation makes yoghurt suitable for people who are moderately lactose intolerant as reported by Akeem *et al.,* (2018).

Milk is the main ingredient used in yoghurt manufacturing. Milk is a normal habitat of a number of lactic acid bacteria, which causes spontaneous souring of milk held at a bacteria growth temperature for appropriate length of time. Depending on the type of lactic acid bacteria gaining entry from the environmental source, the sour milk attains characteristic flavor and texture. Fermentation conserves the vital nutrient of milk, it modifies milk constituent enhancing their nutritional values. Yoghurt is produced from the milk of cows, buffalos, goat, sheep and other mammals. In industrial production of yoghurt, cow milk is the predominant starting material. The viscosity and texture characteristic of yoghurt is related to its moisture content and protein level, protein fractions and their ratios plays a significant role in gel formation and their strength. Milk protein consists of casein and whey protein, which has distinct functional properties. Type of milk to be used depends on the variety or type of the yogurt that would be prepared. For instance, whole milk is used for full fat/regular yoghurt, partially skimmed milk is used for low fat yoghurt and skimmed milk is used for nonfat yogurt. Cream/butter fat is used to adjust the fat content whereas skim milk powder, whey protein concentrate are used to elevate the total solid content (SNF) of the yoghurt mix. According to the Codex Alimentarius Commission, yoghurt should have a minimum protein content of 2.7% and a maximum fat content of 15%. In order to achieve this, the FAO/WHO standard specifies that milk should be standardized with the minimum of SNF and milk fat content of 8.2% and 3% respectively for yoghurt manufacture, according to Chandan, *et al* (2008). Addition of different herbals can improve dairy product consumption, because using various herbs in dairy product production could give different choices to the consumers. Herbal extracts or powder could be used in dairy products, pharmaceuticals, ready-to-drink mixes, dietary foods, confectionery, spices mixes among others. So, fortification of herbs within dairy products may furnish worth addition as, functional dairy product, as reported by Al-Soud*et al* (2020). However since herbs and spices are antimicrobials, these might affect probiotic viability. In-vitro studies that tested herbs and spices on the growth of selected probiotics

showed that herbs and spices significantly enhanced the growth of probiotics while inhibiting pathogens. Herbs do make contributions significantly in health and human nutrition because these include almost all essential human nutrients. However, there are some underutilized indigenous herbs that could be used in place of these exotic fruits and plants. For instance; utazi (*Gongronemalatifolium*) and uziza (*Piper guineense*) could be used in flavouring yoghurt and as natural preservatives and for medicinal purposes, which could be called a herbal yoghurt. Phenolic compounds of herbs and spices are good substitutes for the artificial antimicrobial agents used in food manufacturing. Phenolic compounds such as tea catechins, oleuropein, ferulic acid, ellagic acid and coumaric acid have been found to prevent the growth of some pathogenic bacteria (*Staphylococcus aureus, Salmonella enteritidis and Listeria monocytogenes*) and fungi. Functional applications of herbs are in several forms (that is powder, fresh, extract, essential oils, among others). The health benefits of yoghurts are well-known and several yoghurt based products are consumed by people all over the world as stated by El-Sayed *et al.*(2019). Addition of utazi (*Gongronemalatifolium*) and uziza (*Piper guineense*) will increase the health benefits of yoghurt. Fortification of dairy products with herbs need some requirements based on the following properties; provides natural anti-oxidant, anti-microbial and medicinal properties improve sensory qualities and performs as bio preservative.

2. Materials and methods

Procurement of raw materials

The variety of utazi and uziza leave, whole milk, sugar and stabilizer (CMC) were purchased from Ogige main market in Nsukka local Government area of Enugu state, Nigeria. The starter culture was purchased from department of food science and technology.

Sample preparation

Preparation of Utazi (*Gongronemalatifolium*) extract

The utazi leaves were destalked and sorted to remove discolored and diseased ones, washed using clean water and then weighed out 50g using a digital kitchen scale. Fifty gram of leaves were blended with 200ml of water and the juices were extracted using a muslin cloth.The processing step is illustrated in

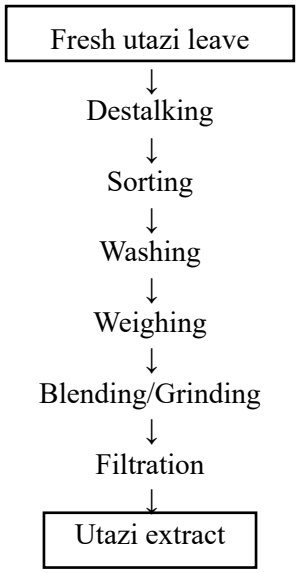


Figure 1: Flow chart diagram on processing of utazi leave to extract

Preparation of Uziza (*Piper guineense*) extract

The uziza leaves were destalked and sorted to remove discolored and diseased ones, washed using clean water and then weighed out 50g using a digital kitchen scale. Fifty gram of leaves were blended with 200ml of water and the juices were extracted using a muslin cloth. The processing step is illustrated in

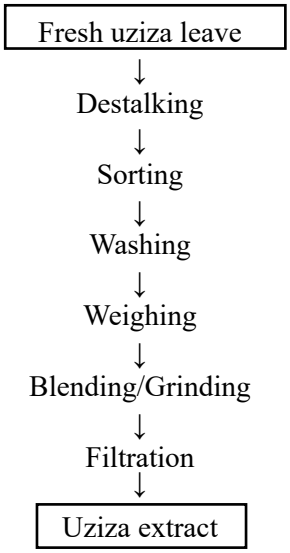
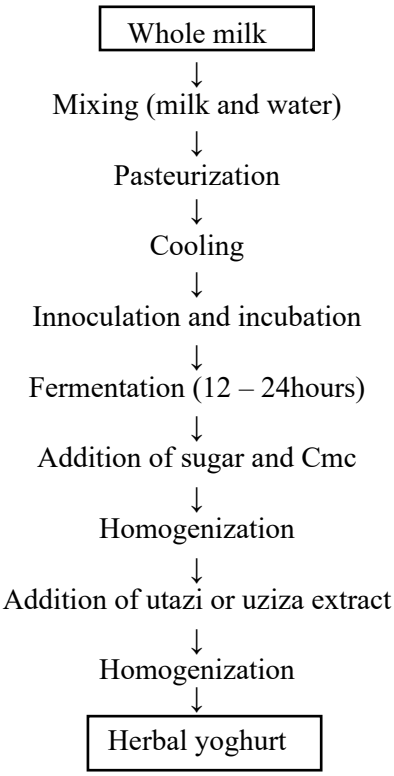


Figure 2: Flow chart diagram on processing of uziza leave to extract

Production of herbal yoghurt

The process of yogurt making is comprised of; weigh the quantity of water and milk needed, mix the milk and water thoroughly, pasteurize the milk liquor using the double steaming method for 30 minutes, allow to cool, inoculate the starter culture and incubate to ferment for 12 to 24 hours, weigh and add the quantity of sugar, herbal extract, and stabilizer, homogenize to get an even texture, package and store. The production steps in manufacture of yoghurt are shown in Figure 3



Packaging and cooling

Figure 3: Flow chart diagram on the production of herbal yoghurt

Table 1: Formulation ratio of herbal yoghurt

Sample code	Yoghurt proportion to extract (ml)	Sample code	Yoghurt proportion to powder (g)
PY	100:0	YUP1	99:1
YUE1	96:4	YUP2	98:2
YUE2	92:8	YUP3	97:3
YUE3	88:12	YUP4	96:4
YUE4	84:16	YUP5	95:5
YUE5	80:20	YUZP1	99:1
YUZE1	96:4	YUZP2	98:2
YUZE2	92:8	YUZP3	97:3
YUZE3	88:12	YUZP4	96:4
YUZE4	84:16	YUZP5	95:5
YUZE5	80:20		

Key: PY= plain yoghurt; YUE1, 2,3,4,5 = yoghurt with utazi extract; YUZE 1, 2,3,4,5 = yoghurt with uziza extract; YUP 1, 2,3,4,5, = yoghurt with utazi powder; YUZP 1, 2,3,4,5, = yoghurt with uziza powder.

Proximate analysis of the formulated yoghurt flavored with Utazi and Uziza extract

Determination of moisture content in the formulated herbal yoghurt:

The moisture content of the samples was determined using the hot oven method of AOAC (2010). Two (2 ml) of each sample was put into a washed and dried crucible dish and placed in a Phoenix oven at a temperature of 70-80°C for 2 hours and at 100-105°C until the weight is constant. The samples were cooled in a desiccator and weighed. The weight loss was obtained as the moisture content and was calculated as:

$$\% \text{ of moisture content} = \frac{W_2 - W_{3X}}{W_2 - W_1} 100 \quad (1)$$

Where; W_1 = initial weight of empty crucible; W_2 = weight of crucible + sample before drying; W_3 = final weight of crucible + sample after drying

Determination of crude protein content of the formulated herbal yoghurt

The crude protein of the samples was determined by the semi-micro Kjeldahl technique described by AOAC (2010). Then, 0.1millilitre of the sample was put into a Kjeldahl flask. Three grams (3g) anhydrous sodium sulphate and one (1g) of hydrated copper sulphate (catalyst) were added into the flask. Then, 20 ml of concentrated tetraoxosulphate (IV) acid (H_2SO_4) was added to digest the sample. The digestion continued under heat until a solution was observed. The clear solution was then cooled and made up to 100 ml with distilled water and a digest of about 5 ml was collected for distillation. Then, 5 ml of 60% sodium hydroxide (NaOH) was put into the distillation flask and distillation was allowed to take place for some minutes. The ammonia distilled off was absorbed by boric acid indicator and this was titrated with 0.01M hydrochloric acid (HCl). The titer value of the end point at which the colour changed from green to pink was taken. The crude protein was calculated as:

$$\% \text{ of crude protein} = \frac{0.0001401 \times T \times 100 \times 6.25}{W \times 5} \quad (2)$$

Where; T= titer value; W= weight of sample dried; 6.25= conversion factor.

Determination of crude fiber content of the formulated herbal yoghurt:

Crude fiber was dome using the method of Kirk and Sawyer (1991). Two milliliters (2ml) of the sample was hydrolyzed in beaker with 299ml of 1.25% sulphuric acid (H_2SO_4) and boiled for 30 minutes. The mixture was filtered, washed with hot distilled water and boiled again for 30 minutes with 200 ml of 1.25% of NaOH. The digested sample was also washed with 1% HCl acid to neutralize the NaOH and then with hot distilled water for several times. The residue was put into weighed crucible and dried at 100 °C for 2 hours in an air oven, after drying the sample was cooled, weighed and then transferred into a muffle furnace for burning at 500°C for 5 hours. The loss in weight was taken and percentage crude fiber was calculated as follow

$$\% \text{ crude fiber} = \frac{\text{loss in weight after ignition}}{\text{Weight of original sample}} \times 100 \quad (3)$$

Determination of crude fat content of the formulated herbal yoghurt

The solvent extraction method as described by AOAC (2010) was used. The extraction flasks were washed with petroleum ether, dried and cooled and weighed. Five milliliters (2 ml) of the sample were weighed into the extraction thimble. It was then placed back in the Soxhlet apparatus. The washed flask was filled to about three quarter of its volume with petroleum ether (that has the boiling temperature range of 40-60^{0}C). The apparatus was then set-up and extraction carried out for a period of 4 - 6 hours after which complete extraction was made. The petroleum ether was recovered leaving only oil in the flask at the end of the extraction. The oil in the extraction flask was dried in the oven, cooled and finally weighed. The fat content was expressed as a percentage of raw materials. The difference in weight of empty flasks and the flask with oil content which was calculated as:

$$\% \text{ of fat content} = \frac{C - B}{A} \times 100 \quad (4)$$

Where; A = Weight of sample; B = Weight of empty flask; C = Weight of flask + Oil.

Determination of ash content of the formulated herbal yoghurt

The ash content of the sample was determined by the method recommended by AOAC (2010). A silica dish was heated to about 60 ^{0}C, cooled in a desiccator and weighed. Five milliliters (5 ml) of the sample was put into the silica dish and transferred to the furnace. The temperature of the furnace was then allowed to reach about 525 ^{0}C after placing the dish in it. The temperature was maintained until whitish-grey colour was obtained indicating that all the organic matter content of the sample has been destroyed. The dish was then brought out from the furnace and cooled in the desiccator and re-weighed. The percentage ash content was the calculated as:

$$\% \text{ of ash content} = \frac{C - A}{B - A} \times 100 \quad (5)$$

Where: A = weight of empty dish; B = weight of empty dish + sample before ashing; C = weight dish + ash.

Determination of carbohydrate of the formulated herbal yoghurt

Carbohydrate was determined as the nitrogen free extraction calculated by difference as described by the method recommended by AOAC (2010).The formula below was used:

$$\% \text{ of carbohydrate} = 100\% - \% \text{ (protein + fat + fiber + ash + ash)} \quad (6)$$

Micronutrient analysis of the formulated herbal yoghurt

Determination of vitamin a content of the formulated herbal yoghurt

Vitamin A content was determined by the method recommended by AOAC (2010). Five milliliters (5 ml) sample was first saponified using an alcoholic solution of potassium hydroxide in the presence of pyrogallol. This freed the vitamins from the food matrix and converted any retinyl ester to retinol. The unsaponified matter containing vitamin A was extracted using a mixture of diethyl ether and petroleum spirit. The extract was evaporated under nitrogen and the residue was dissolved in methanol. The extract was chromatographed using a reverse phase octadecylsilane (ODS) column with the mobile phase consisting of 95% acetonitrile with 5% water. The separated retinol was then quantified using a UV absorbance detector at 328 nm.

Determination of vitamin a content of the formulated herbal yoghurt

The ascorbic acid was determined by the method recommended by AOAC (2010). Two millilitres (2ml) of the sample was weighed and 100 ml of distilled water was added to it. It was then filtered to get a clear solution. Also, 10 ml of the clear solution was pipette into small flask in which 2.5 ml acetone was added. It was then titrated with indophenols solution (2, 6-dicholorophenolindophenol) to a faint pink colour which persists for 15 seconds. The vitamin C content was calculated as:

Vitamin C (mg/ 100ml of sample) = 20 x V x C (7)

Where: V= indophenols solution in titration (ml); C= mg Vitamin C/ml indophenols.

Determination of calcium content of the formulated herbal yoghurt

It was determined by titration method according to Kirk and Sawyer (1991). Two milliliters (2 ml) of the ashed sample was diluted with 3 ml of distilled water and 1 ml of 50% ammonium oxalate. One drop of methyl red indicator was made alkaline with ammonia drops and drops of glacial acetic acid until colour changes to pink. It was stood for 4 hours and centrifuged for 5 minutes, followed by decantation of the supernatant. One milliliter (1 ml) of hydrogen sulphate was added to the residue which was diluted with 4 ml of distilled water. The solution was boiled and titrated with 0.02 N potassium permanganate.

Microbial analysis of the formulated herbal yoghurt

Determination of total viable count of the formulated herbal yoghurt

One Ringer tablet was dissolved in distilled water (500 ml). The clear solution formed was sterilized by autoclaving for 15 minutes at 121^0C and 15lb pressure. The Ringer solution was allowed to cool completely to a temperature of about 28 ± 2^0C. The total viable count test was carried out using the method recommended by AOAC (2010).Using of sample and sterilized quarter strength ringer solution as diluents, 1 ml of the sample and 9ml ringer solution was made serial dilutions (10^{-4}). The diluted sample was pipetted into a marked Petri dish, swirled to mix and incubated at the temperature of about 37°C for 24hours. After incubation, the number of colonies was counted and represented as colony forming unit per milliliter.

Determination of lactic acid bacteria (LAB) of the formulated herbal yoghurt:

The microbial count of lactic acid bacteria (LAB) in the formulated yoghurt was determined using deManRogosa Sharpe (MRS) agar (CM 361) according to Harrigan and McCance (1976). Samples were serially diluted in

duplicates using the surface pour plate method. The plates were incubated under anaerobic conditions at 37ºC for 48 hours.

Determination of pH of the formulated herbal yoghurt:

The pH was determined using a pH meter as described by AOAC (2010). The electrode was dipped into the yoghurt solution and then the pH was recorded.

Determination of total titrable acidity (TTA) of the formulated herbal yoghurt:

The total titrable acidity was determined by the method described by AOAC (2010). Ten milliliters (10 ml) of the sample was measured into a conical flask and about 3 drops of phenolphthalein indicator was added to the sample and titrated with 0.1 N sodium hydroxide (NaOH) until colour change was observed. The end point was taken and the TTA expressed as % lactic acid was given as:

$$\% \text{ of TTA as lactic acid} = \frac{N\ (NAOH) \times 0.09 \times 100}{\text{Volume of sample}} \quad (8)$$

Sensory evaluation of the formulated yoghurt flavored with Utazi and Uziza extract

Sensory parameters of the samples were evaluated by 20 semi-trained panelists from the department of food science and technology, University of Nigeria, Nsukka, who are conversant with yoghurt. The extent of differences between the yoghurt samples for each sensory quality was measured on a nine-point Hedonic scale, where “9” represents extremely like and “1” represents extremely dislike to access each sensory attributes such as appearance, taste, color, mouth feel, consistency, after taste, flavor, and overall acceptability

Experimental design and data analysis

The experimental design was laid out in Completely Randomized Design (CRD). Data obtained from the various analyses were subjected to Analysis of Variance (ANOVA) using Statistical Package for and Service Solution (SPSS).

3.Results and discussion

Pictorial diagram of herbal yoghurt, utazi and uziza extract.

Plate 1: YUE1

Plate 2: YUE2

Plate 3: YUZE1

Plate 4:YUZE3

Plate 5: Utazi aqueous extract

Plate 6:Uziza aqueous extract

Table 2. Proximate composition (%) of the formulated yoghurt flavored with utazi and uziza extract

Sample	Moisture		Protein		Fat		Fibre		Ash		Carbohydrate	
PY	62.48^{b}	±2.48	5.50^{a}	±0.03	0.18^{a}	±0.04	0.04^{b}	±0.04	0.02^{b}	±0.00	31.42^{a}	±2.55
YUE1	60.38^{b}	±0.58	3.53^{cd}	±0.05	0.15^{a}	±0.04	0.02^{bc}	±0.02	0.02^{bc}	±0.00	35.90^{a}	±0.17
YUE2	66.53^{a}	±0.38	4.30^{b}	±0.10	0.07^{a}	±0.05	0.01^{a}	±0.01	0.04^{a}	±0.00	29.05^{b}	±0.03
YUZE1	62.24^{b}	±0.01	3.43^{d}	±0.08	0.18^{a}	±0.03	0.03^{d}	±0.03	0.02^{d}	±0.00	34.12^{a}	±0.06
YUZE3	62.26^{b}	±0.04	3.75^{c}	±0.05	0.11^{a}	±0.00	0.01^{b}	±0.01	0.05^{b}	±0.00	33.88^{a}	±0.09

The moisture content of the formulated yoghurt ranged from 60.38 to 66.53% with sample YUE1 having the lowest moisture content and sample YUE2 having the highest moisture content. Sample PY is the control sample, sample YUE1 and YUE2 are from utazi extract. It was observed that the moisture content of sample YUE1 decreased but increased in sample YUE2 which shows that the higher the utazi extract the higher the moisture content. While sample YUZE1 and YUZE3 are from uziza extract and it was observed that the higher the uziza extract the slight increase in the moisture content.

The crude protein of the herbal yoghurt ranged from 3.43 to 5.50 % with sample YUZE1 having the lowest crude protein and sample PY having the highest crude protein. The control sample had the highest protein content. Sample YUE1 and YUE2 are from utazi extract and it was observed that the higher the extract the higher the protein content. Sample YUZE1 and YUZE3 are from uziza extract and it was observed that the higher the extract the higher the protein content.

The fat content of the herbal yoghurt varied between 0.07 to 0.18% with sample PY and YUZE1 having the highest fat content and sample YUE2 having the lowest fat content. It was observed that increase in the utazi or uziza extract in the yoghurt led to a decrease in the fat content.

The fiber content of the herbal yoghurt varied between 0.01 to 0.04% with sample YUE2 and YUZE3 having the least fiber content and the control sample PY the highest crude fiber. It was observed that the higher the herbal extract the lower the crude fibre content.

The ash content of the herbal yoghurt samples varied from 0.02 to 0.05% with sample YUZE3 having the highest ash content. It was observed that the herbal yoghurt sample YUE2 and YUZE3 had higher ash content than sample PY, YUE1 and YUZE1.

The carbohydrate content of the herbal yoghurt varied from 29.05 to 35.90% with sample YUE2 having the lowest carbohydrate and YUE1 having the highest carbohydrate. The variation in carbohydrate content could be due to the differences in the contents of other components such as protein, fat, fiber, and ash.

Micronutrient composition of the formulated herbal yoghurt using Utazi and Uziza extract

Table 3. Micronutrient composition of formulated herbal yoghurt

Sample	Vitamin C mg/100g		Vitamin B1 mg/100g		Vitamin A mg/100g	
Py	0.03^{d}	±0.00	0.06^{c}	±0.00	0.03^{c}	±0.00
Yue1	0.04^{b}	±0.00	0.05^{d}	±0.00	0.03^{d}	±0.00
Yue2	0.04^{a}	±0.00	0.08^{b}	±0.00	0.03^{c}	±0.00
Yuze1	0.03^{c}	±0.00	0.08^{a}	±0.00	0.04^{b}	±0.00
Yuze3	0.03^{b}	±0.00	0.06^{c}	±0.00	0.03^{c}	±0.00

Table 3 shows the micronutrient of herbal yoghurt formulated using utazi and uziza leaf extract.

The vitamin C content of the herbal yoghurt varied from 0.03 to 0.04 mg/100g. It was observed that vitamin C content increased in the sample containing utazi extract with sample YUE1 and YUE2.

The vitamin A content of the herbal yoghurt varied from 0.03 to 0.04 mg/100g with sample YUZE1 having the highest pro-vitamin A content. There were significant ($p < 0.05$) differences between the control (PY) and all the herbal yoghurt samples.

The vitamin B_1 content of herbal yoghurt varied from 0.05 to 0.08 mg/100g. It was observed that vitamin B_1 increased as the ratio of utazi extract increased in the yoghurt and decreased as the ratio of uziza extract increased. There was significant ($p < 0.05$) differences between the control (PY) and all the herbal yoghurt samples.

Phytochemical composition of the formulated herbal yoghurt using utazi and uziza leaf extract

Table 4 : Phytochemical composition of the formulated herbal yoghurt using utazi and uziza extract

Sample	Flavonoid mg/100g		Tannin mg/100g		Glycosides mg/100g	
Py	5.01^{a}	±0.01	0.02^{c}	±0.01	13.02^{d}	±0.05
Yue1	4.65^{c}	±0.01	0.17^{b}	±0.04	14.51^{c}	±0.02
Yue2	4.23^{d}	±0.02	0.11^{c}	±0.00	13.06^{d}	±0.01
Yuze1	4.76^{b}	±0.01	0.26^{a}	±0.03	18.48^{a}	±0.06
Yuze3	4.59^{c}	±0.03	0.24^{ab}	±0.01	16.51^{b}	±0.42

From Table 3, the flavonoid composition of herbal yoghurt varied from 5.01 to 4.23mg/100g with the control sample PY having the highest flavonoid content. There were significant ($p < 0.05$) differences between the control (PY) and all the herbal yoghurt samples. It was observed that the flavonoid content of the utazi extract sample (YUE1 and YUE2) decreased while the extract increased in the yoghurt; same was applied to uziza extract samples (YUZE1 and YUZE3). It was observed that herbal yoghurt samples containing uziza extract had the highest flavonoid composition.

The tannin composition of herbal yoghurt varied from 0.02 to 0.26mg/100g with the control sample PY having the lowest tannin content while sample YUZE3 had the highest tannin content. There were significant ($p < 0.05$) differences between the control (PY) and all the herbal yoghurt samples. It was observed that the tannin content of the utazi extract sample (YUE1 and YUE2) decreased with increase in the ratio of the extract to the yoghurt; same thing was applied to uziza extract samples (YUZE1 and YUZE3). It was observed that herbal

yoghurt samples containing uziza extract had higher tannin content. The glycosides content of herbal yoghurt varied from 13.02 to 18.48mg/100g with the control sample PY having the lowest glycosides content while sample YUZE1 had the highest glycosides content. There were significant ($p < 0.05$) differences between the control (PY) and all the herbal yoghurt samples. It was observed that the glycosides content of the utazi extract sample (YUE1 and YUE2) decreased while the extract increased; a similar trend was applied to uziza extract samples (YUZE1 and YUZE3). It was observed that herbal yoghurt samples containing uziza extract has higher glycosides content.

Physico-chemical composition of formulated herbal yoghurt from utazi and uziza leaf extract

Table 5: Physico-chemical composition of the formulated herbal yoghurt using utazi and uziza extract

Sample	pH		TTA	
Py	4.70[a]	±0.20	0.02[a]	±0.01
Yue1	3.95[abc]	±0.05	0.02[a]	±0.00
Yue2	3.20c	±0.40	0.02[a]	±0.00
Yuze1	3.80[bc]	±0.10	0.03[a]	±0.00
Yuze3	4.05[ab]	±0.05	0.03[a]	±0.00

The pH of the herbal yoghurt varied from 3.20 to 4.70 with the control sample PY having the highest pH and sample YUE2 having the lowest pH. There were significant ($p < 0.05$) differences between the control (PY) and all the herbal yoghurt samples. It was observed in yoghurt sample containing utazi extract (YUE1 and YUE2) that the higher the extract the lower the pH while in yoghurt sample containing uziza extract (YUZE1 and YUZE3) the higher the extract the higher the pH.

The titrable acidity of the herbal yoghurt varied from 0.02 to 0.03 with the yoghurt sample contain uziza extract (YUZE1 and YUZE3), having the highest titrable acidity. There was no significant ($p>0.05$) difference between the control yoghurt sample PY and samples containing utazi extract (YUE1 and YUE2) but significant ($p < 0.05$) difference existed between the control (PY) and the herbal yoghurt samples containing uziza extract (YUZE1 and YUZE3).

Microbial count of formulated herbal yoghurt from utazi and uziza leaf extract

Table 6: Microbial count of the formulated herbal yoghurt flavoured with utazi and uziza extract

Sample code	**TVC**	**LAB**	**Coliform count**	**Mould Count**
PY	5.50×10^2	4.50×10^2	ND	ND
YUE1	9.40×10^2	6.40×10^2	ND	ND
YUE2	1.34×10^3	9.20×10^2	1.0 x 10	ND
YUZE1	1.86×10^3	1.12×10^3	ND	ND
YUZE3	2.42×10^3	1.43×10^3	ND	ND

Values are means of duplicate determinations. ND= not detected

The total viable count (TVC) of the formulated herbal yoghurt varied from 5.50 x 10^2 to 2.42 x 10^3 cfu/ml. The result shows that sample PY (plain yoghurt) which served as the control had the lowest total viable count of 5.5x10^2cfu/ml while sample YUZE3 had the highest total viable count of 2.42 x 10^3cfu/ml.

The lactic acid bacteria of the formulated herbal yoghurt varied from 4.50 x 10^2 to 1.43 x 10^3. It was observed from the result that the most viable microorganism present in the herbal yoghurt is the lactic acid bacteria. It was also observed that LAB is more in the herbal yoghurt but more dominant in the herbal yoghurt containing uziza extract (YUZE1 and YUZE3). These could be due to the anti microbial and preservative effect of the herb extract.

The coliform count present in the formulated herbal yoghurt was less than 1.0 x 10cfu/ml. There were no coliform detected in the herbal yoghurt except sample YUE2 which could be as a result of contamination from the environment during processing or through the packaging material. Studies have shown that the contaminated sample was within the acceptable range of coliform count for dairy product.

There was no mould count detected in the formulated herbal yoghurt making the yoghurt safe for consumption and longer shelf stability.

Sensory scores of the formulated yoghurt flavored with Utazi and Uziza extract

The sensory scores for appearance of herbal yoghurt varied from 7.95 to 3.15. The highest score was recorded in sample PY (plain yoghurt) while the lowest score was recorded in sample YUP3.The herbal yoghurt samples with plant extract was more appetizing than the yoghurt sample containing powdered herb. The sensory scores for colour of herbal yoghurt varied from 7.85 to 2.45. The highest score was recorded in sample PY (plain yoghurt) while the lowest score was recorded in sample YUP5.The herbal yoghurt samples with extract had a light green colour and this could be attributed to the chlorophyll present in the plant extract while the yoghurt samples with powdered herbs had a dark green colour. The sensory scores for flavor of herbal yoghurt varied from7.75to2.30. The highest was recorded in sample PY (plain yoghurt) while the lowest score was record. ed in sample YUP5. The flavor of the herbal yoghurt became pronounced as the herbal extract or powder increased in the yoghurt.

The sensory scores for taste of herbal yoghurt varied from 7.65 to 1.90. The highest score was recorded in sample YUE1 (yoghurt with 4ml of utazi extract) while the lowest score was recorded in sample YUP2. The addition of the plant powder increased the bitter taste of the yoghurt due to certain concentration of aromatic compounds. The addition of plant extract makes the yoghurt to have a slight bitter taste, therefore the higher plant extract or powder the increase in the bitter taste of the yoghurt. The sensory scores for consistency of herbal yoghurt varied from 7.45 to 3.20. The highest score was recorded in sample PY (plain yoghurt) while the lowest score was recorded in sample YUP4.The herbal yoghurt samples with herbal extract had as light watery consistency compared to the plain yoghurt. This can be attributed to the increased moisture content of the plant extract while the yoghurt sample with herbal powder had a slight thick consistent. The sensory scores for aftertaste of herbal yoghurt varied from 7.60 to 2.25. The highest score was recorded in sample PY (plain yoghurt) while the lowest score was recorded in sample YUP5. The yoghurt sample with herbal powder has an intense bitter aftertaste which is the panelists found undesirable. The yoghurt sample with the herbal extract was more preferred due to the bitter after taste was in tolerant level.

The sensory scores for aroma of herbal yoghurt varied from 7.75 to 2.95. The highest score was recorded in sample PY (plain yoghurt) while the lowest score was recorded in sample YUP5.

The sensory scores for overall acceptability of herbal yoghurt varied from 8.00 to 2.10. The highest score was recorded in sample PY (plain yoghurt) while the lowest score was recorded in sample YUP5. The panelists were not accustomed to the yoghurt containing herbal extract thus rated the plain yoghurt higher than the herbal yoghurts in terms of overall acceptability. However, the herbal yoghurt sample YUE1 and YUZE1 were the most accepted followed by YUE2 and YUZE3 which formed the basis of carrying out further analysis to determine its proximate, physicochemical and nutritional composition.

4. Conclusion

Apart from the basic use of utazi and uziza leaves in locally processed foods, these are consumed mostly as vegetable or spices. Both have been greatly under-utilized in the food industry. Therefore, its incorporation in yoghurt would create added value to the herbs and it would improve therapeutic or health benefit of the product. Furthermore, the inexpensive and available nature of the herbs would go a long way in reducing cost of production of yoghurt. Also it would help increase availability in varieties of yoghurt. Phenolic compounds of herbs and spices are good substitutes for the artificial antimicrobial agents used in food manufacturing; therefore herbal extract could be used as a preservative agent in yoghurt. Extracts of utazi leaf and uziza leaf in herbal yoghurt production does not only improves the nutritional and health benefits of the consumers but also commercially acceptable.

Recommendation

It is recommended that further research should be carried out on the preservative effect of these herbs in yoghurt. Also more research should be done on extraction yield of utazi and uziza leaf and why utazi leaf had more yield than uziza leaf.

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Effect of Addition of Diced African Bush Mango (*Irvingia gabonensis*) Pulp in Formulated Spoonable Yoghurt

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Abstract

Introduction and Aim: Yoghurt is a semi-solid fermented milk product that has been given different names and forms. To modify certain properties of yoghurt, it can be fortified with milk powder, proteins, vitamins, minerals or fruits. This study investigated the production and evaluation of spoonable yoghurt flavored with diced African bush mango (*Irvingia gabonensis*) pulp.

Method: Yoghurt was produced and flavoured with diced African bush mango pulp by substituting 0, 5, 10 and 15% of yoghurt before and after fermentation. The samples before fermentation were coded as SYFI (95:5), SYFI (90:10) and SYFI (85:15) while samples after fermentation were coded as SYFP (95:5), SYFP (90:10) and SYFP (85:15). Sample SYF (100:0) served as the control. The proximate, micronutrient, physicochemical, sensory and microbiological properties of the yoghurts were determined using standard procedures.

Results: From the study, the vitamin C content ranged from 18.00 to 28.60 mg/100mg and calcium content ranged from 20.00 to 34.00 mg/100mg. The vitamin A and phosphorus decreased with increased level of diced African bush mango pulp in the yoghurt. The flavoured yoghurt had pH (4.90 – 5.20), total titratable acidity (0.36 – 0.44) and viscosity (1.93 – 3.03mpas). The total viable count and lactic acid bacteria ranged from 1.4×10^4 to 2.8×10^4cfu/ml and 1.2×10^4 to 1.8×10^4cfu/ml, respectively. Sample SYF (100:0) had the highest mean for all sensory attributes with an overall acceptability of 7.30.

Conclusion: The use of African bush mango improved the nutritional and sensory properties of the developed yoghurt.

Keywords: Spoonable yoghurt, African bush mango, sensory properties

1. Introduction

Yoghurt is a semi-solid fermented milk product that has been given different names and forms (Tamine and robbinson,2007). Yoghurt is a food staple that can be enjoyed in many different ways. It is a fermented coagulated milk product that is obtained through an anaerobic fermentation of lactose in milk by relevant microorganisms which are classified as probiotic. Fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give yoghurt its texture and characteristic tang (Xue et al., 2016). There are numerous advantages of consuming fermented dairy products containing probiotic bacteria. A high population of probiotic organisms in the colon contributes to good intestinal health. Consequently, consumption of products such as yoghurt containing viable probiotic organisms adds benefit to human gut health. Moreover, yoghurt supplies good quality proteins, also an excellent source of calcium, phosphorus, potassium and contains vitamins A, D B_2 and B_{12} (Drewnowski, 2018).

However, milk, the major ingredient in yoghurt production, is manufactured by a number of animals, although in terms of commercial quantity milk from cow is the most popular.

To modify certain properties of yoghurt, it can be fortified with milk powder, proteins, vitamins, minerals or fruits. Ingredients like fruits are added to increase the nutritional properties. Presently, during the production of yoghurt only exotic fruits such are raspberry, banana, peach, vanilla, and strawberry are commercially available. However, there are some underutilized indigenous fruits that can be used in place of these exotic ones for instance; African bush mango "Ugiri" (*Irvingia gabonensis*). *Irvingia gabonensis* belongs to the family Irvingiaceae. The fruit pulp is juicy and the taste varies between sweet and bitter (Etebu, 2012). The fruit is rich in vitamin C, minerals and phytochemicals such as flavonoids, alkaloids and tannins[5]. The tasty mesocarp is widely consumed raw as dessert fruit or snack throughout Western and Central Africa for its health promoting potential (Manach et al., 2004). The health enhancing potential of flavonoids contributes to the health of locals in the villages across Nigeria who regularly eats fresh Irvingia fruits as snack(Ngondi et al., 2005; Ogbonna et al., 2013). Although the pulp is consumed to a considerable extent among the locals in the rural areas, large quantities are usually wasted. This is as a result of inadequate facilities for processing, poor storage facilities and preservation capacity, and poor road network.

Therefore, the production of yoghurt flavoured with diced African bush mango would help preserve the fruit and reduce post-harvest losses and create a stable market for farmers thereby improving the economy of the country. Also, the study has shown that the use of diced African bush mango as a flavourant would improve the organoleptic properties and health benefits of the yoghurt. The broad main aim of the study is to produce and evaluate the quality characteristics of spoonable yoghurt flavoured with diced African bush mango pulp.

2. Materials and methods

Study area: the project was carried out in the department of food science and technology, university of Nigeria, in the month of July- October, 2020

Procurement of raw materials

The sweet variety of African bush mango, "ugiri" (*Irvingia gabonensis*), skimmed milk, sugar and stabilizer (CMC) were purchased from Ogige main market in Nsukka local Government area of Enugu State, Nigeria. The starter culture (CHR HANSEN) was purchased from Onitsha main market, Anambra State.

Sample preparation

Processing of African bush mango pulp

African bush mango was processed by the modified method of Akubor (2017). The Fresh ripe African bush mango fruits were sorted to remove the bad ones, washed, peeled, sliced (1.0 mm) and diced (1.0 mm) using a knife and transparent meter rule to obtain uniform dices.

Production of yoghurt

The yoghurt was produced by the modified method described by Lee and Lucey (2010). Diced African bush mango flavoured spoonable yoghurt was produced. The raw materials (skimmed milk, sugar and stabilizer) were approximately mixed with portable water. The mixed product was then homogenized to obtain a creamy and uniform product. Pasteurization was then carried out at 85^0C for 30 minutes to destroy the undesirable microorganism (pathogenic and spoilage microorganisms) in the raw materials (skimmed milk, sugar and stabilizer) to provide a favourable environment free from competition for the growth of the starter culture. The yoghurt mix was then cooled to a temperature of $42 \pm 2\ ^0$C which is the ideal growth temperature of the starter culture. The starter was inoculated and fermentation was carried out for 12 hours after which the yoghurt was set. The diced African bush mango pulp was added before and after fermentation at different blends. Table 1 shows the proportion for the processing of spoonable yoghurt flavoured using diced African bush mango pulp.

Table 1. Proportion to produce spoonable yoghurt flavoured with diced African bush mango pulp

Sample	Yoghurt (ml)	African Bush mango Pulp
SYF (100:0)	100	0
SYFB (95:5)	95	5
SYFB (90:10)	90	10
SYFB (85:15)	85	15
SYF (100:0)	100	0
SYFA (95:5)	95	5
SYFA (90:10)	90	10
SYFP (85:15)	85	15

Key: SYF = Control; SYFB = Spoonable yoghurt flavored before fermentation; SYFA = Spoonable yoghurt flavored after fermentation

Experimental Analysis

Determination of proximate composition

Moisture content, crude fibre, ash, protein, fat were determined using AOAC (2010) method while carbohydrate content was determined by difference.

Micronutrient analysis of the formulated spoonable yoghurt flavoured with diced African bush mango pulp

Determination of vitamin A content

Vitamin A content was determined according to standard methods of AOAC (2005) procedure and calculated

using the equation:

$$\text{vitamin A} = \frac{\text{absorbance test} \times \text{dilution factor}}{\text{slope (from standard curve)}}$$

Determination of vitamin C content

The ascorbic acid was determined using the method described by Onwuka (2005). The vitamin C content was calculated as:

Vitamin C (mg/ 100ml of sample) = 20 x V x C

Where: V= indophenols solution in titration (ml); C= mg Vitamin C/ml indophenols

Determination of phosphorus content

Phosphorus content in the sample was determined according to Onwuka (2018) by molybdate method using hydroquinone as a reducing agent and calculated using the equation

$$\text{phosphorus} = \frac{\text{absorbance test} \times \text{dilution factor}}{W \times 5}$$

Determination of calcium content

The calcium content was determined following the methods described by the AOAC (2010)

Microbial analysis of the formulated spoonable yoghurt flavoured with diced African bush mango pulp

Determination of lactic acid bacteria (LAB)

The lactic acid bacteria (LAB) in the formulated yoghurt were determined using deMan Rogosa Sharpe (MRS) Agar (CM 361). After incubation, the number of colonies were counted using the colony counter and represented as colony forming unit per millilitre (cfu/ ml).

Cfu/ ml = average count × dilution factor (D.F)

Determination of total viable count

The total viable count was determined by the method of Pour Plate Count described by Prescott *et al* (2005). The method involved weighing the sample (1 g) into a sterile test tube. A $^{1}/_{4}$ strength Ringers solution (9 ml) was poured into it and also into other test tube arranged for serial dilution. The sample with the solution was homogenized by shaking. The sample with solution was pipetted (1 ml) into test tube containing Ringers solution (9 ml). Then, 1 ml of different dilution factor was transferred into the sterile petri dishes and sterile nutrient agar was poured into the same petri dish and was mixed by rocking. When they solidified, they were turned upside down and cultured by incubation for 24 h at temperature of 37 °C. At the end of the incubation period, the colonies were counted using the colony counter (Gallenkamp colony counter, CWN 330- 010X) and the number of colonies recorded appropriately.

Determination of mould count

The mould count was determined using Sabouraud Dextrose Agar (SDA) as the plating medium. The sample (1 g) was weighed and put in a test tube prepared for serial dilution. The ringer solution (9 ml) was aseptically transferred serially into other test tubes. Serial dilution of 10^{-1} was used for mould count determination. Appropriate diluent (1 ml) was transferred into the sterile petri dishes. Sabouraud Dextrose Agar was used for culturing the organism for 48 hours at room temperature. The mould colonies were enumerated and calculated as colony forming units (cfu)/g of the sample.

Cfu/g = Number of colonies × reciprocal of dilution factor

Physico-chemical analysis of the formulated spoonable yoghurt flavoured with diced African bush mango pulp

Determination of total titrable acidity

The titrable acidity was determined by the method described by AOAC (2010) and calculated using the equation

$$\%\ \text{titratable acidity} = \frac{\text{M (NaOH)} \times N\ \text{(NaOH)} \times 0.09 \times 100}{\text{volume of sample}}$$

Determination of pH

A standard pH meter (model 20 pH conductivity meter, Denver Instrument, United Nations Inventory Database) was standardized using buffer solutions of pH 4.0 and 9.0. The pH electrode was dipped into the yoghurt and after a few minutes of equilibration, the pH of the yoghurt sample was taken (AOAC, 2005).

Determination of apparent viscosity

Sample viscosity was determined by using a ferrantic portable viscometer according to AOAC (2010). The apparent viscosity was calculated in centipoises using the formula below:

$$\text{apparent viscosity (cP)} = \frac{n2 \times e1 \times t1}{e2 \times t2}$$

Sensory evaluation

The flavored yoghurt was assessed by a 20- man semi- trained panelist selected randomly from among students of the Department of Food Science and Technology, University of Nigeria, Nsukka. The samples were evaluated for colour, appearance, mouthfeel, aroma, taste, texture and general acceptability on a 9 - point Hedonic scale as described by (Sukanya and Michael,2014). The samples were presented in coded plates. The order of presentation of samples was randomized Water was served to the panelist to rinse their mouths in-between sample evaluation.

Data analysis and Experimental design

The experimental design that was used is Completely Randomized Design and the mean values were subjected to analysis of variance (ANOVA) using Duncan's Multiple Range Test (DMRT) and SPSS (Statistical Product and Service Solution) version 20 computer was used. Significance was accepted at $p < 0.05$.

3. Results

Proximate composition (%) of the formulated spoonable yoghurt flavoured with diced African bush mango pulp

Table 2 shows the proximate composition (%) of the formulated spoonable yoghurt flavoured with diced African bush mango pulp. The moisture content of the flavoured yoghurt samples ranged from 77.59 □ 79.53 %. There was significant ($p < 0.05$) difference in the moisture content of the flavouredyoghurt. The control had the lowest moisture content while sample SYFB (85:15) yoghurt flavoured before fermentation had the highest moisture content. This showed that the moisture levels increased with increase in the concentration of diced African bush mango pulp. This could be because of the high moisture content of African bush mango pulp. The higher moisture content observed in yoghurt flavoured before fermentation is attributed to the reduction of water holding capacity of the milk by the pulp before fermentation.

The crude protein content of the flavoured yoghurt ranged from 3.03 – 3.66% (Table 2). Sample SYF (100:0) had the highest crude protein content while sample SYFB (85:15) yoghurt flavoured before fermentation had the lowest crude protein content. The protein content of the flavoured yoghurt decreased with increase in the level of diced African bush mango pulp. The decrease could be attributed to the lower protein content of African bush mango pulp compared to milk.

The ash content of the flavoured yoghurt samples ranged from 1.27 – 2.00 % (Table 2). There were significant ($p < 0.05$) differences in the ash content of the flavoured yoghurt. Sample SYF (100:0) had the highest ash content while sample SYFA (90:10) yoghurt flavoured after fermentation had the lowest ash content. The ash content of the samples was observed to decrease with increasing level of diced African bush mango pulp added. This could probably be as a result of the decrease in the volume of milk due to the high concentration of African bush mango pulp. This could also be because milk is highly rich in minerals some of which are not found in African bush mango pulp. The ash of the flavoured yoghurt obtained in this study was higher than the value 0.18 – 0.30 % of yoghurt flavoured with African bush mango juice and pulp as reported by Mbaeyi-Nwaoha *et al* (2017b).

The crude fat content of the flavoured yoghurt samples ranged from 3.37 – 4.61% (Table 2). There were significant ($p < 0.05$) differences between all the samples. Sample SYF (100:0) had the highest crude fat content while sample SYFB (85:15) yoghurt flavoured before fermentation had the lowest crude protein content. This showed that the crude fat levels decreased with increase in the concentration of diced African bush mango pulp. Generally, African bush mango pulp contains low level of fat therefore addition of the pulp might have decreased the percent of flavoured yoghurt.

Table 2 shows the crude fiber content of the flavoured yoghurt samples which ranged from 1.55 to 2.21 %. There were significant ($p < 0.05$) differences in the crude fiber content of the flavoured yoghurt. Sample SYF (100:0) had the highest crude fiber content (2.21) while sample SYFA (90:10) yoghurt flavoured after fermentation had the lowest crude fiber content (1.55). The crude fiber content of the samples was observed to decrease with increasing level of diced African bush mango pulp added. This

could be as a result of low fiber content of the African bush mango pulp and also a result of the pulp not fully disintegrated into the yoghurt samples.

The carbohydrate content of the flavoured yoghurt samples ranged from 9.94 – 11.25 % (Table 2). The control had the lowest carbohydrate content while sample SYFA (85:15) yoghurt flavoured after fermentation had the highest carbohydrate content. The carbohydrate content increased with increase in concentration of diced African bush mango pulp in the flavoured yoghurt. This could be as a result of high carbohydrate content of African bush mango pulp 15.7g in 100g.

Table 2: Proximate composition (%) of the formulated spoonable yoghurt flavoured with diced African bush mango pulp

Sample	Moisture	Protein	Ash	Fat	Fibre	CHO
SYFB (95:5)	$78.51^{bc} \pm 0.04$	$3.44^{b} \pm 0.03$	$1.81^{b} \pm 0.01$	$4.04^{d} \pm 0.06$	$1.88^{b} \pm 0.03$	$10.33^{ab} \pm 0.10$
SYFB (90:10)	$79.44^{d} \pm 0.01$	$3.28^{c} \pm 0.02$	$1.53^{c} \pm 0.01$	$4.01^{d} \pm 0.06$	$1.80^{bc} \pm 0.28$	$9.95^{a} \pm 0.21$
SYFB (85:15)	$79.53^{d} \pm 0.35$	$3.03^{d} \pm 0.04$	$1.73^{b} \pm 0.01$	$3.37^{f} \pm 0.03$	$1.83^{bc} \pm 0.09$	$10.52^{b} \pm 0.24$
SYF (100:0)	$77.59^{a} \pm 0.06$	$3.66^{a} \pm 0.04$	$2.00^{a} \pm 0.01$	$4.61^{a} \pm 0.03$	$2.21^{a} \pm 0.03$	$9.94^{a} \pm 0.08$
SYFA (95:5)	$78.21^{b} \pm 0.06$	$3.45^{b} \pm 0.08$	$1.40^{cd} \pm 0.01$	$4.17^{c} \pm 0.04$	$1.61^{bc} \pm 0.02$	$11.17^{c} \pm 0.06$
SYFA (90:10)	$78.61^{c} \pm 0.06$	$3.55^{ab} \pm 0.06$	$1.27^{d} \pm 0.02$	$3.78^{e} \pm 0.01$	$1.55^{c} \pm 0.21$	$11.25^{c} \pm 0.37$
SYFA (85:15)	$78.69^{c} \pm 0.06$	$3.20^{c} \pm 0.12$	$1.53^{c} \pm 0.20$	$4.35^{b} \pm 0.06$	$1.76^{bc} \pm 0.05$	$10.48^{ab} \pm 0.37$

Values are means ± standard deviation of duplicate readings. Means on the same column with different superscripts are significantly ($p < 0.05$) different.

Key: SYF = Control; SYFB = Spoonable yoghurt flavored before fermentation; SYFA = Spoonable yoghurt flavored after fermentation CHO= Carbohydrate

Micronutrient composition of the formulated spoonable yoghurt flavoured with diced African bush mango pulp

The composition of some selected vitamins and mineral of the formulated spoonable yoghurt flavoured with diced African bush mango pulp are shown in Table 3. The vitamin A content of the flavoured yoghurt samples ranged from 52.50 – 72.50 IU (Table 3). There was no significant ($p > 0.05$) difference in the vitamin A content of the control and sample SYFA (95:5). Sample SYFB (95:5) and SYFA (85:15) also showed the similar trend that there is no significant ($p > 0.05$) difference in the vitamin A content of the flavoured yoghurt samples. This is probably due to the decrease in milk content which is rich in vitamin A and also the low content of vitamin A in African bush mango pulp.

The vitamin C content of the flavoured yoghurt samples ranged from 18.00 – 28.60 mg/100mg (Table 3). The control had the lowest vitamin C content while sample SYFA (85:15) yoghurt flavoured after fermentation had the highest moisture content. This showed that the vitamin C content increased with increase in the concentration of diced African bush mango pulp. This could be because of the high vitamin C content of African bush mango pulp. The value (10.01 – 16.71 mg/100mg) of Vitamin C

(ascorbic acid) content of formulated yoghurt samples flavoured with black velvet tamarind reported by Mbaeyi-Nwaoha and Onwe (2019) was lower than values obtained in this study.

Table 3 shows the calcium content of the flavoured yoghurt samples ranged from 20.00 – 34.00 mg/100mg There was significant ($p < 0.05$) difference in the moisture content of the flavoured yoghurt. Sample SYFB (95:5) had the lowest calcium content while sample SYFA (90:10) yoghurt flavoured after fermentation had the highest calcium content. This showed that the calcium content increased with increase in the concentration of diced African bush mango pulp. This could be because of the calcium content of African bush mango pulp.

The phosphorus content of the flavoured yoghurt samples ranged from 198.50 – 235.00 mg/100mg (Table 3). There was no significant ($p > 0.05$) difference in the phosphorus content of the flavoured yoghurt samples SYFB (85:15) and SYFA (85:15). There was also no significant difference ($p > 0.05$) in the phosphorus content of the flavoured yoghurt samples SYFB (95:5), SYFB (90:10), SYFA (95:5) and SYFA (90:10) respectively. This showed that the phosphorus content decreased with increase in the concentration of diced African bush mango pulp. The value (153.03 – 201.53 mg/100mg) of phosphorus of formulated yoghurt samples stabilized with Achi and Ofor reported by Mbaeyi-Nwaoha *et al* (2017a) concurred with the values obtained in this study. The result range observed in this study was also in close agreement with the value (136.35 – 173.09 mg/100mg) reported by Mbaeyi-Nwaoha and Onwe (2019).

Table 3: Micronutrients composition of the formulated spoon able yoghurt flavoured with diced African bush mango pulp

Sample	Vitamin A (IU)	Vitamin C mg/100mg)	Calcium mg/100mg)	Phosphorus mg/100mg)
SYF(100:0)	$72.50^{a} \pm 0.00$	$18.00^{d} \pm 0.02$	$24.67^{e} \pm 0.00$	$235.00^{a} \pm 0.01$
SYFB (95:5)	$56.50^{bc} \pm 0.00$	$22.00^{c} \pm 0.01$	$20.00^{f} \pm 0.00$	$212.50^{ab} \pm 0.00$
SYFB (90:10)	$54.50^{c} \pm 0.01$	$25.00^{abc} \pm 0.01$	$26.00^{d} \pm 0.00$	$210.00^{ab} \pm 0.14$
SYFB (85:15)	$52.50^{c} \pm 0.00$	$27.00^{b} \pm 0.00$	$28.00^{c} \pm 0.00$	$198.50^{b} \pm 0.00$
SYF (100:0)	$72.50^{a} \pm 0.00$	$18.00^{d} \pm 0.02$	$24.67^{e} \pm 0.00$	$235.00^{a} \pm 0.01$
SYFA (95:5)	$69.50^{a} \pm 0.00$	$22.80^{bc} \pm 0.03$	$29.33^{b} \pm 0.00$	$221.50^{ab} \pm 0.00$
SYFA (90:10)	$66.50^{ab} \pm 0.00$	$26.00^{ab} \pm 0.04$	$34.00^{a} \pm 1.41$	$205.00^{ab} \pm 0.01$
SYFA (85:15)	$59.00^{bc} \pm 0.00$	$28.60^{a} \pm 0.01$	$23.67^{e} \pm 0.00$	$200.00^{b} \pm 0.03$

Values are means ± standard deviation of duplicate readings. Means on the same column with different superscripts are significantly ($p < 0.05$) different.

Key: SYF = Control; SYFB = Spoonable yoghurt flavoured before fermentation; SYFA = Spoonable yoghurt flavoured after fermentation

Physicochemical composition of the formulated spoonable yoghurt flavoured with diced African bush mango pulp

Table 4 shows some selected physicochemical of the formulated spoonable yoghurt flavoured with diced African bush mango pulp. Table 4 showed that the pH of the flavoured yoghurt samples ranged from 4.90 – 5.20. The pH of sample SYF (100:0) was significantly ($p < 0.05$) different from other yoghurt samples flavoured with African bush mango pulp. The pH value decreased with increase in the concentration of diced African bush mango pulp. This could be because of the African bush mango pulp is slightly acidic content of African bush mango pulp. The pH of the formulated samples observed in this study was higher than the normal pH of yoghurt which is between 4.0 – 4.5. This could probably be because of the period of fermentation. Higher period of fermentation would have resulted to higher acidity of the flavoured yoghurt samples.

The titratable acidity of the flavoured yoghurt samples ranged from 0.36 – 0.44 (Table 4). There was no significant ($p < 0.05$) difference in the titratable acidity of the flavoured yoghurt samples SYFB (95:5) and SYFB (85:15). There was also no significant ($p<0.05$) difference in the titratable acidity of the flavoured yoghurt samples SYFA (95:5), SYFA (90:10) and SYFA (85:15) respectively. The titratable acidity decreased with decrease in the pH values.

The viscosity of the flavoured yoghurt samples ranged from 1.93 – 3.03 mpas (Table 4). There was significant ($p < 0.05$) difference in the viscosity of the flavoured yoghurt. Sample SYF (100:0) had the highest viscosity while sample SYFB (85:15) yoghurt flavoured before fermentation had the lowest viscosity. This showed that the viscosity decreased with increase in the concentration of diced African bush mango pulp. The decrease in viscosity could be attributed to the fact that African bush mango pulp contains high moisture content.

Table 4: Physicochemical composition of the formulated spoonable yoghurt flavoured with diced African bush mango pulp

Sample	pH	Total Titratable Acidity	Viscosity (mpas)
SYF (100:0)	$5.20^{a} \pm 0.00$	$0.44^{a} \pm 0.00$	$3.03^{a} \pm 0.04$
SYFB (95:5)	$4.90^{d} \pm 0.00$	$0.36^{d} \pm 0.00$	$2.37^{de} \pm 0.10$
SYFB (90:10)	$4.95^{cd} \pm 0.00$	$0.38^{c} \pm 0.01$	$2.21^{e} \pm 0.08$
SYFB (85:15)	$4.90^{d} \pm 0.00$	$0.37^{d} \pm 0.01$	$1.93^{f} \pm 0.18$
SYF (100:0)	$5.20^{a} \pm 0.00$	$0.44^{a} \pm 0.00$	$3.03^{a} \pm 0.04$
SYFA (95:5)	$5.05^{bc} \pm 0.07$	$0.41^{b} \pm 0.01$	$2.51^{cd} \pm 0.02$
SYFA (90:10)	$5.05^{bc} \pm 0.07$	$0.41^{b} \pm 0.01$	$2.63^{bc} \pm 0.06$
SYFA (85:15)	$5.10^{ab} \pm 0.00$	$0.41^{b} \pm 0.00$	$2.83^{ab} \pm 0.18$

Values are mean ± standard deviation of duplicate readings. Means on the same column with different superscripts are significantly ($p <0.05$) different.

Microbial count (cfu/ml) of the formulated spoonable yoghurt flavoured with diced African bush mango pulp

Table 5 shows the microbial population of the formulated spoonable yoghurt flavoured with diced African bush mango pulp. Table 5 showed that the total viable count of the flavoured yoghurt samples ranged from 1.4×10^4 to 2.8×10^4 cfu/ml. The total viable count was observed to decrease with increase in diced African bush mango pulp. This could attribute to the antimicrobial effect of African bush mango. African bush mango has inhibitory activity against several bacteria and fungi with its potential mechanisms action which include membrane disruption by terpenoids and inactivation of microbial adhesion, enzymes, and cell envelope transport proteins by ellagic acids-like compound (Fadere and Ajaiyeoba, 2008).

The lactic acid bacteria of the flavoured yoghurt samples ranged from 1.2×10^4 to 1.8×10^4 cfu/ml (Table 5). The lactic acid bacteria (LAB) count was also observed to decrease with increase in diced African bush mango pulp concentration. This could be as a result of the decrease in milk content which contains the lactose which acts as a substrate for the growth and multiplication of the LAB.

Table 5: Microbial count (cfu/ml) of the formulated spoonable yoghurt flavoured with diced African bush mango pulp

Sample	TVC (cfu/ml)	LAB (cfu/ml)	Mould count (cfu/ml)
SYF (100:0)	2.8×10^4	1.8×10^4	ND
SYFI (95:5)	1.5×10^4	1.2×10^4	ND
SYFI (90:10)	1.4×10^4	1.3×10^4	ND
SYFI (85:15)	1.5×10^4	1.4×10^4	ND
SYF (100:0)	2.8×10^4	1.8×10^4	ND
SYFP (95:5)	2.2×10^4	1.6×10^4	ND
SYFP (90:10)	2.3×10^4	1.7×10^4	ND
SYFP (85:15)	2.0×10^4	1.5×10^4	ND

Key: SYF = Control; SYFI = Spoonable yoghurt flavoured before fermentation; SYFP = Spoonable yoghurt flavoured after fermentation; TVC = Total viable count; LAB = Lactic acid bacteria; cfu = coliform forming unit per millimeter; ND = not detected

Sensory scores of the formulated spoonable yoghurt flavoured with diced African bush mango pulp

Table 6 shows the sensory scores of yoghurt samples fortified with African bush mango pulp**.** The mean scores for colour ranged from 6.50 – 7.55. Sample SYF (100:0) had the highest score for colour but there was no significant ($p > 0.05$) difference in the colour of SYF (100:0) and the flavoured yoghurt samples. Sample SYFA (85:15) yoghurt flavoured after fermentation had the lowest score for colour.

The mean scores for appearance ranged from 5.80 – 7.25 (Table 6). There was significant ($p < 0.05$) difference in the appearance of the flavoured yoghurt samples. Sample SYFB (95:5) had the highest score for appearance but there was no significant ($p > 0.05$) difference in the colour of SYFB (95:5) and sample SYF (100:0). Sample SYFA (85:15) yoghurt flavoured after fermentation had the lowest score for appearance.

The mean scores for aroma ranged from 6.50 – 7.45 (Table 6). Sample SYF (100:0) had the highest score for aroma but there was no significant ($p > 0.05$) difference in the colour of SYF (100:0) and the flavoured yoghurt samples. Sample SYFA (85:15) yoghurt flavoured after fermentation had the lowest score for aroma.

The mean scores for taste ranged from 6.25 – 7.30 (Table 6). Sample SYF (100:0) had the highest score for taste but there was no significant difference ($p > 0.05$) in the taste of SYF (100:0) and the flavoured yoghurt samples. Sample SYFA (95:5) yoghurt flavoured after fermentation had the lowest score for taste.

The mean scores for flavour ranged from 6.25 – 7.25 (Table 6). There was significant ($p < 0.05$) difference in the flavour of the flavoured yoghurt samples. Sample SYFI (95:5) had the highest score for flavour. The preference for the flavor of the flavoured yoghurt samples decreased with an increasing level of diced African bush mango addition. There was no significant ($p > 0.05$) difference in the flavour of sample SYFB (85:15) yoghurt flavoured before fermentation and sample SYFA (85:15) yoghurt flavoured after fermentation.

The mean scores for mouth feel ranged from 6.30 – 6.80 (Table 6). Sample SYF (100:0) had the highest score for mouth feel but there was no significant ($p > 0.05$) difference in the colour of SYF (100:0) and the flavoured yoghurt samples. Sample SYFA (95:5) yoghurt flavoured after fermentation had the lowest score for mouth feel.

The mean scores for consistency ranged from 5.75 – 6.95 (Table 6). Sample SYF (100:0) had the highest score for consistency but there was no significant ($p > 0.05$) difference in the consistency of SYF (100:0) and the flavoured yoghurt samples. Sample SYFB (85:15) yoghurt flavoured before fermentation had the lowest score for consistency.

The mean scores for aftertaste ranged from 5.90 – 7.25 (Table 6). There was significant ($p < 0.05$) difference in the aftertaste of the flavoured yoghurt samples. Sample SYF (100:0) had the highest score for aftertaste while sample SYFB (85:15) yoghurt flavoured before fermentation had the lowest score for aftertaste. The preference for the aftertaste of the fortified yoghurt samples decreased with an increasing level of diced African bush mango addition.

The mean scores for overall acceptability ranged from 6.25 – 7.30 (Table 6). Sample SYF (100:0) and SYFB (95:5) yoghurt before fermentation had the highest score for overall acceptability but there was no significant ($p < 0.05$) difference in the overall acceptability of the two samples and the other flavoured yoghurt samples. Sample SYFA (95:5) yoghurt after fermentation had the lowest score for overall acceptability. SYFB (95:5) yoghurt before fermentation had the highest mean for general acceptability (7.30) and overall preference compared to other flavoured yoghurt samples. SYFA (90:10) yoghurt flavoured after fermentation was second in general acceptability (6.90) and overall preference compared to other flavoured yoghurt samples. The sensory scores implied that yoghurt flavoured with 5 and 15% African bush mango pulp could be produced without having a negative impact on the consumer acceptability of the product. This study shows that yoghurt flavoured with more than 15% could have a

negative impact on the consumer acceptability of the product. This could be attributed to the sweet bitter taste accustomed to the African bush mango. This study agrees with FAO/WHO recommendations for fruit yoghurt which is between 5 and 15% (Sarmini et al., 2014).

Table 6: Sensory scores of the formulated spoonable yoghurt flavoured with diced African bush mango pulp

Sample	Colour	Appearance	Aroma	Taste	Flavour	Mouthfeel	Consistency	Aftertaste	Acceptability
SYF (100:0)	$7.55^{a} \pm 1.36$	$7.10^{a} \pm 1.52$	$7.45^{a} \pm 1.10$	$7.30^{a} \pm 1.72$	$7.25^{a} \pm 1.41$	$6.80^{a} \pm 1.76$	$6.95^{a} \pm 1.76$	$7.25^{a} \pm 1.45$	$7.30^{a} \pm 1.30$
SYFB (95:5)	$7.45^{a} \pm 1.36$	$7.25^{a} \pm 1.59$	$7.40^{a} \pm 1.14$	$7.15^{a} \pm 1.18$	$7.00^{ab} \pm 1.08$	$6.75^{a} \pm 1.37$	$6.60^{a} \pm 1.67$	$6.70^{ab} \pm 1.98$	$7.30^{a} \pm 1.38$
SYFB (90:10)	$6.70^{a} \pm 1.53$	$6.25^{ab} \pm 1.71$	$6.70^{a} \pm 1.56$	$6.90^{a} \pm 1.41$	$6.80^{ab} \pm 1.32$	$6.50^{a} \pm 1.40$	$5.95^{a} \pm 1.50$	$6.55^{ab} \pm 1.50$	$6.80^{a} \pm 1.36$
SYFB (85:15)	$6.75^{a} \pm 1.41$	$6.10^{ab} \pm 2.02$	$6.75^{a} \pm 1.33$	$6.45^{a} \pm 1.39$	$6.25^{b} \pm 1.29$	$6.35^{a} \pm 1.60$	$5.75^{a} \pm 1.71$	$5.90^{b} \pm 1.48$	$6.50^{a} \pm 1.30$
SYF (100:0)	$7.55^{a} \pm 1.36$	$7.10^{a} \pm 1.52$	$7.45^{a} \pm 1.10$	$7.30^{a} \pm 1.72$	$7.25^{a} \pm 1.41$	$6.80^{a} \pm 1.76$	$6.95^{a} \pm 1.76$	$7.25^{a} \pm 1.45$	$7.30^{a} \pm 1.30$
SYFA (95:5)	$6.95^{a} \pm 1.57$	$5.85^{b} \pm 1.79$	$6.55^{a} \pm 2.04$	$6.25^{a} \pm 1.88$	$6.40^{ab} \pm 1.64$	$6.30^{a} \pm 1.98$	$6.00^{a} \pm 1.97$	$6.15^{ab} \pm 1.81$	$6.25^{a} \pm 2.12$
SYFA (90:10)	$7.10^{a} \pm 1.29$	$6.25^{ab} \pm 1.33$	$6.95^{a} \pm 1.28$	$7.00^{a} \pm 1.21$	$6.90^{ab} \pm 1.07$	$6.65^{a} \pm 1.31$	$6.25^{a} \pm 1.48$	$6.65^{ab} \pm 1.14$	$6.95^{a} \pm 1.90$
SYFA (85:15)	$6.50^{a} \pm 1.73$	$5.80^{b} \pm 1.94$	$6.50^{a} \pm 1.43$	$6.45^{a} \pm 1.82$	$6.25^{a} \pm 1.68$	$6.60^{a} \pm 1.82$	$5.95^{a} \pm 1.57$	$6.40^{ab} \pm 1.57$	$6.75^{a} \pm 1.41$

Values are mean ± standard deviation of 30 panelists. Means on the same column with different superscripts are significantly ($p < 0.05$) different.
Key: SYF = Control; SYFB = Spoonable yoghurt flavoured before fermentation; SYFA = Spoonable yoghurt flavoured after fermentation

4.Conclusion

From the finding of this study, it was observed that the addition of diced African bush mango pulp improved the micronutrients, proximate, physicochemical and sensory properties of the formulated yoghurt product. The flavoured yoghurt contained an appreciable amount of vitamin C, which is vital in iron metabolism and subsequent fight against iron deficiency anemia (IDA). The flavoured yoghurt also contained a good amount of calcium, which promotes bone health and reduce risk of osteoporosis (a degenerative bone disease). The use of underutilized natural fruit (African bush mango) helps in creating variety of yoghurt as well as increases the nutritional as well as medicinal value of yoghurt. From the results obtained in this study, it can be concluded that yoghurt flavoured with diced African bush mango before fermentation at a ratio of 95:5 had highest mean for general acceptability (7.30) and overall preference. Yoghurt flavoured with diced African bush mango after fermentation at a ratio of 90:10 was second in general acceptability (6.90) and overall preference. Significance of statement: this study discovered the use of African bush mango in the production of flavoured spoonable yoghurt. These are used because they are readily available, containing latent potentials and underutilized. We did the research in order to showcase the latent potentials of these genetically modified raw materials in new food product development. This study will help the researchers to uncover the potentials embedded in African bush mango, thereby encouraging local producers and industrialists to understand the potentials embedded in the indigenous tropical farm produce as compared to the exotic ones.

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Microbial, Functional and Sensory Properties of Herbal Yoghurt Formulated with Broccoli (*Brassica oleracea var. italica*) and Garden Egg Leaf (*Solanum aethiopicum*) Extract

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Abstract

Introduction and Aim: Yoghurt is a dairy product obtained from the lactic acid fermentation of milk. Vegetables present a valuable source of nutrients and are low in calories. They are rich in dietary fibre, minerals as well as many bioactive compounds. Fortification of yoghurt with biological active substances such as broccoli and garden egg leaves is one possibility for producing yoghurt with high nutritional value and functional properties.

Methods: Broccoli *(Brassica oleracea var. italica)* and garden egg leaves *(Solanum aethiopicum)* were processed to formulate herbal yoghurt in the following ratios (ml) 99:1, 98:2, 97:3. Yoghurt without the broccoli and garden egg leaves extracts (100:0ml) served as the control. Based on preliminary studies, the best samples were subjected to physicochemical, phytochemical, microbial, and selected micronutrient analysis using standard methods.

Results: From the results, herbal yoghurt samples showed an increase in the protein content (15.62 to18.12%) than the control (14.28%). The ash content ranged from 0.59% – 2.71%, while the addition of broccoli and garden egg leaves extracts to the yoghurt samples caused the pH to drop from 4.73 in the control sample to 3.01 in the herbal yoghurt samples. The total solid content (%) of the yoghurt samples ranged from 16.11 – 20.50, flavonoid content (mg QE/g) ranged from 9.58 – 20.81 and total phenol content (mg GAE/g) of the yoghurt ranged from 15.09 – 32.68. Sodium content (mg/100g) ranged from 6.03-8.39, while calcium content (mg/100g) ranged from 24.34 – 30.34. The vitamin A content (mg/100g) of the yoghurt samples ranged from 0.52 to 10.92, while the vitamin C content (mg/100g) ranged from 1.04 – 3.03. The lactic acid bacteria content of the yoghurt samples ranged from 1.66 x10^5Cfu/ml – 2.58 x 10^5Cfu/ml.

Conclusion: The best yoghurt samples were those formulated with the mixture of liquid broccoli and garden egg leaves extracts having ratio (ml) 99:1 and 98:2.

Keywords: Broccoli, bioactive compounds, functional foods, yoghurt

1. Introduction

Yogurt is a dairy product produced by fermenting milk with specific bacteria, known as yogurt cultures (US Food and Drug Administration, 2016). It is regarded as a nutrient-dense food because of its rich nutrient profile and is particularly high in calcium, which is available in a form that is easily absorbed by the body (Adolfsson *et al*., 2004). There are various categories of Yoghurt classified according to its physical and textural properties. The categories include stirred, drinkable, set, smoked, frozen, concentrated and herbal yoghurt. This category comes under the manufacturing method applied while plain, fruit and flavored yoghurt is categorized based on the flavor of the yoghurt (National Yoghurt Association, 2013). Fruits are the most natural flavor used in the production of yoghurt. However, Vegetables presents a good alternative to fruits because they have a valuable source of nutrients, low in calories, rich in dietary fibre, minerals and a good source of bioactive compounds including antioxidants. Some examples of the antioxidant include ascorbic acid, phenolic substances, carotenoids and tocopherols. Biological antioxidants are known to have the ability to delay or prevent oxidative damage of various bio-molecules which are connected to various cardiovascular diseases such as cancer, diabetes, inflammation, liver diseases, Alzheimer's disease, aging, arthritis, Parkinson's disease, atherosclerosis and AIDS. Fortification of yoghurt with vegetables that are rich in antioxidants would provide additional health properties to it, thereby resulting in the production of a novel functional food. It is to be noted that yoghurt itself is a source of bioactive peptides which are produced during fermentation, but generally the antioxidant activity is limited. As a result, there have been various attempts to produce yoghurts that are fortified with natural antioxidants obtained from natural sources thereby presenting a novel approach for product development (Gahruie *et al.,* 2015; Caleja *et al.,* 2016). With respect to this, extracts obtained from plants, herbs, fruits, and mushrooms that are rich in bioactive compounds are used as additive in yoghurt production to improve its nutritional and functional properties. Broccoli (*Brassica oleracea var. italica*) is a leafy vegetable, belonging to the flowering plant family Brassicaceae (formerly Cruciferae), it is known for its fleshy green flower heads which are arranged in a tree-like fashion with branches sprouting from a thick edible stalk (Geetha and Amit, 2020). Broccoli is rich in minerals such as iron, calcium, potassium, zinc and magnesium, a good source of fibre, and high in vitamins such as vitamin C, vitamin A, carotene and vitamin B. It is believed to play a crucial role in preventing certain types of cancer, as well as diabetes, heart disease, osteoporosis, and high blood pressure.

Garden egg leaves *(Solanum aethiopicum)* also known as African eggplant leaves are native to West Africa, primarily cultivated in Nigeria, and are more commonly grown and consumed in the southern part of Nigeria (Finelib, 2021). Garden egg leaves are recognized for their healing and nutritional benefits, frequently used as a natural treatment for certain conditions and a substitute for medical supplements. They are packed with vitamin B and C, potassium and calcium, which contribute to numerous health advantages including improving kidney health, supporting pregnancy, boosting fertility, preventing cancer, aiding in weight loss, functioning as a blood tonic, regulating heart rhythm, reducing blood sugar levels, enhancing digestion and preventing stomach ulcers (Deljayl, 2020). Integrating antioxidant-rich herbs like broccoli and garden egg leaves into yoghurt formulations shows great potential for development as a functional food. The aim of the research was to produce acceptable herbal yoghurt formulated with broccoli and garden egg leaves, and evaluate the microbial, phytochemical and sensory properties of the yoghurt.

2. Methods

Raw materials

Skimmed milk, yoghurt starter culture, fresh broccoli, fresh garden egg leaves, stabilizer and sugar.

Sample procurement

Powdered skim milk and commercial yoghurt culture (YC-180 DVS) was obtained from Foodco supermarket in Ibadan, Oyo state. Fresh broccoli was purchased from Shoprite in Ibadan, Oyo, and fresh garden egg leaves were harvested from International Institute of Tropical Agriculture (IITA) vegetable farm in Ibadan, Oyo state. Carboxylmethyl cellulose (CMC) stabilizer and sugar was purchased from Ojoo market in Ibadan, Oyo state, Nigeria.

Processing of broccoli and garden egg leaves into liquid extracts

Fresh broccoli and garden egg leaves were rinsed with distilled water and cut into small pieces, into different containers, and then put into blenders. The leaves were blended using two milliliters of distilled water in a blender. Muslin cloth was used to strain the liquor. The liquid extract (which is the filtrate) was stored in a container and refrigerated.

Processing of broccoli and garden egg leaves into powdered extracts

Fresh broccoli and garden egg leaves extract were placed-on oven trays. The leaves were conveyed into an oven and then oven dried at a temperature of 125^{o}C for 2 hours. The dried leaves were milled into fine powder.

Formulation of herbal yoghurt with broccoli and garden egg leaves extracts

Table 1 shows the proportion of the broccoli *(Brassica oleracea var. italica)* and garden egg leaves *(Solanum aethiopicum)* used in the formulation of herbal yoghurt.

Table 1: Formulation of herbal yoghurt fermented with broccoli and garden egg leaves.

S/N	Sample Code	Yoghurts Proportions to Extracts (Ml)
1.0	CYS	100:0
2.0	YLBE 1	99:1
3.0	YLBE 2	98:2
4.0	YLBE 3	97:3
5.0	YLGE 1	99:1
6.0	YLGE 2	98:2
7.0	YLGE 3	97:3
8.0	YPBE 1	99:1
9.0	YPBE 2	98:2
10.0	YPBE 3	97:3
11.0	YPGE 1	99:1
12.0	YPGE 2	98:2
13.0	YPGE 3	97:3
14.0	YLBGE 1	99:1
15.0	YLBGE 2	98:2
16.0	YLBGE 3	97:3
17.0	YPBGE 1	99:1
18.0	YPBGE 2	98:2
19.0	YPBGE 3	99:3

Keys: CYS: Control yoghurt sample (Yoghurt sample without the addition of broccoli and garden egg leaves extracts), YLBE: Yoghurt samples formulated with liquid broccoli extracts, YLGE: Yoghurt samples formulated with liquid garden egg leaves extracts, YPBE: Yoghurt samples formulated with powder broccoli extracts, YPGE: Yoghurt samples formulated with powder garden egg leaves extracts, YLBGE: Yoghurt samples formulated with liquid broccoli ad garden egg leaves extracts, YPBGE: Yoghurt samples formulated with powder broccoli and garden egg leaves extracts.

Production of herbal yoghurt formulated with broccoli and garden egg leaf extracts

Preparation of the herbal yogurt samples:

The yogurt samples were produced using skimmed milk. To achieve this, 1500grams of powdered skimmed milk was mixed with 6 liters of water and homogenized. Stabilizer was added to the milk, and thereafter pasteurized at 90°C for 30 min, cooled to inoculation temperature of 45±2°C. Four table spoons of yoghurt culture consisting of *Lactobacillus bulgaricus, Streptococcus thermophilus and Lactobacillus acidophilus* were added to 6000 ml of the milk. The milk sample was left for 10 hours at room temperature in order to allow for fermentation and aid in curd formation. The fermented milk sample was then sieved, homogenized and smoothened, and divided into proportions. The extracts obtained from Broccoli and garden egg leaf were added into each of the proportions of the sample aside from the control sample which had no extract. The prepared samples were poured into yogurt containers and incubated at 40°C until reaching the pH value of 4.6. These were, subsequently, cooled down until 5°C.

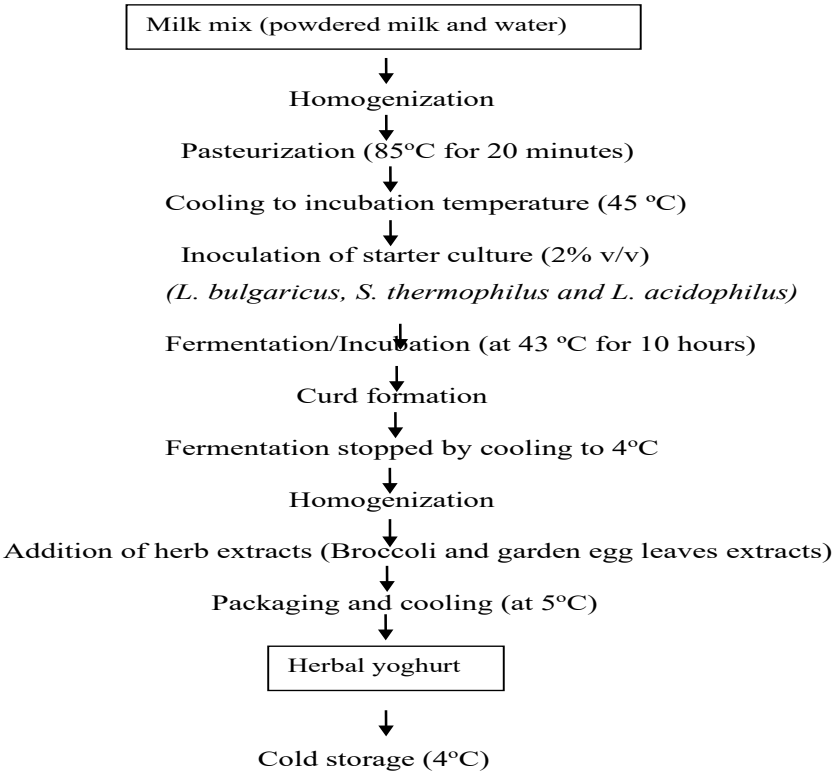


Figure 1: Production of herbal yoghurt formulated with broccoli and garden egg leaves extracts.

Analysis

Analysis was carried out on the control, the herbal yoghurts formulated with broccoli and garden egg leaf extracts, the broccoli and garden egg leaf aqueous and powdered extracts, and the broccoli leaves and the fresh garden egg fresh leaves

Physico-chemical analysis of formulated yoghurt and extracts of broccoli and garden egg leaf

Determination of pH

This was carried out using the method described by Onwuka (2005). The pH meter was standardized using a buffer solution pH 4.0 and 7.0. Ten minutes was allowed for standardization before the reading was taken. Ten percent (10%) w/v suspension of the sample was prepared using the distilled water. The mixture was mixed

vigorously by shaking manually, the pH was measures with functional pH meter (Extech instruments, model DO700, China).

Determination of total titratable acidity (TTA)

The total titratable acidity was determined by the method described by AOAC (2010). Ten milliliter of the samples was measured into a conical flask and 3 drops of phenolphthalein indicator was added to the samples and titrated with 0.1 N sodium hydroxide (NaOH) until colour change was observed. The end point was taken and the TTA expressed as % lactic acid calculated using the relationship.

$$\%\text{TTA as lactic acid} = \frac{n(NaOH) \times N(NaOH) \times 0.09}{\text{Volume of sample}} \times \frac{100}{1} \quad \ldots\ldots 1$$

Where; n= volume of titre, N=number of moles

Determination of total solids

The total solid content of samples was determined by drying 5ml 0f the samples to constant weight in hot air oven (Gallen Kamp) at 130°C. The total solid content was observed as percentage (%) total solids (AOAC, 2010).

$$\%\ \text{Total solids} = \frac{\text{Weight of dried samples}}{\text{Weight of sample}} \times \frac{100}{1} \quad \ldots\ldots 2$$

Proximate analysis of formulated yoghurt and extracts from broccoli and garden egg leaf

Determination of moisture content

The moisture content of the samples was determined according to the standard method of association of official analytical chemist (AOAC, 2010). The crucible was washed and dried in an oven at 100°C for 1 hour (W_1). The hot dried crucible was cooled in the desiccators. The weight was taken and cooled. Two milliliters of samples were weighed into the crucible (W_2) and then placed inside the oven (zitalo Z050P, Nigeria) at 100°C for 4 hours. The crucible and content were weighed (W_3).

$$\%\ \text{Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1} \quad \ldots\ldots 3$$

Where W_1= Initial weight of empty crucible, W_2= Weight of crucible + Weight of sample before drying, W_3= Weight of dish + eight of sample after drying.

Determination of ash content

The ash content of the samples was determined according to the standard method of Association of Analytical Chemists (AOAC, 2010). Two milliliters (2 ml) of the sample (W_1) were weighed into a preheated cooled crucible (W_2). The sample was charred on a Bunsen flame aside a fume cupboard. The sample was transferred into a preheated muffle furnace at 550°C for 2 hours until a white or light grey ash was obtained (W_3). It was cooled in a desiccator and weighed. The ash content was calculated mathematically as follows:

$$\%\ \text{Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1} \quad \ldots\ldots 4$$

Where; W_1= weight of empty crucible; W_2= weight of crucible + weight of sample before ashing, W_3= weight of crucible + weight of samples after ashing.

Determination of crude protein

The protein content of the herbal yoghurt was determined according to the standard method of AOAC (2010), using Kjeldahl method. The sample (2 ml) was weighed into Kjeldahl flask. Anhydrous sodium sulphate (5g or

4 tablet of Kjeldahl catalyst) was added to the flask. Also, 25 milliliters (25 ml) of concentrated tetraoxosulphate (VI) acid (H_2SO_4) were added with few boiling chips. The flask with the content was heated in the fume chamber until solution became clear, cooled at room temperature, transferred into 250 ml volumetric flask (receiving flask) containing 5 ml of 2% boric acid solution with few drops of methyl red indicator was placed under the condenser. Then, 5ml of the digest was pipetted into the apparatus through the small funnel and washed down with distilled water. Five milliliters of 60% NaOH (sodium sulphate) was collected in the receiving flask. The solution in the receiving flask was titrated with 0.049 M H_2SO_4 to a pink-coloured end point. A blank with filter paper was subjected to the same procedure.

$$\% \text{ Nitrogen of sample (\%N)} = \frac{Vs - Vb \times N \text{ acid} \times 0.01401}{W} \times \frac{100}{1} \quad \ldots\ldots\ldots\ldots\ldots\ldots 5$$

Where: Vs = Volume (ml) of acid required to titrate the sample; Vb= volume(ml) of acid required to titrate the blank; N acid = Normality of acid (0.1N); W = weight of sample in gram % crude protein = %N X 6.25 (conversion factor).

Determination of fat content

The fat content of the samples was determined using the standard method of AOAC (2010). A Soxhlet extractor with a reflux condenser and a 500 ml round bottom flask was fixed. The extraction thimble was sealed with cotton wool. The Soxhlet apparatus after assembling was allowed to reflux for about 6 hours. The thimble was removed with care and petroleum ether (boiling point 40-60^{0}C) collected in the top and drained into a container to reuse. When the flask was free of ether, it was removed and dried at 105^{0}C for 1 hour in an oven. It was cooled in a desiccator and then weighed.

$$\%\text{Fat} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times \frac{100}{1} \quad \ldots\ldots\ldots\ldots\ldots\ldots 6$$

Determination of crude fibre

The crude fiber content of the sample was determined using standard method of AOAC (2010). Petroleum ether (boiling point of 40-60^{o}C) was used to defat 2 ml of sample. This was put in boiled 200 ml of 1.25% H_2SO_4 and boiled for 30 minutes. The solution was filtered through linen or muslin cloth in a fluted funnel. It was washed with boiling water until it is free from acid. The residue was returned into 200 ml NaOH and allowed for 30 minutes. It was further washed with 1 % HCl, boiling water, to free it of acid. The final residue was drained and transferred to silica ash crucible (porcelain crucible), dried in oven at 100^{0}C for 2 hours and cooled, until a constant weight is obtained. The cooled sample was incinerated or washed in a muffle furnace at 600^{o}C for 5 hours, cooled in a desiccator and weighed.

$$\% \text{ Crude fiber} = \frac{\text{Loss of weight after ignition}}{\text{Weight of original sample}} \times \frac{100}{1} \quad \ldots\ldots\ldots\ldots\ldots\ldots 7$$

Determination of carbohydrate content

Using the standard method of AOAC (2010), carbohydrate content of the sample was determined by difference as follows:

% Carbohydrate = 100 – (% moisture + % protein + % crude fiber + %fat + % ash) ..…….. 8

Phytochemical analysis

Determination of total phenolic content

Total phenolic concentrations of the sample were determined by the Folin-Ciocalteu method according to John *et al*. (2014). With slight modification, 1 mL of each concentration series from the sample was taken and then 0.4 mL of the Folin-Ciocalteu reagent was added. After that, it was shaken, left for 8 mins, then 4 mL

of Na_2CO_3 7% was added. The mixture was shaken to homogeneity, and then methanol was added to make 10 mL. The mixture was allowed to stand for 30 minutes. After that, absorbance was measured using UV-VIS spectrophotometer at a wavelength of 647 nm. Measurement of each sample concentration series was conducted two times for replication. The total phenolic content was expressed as gallic acid equivalent (mg GAE) per gram of the sample.

Total flavonoid content determination

Total flavonoid content was measured using the colorimetric method (John *et al.,* 2014; Vyas *et al.,* 2015). With slight modification, 10 mg of sample was dissolved with methanol p.a to 10 mL. Next, 1 mL and 3 mL of pure methanol were added, and then 0.2 mL of 10% aluminum chloride and 0.2 mL of 1 M potassium acetate were also added. Distilled water was added to make 10 mL. The mixture was incubated for 30 mins. Then, the absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 439 nm. Measurement was conducted twice and the mean value was obtained times. The total flavonoid content was expressed in terms of quercetin equivalent (mg QE/g of sample).

Determination total saponin

Total saponin of the sample was determined by using a spectrophotometric method proposed by Hiai *et al.* (1975). This method is known as the vanillin-sulfuric acid assay. This was done by incubating 0.25 mL of sample, standards or reagent blank with 0.25 mL of 8% (w/v) vanillin in ethanol and 2.50 mL of 72% (v/v) sulphuric acid in water for 15 min at 60 ◦C in a shaking water bath, with the standards and the reagent blank made up with the solvent used for extracting the samples (extraction solvent). After cooling in water at the ambient temperature for 5 min, the absorbance of the standards and samples were measured at 560 nm using a Cary 50 UV–VIS spectrophotometer after zeroing it with the reagent blank. The total saponin content of the samples was then expressed in mg of standard equivalents per gram of sample (mg SE g−1).

Micronutrient analysis of formulated yoghurt and extracts from broccoli and garden egg leaf.

Determination of calcium, sodium, iron and manganese

Mineral composition of the yoghurt samples and extracts was determined using the instrument ICP-OES. A digest of the yoghurt sample and liquid extract was made using the method described by Greenberg (1992). The plant samples are mixed and a suitable amount was transferred to a 50ml digestion tube. Five milliliters of concentrated HNO_3 was added, then brought to a low boil and evaporated on a hot digestion block to lowest possible (5ml) before precipitation occurred. It was continuous heated and conc. Nitric acid was added as necessary until a light-coloured clear solution was seen, indicating that digestion was complete. The sample was allowed to dry during digestion. Ultra-pure water was added into the digestion tube until it marked 50ml, and then mixed thoroughly and centrifuged. The digested sample was transferred into clean ICP-OES vials and then calcium, sodium, manganese, and iron content determination using the ICP-OES instrument.

A digest of the powdered extracts and fresh broccoli and garden egg leaves were made using the method described by Benton (1990), and Hunter (1984). 1.2 liters of distilled water is added into a 2-liter volumetric flask, and conc. 400 ml of Hydrochloric acid was added into the flask. Also, 133 ml of 70 % nitric acid was added to dilute it to 2 liters. Ashing is then done. 0.48-0.52 g of the sample was put into a clean porcelain crucible. And the weight was recorded to the nearest 0.001g. One empty crucible was included as blank. The crucibles were placed in a cool muffle furnace of temperature 500°C over a period of 2 hours. It was allowed to remain at 500°C for an additional 2 hours. It was then allowed to cool down in an oven. The sample was removed from the oven while making sure that the environment is free from breeze. The ashed sample was then poured into an already labelled 50 ml centrifuge tube. The crucible was rinsed with 5 ml of distilled

water into the centrifuge tube and the rinsed again with 5 ml aqua regia. This was done again to make a total volume of 20 ml. The sample was vortexed for proper mixing, and then centrifuged for 10 minutes at 300 rpm. The supernatants were then decanted into clean ICP-OES vials, and the calcium, sodium, manganese and iron composition were determined using Perkin Elmer Optima 8000 ICP-OES instrument.

The digested samples were then transferred into a set of ICP-OES plastic vials of 5ml volume and labelled, then arranged on an autosampler. The autosampler was which was interphased with proportioning pump picked each sample to the plasma through the nebular, then to the sample chamber and finally draw through the touch into the plasma. At the plasma, the sample was desolvated, evaporated, atomized, and ionized. The emitted spectral lines of the elements of interest are focused on the CCD (Cation Cathode Detector). The spectral lines were then amplified and displayed on the readout. Since the instrument was interphased with a computer unit with Winelab 32 result processing software, the software used the necessary data/information imputed on the software to calculate the results of the analysis. The result coming out of the system was the intensity of the element of interest. It didn't give the final result. The final result was determined using the formular;

Final results (ppm) = Intensity of the element of interest in ICP-OES x

$$\frac{\text{Volume of extracts/digest x Dilution factor}}{\text{Weight of the samples}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 9$$

1ppm = 0.1mg/100g.

Determination of vitamin C content

The dichlorophenol titrimetric method as described by AOAC (2010) was adopted. Two milliliters (2ml) of sample were extracted by homogenizing sample in acetic acid solution.

Procedure:

The standard solution was prepared by dissolving 50 mg standard ascorbic acid tablet in 100 ml in a volumetric flask with water. The solution was filtered to get clear solution. A 10 ml of the filtrate was added into a flask in which 2.5 ml acetone has been added. This was titrated with indophenol solution (dye 2, 6, dichlorophenol indophenol) to a faint pink colour which persist for 115 seconds. The standard was treated identically.

$$\text{Mg ascorbic acid 1g} = C \times V \times \frac{DF}{WT} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 10$$

Where; C = mg ascorbic acid 1 ml dye; V = volume of dye used for titrate of dilute sample; DF = Dilution factor; WT = Weight of sample in ml.

Determination of pro-vitamin A content

Pro- vitamin A was determined using AOAC (2010). Five milliliters (5 ml) of the sample were pipetted in duplicate into a glass stoppered test tube and equal volume of ethanol was added drop wise with mixing to give 50% solution (v/v). At this concentration, the protein precipitated and free from retinol and retinly esters was extracted by addition of 3 ml hexane. The tube was stoppered and the content mixed vigorously on the vortex for 2 minutes to ensure complete extraction of the carotene for 5 – 10 minutes at 600 – 100 g to obtain a clean separation of phases. Then, 2 mg/ml of the upper hexane extract was pipetted. Absorbance due to carotenoids at 450 nm was used against a hexane blank (A450). A standard curve was plotted from the A620 value on ordinary rectangular coordinate paper, where the ordinate was at the A620 value and the abscissa was the µg vitamin A/ tube and a factor (FA620) calculated.

FA620 = µg vitamin A/tube …….. 11
A620

Pro-vitamin A was calculated using the formular:

Total carotenoid (as lycopene/dl) = A620 x Fc450 x 150 ……..……..12

Where, Fc450 = Constant determined on the laboratory, 150 = dilution factor

Likewise, pro-vitamin A (as µg retinol/dl) was calculated:

(As µg retinol/dl) [A620 – 2 x A450 x Fc450] x FA620 x 75 ……..13
FC620

Microbial analysis of formulated yoghurt and extracts from broccoli and garden egg leaf.

Microbial analysis of the yoghurt samples and the extracts were carried out using the pour plate method.

Determination of total viable count

The total viable count was determined using the method described by Atallah (2015). The fermenting slurry (1 ml) was dissolved into 9ml of Ringer's solution in a test tube and mixed thoroughly by shaking. This was a 10^{-1} dilution; one milliliter (1 ml) of the mixture was pipetted into another 9ml of Ringer's solution to give 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilution. Also, 1 ml aliquot from different dilutions (10^{-3} and 10^{-4}) was used to check the total viable count per ml on nutrient agar media. The petri dishes were made in triplicate to each sample and each plate, 15 ml of the sterile nutrient agar medium was added and 1 ml of each sample dilution was pipette into each medium containing plate respectively. This was followed by shaking and rocking in a circular movement for about 10 seconds to uniform homogenization. The plates were counted and recorded, and the total viable count was measured in colony forming unit per gram (cfu/ml) ogf the sample (Lin *et al.,* 2006).

No of colonies (cfu/ml) = average count X dilution factor (Df) ……..……..14

Mould count determination

This was determined using the method described by Obasi *et al.,* (2014) using the potato dextrose agar (PDA) as the nutrient medium. Ringer's solution was prepared by dissolving a tablet of quarter strength Ringer's tablet in 500 ml of distilled water and autoclaved at 121^0C for 15 minutes at 15 psi. Then, 2 ml of the sample was taken and put into serial dilution bottles which had been previously autoclaved and shaken for 2 minutes. Following this, 1ml of the appropriate diluent was pipetted into the sterile petri dish and potato dextrose agar was used for plating and the set up left in an incubator for 72 hours. Then, count was determined and expressed as colony forming unit per gram (cfu/ml) sample.

Lactic acid bacterial determination

The lactic acid bacterial (LAB) count in formulated yoghurt were determined using deMan Rogosa Sharpe (MRS) Agar (CM 361) as described by Kailasapathy *et al*., 2008. Samples were serially diluted in triplicate and inoculated using the surface pour plate method. The plates were incubated under anaerobic condition at 37^0C for 48 hours. After incubation, the number of colonies were counted and presented as colony forming unit per milliliter (cfu/ml) (Sivakumar and Kalaiarasu, 2010).

Cfu/ml = average count x dilution factor (D.F). ……..……15

Sensory evaluation

Sensory properties of the samples were evaluated by 20 semi-trained panelists who consisted of students of University of Nigeria Nsukka for various sensory attributes (colour, taste, flavour, mouthfeel, consistency, aftertaste, and overall acceptability). The extent of difficulties of differences between the yoghurt samples for

each sensory qualities was measured on nine- point Hedonic scale, (where "9" represents extremely like and "1" represents extremely dislike), according to Obi *et al*. (2010).

Data analysis and experimental design

The data generated was subject to a one-way analysis of variance (ANOVA) under split-plot in completely randomized design using Statistical Product for Service Solution (SPSS) version 20.0 computer programme. Mean separation was by the Duncan's new multiple range tests. Sgnificant difference was accepted at $p>0.05$ by Omola *et al.* (2014).

3.Results and discussion

Yoghurt samples formulated with broccoli leaves *(Brassica oleracea var. italica)* and garden egg leaves *(Solanum aethiopicum).*

Plate 1-6 shows the formulated herbal yoghurts, while plate 7-8 shows the extracts from broccoli and garden egg leaf.

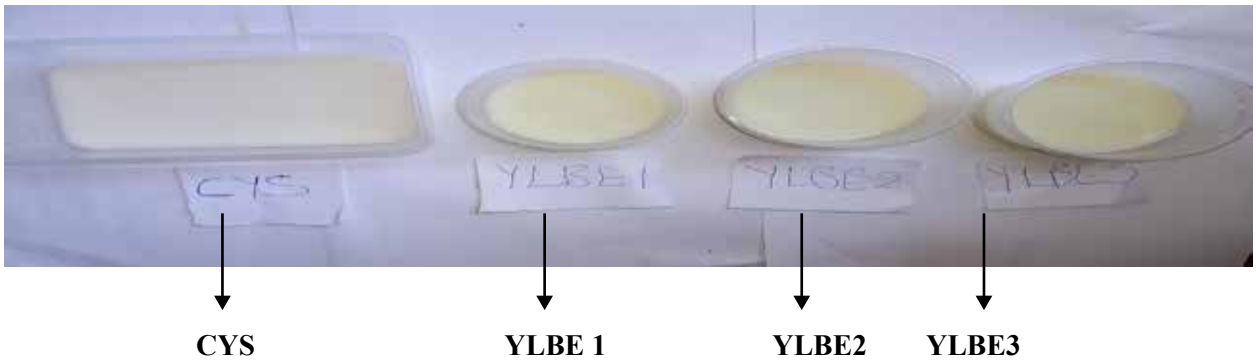


Plate 1: Yoghurt samples formulated from graded levels of liquid extracts of broccoli leaves.

Keys: CYS= Control yoghurt sample, YLBE 1= Yoghurt sample formulated with 1 ml of liquid broccoli extracts, YLBE 2= Yoghurt sample formulated with 2 ml of liquid broccoli extracts, YLBE 3= Yoghurt sample formulated with 3 ml of liquid broccoli extracts.

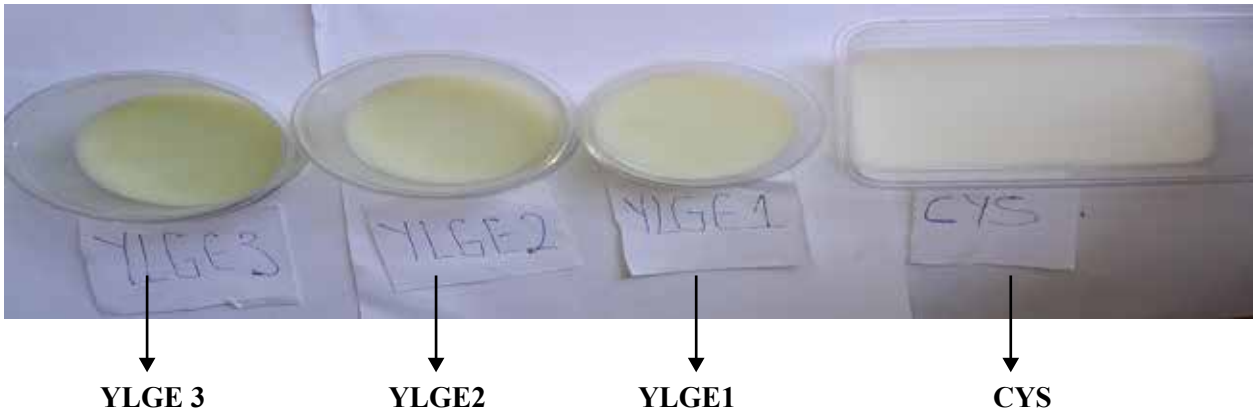
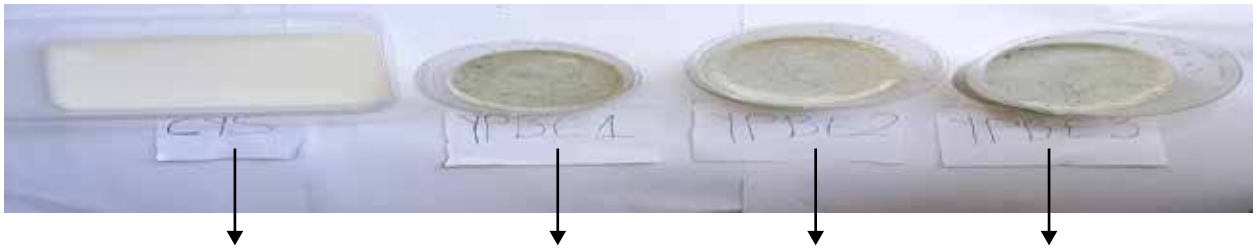


Plate 2: Yoghurt samples formulated from graded levels of liquid extracts of garden egg leaves.

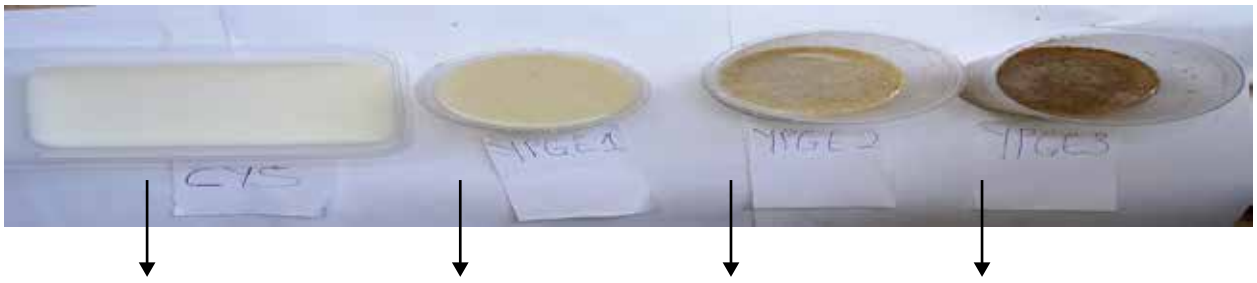
Keys: CYS= Control yoghurt sample, YLGE 1= Yoghurt sample formulated with 1 ml of liquid garden egg leaves extracts, YLGE 2= Yoghurt sample formulated with 2 ml of liquid garden egg leaves extracts, YLGE 3= Yoghurt sample formulated with 3 ml of liquid garden egg leaves extracts.



CYS **YLBE1** **YLBE2** **YLBE3**

Plate 3: Yoghurt samples formulated from graded levels of powdered extracts of broccoli leaves.

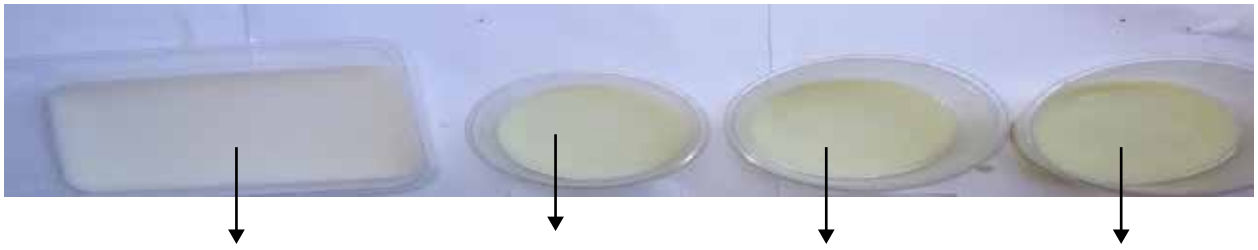
Keys: CYS= Control yoghurt sample, YLBE 1= Yoghurt sample formulated with 1 ml of powdered broccoli extracts, YLBE 2= Yoghurt sample formulated with 2 ml of powdered broccoli extracts, YLBE 3= Yoghurt sample formulated with 3 ml of powdered broccoli extracts.



CYS **YPGE1** **YPGE2** **YPGE3**

Plate 4: Yoghurt samples formulated from graded levels of powdered extracts of garden egg leaves.

Keys: CYS= Control yoghurt sample, YPGE 1= Yoghurt sample formulated with 1 ml of powdered garden egg leaves extracts, YPGE 2= Yoghurt sample formulated with 2 ml of powdered garden egg leaves extracts, YPGE 3= Yoghurt sample formulated with 3 ml of powdered garden egg leaves extracts.



CSY **YLBGE 1** **YLBGE2** **YLBGE3**

Plate 5: Yoghurt samples formulated from graded levels of liquid mixture of extracts of broccoli and garden egg leaves.

Keys: CYS= Control yoghurt sample, YLBGE 1= Yoghurt sample formulated with 1 ml of liquid mixture of broccoli and garden egg leaves extracts, YLBGE 2= Yoghurt sample formulated with 2 ml of liquid mixture of broccoli and garden egg leaves extracts, YLBGE 3= Yoghurt sample formulated with 3 ml of liquid mixture of broccoli and garden egg leaves extracts.

CYS YPBGE1 YPBGE2 YPBGE3

Plate 6: Yoghurt samples formulated from graded levels of powdered mixture of extracts of broccoli and garden egg leaves.

Keys: CYS= Control yoghurt sample, YPBGE 1= Yoghurt sample formulated with 1 ml of liquid mixture of broccoli and garden egg leaves extracts, YPBGE 2= Yoghurt sample formulated with 2 ml of liquid mixture of broccoli and garden egg leaves extracts, YPBGE 3= Yoghurt sample formulated with 3 ml of liquid mixture of broccoli and garden egg leaves extracts.

Powdered broccoli leaves extract. Powdered Garden egg leaves extracts

Plate 7: Powdered Broccoli and garden egg leaves extracts

Liquid broccoli leaves *(Brassica oleracea var. italica).* Liquid Garden egg leaves *(Solanum aethiopicum).*

Plate 8: Liquid broccoli and garden egg leaves extracts.

Sensory scores for herbal yoghurts formulated with broccoli and garden egg leaves extract.

The sensory scores of the herbal yoghurts formulated with broccoli and garden egg leaves extracts, and the plain yoghurt (control yoghurt) is shown in Table 2.

Table 2 shows the sensory scores of herbal yoghurts formulated with broccoli and garden egg leaves extracts. The sample containing 2 tbsp of the mixture of liquid broccoli and garden egg leaves extracts (YLBGE 2 = 7.45) was recorded as the best in overall acceptability. This could be due to the fact that it had the best record for mouthfeel, taste and the second-best record for flavour and consistency according to judgement from the

semi-trained panelists. There was no significant ($p<0.05$) difference in the colour of CYS, YLBE1, YLBE2, YLBE3, YLGE1, YLGE2, YLBGE1, YLBGE2, and YLBGE3, as they had values of 7.90, 7.60, 7.70, 7.05, 7.60, 7.25, 7.70, 7.65, and 7.25 respectively, with control having the highest value (7.90). The flavour of the yoghurt samples also followed this trend. This agreed with the work of Honest *et al.,* (2018) on flavour and taste score of probiotic herbal yoghurt formulated with ginger extracts, having their values as T_0 (6.75), T_1 (7.68), T_2 (7.63), T_3 (7.63), T_4(7.60), where T_0 is the control yoghurt sample.

There was reduction in the overall acceptability of the yoghurt samples as the concentration of the extracts increased except in YLBGE 1 and YLBGE 2 which had values 7.25 and 7.45 respectively. This could be as a result of the nice combination of the taste, flavour and colour of the combination of the broccoli and garden egg leaves in the yoghurt samples. Samples YLBE1, YLBE2, YLBE3, YLGE1, YLGE2, and YLGE3 having values 6.55, 6.35, 6.75, 6.60, 6.40, and 6.50, respectively compares well with the control yoghurt sample (6.75) and there was no significant ($0<0.05$) difference between them.

There was reduction in the mouthfeel, consistency and aftertaste of the herbal yoghurt samples as the concentration of the extracts increased except in YLBGE3 which had the highest mouthfeel and taste of 7.35, and 7.40 respectively. The mouthfeel of the samples YLBE1, YLBE2, YLBE3, YLGE1, YLGE2, YLGE3 and YLBGE1 having values of 6.60, 7.15, 7.15, 6.85, 6.60, 6.20, and 7.25 respectively compares well with the control (7.25). This agrees with the results of Honest *et al.* (2018).

Table 2: Sensory scores of the herbal yoghurt formulated with broccoli and garden egg leaves extracts, and the plain yoghurt.

Samples	Colour	Flavour	Mouthfeel	Consistency	Taste	Aftertaste	Overall acceptability
CYS	$7.90^{de}\pm0.85$	$6.95^{d}\pm1.82$	$7.25^{ef}\pm1.29$	$7.60^{g}\pm1.31$	$5.60^{cd}\pm2.09$	$6.20^{cd}\pm1.28$	$6.75^{de}\pm1.16$
YLBE1	$7.60^{de}\pm1.47$	$6.75^{d}\pm1.25$	$6.60^{ef}\pm1.57$	$6.85^{fg}\pm1.31$	$6.00^{cd}\pm1.49$	$5.75^{c}\pm1.45$	$6.55^{de}\pm1.15$
YLBE2	$7.70^{de}\pm1.49$	$6.35^{d}\pm1.76$	$7.15^{ef}\pm1.35$	$6.70^{fg}\pm1.56$	$5.85^{cd}\pm2.08$	$5.70^{c}\pm1.90$	$6.35^{de}\pm1.87$
YLBE3	$7.05^{de}\pm1.85$	$6.50^{d}\pm1.64$	$7.15^{ef}\pm1.53$	$6.75^{fg}\pm1.94$	$6.05^{cd}\pm2.06$	$6.20^{cd}\pm1.77$	$6.75^{de}\pm1.68$
YLGE1	$7.60^{de}\pm1.05$	$6.45^{d}\pm1.50$	$6.85^{ef}\pm1.09$	$6.70^{fg}\pm1.49$	$6.50^{efg}\pm1.47$	$6.20^{cd}\pm1.64$	$6.60^{de}\pm1.60$
YLGE2	$7.25^{de}\pm1.86$	$6.65^{d}\pm1.27$	$6.60^{ef}\pm1.00$	$6.05^{ef}\pm2.16$	$6.20^{cde}\pm1.79$	$6.25^{cd}\pm1.29$	$6.40^{de}\pm1.54$
YLGE3	$6.65^{d}\pm1.50$	$6.30^{cd}\pm1.26$	$6.20^{ef}\pm1.24$	$5.55^{de}\pm2.01$	$6.10^{cd}\pm1.29$	$6.15^{cd}\pm1.09$	$6.50^{de}\pm1.19$
YPBE1	$4.05^{ab}\pm1.96$	$4.05^{a}\pm2.09$	$4.75^{bc}\pm1.68$	$4.75^{bcd}\pm1.89$	$3.85^{b}\pm1.73$	$3.65^{a}\pm1.73$	$4.10^{b}\pm1.71$
YPBE2	$3.10^{a}\pm1.83$	$3.90^{a}\pm2.02$	$4.05^{ab}\pm1.70$	$3.15^{a}\pm1.73$	$3.25^{ab}\pm1.59$	$3.10^{a}\pm1.52$	$3.40^{ab}\pm1.47$
YPBE3	$3.35^{a}\pm1.66$	$3.75^{a}\pm1.65$	$4.00^{ab}\pm1.62$	$4.10^{ab}\pm1.65$	$3.45^{ab}\pm1.36$	$3.20^{a}\pm1.64$	$3.80^{ab}\pm1.54$
YPGE1	$5.45^{c}\pm1.57$	$5.25^{bc}\pm1.77$	$5.30^{cd}\pm1.90$	$5.30^{cde}\pm1.42$	$5.05^{c}\pm1.64$	$4.65^{b}\pm1.63$	$5.30^{c}\pm1.45$
YPGE2	$4.05^{ab}\pm1.36$	$4.15^{a}\pm1.27$	$4.30^{abc}\pm1.63$	$4.05^{ab}\pm1.57$	$3.60^{ab}\pm1.27$	$3.40^{a}\pm1.50$	$3.85^{ab}\pm1.27$
YPGE3	$3.80^{ab}\pm1.20$	$4.05^{a}\pm1.23$	$4.00^{ab}\pm1.81$	$3.70^{ab}\pm1.78$	$3.30^{ab}\pm1.59$	$3.15^{a}\pm2.08$	$3.65^{ab}\pm1.69$
YLBGE1	$7.70^{de}\pm1.42$	$6.85^{d}\pm1.87$	$7.25^{ef}\pm1.12$	$7.35^{g}\pm1.39$	$7.30^{efg}\pm1.49$	$7.10^{d}\pm1.33$	$7.25^{de}\pm1.45$
YLBGE2	$7.65^{de}\pm1.42$	$6.85^{d}\pm1.84$	$7.35^{f}\pm0.88$	$7.35^{g}\pm1.23$	$7.40^{f}\pm1.19$	$7.05^{d}\pm1.15$	$7.45^{e}\pm1.00$
YLBGE3	$7.25^{de}\pm1.41$	$6.85^{d}\pm1.87$	$6.65^{de}\pm1.57$	$6.95^{fg}\pm1.40$	$6.70^{efg}\pm1.45$	$6.70^{cd}\pm1.38$	$6.95^{d}\pm1.19$
YPBGE1	$4.65^{bc}\pm1.60$	$4.55^{ab}\pm1.28$	$4.35^{abc}\pm1.42$	$4.30^{abc}\pm1.56$	$4.00^{b}\pm1.89$	$3.35^{a}\pm1.46$	$4.15^{b}\pm1.35$
YPBGE2	$3.75^{ab}\pm1.45$	$3.95^{a}\pm1.28$	$4.10^{ab}\pm1.92$	$3.90^{ab}\pm1.86$	$3.60^{ab}\pm1.57$	$3.30^{a}\pm1.84$	$3.90^{ab}\pm1.52$
YPBGE3	$3.35^{a}\pm1.57$	$3.55^{a}\pm1.54$	$3.45^{a}\pm2.14$	$3.50^{a}\pm2.09$	$2.60^{a}\pm1.70$	$2.55^{a}\pm1.73$	$3.00^{a}\pm1.78$

Values are means ± 20 panelists. Sample means on the same column with different superscripts are significantly ($p<0.05$) different. **Keys:** CYS= Control yoghurt samples; YLBE 1= Yoghurt samples formulated with 1 ml of liquid broccoli extracts; YLBE 2 = Yoghurt samples formulated with 2 ml of liquid broccoli extract; YLBE 3 = Yoghurt samples formulated with 3 ml liquid broccoli extract; YLGE 1= Yoghurt samples formulated with 1 ml of liquid garden egg leaves extract; YLGE 2 = Yoghurt samples formulated with 2 ml liquid garden egg leaves extract; YLGE 3 = Yoghurt samples formulated with 3 ml of liquid garden egg leaves; YPGE 1 = Yoghurt samples formulated with 1 ml of powdered garden egg leaves extracts; YLBGE 1 =Yoghurt samples formulated with 1 ml of liquid mixture of broccoli and garden egg leaves extracts; YLBGE 2 = Yoghurt samples formulated with 2 ml of liquid mixture of broccoli and garden egg leaves extracts; YLBGE 3 = Yoghurt samples formulated with 3 ml of liquid mixture of broccoli and garden egg leaves extracts; YLPBGE1 == Yoghurt samples formulated with 1 ml of powdered mixture of broccoli and garden egg leaves extracts; YPBGE2 = Yoghurt samples formulated with 2 ml of powder mixture of broccoli and garden egg leaves extracts; YPBGE3 = Yoghurt samples formulated with 3 ml of powder mixture of broccoli and garden egg leaves extracts; LBE = Liquid broccoli leaves extracts; LGE = Liquid garden egg leaves extracts; PBE = Powdered broccoli leaves extracts; PGE=Powdered garden egg leaves extracts; BL =Broccoli leaves; GL =Garden egg leaves.

The taste of the yoghurt samples increased with increase in the concentration of the extracts. In present studies, yoghurt formed may be characterized physically by its smooth viscous gel structure or organoleptically by its taste and flavor. Under normal fermentation condition, the main products of metabolism are lactic acid, acetic acid, acetaldehyde, ethanol and diacetyl, all of which contributed to the specific sour taste and flavour

of fermented yoghurts. Herbs contain phytochemicals and this may play important role in the organoleptic properties of herbal yogurts. This is because most herbs contain a unique richness and diversity of metabolites responsible for their taste and flavor. Yoghurts formulated with liquid broccoli and garden egg leaves extracts was increasing in overall taste acceptability in comparison to plain yogurts (control yoghurt) because, broccoli and garden egg leaves (gilo group) have unique organoleptic taste and flavour. The present study was supported by Honest *et al*. (2018) who have overall taste acceptability of herbal yoghurt formulated with ginger extracts, having values of T0 (6.93), T1 (7.13), T2 (7.66), T3 (7.88), and T4 (8.06), where T0 is the control yoghurt sample.

The overall acceptability of the herbal yoghurt samples compared well with the control except the YLBE2, YLBE3, and the yoghurt samples formulated with powdered extracts. YLBE2 had the highest overall acceptability. It was observed that yogurt samples formulated with powdered broccoli and garden egg leaves extracts had the low acceptability in colour, flavour, taste, aftertaste, mouthfeel, consistency and overall acceptability. This could be as a result of the lack of smoothness, flavour and taste of the extracts due to the production process used.

Physicho-chemical composition of the plain yoghurt samples, the herbal yoghurt samples, the broccoli and garden egg leaves extracts, and the fresh broccoli and garden egg leaves.

Table 3 shows the physico-chemical composition of herbal yoghurt samples formulated with broccoli and garden egg leaves extracts and plain yoghurt sample (control yoghurt). There was significant ($p<0.05$) difference in the pH value between the herbal yoghurt samples and the plain yoghurt sample. There was no significant ($p<0.05$) difference between the control yoghurt sample (4.73) and YPGE1 (4.70), and there was no significant ($p<0.05$) difference between samples YLBE2 (3.64) and YLBGE2 (3.47). It was observed that as the concentration of extracts increased, the pH decreased.

Table 3: Physico-chemical composition of the plain yoghurt samples, the herbal yoghurt samples, the broccoli and garden egg leaves extracts, and the fresh broccoli and garden egg leaves.

Sample	Total solids (%)	pH	Total Titratable acidity
CYS	$16.11^{e}\pm0.03$	$4.58^{g}\pm0.03$	$0.51^{d}\pm0.03$
YLBE1	$17.18^{f}\pm0.01$	$4.27^{e}\pm0.02$	$0.57^{def}\pm0.01$
YLBE2	$18.20^{g}\pm0.08$	$3.64^{c}\pm0.02$	$0.62^{gh}\pm0.03$
YLBE3	$19.33^{h}\pm0.03$	$3.10^{b}\pm0.07$	$0.69^{gh}\pm0.00$
YLGE1	$17.21^{f}\pm0.04$	$4.53^{fg}\pm0.03$	$0.59^{ef}\pm0.01$
YLGE2	$18.24^{g}\pm0.01$	$4.06^{d}\pm0.06$	$0.66^{gh}\pm0.03$
YLGE3	$19.34^{h}\pm0.02$	$3.10^{b}\pm0.04$	$0.82^{i}\pm0.04$
YPGE1	$20.50^{i}\pm0.02$	$4.58^{g}\pm0.06$	$0.54^{de}\pm0.05$
YLBGE1	$17.16^{f}\pm0.06$	$4.07^{d}\pm0.07$	$0.72^{hi}\pm0.05$
YLBGE2	$19.24^{h}\pm0.01$	$3.47^{c}\pm0.02$	$0.76^{i}\pm0.01$
YLBGE3	$20.32^{i}\pm0.03$	$3.01^{b}\pm0.11$	$0.84^{j}\pm0.02$
LBE	$3.73^{b}\pm0.04$	$4.40^{ef}\pm0.28$	$0.53^{cd}\pm0.01$
LGE	$1.24^{a}\pm0.06$	$5.20^{h}\pm0.14$	$0.36^{b}\pm0.01$
PBE	$51.21^{k}\pm0.08$	$N.D^{a}$	$N.D^{a}$
PGE	$54.08^{l}\pm0.06$	$N.D^{a}$	$N.D^{a}$
BL	$8.52^{c}\pm0.03$	$5.75^{i}\pm0.06$	$0.32^{bc}\pm0.01$
GL	$9.02^{d}\pm0.02$	$6.68^{j}\pm0.03$	$0.29^{b}\pm0.04$

Values are presented in mean ± standard deviation. Mean values on the same column with different superscripts are significantly ($p<0.05$) different. **Keys:** CYS= Control yoghurt samples, YLBE 1= yoghurt samples formulated with 1ml of liquid broccoli extract, YLBE 2 = yoghurt samples formulated with 2ml of liquid broccoli extract , YLBE 3 = yoghurt samples formulated with 3ml liquid broccoli extract , YLGE 1= yoghurt samples formulated with 1ml of liquid garden egg leaves extract, YLGE 2 = yoghurt samples formulated with 2ml liquid garden egg leaves extract, YLGE 3 = yoghurt samples formulated with 3ml of liquid garden egg leaves, YPGE 1 = yoghurt samples formulated with 1ml of powdered garden egg leaves extracts, YLBGE 1 =yoghurt samples formulated with 1ml of liquid mixture of broccoli and garden egg leaves extracts, YLBGE 2 = yoghurt samples formulated with 2ml of liquid mixture of broccoli and garden egg leaves extracts 2, YLBGE 3 = yoghurt samples formulated with 3ml of liquid mixture of broccoli and garden egg leaves extracts, YLPBGE1 == yoghurt samples formulated with 1ml of powdered mixture of broccoli and garden egg leaves extracts, YPBGE2 = yoghurt samples formulated with 2ml of powder mixture of broccoli and garden egg leaves extracts, YPBGE3 = yoghurt samples formulated with 3ml of powder mixture of broccoli and garden egg leaves extracts, LBE = Liquid broccoli leaves extracts , LGE = Liquid garden egg leaves extracts , PBE = Powdered broccoli leaves extracts , PGE=Powdered garden egg leaves extracts , BL =Broccoli leaves, GL =Garden egg leaves.

This is attributed to the presence of acid in the extracts. Hence, the more the addition of extracts to the yoghurt samples, the more the pH values increased towards acidity. This result is in agreement with the work of Matela et al. (2019), whom reported pH trend values of 4.22, 4.20 and 4.12. All pH results are in accordance with FDA Standards specification which states that yoghurt should have maximum pH value of 4.6 (Weerathilake *et al.,* 2014).

The titratable acidity of the yoghurt samples was expressed as percentage of lactic acid present in the yoghurt

samples. There was significant ($p<0.05$) difference between the yoghurt samples. The percentage of titratable acidity were found to be increasing as pH decreased, having values of 0.51, 0.57, 0.62, 0.69, 0.59, 0.66, 0.82, 0.54, 0.72, 0.76, 0.84 for CYS, YLBE1, YLBE2, YLBE3, YLGE1, YLGE2, YLGE3, YPGE1, YLBGE1, YLBGE2 and YLBGE3 respectively. This could be attributed to the addition of extracts in the yoghurt samples. Broccoli and garden egg leaves are slightly acidic. According to George (2022), broccoli contains maleic and citric acid in the ratio of (3:2), while garden egg leaves contain folic acid. Hence, the acid content in the extracts added to the acid content in the yoghurt samples. Mataela *et al.* (2019) reported a total titratable acidity of 0.69%, 0.79% and 0.76%, for Y1, Y2 and Y3 respectively. Samples YLGE3, YLBGE1, YLBGE2 and YLBGE3 with titratable acidity values of 0.82, 0.72, 0.76, and 0.84 respectively complied with FDA specification which states that yoghurts should have a minimum titratable acidity of 0.7% (Weerathilake *et al.,* 2014).

There was significant ($p<0.05$) difference between the yoghurt samples for total solid content, except for samples YLBE1, YLGE1, and YLBGE1 which had total solid content values of 17.18, 17.21 and 17.16 respectively. The herbal yoghurt samples contained more total solids than the control yoghurt samples with the highest being YPGE1 having total solid value of 20.50.

Table 4: Proximate composition (%) of the plain yoghurt samples, the herbal yoghurt samples, the broccoli and garden egg leaves extracts, and the fresh broccoli and garden egg leaves.

Sample	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Fibre (%)	Energy (kcal)
CYS	64.54^{cd}±0.06	0.59^{c}±0.01	0.43^{b}±0.04	14.28^{h}±0.04	19.55^{p}±0.06	ND	139.17^{n}±0.30
YLBE1	66.06^{e}±0.06	0.64^{cd}±0.01	0.62^{d}±0.03	15.62^{l}±0.02	16.46^{n}±0.06	ND	133.88^{m}±0.57
YLBE2	71.14^{g}±0.04	0.70^{de}±0.01	0.88^{f}±0.01	16.17^{k}±0.01	10.60^{k}±0.01	ND	114.98^{j}±0.21
YLBE3	75.17^{h}±0.03	0.76^{ef}±0.02	1.03^{g}±0.04	17.87^{o}±0.03	4.58^{f}±0.03	ND	99.07^{h}±0.61
YLGE1	65.11^{d}±1.40	0.63^{cd}±0.03	0.54^{c}±0.03	15.56^{i}±0.01	18.53^{o}±0.00	ND	141.22^{o}±0.31
YLGE2	70.13^{f}±0.02	0.68^{d}±0.01	0.66^{d}±0.02	16.01^{j}±0.01	11.69^{l}±0.01	ND	116.70^{k}±0.30
YLGE3	75.16^{h}±0.01	0.78^{f}±0.04	0.73^{d}±0.03	17.73^{n}±0.03	5.27^{g}±0.04	ND	98.57^{h}±0.31
YPGE1	64.04^{c}±0.01	2.71^{h}±0.03	0.49^{e}±0.01	10.14^{g}±0.04	7.17^{i}±0.03	15.36^{d}±0.04	73.65^{g}±0.16
YLBGE1	65.13^{d}±0.03	0.67^{d}±0.01	0.76^{c}±0.04	16.96^{l}±0.01	16.11^{m}±0.01	ND	139.08^{n}±0.21
YLBGE2	70.13^{f}±0.01	0.78^{f}±0.03	1.54^{a}±0.03	17.63^{m}±0.08	9.56^{j}±0.01	ND	122.60^{l}±0.11
YLBGE3	75.20^{h}±0.02	0.85^{g}±0.03	1.97^{h}±0.01	18.12^{p}±0.01	3.89^{d}±0.04	ND	105.77^{i}±0.10
LBE	95.66^{k}±0.03	0.41^{b}±0.04	0.62^{i}±0.03	2.57^{e}±0.03	0.71^{c}±0.04	ND	18.70^{d}±0.31
LGE	97.86^{j}±0.01	0.30^{a}±0.01	0.43^{d}±0.01	1.46^{c}±0.03	0.47^{b}±0.01	ND	11.59^{c}±0.04
PBE	40.71^{a}±0.02	32.12^{l}±0.08	0.11^{a}±0.03	0.34^{d}±0.03	0.15^{a}±0.03	16.25^{e}±0.02	2.95^{b}±0.48
PGE	45.93^{b}±0.04	29.71^{k}±0.01	0.06^{a}±0.01	0.15^{a}±0.04	0.17^{a}±0.01	14.03^{c}±0.04	1.80^{a}±0.21
BL	82.36^{i}±0.02	9.13^{j}±0.04	0.77^{e}±0.03	3.1^{2f}±0.02	6.53±^{h}0.06	4.11^{b}±0.01	45.51^{f}±0.11
GL	84.13^{j}±0.01	8.27^{i}±0.03	0.54^{c}±0.01	2.41^{d}±0.01	4.43^{e}±0.01	2.29^{a}±0.02	32.22^{e}±0.24

Values are presented in mean ± standard deviation. Mean values on the same column with different superscripts are significantly ($p<0.05$) different. **Keys:** CYS= Control yoghurt samples, YLBE 1= yoghurt samples formulated with 1ml of liquid broccoli extract, YLBE 2 = yoghurt samples formulated with 2ml of liquid broccoli extract , YLBE 3 = yoghurt samples formulated with 3ml liquid broccoli extract , YLGE 1= yoghurt samples formulated with 1ml of liquid garden egg leaves extract, YLGE 2 = yoghurt samples formulated with 2ml liquid garden egg leaves extract, YLGE 3 = yoghurt samples formulated with 3ml of liquid garden egg leaves, YPGE 1 = yoghurt samples formulated with 1ml of powdered garden egg leaves extracts, YLBGE 1 =yoghurt samples formulated with 1ml of liquid mixture of broccoli and garden egg leaves extracts, YLBGE 2 = yoghurt samples formulated with 2ml of liquid mixture of broccoli and garden egg leaves extracts 2, YLBGE 3 = yoghurt samples formulated with 3ml of liquid mixture of broccoli and garden egg leaves extracts, YLPBGE1 == yoghurt samples formulated with 1ml of powdered mixture of broccoli and garden egg leaves extracts, YPBGE2 = yoghurt samples formulated with 2ml of powder mixture of broccoli and garden egg leaves extracts, YPBGE3 = yoghurt samples formulated with 3ml of powder mixture of broccoli and garden egg leaves extracts, LBE = Liquid broccoli leaves extracts , LGE = Liquid garden egg leaves

Table 4 shows the proximate composition of the yoghurt samples, and the extracts. There was significant ($p<0.05$) difference in the proximate composition between the herbal yoghurt samples and the plain yoghurt sample (control). In the moisture content, there was no significant ($p<0.05$) difference between samples YLGE1 (65.11) and YLBGE1 (65.13), and between the samples YLBE3 (75.17), YLGE3 (75.16), and YLBGE3 (75.20). The high moisture content of the yoghurt samples might be as a result of dilution (reconstitution) of the milk prior to fermentation. This corresponds with the work of Ihemeje *et al.* (2015), who had moisture content of 84.67 in their yoghurt sample.

The fat content ranged from 0.43 to 1.97%. There was significant ($p<0.05$) difference in the fat content of the herbal yoghurt samples (YLBE1=0.62, YLBE2= 0.88, YLBE3=1.03, YLGE1=0.54, YLGE2=0.66,

YLGE3=0.73, YPGE1= 0.49, YLBGE1= 0.76, YLBGE2= 1.54, and YLBGE3=1.97) and the plain yoghurt sample (0.43). In all, low fat content was recorded. The low fat value could be attributed to the low oil content in the milk (skimmed milk) and extracts used. This is in agreement with the work of Ihemeje *et al.* (2015) who stated categorically that yoghurt manufactured from skimmed milk will likely have a very low-fat content of 2% and below, while those produced from full cream milk will have fat content in the range of 4% or slightly above.

The ash content ranged from 0.59 to 2.71%. There was significant ($p<0.05$) difference in the ash content between the herbal yoghurt samples (YLBE1=0.64, YLBE2=0.70, YLBE3=0.76, YLGE1=0.63, YLGE2=0.68, YLGE3=0.78, YPGE1=2.71, YLBGE1=0.67, YLBGE2=0.78, and YLBGE3=0.85) and the plain yoghurt sample (0.59). The fibre content of the yoghurt samples was not detectable except in YPGE1. This could be due to the fact that yoghurt generally has poor fiber content because they are milk and water-based products, and also, the extracts were properly filtered using a very compact diameter muslin cloth, hence, no fiber could penetrate through the cloth. The presence of fiber in YPGE1 was as a result of the addition of powdered garden egg leaves extracts in the yoghurt sample. This result agrees with observation of Ihemeje *et al.* (2015) who had ash and fibre of 0.44 and 0.32 respectively in their yoghurt sample.

There was significant ($p<0.05$) difference in the carbohydrate content of all the yoghurt samples. The carbohydrate content of some of the yoghurt samples was relatively on the high side. This could be due to the moisture content of the yoghurt samples. The moisture content of some of the yoghurt samples were mildly above average, hence, going by calculation, the carbohydrate level of those samples was relatively high. This did not agree with the work of Ihemeje *et al.* (2015), as it was stated that carbohydrate (lactose) being the major constituents of milk, is converted to lactic acid during fermentation process. So, the fermentation and conversion of lactose to lactic acid should account for the low content of carbohydrate of yoghurt as observed in the results (1.70).

The protein content ranged from 14.28 to 17.87%. There was significant ($p<0.05$) difference between the herbal yoghurt samples (YLBE1=15.62, YLBE2=16.17, YLBE3=17.87, YLGE1=15.56, YLGE2=16.01, YLGE3=17.73, YPGE=10.14, YLBGE1=16.96, YLBGE2=17.63, and YLBGE3=18.12) and the plain yoghurt sample (14.28). It was observed that the protein content of the yoghurt samples compare favourably with commercial standard stated by National yoghurt Association (2000), that commercial yoghurt should have 11-18% protein.

Mineral composition of the plain yoghurt samples, the herbal yoghurt samples, the broccoli and garden egg leaves extracts, and the fresh broccoli and garden egg leaves.

Table 5 shows the mineral composition of the yoghurt samples and the extracts. There was significant ($p<0.05$) difference in the mineral content of the herbal yoghurt samples and the plain yoghurt sample except in the herbal yoghurt sample YPGE1. This could be due to the method used in the production of the garden egg leaves powdered extract. Oven was used to dry the leaves, destroying the minerals contained in the leaves. Hence, when used to formulate herbal yoghurt, there won't be significant ($p<0.05$) difference in the mineral content of the plain yoghurt and the herbal yoghurt samples. This was also applicable to the vitamin, proximate and microbial content of the yoghurt samples. There was no significant ($p<0.05$) difference in the sodium content between samples YLBE1(6.49) and YLBE2 (6.54). Mineral analysis of the plain yoghurt had values for calcium (24.34 mg/100g), manganese (0.09 mg/100g), sodium (6.03 mg/100g) and iron (1.14 mg/100g) content. The result justifies the assertion of Gray (2007) that yoghurt is a very good source of essential minerals needed for human metabolism or functionality of cells. The addition of broccoli and garden egg leaves extracts

resulted in a slight increase in calcium, manganese, sodium, and iron content of the yoghurt samples. A similar increase in minerals was also observed by Ihemeje *et al.* (2013), where pepper fruit was used in Zobo drink production, having phosphorus trend values of PY=158, PFSY=158.12, GSY=158.33, CFY=158.04, and PFY=158.10, where PY was the plain yoghurt sample.

Table 5: Mineral composition of the plain yoghurt samples, the herbal yoghurt samples, the broccoli and garden egg leaves extracts, and the fresh broccoli and garden egg leaves.

Samples	Na (mg/100 g)	Mn (mg/100 g)	Fe (mg/100 g)	Ca (mg/100 g)
CYS	$6.03^{f}\pm0.01$	$0.09^{a}\pm0.04$	$1.14^{b}\pm0.01$	$24.34^{f}\pm0.03$
YLBE1	$6.49^{g}\pm0.04$	$0.96^{f}\pm0.01$	$1.56^{d}\pm0.01$	$26.35^{g}\pm0.01$
YLBE2	$6.94^{h}\pm0.04$	$1.50^{i}\pm0.03$	$2.38^{g}\pm0.01$	$28.40^{l}\pm0.06$
YLBE3	$7.45^{j}\pm0.03$	$2.18^{j}\pm0.06$	$3.45^{k}\pm0.01$	$30.53^{n}\pm0.06$
YLGE1	$6.54^{g}\pm0.01$	$0.36^{c}\pm0.01$	$1.49^{c}\pm0.01$	$24.75^{g}\pm0.16$
YLGE2	$7.22^{i}\pm0.04$	$0.55^{d}\pm0.03$	$2.28^{f}\pm0.01$	$25.23^{h}\pm0.08$
YLGE3	$7.67^{k}\pm0.03$	$0.86^{e}\pm0.01$	$2.86^{i}\pm0.03$	$25.92^{i}\pm0.06$
YPGE1	$6.07^{f}\pm0.07$	$0.08^{a}\pm0.03$	$1.16^{b}\pm0.03$	$24.35^{f}\pm0.01$
YLBGE1	$6.96^{h}\pm0.01$	$1.22^{gh}\pm0.04$	$1.94^{e}\pm0.01$	$26.91^{k}\pm0.08$
YLBGE2	$7.77^{l}\pm0.01$	$2.81^{k}\pm0.04$	$2.53^{h}\pm0.01$	$28.83^{m}\pm0.08$
YLBGE3	$8.39^{m}\pm0.03$	$3.72^{l}\pm0.04$	$3.48^{k}\pm0.01$	$30.34^{n}\pm0.33$
LBE	$1.07^{n}\pm0.03$	$9.72^{m}\pm0.03$	$4.56^{l}\pm0.01$	$4.18^{d}\pm0.08$
LGE	$1.26^{d}\pm0.01$	$1.18^{g}\pm0.03$	$2.84^{i}\pm0.01$	$1.17^{b}\pm0.08$
PBE	$0.33^{a}\pm0.01$	$1.28^{h}\pm0.01$	$1.14^{b}\pm0.01$	$0.45^{a}\pm0.01$
PGE	$0.53^{b}\pm0.02$	$0.22^{b}\pm0.01$	$0.48^{a}\pm0.01$	$0.28^{a}\pm0.01$
BL	$1.20^{d}\pm0.01$	$10.80^{n}\pm0.04$	$5.04^{m}\pm0.01$	$4.73^{k}\pm0.03$
GL	$1.52^{e}\pm0.01$	$1.48^{i}\pm0.01$	$3.29^{j}\pm0.01$	$1.56^{c}\pm0.04$

Values are presented in mean ± standard deviation. Mean values on the same column with different superscripts are significantly ($p<0.05$) different. **Keys:** CYS= Control yoghurt samples, YLBE 1= yoghurt samples formulated with 1ml of liquid broccoli extract, YLBE 2 = yoghurt samples formulated with 2ml of liquid broccoli extract , YLBE 3 = yoghurt samples formulated with 3ml liquid broccoli extract , YLGE 1= yoghurt samples formulated with 1ml of liquid garden egg leaves extract, YLGE 2 = yoghurt samples formulated with 2ml liquid garden egg leaves extract, YLGE 3 = yoghurt samples formulated with 3ml of liquid garden egg leaves, YPGE 1 = yoghurt samples formulated with 1ml of powdered garden egg leaves extracts, YLBGE 1 =yoghurt samples formulated with 1ml of liquid mixture of broccoli and garden egg leaves extracts, YLBGE 2 = yoghurt samples formulated with 2ml of liquid mixture of broccoli and garden egg leaves extracts 2, YLBGE 3 = yoghurt samples formulated with 3ml of liquid mixture of broccoli and garden egg leaves extracts, YLPBGE1 == yoghurt samples formulated with 1ml of powdered mixture of broccoli and garden egg leaves extracts, YPBGE2 = yoghurt samples formulated with 2ml of powder mixture of broccoli and garden egg leaves extracts, YPBGE3 = yoghurt samples formulated with 3ml of powder mixture of broccoli and garden egg leaves extracts, LBE = Liquid broccoli leaves extracts , LGE = Liquid garden egg leaves extracts , PBE = Powdered broccoli leaves extracts , PGE=Powdered garden egg leaves extracts , BL =Broccoli leaves, GL =Garden egg leaves.

Table 6: Vitamin content of the plain yoghurt samples, the herbal yoghurt samples, the broccoli and garden egg leaves extracts, and the fresh broccoli and garden egg leaves.

Sample	Vitamin C (mg/100g)	Vitamin A (mg/100g)
CYS	$1.04^{a}\pm0.04$	$0.52^{a}\pm0.06$
YLBE1	$1.52^{b}\pm0.10$	$1.14^{b}\pm0.02$
YLBE2	$2.12^{e}\pm0.04$	$3.65^{c}\pm0.06$
YLBE3	$3.03^{i}\pm0.10$	$5.73^{e}\pm0.06$
YLGE1	$1.11^{a}\pm0.01$	$3.71^{c}\pm0.08$
YLGE2	$1.87^{d}\pm0.04$	$6.78^{f}\pm0.08$
YLGE3	$2.68^{g}\pm0.01$	$10.88^{i}\pm0.04$
YPGE1	$1.06^{a}\pm0.08$	$0.52^{a}\pm0.04$
YLBGE1	$1.68^{c}\pm0.04$	$4.67^{d}\pm0.03$
YLBGE2	$2.09^{e}\pm0.03$	$8.85^{g}\pm0.04$
YLBGE3	$2.90^{h}\pm0.06$	$10.92^{i}\pm0.05$
LBE	$3.49^{j}\pm0.08$	$10.50^{h}\pm0.06$
LGE	$1.85^{d}\pm0.03$	$48.12^{k}\pm0.01$
BL	$6.18^{k}\pm0.03$	$12.59^{j}\pm0.00$
GL	$2.35^{f}\pm0.01$	$53.56^{l}\pm0.01$

Values are means ± standard deviation of different determinations. Mean values on the same column with different superscripts are significantly ($p<0.05$) different. **Keys:** CYS= Control yoghurt samples, YLBE 1= Yoghurt samples formulated with 1ml of liquid broccoli extract; YLBE 2 = Yoghurt samples formulated with 2ml of liquid broccoli extract; YLBE 3 = Yoghurt samples formulated with 3ml liquid broccoli extract; YLGE 1= Yoghurt samples formulated with 1ml of liquid garden egg leaves extract; YLGE 2 = Yoghurt samples formulated with 2ml liquid garden egg leaves extract; YLGE 3 = Yoghurt samples formulated with 3ml of liquid garden egg leaves; YPGE 1 = Yoghurt samples formulated with 1ml of powdered garden egg leaves extracts; YLBGE 1 =Yoghurt samples formulated with 1ml of liquid mixture of broccoli and garden egg leaves extracts; YLBGE 2 = Yoghurt samples formulated with 2ml of liquid mixture of broccoli and garden egg leaves extracts; YLBGE 3 = Yoghurt samples formulated with 3ml of liquid mixture of broccoli and garden egg leaves extracts; YLPBGE1 == Yoghurt samples formulated with 1ml of powdered mixture of broccoli and garden egg leaves extracts; YPBGE2 = Yoghurt samples formulated with 2ml of powder mixture of broccoli and garden egg leaves extracts; YPBGE3 = Yoghurt samples formulated with 3ml of powder mixture of broccoli and garden egg leaves extracts; LBE = Liquid broccoli leaves extracts; LGE = Liquid garden egg leaves extracts; BL =Broccoli leaves; GL =Garden egg leaves.

Table 6 shows the vitamin A and C content of the herbal yoghurt samples, the plain yoghurt sample and the extracts. There was no significant ($p<0.05$) difference in the vitamin A and C content between the herbal yoghurt samples and the plain yoghurt sample, except in samples YPGE1 and YLGE1. The plain yoghurt had vitamin A and C content of 0.52 and 1.04 respectively. Addition of broccoli and garden egg leaves extracts respectively caused improvement in vitamin A content of the yoghurt samples YLBE1=1.14, YLBE2 = 3.65, YLBE3 = 5.73, YLGE1 = 3.71, YLGE2 = 6.78, YLGE3 = 10.88, YPGE1 = 0.52, YLBGE1 = 4.67, YLBGE2 = 8.85, and YLBGE3 = 10.92, and the vitamin C content of the samples YLBE1=1.52, YLBE2=2.12, YLBE3=3.03, YLGE1 = 1.11, YLGE2 = 1.87, YLGE3 = 2.68, YPGE1 = 1.06, YLBGE1 = 1.68, YLBGE2

= 2.09, and YLBGE3 = 2.90. Similar trend of increase in vitamin content of flavoured and spiced yoghurts was respectively recorded by Ihemeje *et al.* (2015), having values of the vitamin A and C content of the plain yoghurt as 5.87 mg and 3.90 mg respectively, and the vitamin A and C content of the formulated yoghurts as (CFY=0.18, PFSY=0.79 and GSY=0.05), and (CFY =0.11, PFSY=0.35 and GSY=0.58) respectively.

Phytochemical content of the plain yoghurt samples, the herbal yoghurt samples, the broccoli and garden egg leaves extracts, and the fresh broccoli and garden egg leaves.

Table 7 shows the total phenol, flavonoids and saponin content of the herbal yoghurt samples, plain yoghurt sample and the extracts. There was significant ($p<0.05$) difference in the flavonoids and phenol content among all the yoghurt samples. There was also significant ($p<0.05$) difference in the saponin content among all the yoghurt samples except between YLBE2 (21.73 DAE/g) and YLBGE2 (17.66 DAE/g). The plain yoghurt sample had total flavonoid, total saponin and total phenol content of 9.58 mg QE/g, 3.11 mg DAE/g, and 15.09 mg GAE/g respectively. It was observed that as the concentration of the extracts increased, there was an increase in the saponin, flavonoid and phenol content of the yoghurt samples. This could be as a result of the high phytochemical content in the broccoli and garden egg leaves. This work agreed with the work of Ezeoke (2018), who worked on enriching yoghurt with passion fruits. He reported a trend for phenolic content of the yoghurt to be NY(0.06), Is1(0.09), Ks1 (0.08), and Ip1 (0.10), where NY is the plain yoghurt sample (control yoghurt).

Table 7: Phytochemical content of the plain yoghurt samples, the herbal yoghurt samples, the broccoli and garden egg leaves extracts, and the fresh broccoli and garden egg leaves.

Samples	Total flavonoids (mg QE/g)	Total Saponins (mg DAE/g)	Total Phenol (mg GAE/
CYS	$9.58^{c}\pm0.08$	$3.11^{d}\pm0.06$	$15.09^{b}\pm0.06$
YLBE1	$12.89^{f}\pm0.08$	$7.29^{h}\pm0.06$	$18.27^{f}\pm0.06$
YLBE2	$16.31^{i}\pm0.06$	$15.55^{a}\pm0.06$	$24.49^{j}\pm0.06$
YLBE3	$19.58^{l}\pm0.03$	$21.73^{n}\pm0.07$	$28.57^{m}\pm0.08$
YLGE1	$11.77^{e}\pm0.08$	$5.18^{g}\pm0.02$	$17.14^{e}\pm0.03$
YLGE2	$14.17^{h}\pm0.06$	$10.19^{j}\pm0.06$	$22.18^{i}\pm0.08$
YLGE3	$17.31^{j}\pm0.06$	$16.32^{l}\pm0.06$	$27.40^{k}\pm0.08$
YPGE1	$10.68^{d}\pm0.01$	$3.79^{e}\pm0.06$	$15.40^{c}\pm0.08$
YLBGE1	$13.96^{g}\pm0.04$	$9.38^{i}\pm0.01$	$18.41^{h}\pm0.06$
YLBGE2	$17.49^{k}\pm0.00$	$17.66^{n}\pm0.01$	$28.49^{l}\pm0.08$
YLBGE3	$20.81^{m}\pm0.07$	$23.86^{o}\pm0.02$	$32.68^{n}\pm0.04$
LBE	$47.58^{p}\pm0.06$	$49.73^{m}\pm0.01$	$27.72^{o}\pm0.01$
LGE	$21.11^{n}\pm0.04$	$4.55^{c}\pm0.01$	$10.56^{d}\pm0.03$
PBE	$2.63^{b}\pm0.06$	$8.50^{b}\pm0.06$	$5.27^{g}\pm0.01$
PGE	$1.19^{a}\pm0.06$	$0.19^{a}\pm0.02$	$0.59^{a}\pm0.01$
BL	$50.22^{q}\pm0.06$	$56.81^{p}\pm0.04$	$39.25^{p}\pm0.06$
GL	$28.10^{o}\pm0.03$	$15.47^{f}\pm0.01$	$16.51^{j}\pm0.06$

Values are presented in mean ± standard deviation. Mean values on the same column with different superscripts are significantly ($p<0.05$) different. **Keys:** CYS= Control yoghurt samples, YLBE 1= yoghurt samples formulated with 1ml of liquid broccoli extract, YLBE 2 = yoghurt samples formulated with 2ml of liquid broccoli extract , YLBE 3 = yoghurt samples formulated with 3ml liquid broccoli extract , YLGE 1= yoghurt samples formulated with 1ml of liquid garden egg leaves extract, YLGE 2 = yoghurt samples formulated with 2ml liquid garden egg leaves extract, YLGE 3 = yoghurt samples formulated with 3ml of liquid garden egg leaves, YPGE 1 = yoghurt samples formulated with 1ml of powdered garden egg leaves extracts, YLBGE 1 =yoghurt samples formulated with 1ml of liquid mixture of broccoli and garden egg leaves extracts, YLBGE 2 = yoghurt samples formulated with 2ml of liquid mixture of broccoli and garden egg leaves extracts 2, YLBGE 3 = yoghurt samples formulated with 3ml of liquid mixture of broccoli and garden egg leaves extracts, YLPBGE1 == yoghurt samples formulated with 1ml of powdered mixture of broccoli and garden egg leaves extracts, YPBGE2 = yoghurt samples formulated with 2ml of powder mixture of broccoli and garden egg leaves extracts, YPBGE3 = yoghurt samples formulated with 3ml of powder mixture of broccoli and garden egg leaves extracts, LBE = Liquid broccoli leaves extracts , LGE = Liquid garden egg leaves extracts , PBE = Powdered broccoli leaves extracts , PGE=Powdered garden egg leaves extracts , BL =Broccoli leaves, GL =Garden egg leaves.

Table 8: Microbial counts of the herbal yoghurt samples, the broccoli and garden egg leaves extracts, and the fresh broccoli and garden egg leaves.

Samples	TVC (Cfu/ml)	LAB (Cfu/ml)	Mould (Cfu/ml)
CYS	1.56×10^3	2.58×10^5	N.D
YLBE 1	1.43×10^3	2.27×10^5	2×10^0
YLBE 2	1.39×10^3	2.11×10^5	4×10^0
YLBE 3	1.28×10^3	2.05×10^5	6×10^0
YLGE 1	1.51×10^3	2.10×10^5	3×10^0
YLGE 2	1.46×10^3	2.47×10^5	5×10^0
YLGE 3	1.38×10^3	2.36×10^5	7×10^0
YPGE 1	1.55×10^3	2.57×10^5	N.D
YLBGE 1	1.41×10^3	2.18×10^5	4×10^0
YLBGE 2	1.31×10^3	1.97×10^5	7×10^0
YLBGE 3	1.24×10^3	1.66×10^5	9×10^0
LBE	2.2×10^1	N.D	4×10^0
LGE	3.6×10^1	N.D	6×10^0
PBE	N.D	N.D	N.D
PGE	N.D	N.D	N.D
BL	0.5×10^2	N.D	1.0×10^1
GL	1.2×10^2	N.D	1.5×10^1

Keys: CYS= Control yoghurt samples, YLBE 1= yoghurt samples formulated with 1ml of liquid broccoli extract, YLBE 2 = yoghurt samples formulated with 2ml of liquid broccoli extract , YLBE 3 = yoghurt samples formulated with 3ml liquid broccoli extract , YLGE 1= yoghurt samples formulated with 1ml of liquid garden egg leaves extract, YLGE 2 = yoghurt samples formulated with 2ml liquid garden egg leaves extract, YLGE 3 = yoghurt samples formulated with 3ml of liquid garden egg leaves, YPGE 1 = yoghurt samples formulated with 1ml of powdered garden egg leaves extracts, YLBGE 1 =yoghurt samples formulated with 1ml of liquid mixture of broccoli and garden egg leaves extracts, YLBGE 2 = yoghurt samples formulated with 2ml of liquid mixture of broccoli and garden egg leaves extracts 2, YLBGE 3 = yoghurt samples formulated with 3ml of liquid mixture of broccoli and garden egg leaves extracts, YLPBGE1 == yoghurt samples formulated with 1ml of powdered mixture of broccoli and garden egg leaves extracts, YPBGE2 = yoghurt samples formulated with 2ml of powder mixture of broccoli and garden egg leaves extracts, YPBGE3 = yoghurt samples formulated with 3ml of powder mixture of broccoli and garden egg leaves extracts, LBE = Liquid broccoli leaves extracts , LGE = Liquid garden egg leaves extracts , PBE = Powdered broccoli leaves extracts , PGE=Powdered garden egg leaves extracts , BL =Broccoli leaves, GL =Garden egg leaves.

Table 8 shows the microbial load of yoghurts formulated with broccoli and garden egg leaves extracts, the plain yoghurt (control yoghurt), the broccoli and garden egg leaves extracts, and the fresh broccoli and garden egg leaves. The total viable count of the microbial analysis of the herbal yoghurt and the plain yoghurt samples ranged from 1.24×10^3 cfu/ml to 1.56×10^3 cfu/ml. High bacteria count was expected because of the presence of starter cultures, mainly lactic acid bacterial (Dirisu *et al.,* 2015). The standard count is $10^6 – 10^7$ (Codex alimentarium, 2003; and Ezeoke, 2018). Very high count however is used as an indication of post-pasteurization contamination (Tamine and Robinson, 2004). The plain yoghurt samples had higher viable counts than the formulated yoghurt samples. Microorganism used as starter culture might have contributed to the total viable count of the yoghurt samples. However, the decrease in the total viable count in the herbal yoghurt samples was as a result of the antibacterial activity of the broccoli and garden egg leaves in the yoghurt

samples (Sammah and Ahmmed, 2019).

Mould count ranged from 0 cfu/ml to 0.9 x 10^1 cfu/ml. The control yoghurt sample had no detectable mould count, while the herbal yoghurt samples had detectable mound counts ranging from 0.2 x 10^1 cfu/ml to 0.9 x 10^1 cfu/ml. The plain yoghurt sample conformed to standards of Codex Alimentarius (2003). Yoghurt should contain no greater than 1 yeast cell per gram (10cfu), but mould counts was recorded in the herbal yoghurt samples. This was as a result of cross contamination of the yoghurt samples by the broccoli and garden egg leaves extracts, due to insufficient hygienic practices during the processing of the broccoli and garden egg leaves extracts.

The lactic acid bacterial of the yoghurt were least in sample YLBGE3 (1.66 x 10^5 cfu/ml). This was because broccoli and garden egg leaves have antibacterial properties and could have rendered some lactic acid in the yoghurt non-viable. The mould count in YLBGE3 was also relatively high and could have also suppressed some lactic acid bacterial in the yoghurt (Sammah and Ahmmed, 2019).

4.Conclusion

The results of this study shows that addition of broccoli and garden egg leaf extracts to yoghurt as herbal agent improved the functional and sensory properties of yoghurt, especially when a combination of liquid extracts of garden egg and broccoli leaves was used. The addition of broccoli and garden egg leaf extracts improved the flavour, taste, mouthfeel and overall acceptability of the yoghurt.

The utilization of broccoli and garden egg leaf extracts as a natural herbal agent improved the nutritional properties of the yoghurt. The yoghurts formulated with liquid broccoli and garden egg leaf extracts contained higher protein and fat content than the plain yoghurt (control yoghurt). But the plain yoghurt had the highest carbohydrate content than the yoghurt formulated with broccoli and garden egg leaf extracts. The yoghurt samples formulated with broccoli and garden egg leaf extracts had more mineral content than the plain yoghurt sample. The high nutrient content of the yoghurt makes it a very nutritious drink. The phytochemical content of the yoghurt was higher in those formulated with broccoli and garden egg leaf extracts than the plain yoghurt sample. Hence, it made it an ideal drink for all classes of people in the world: children, aged, sick, pregnant women, amongst others.

Contribution to knowledge

The community: This research work provided information on the microbial, antinutrients, nutritional, physicochemical, and sensory properties of herbal yoghurts formulated with broccoli and garden egg leaf extracts. There is little or no information on herbal yoghurts formulated with broccoli and garden egg leaf extracts in Nigeria. Therefore, this study would generate information on herbal yoghurts formulated with broccoli and garden egg leaf which would aid in further research.

The policy: This piece of work provided information on how to set up standards that would guide food processors in the production of herbal yoghurts formulated with broccoli and garden egg leaf extracts. This research work would aid the government/ policy makers to create awareness and educate the masses, especially farmers on the existence of broccoli, and also on various possible ways to properly utilize garden egg leaves, to avoid postharvest losses in Nigeria.

The consumers: This research work would enlighten the masses on the health benefit of consuming herbal yoghurts formulated with broccoli and garden egg leaf and its nutritional content. It also provides information on how safe herbal yoghurt formulated with broccoli and garden egg leaf extracts is for consumption. The physicochemical, phytochemical content among others, are very important as the combination of broccoli and garden egg leaves is envisaged to form a healthy and nutritious product.

The food industry: Broccoli and garden egg leaves has been shown to be a herbal agent and hence, it has widened the food base of herbs/vegetables used in formulating herbal yoghurts and other food products in the food industry. This study has proven the suitability of yoghurt formulated with broccoli and garden egg leaves extracts produced under good hygienic conditions in the manufacture of acceptable yoghurt of excellent nutritional and sensory qualities. The results obtained could be used to produce yoghurts formulated with broccoli and garden egg leaves extracts in food industries. Efforts should therefore be intensified towards the commercial production of herbal yoghurts.

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High World Happiness Record of Finland Seven Consecutive Times: Could Commitment to Sustainable Development Goals be the Reason behind this Great Success?

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Abstract

Our aging world is struggling with a bulk of problems: climate change, environmental disasters such as earthquakes, floods, tornadoes, food shortage, pandemics, increasing gap between the rich and poor segments of society, corruption, massive migration with the hope of settling down in wealthy nations, lost lives and hopes in the meantime. One thing for sure is that happiness is just a dream for people who suffer from these poor living conditions.

Happiness is a legitimate human right. Every person has the right to be happy in their lives, at least from time to time if not all the time. It is the governments' responsibility to create the conditions for their citizens to be happy. Large business firms also have a responsibility in the creation of happy citizens.

The World Happiness Report (WHR) is prepared jontly by United Nations (UN), Oxford University and Gallup. According to the report Finland has the highest happiness score for the seventh year in a row followed by Denmark, Iceland, Sweden, Netherlands and Germany.

As a solution to these problems the UN proposed 17 Sustainable Development Goals (SDGs), Agenda 2030. The question is whether Finland's high happiness score has any connection to Finland Government's commitment to achieving SDGs. An extensive literature survey including UN's SDGs, governmental papers regarding SDG performance of Finland, the role of multinational companies (MNCs) is conducted to find out Finland's performance towards achieving SDGs.

As a first step the concept of happiness and its measurement is reviewed. Then the meaning of meeting SDGs for a country's government is investigated. Finally, Finland Government's performance in achieving SDGs and the role of MNCs in this endevour are evaluated. The findings are expected to provide guidance to governments and businesses in their efforts to increase the happiness of their citizens.

Keywords: Finland, World Happiness Report, Sustainable Development Goals, multinational companies.

1. Introduction

In 2015 the UN adopted 17 Sustainable Development Goals (SDGs) with the objective to eradicate poverty, protect the earth and ensure that everyone can enjoy peace and prosperity by the year 2030. Among the various goals of SDGs are to end hunger and poverty, AIDS and similar diseases as well discrimination against women and children.

These goals require universal action among all governments, civil society and major businesses. The goals are interrelated; thus action in one area will influence the results in others. Consequently, it is necessary to reach a balance among economic, social and environmental sustainability for any progress to be achieved. To achieve the goals in every context; tehnology, finance, knowhow and creativity from the whole society is required (Sightsavers, 2024).

Collaboration between civil society, governments and private sector; accompanied with the necessary resources, partnerships and innovation capacity encourage implementation are basic requirements (UNDP, 2023). The achievement of the goals require a major transformation and business has an important position. To achieve the SDGs, the business must increase its positive impacts and reduce its negative ones.

Especially multinational enterprises (MNEs) can be crucial partners in achieving SDGs. These large businesses deal with large shares of global trade, employment, production, investment, innovation and research. Their decisions affect their employees, customers, their business partners and their competitors as well as the economies and societies of the places in which they are located. Multinational firms can be great actors in promoting local sustainable development while they achieve the development in SDGs (Foley et al., 2021:3).

As a result of all body of knowledge presented up to this point, it seems reasonable to think that local people might catch happiness when a nation has reached a state that SDGs aforementioned have been achieved to a satisfactory level. The purpose of the research study is to investigate whether there can be any connection between the achievement of a satisfactory progress towards the SDGs and happiness of citizens in a nation. Finland is selected to conduct the analysis since it is the country with the highest happiness score in the Gallup researches for seven consecutive years.

2. Happiness Concept

Happiness concept is related to a person's feeling well. In psychology, happiness is a person's emotional well-being or happening of good things at a certain time. Defined more broadly, happiness is a positive evaluation of one's overall achievements and life. One can achieve happiness both from positive emotions such as interest, excitement and affection; as well as negative emotions like anger, fear and sorrow (Encyclopedia Britannica). Happiness can also be defined as being satisfied when something is good or right (Oxford English Dictionary).

Every decent person has a right to be happy. Indeed there are some people who are quite satisfied with their achievements and living conditions and happiness is a natural gift for them. However, this is not the case for an abundance of people. Those people who tackle with many problems like hunger, diseases, unemployment, inequality in every sense, poor living conditions, etc. consider happiness as an ideal with no hope of reaching. No wonder we hear lots of tragic news every day about these people who struggle in search of a better world, in the hope of reaching happiness.

A diligent health care program, employment for all, healthy nutrition, decent incomes, gender equality, measures to preserve the environment and waste management are among the major topics of governmental programs and this can be considered as an address to a happy nation or happy citizens. In fact, United Nations' SDGs guide the nations to happiness through achieving the 17 goals and 169 targets.

3. World Happiness Report

Happiness rankings of nations in the World Health Report (2024) are collected on the basis of **life evaluations**, which reflect a more stable indicator, the quality of people's lives. The data in the report is provided by Gallup World Poll.

In terms of life evaluations, respondents are asked to evaluate their present life as a whole within the context of a ladder image, within a scale of 0 to 10. For each country, approximately one thousand responses are gathered every year. For each year, construct population-representative national averages in each country are constructed. Rankings of happiness are generated using three-year averages of life evaluations. The evaluations are calculated using the following six parameters:

Gross Domestic Product (GDP) per person, life expectancy in healthy conditions, having a dependable friend and/or relative, independence to make life choices, freedom from corruption and generosity.

Positive emotions (Positive affect) is suplied by the average of individual yes or no answers regarding laughter, interest and enjoyment.

Negative emotions (Negative affect) is supplied by the average of individual yes or no answers about anger, sorrow and anxiety.

Happiness ranking scores are calculated on the persons' own evaluation of their lives, especially their responses to the 0 to 10 grading question. Estimates of the six variables' relationships with the evaluations were helpful in comparing the variation of life evaluations between different countries. This is quite similar to estimating the extent to which estimated life span is influenced by diet, exercise and smoking (Helliwell et al., 2024: 13-14).

Researh results indicate that Finland leads all other countries in 13 of the 17 SDGs-World Happiness Report (Helliwell et al., 2024). It can be observed that there is a correlation between the unhappiness and poor national wealth. A high correlation also exists between the national wealth and several social indicators including human rights, longevity and democratic governance, which may partly indicate an association with subjective well-being (Diener et al., 2009: 12).

1. The UN SDGs

UN's SDGs probe the fundamental causes of poverty and the universal need for development. The content of each SDGs are as follows (Global Sustainable Development Report 2023):

1. Eradicate poverty gllobally

2. Promote sustainable agriculture, terminate hunger, achieve improved nutrition and food security

3. Promote well-being at all ages for everyone and ensure healthy lives

4. Ensure equitable and inclusive quality education, promote lifelong learning opportunities for everybody

5. Empower all girls and women, ahieve gender equality

6. Ensure sanitation, sustainable management and availability of water for everybody

7. Ensure access to reliable, modern, sustainable and affordable energy for everybody

8. Promote sustainable, inclusive economic growth, decent work, productive and full employment for all

9. Foster innovation, promote inclusive and sustainable industrialization and build resilient infrastructure

10. Minimize inequality within and among countries

11. Make cities and human settlements safe, sustainable, resilient and inclusive

12. Ensure sustainable production and consumption patterns

13. Take immediate action to fight climate change and its impacts

14. Sustainably use and conserve marine resources, seas and oceans

15. Protect, promote and restore sustainable use of terrestrial ecosystems, manage forests sustainably, stop

biodiversity loss, fight desertification, stop and reverse degradation of land

16. Promote inclusive and peaceful societies for sustainable development; build accountable, inclusive and effective institutions at all levels; provide access to justice for everybody

17. Revitalize global partnership for sustainable development and reinforce the means of implementation

There is a close connection between the progress in each goal and happiness of citizens. Happiness might be a reality for all if people are healthy in their life span, do not suffer from poverty, have no difficulty in finding secure and nutritious food, access clean air and water easily, reach quality education and employment without being exposed to gender inequality, injustice, corruption and violence.

5. Facts about Finland

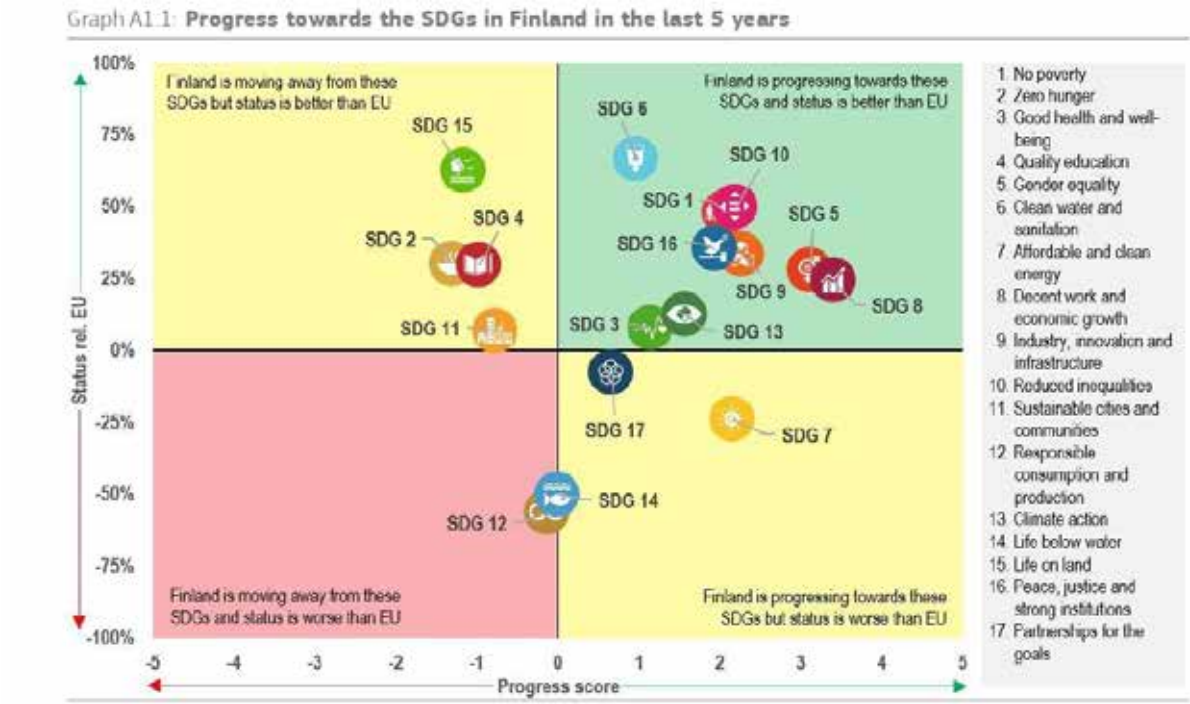
Per capita income in Finland is among the highest in Western Europe. The main economy was based on farming and forestry. At the present it is a modern, diversified industrial economy. Finland became a member of the European Union in 1995 and joined the Euro currency at the beginning of 1999. Following the start of the Ukraine-Russia conflict in 2022, Finland applied to NATO and became a member in 2023 April. In the 21st century, the key features of Today Finland has a strong national social welfare system which is facing the issues of fluctuations and an aging population (CIA gov, 2024).

5.1. Finland's Performance in Meeting SDGs

SDR 2024 (Sustainable Development Report) provides information countries' performance regarding the SDGs. Three Nordic countries, namely Finland, Sweden, and Denmark are the top countries in this year's Index. However, even these countries are faced with major challenges with regards to achieving some of the SDGs. Included in the SDR is the International Spillover Index. The ISI emphasizes the fact that rich countries can generate negative environmental and socioeconomic spillovers, some of which are the result of unsustainable trade and supply chains.

Finland is ranked first in the 2024 SDG Index and also in the World Happiness Report (Helliwell et al., 2024). However, Finland and the closely following Nordic countries face significant challenges in achieving several SDGs, especially goals 2, 12, 13 and 15; namely zero hunger, responsible consumption and production, climate action and life on land. These result in part from negative international spillover effects and unsustainable consumption patterns. It is estimated that only 16% of the SDG targets are on track to be achieved. The remaining 84% of the targets show either limited progress (unlikely to reach the target by 2030) or negative progress. Most of the targets which are off-track are regarding sustainable land use, food systems, biodiversity, peace and strong institutions (Sachs et al., 2024:17).

After five years following their adoption; there are still serious gaps in official statistics in terms of country coverage and timeliness for many SDGs. Especially goals 4, 5, 12, 13 and 14, i.e. quality education, gender equality, responsible production and consumption, climate action and life below water (Sachs et al., 2021: IX).



Source: Institutional paper 250, 2023: 24.

There are four dimensions of sustainability; fairness, environmental sustainability, macroeconomic stability and productivity. According to European Commission's 2023 Country Report, Finland's performance on all dimensions of sustainability is good. Also, Finland is ranked above the EU average on 12 of the 16 SDGs. However, it must be noted that Finland is shifting away from the targets about goals 2, 11 and 15; which are zero hunger, sustainable communities and cities, and life on land. Finland's performance on goals 7, 12 and 14 (clean and affordable energy, responsible production and consumption, life below water) are below the EU average but there is progress in these areas (European Union, 2023:7).

The Food, Agriculture, Biodiversity, Land-Use and Energy Consortium (FABLE) consists of 80 researchers from 22 countries. FABLE worked on how 16 targets involving biodiversity, climate mitigation, food security, water quality and conservation could be realized by 2030 and 2050. According to FABLE's 'global sustainability' pathway, it is possible to make significant progress. The solution suggested will necessitate several major changes in the form of:

1) Limit animal-based protein consumption through dietary shifts that are compatible with cultural preferences and avoid overconsumption;

2) Invest to protect productivity, especially for areas and products with high demand growth;

3) Implement strong, transparent and inclusve monitoring systems to stop deforestation.

This procedure and methodology is expected to avoid deforestation of an area as much as 100 million hectares by the year 2030 and reduce CO2 emissions by 100 gigatons by the year 2050. Additional measures will be required to avoid trade-offs with on-farm employment and water pollution due to excessive fertilizer usage, and to ensure that no one is left behind, particularly in the fight to end hunger (Sachs et al., 2024: IX).

5.2. Finland's MNCs

Corporate organizations that operate in more than one country besides their home country are named multinational companies or multinational corporations. Their head office is located in the home country and facilities, offices, factories and other assets are located in other countries.

Multinational corporations contribute to employment, innovation, investment and productivity by integrating

their operations globally. Policymakers should support those activities of MNCs which contribute to rising living standards whereas discourage competition in a nation. Following are the principles that have been formulated to guide public policy toward multinational corporations (Foley et al., 2021: 2-7):

Principle1: Governments should avoid barriers such as tariffs, punitive regulations and taxes to take advantage of benefits that multinational corporations offer modern economies and other restrictions on the movement of data, goods, investment and people across borders. Opening the doors to direct foreign investment may be the best way to spread innovation, spur market competition and create good jobs.

Principle 2: All sections of society should receive some benefit from globalization and the activities of multinational corporations through the adoption of joint policies and practices of governments and multinational corporations.

The net effects of globalization and the activities of multinational corporations are generally positive But there are costs and especially less-educated workers suffer from these costs in developed countries - particularly workers who perform routine tasks that are automated or easily moved offshore, resulting in a worrisome increase in inequality within countries in recent decades.

Public authorities should allocate funds for programs such as flexible training subsidies, wage insurance and other measures aimed at individual persons and communities who have missed the benefits of globalization. Executives of MNCs should be willing to pay taxes and provide support to help finance such public programs. They also should invest more in training to better equip their employees to cope with a rapidly changing economy.

Principle 3: Governments should work together in designing cohesive and consistent policy frameworks because what happens in one place influences the other places.

Climate change especially presents is a challenging issue that reqires a joint, collaborated work. . No one company or one country alone can be successful in reducing the dangers of climate change. Private actors that contributed to greenhouse gas emissions should be made to share the costs - like cap-and-trade permit systems. and carbon taxes - through designing public policies. MNCs, many of which have announced climate-related goals, should give support and help in designing such government efforts.

Principle 4: Since innovation is an important contributor to prosperity, there should be government policies that encourage MNCs to pursue innovations and share the benefits with the world. MNCs are major innovators since they embody the contributions of the best minds from every part of the world, rich and poor countries alike. Policies that support the free flow of goods, services, human capital, and data are required for supporting innovative activity. It is also necessary to design strong and strictly enforced intellectual property rights as an incentive for MNCs to invest in innovation and make it easy for them to transfer technology to their foreign affiliates.

There are 11 MNCs headquartered in Finland: Amer Sports, Anora Group, Frosmo, Kone, Konecranes, Nokia, Stora Enso, UPM, Valamis, Wärtsilä, YIT. Companies like Kone, Metso, Neste Oil, Nokia, Stora Enso, UPM-Kymmene and Wärtsilä are globally known and they are a source of pride for Finland.

The number of Finnish companies which are in the process of integrating SDGs into their businesses is increasing. After Finland's first Voluntary National Review (VNR) in 2016, the 2030 Agenda has created a breakthrough in the business sector in Finland Many of them have started to integrate SDGs into their strategies. According to The FIBS "Sustainability in Finland 2019" survey, the level of attention paid to the SDGs as part of business operations is increasing. In 2019, more than 50% of the large companies reported that they were taking the SDGs into account in their business, showing a significant increase compared with previous years. The fact that big companies are paying increasingly more attention to the goals shows the level of attention paid to the SDGs as part of business operations (VNR FINLAND, 2020).

Many MNCs declare climate related goals and they are expected to support and help design governments' efforts in dealing with climate change since effectively reducing the dangers of climate change necessitates a joint effort (Foley et al., 2021:2-7). Neste, one of the MNCs of Finland, creates solutions for fighting with cli

mate change and accelerating a shift to a circular economy. The 2023 Annual Report of Neste provides a good indication of the company's commitment to sustainability (Neste Annual Report, 2023):

Neste sustainability vision guides the corporation's activities. The vision includes aspirational targets for biodiversity, climate change, human rights, sustainability, supply chain and raw materials – issues that are all increasingly interlinked. Joined with business partners, Neste is aiming for a carbon neutral and nature positive value chain by 2040. With the ambitious sustainability commitments and solutions, available already today, Neste is committed to limiting global warming to 1,5°*C* and meeting the Paris Agreement objectives.

Part of the Neste strategy is sustainability vision. At corporate level, sustainability is managed by a crossfunctional Sustainability Leadership Team under the chairmanship of VP of Sustainability. The team consists of members with different objectives and functions such as Climate Change and Circular Economy, Human Rights, Innovation and Communications, Supply Chain Sustainability, Sustainability Reporting and Engagement. The Team meets monthly to prepare the sustainability priorities and proposals to be presented to the Executive Committee by the EVP, Renewables Supply Chain and Sustainability, with the relevant sustainability experts (Neste Annual Report, 2023).

Neste identified nine priority goals as goals to which most significantly contributed and the SDG Compass is used to determine them. To understand and prioritize the most relevant goals fort he corporation, both the positive and negative impacts the business has on the SDGs throughout the value chains are checked. For instance goal 11 (Sustainable cities and communities) is the goal Finland is moving away from the target. This goal is in Neste's 2023 Annual Report with the following activities:

• Neste helps clients reduce greenhouse gas emissions by supplying lower-emission renewable fuels for land transport and aviation. Neste's renewable products help reduce emissions related to transportation and improve local air

• Neste assists urban centers in their fight against climate change by reducing Green House Gas emissions. The company supplies renewable diesel fuel to the west coast states of USA and jointly with PetroCard provides access to renewable diesel in the Pacific Northwest region of the USA.

• Neste's "MY Sustainable Aviation Fuel™" is used in Sweden's Trollhättan-Vänesborg Airport, which is the world's first airport to use sustainable aviation fuel on all flights, together with Västflyg Airlines.

• Neste introduced renewable diesel to the French and Danish markets.

•Neste provides public high-power charging for electric vehicles at service stations in Finland. The first charging stations in the Baltics were opened in 2023 and their number in Finland is increasing.

• Neste is a participant in Finland's nationwide energy-saving campaign "Down a Degree" and is reducing energy consumption at its facilities.

• Households in Finland are encouraged to recycle Christmas waste fats into renewable diesel by the Ham Trick campaign.

5.3. Public Administration in Finland

The public administration system of Finland consistently scores the most effective in the EU. There are many features of the public administration system of Finland that contribute positively to happiness of its people. A few examples will be given from this system, based on The 2023 Country Report of the European Commission on Finland.

5. 3. 1. Health System: An efficient health system being a prerequisite for a sustainable economy and society also provides the address for a healthy population. In Finland, life expectancy is higher than the other nations in the EU as a whole. Treatable mortality being low is an indication of an overall effective health system. Health expenditure in Finland is slightly less than the EU average and nearly 80% of it was publicly funded in 2020. Although spending on inpatient care, pharmaceuticals and medical devices is below the EU average, spending on outpatient care is lot higher than the EU average.

Finland Parliament has accepted a proposal on tightening the guarantee to better access to nonurgent primary care through reducing the waiting time from the current 3 months to 7 days. The national target of Finland is 100 000 fewer people at risk of poverty or social exclusion by 2030, out of which at least one third should be children.

5.3.2. The Housing First policy:

In 2008 Finland implemented the "Housing First" policy which was an innovation in poverty eradication with a focus on addressing homelessness. By means of this policy the number of homeless people has decreased from more than 8,000 to 3,686 in 2022. This is a reduction of 50% in 14 years. Within the framework of policy, homeless people are provided access to long-term housing as opposed to the more common temporary shelters. These rental units are financially viable and supply the homeless with crucial social support, such as better employment opportunities.

5. 3. 3. Digitalization: A resilient and competitive economy is based on digital transformation. The focus of Finland's Recovery and Resilience Plan will be on the digital transformation of employment, migration, health and social public services that will involve the development of an operating model through which the public companies and public administration can share data more systematically.

In Finland, the number of citizens interacting with government via the internet is one of the highest in the EU and it goes on increasing. Other channels like telephone and service points are encouraged by the government to make services more accessible. E-government, open data and portal maturity of Finland is well above the EU average.

5. 3. 4. Business Incentives: A good environment for doing business exists in Finland. In world competitiveness rankings organized by The International Institute for Management Development, Finland was placed 8th, up three places from the previous year. The improvement is caused by the advances in government and business. From the point of accessing to finance which is a critical issue especially for Small and Medium Enterprizes (SMEs), Finnish SMEs experienced relatively favorable financing conditions. Finland also possesses one of the best corruption perception scores.

6. Conclusion and Recommendations

Achieving SDG goals and targets necessitates hard work on part of the nations and success in this endevour is determined by the joint efforts of governments, business and civil society. The purpose of the research was to find out whether the high world happiness record of Finland in seven consecutive years can be associated with the nation's commitment to Sustainable Development Goals.

It has been found that Finland shows a consistent effort to achieve SDGs. According to European Commission's 2023 Country Report, Finland performs well on all dimensions of sustainability, namely environmental sustainability, fairness, macroeconomic stability and productivity.

Finland's performance is above the EU average on 12 out of the 16 SDGs for which there is adequate data. Finland is also showing improvement on 3 goals where it performs below the EU average: SDGs 7, 12 and 4 (affordable and clean energy, responsible production and consumption, life below water).

Considering the estimate of SDG Index that only about 16% of the SDG targets are on track to be achieved and 84% of the SDG targets either show limited progress (insufficient to achieve the target by 2030) or even a reversal of progress, Finland's performance is good, possibly explaining the high happiness record of her citizens.

After all, what are the basics of happiness? Health should be mentioned in the first order since money can be far from being adequate for achieving good health. A poor health system in a nation is quite possible to lead to unhappy citizens. It has been discussed that life expectancy in Finland is higher than the other nations in the EU as a whole, health expenditure in Finland is slightly less than the EU average and nearly 80% of it was publicly funded in 2020.

Finland's per capita income is among the highest in Western Europe. Of course money is important to satisfy

all levels of needs starting from the lowest but most urgent to the higher level needs that affect the happiness of people positively.

MNCs in Finland provide a good support to the government in its challenging work to improve SDG performance. The 2023 Annual Report of Neste, one of these MNCs, and the firm's activities for SDG 11 (Sustainable cities and communities) - the goal Finland is moving away from the target – is a good example of business support in achieving SDGs. Governments are advised to provide an environment for businesses without limitations and thus possibly get the benefit of innovation and flourish their citizens' happiness.

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Food Allergies Among Adolescents and Adults: A Review

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Abstract

Introduction and Aim: An allergy is an overreaction of the immune system to a substance that is typically harmless to most people. In the last 10 years, there has been an increase in allergic diseases, including food allergies. Food allergies affect more than 1% to 2% and less than 10% of the population. Data on food allergy prevalence rates in Africa is limited. Prevalence data are important in helping to identify relationships between risk determinants and food allergies. The article reviewed the prevalence of food allergies among adolescents and adults.

Materials and Method: Several literatures on different food allergens, symptoms experienced from food allergies and means of treatment among adolescents and adults were critically reviewed. International initiatives such as the InformAll database and EuroPrevall, a European Union funded project which focused on the prevalence, cost and basis of food allergy in Europe have provided very useful data on prevalence rates across Europe. Results: The review showed that diet elimination method comprising of an individualized allergen avoidance management plan is the most suitable treatment. This have helped to raise awareness of food allergies which has resulted in national and international legislation and recommendation for the identification of priority allergens when present in foods

Conclusion: It will be increasingly important to understand and assess the interplay between food allergy and nutrition to protect and identify appropriate sources of foods for sensitized sub-populations. The identification of susceptibility to food allergens plays an important role in the prevention and treatment of allergic diseases.

Keywords: Immune system, allergies, food, adolescents and adults

1. Introduction

With allergies, the body's immune system treats the substance, called an allergen, as an invader and reacts inappropriately – resulting in harm to the person. (Stephen, 2001)

For most people with allergies, the first exposure to an allergen prompts their immune system to produce an antibody called immunoglobulin E (IgE). With each subsequent exposure, their body produces more IgE, which attaches itself to two types of cells in the body– mast cells and basophils. When the allergen attaches to the IgE, the mast cells and basophils are activated to release histamine and other chemicals to defend against the allergen "invader." The release of these chemicals causes allergic reactions, as the person's body attempts to rid itself of the allergen "invader."

Common allergies include those to food and airborne allergens such as pollen, mold, dust mites and animal dander, urine and saliva. Allergies can be seasonal, like pollen or certain molds, or year-round, like dust mites. Different types of allergens are more prevalent in different parts of the country or the world.

Food allergies among adolescent students present a uniquely challenging situation, since many young adults become independent and unsupervised during these years. They are now responsible for their own health, including deciding which foods to eat and whether to take or carry medications. Young adults are more likely to take risks with regard to food allergies, placing them at higher risk for severe food-induced allergic reactions (Sampson et al., 2006).

Many adolescent students are young adults who encounter numerous health risks along the path to adulthood, many of which affect quality of life and life expectancy.

The elimination of essential foods has the potential to reduce the intake of vital nutrients, which in turn can affect overall health. They are most likely to be at nutritional risk if they are avoiding certain foods due to suspected or actual food allergies. The identification of susceptibility to food allergens plays an important role in the prevention and treatment of allergic diseases.

Food allergies consist of both immunoglobins E (IgE) mediated reactions. As well as non-IgE mediated reactions. IgE mediated reactions typically involve the 14 major food allergens (celery, gluten, crustaceans, eggs, fish, lupin, milk, molluscs, mustard, nuts, peanuts, sesame seeds, soya and sulphites). They can be characterized by a rapid onset of symptoms, whereby adverse reactions, typically hives, vomiting and anaphylaxis, occur within minutes of ingesting the offending food, with abdominal discomfort, vomiting and diarrhea, the most severe of symptoms. A classic example of this for this type of reaction would be coeliac disease (Nowak-Wegrzyn et al., 2015). It is understood that both IgE and non-IgE mediated reactions, can both cause adverse immune responses to certain foods. However, with IgE mediated reactions responsible for a staggering 90% of all food allergies worldwide, it is clear that this particular type of reaction, with its potentially life-threatening properties, is perhaps the most feared (Manea et al., 2016).

Table 1: Food allergy prevalence of adolescents and adults in different countries.

Country	Year of study	Method of diagnosis	Age	Prevalence of food allergy (%)	Reference
USA	2015-2016	Self-reported	>18 years	10.8	Gupta et al. (2019)
Canada	2010	Self-reported	>18 years	6.6	Soller et al. (2012)
U.K	2002-2003	Skin prick tests and oral food challenges	11-15 years	2.3	Pereira et al. (2015)
Australia	2002	Medical history and skin prick testing	20-45 years	1.3	Woods et al. (2002)
India	2005-2010	Serum Specific IgE	20-54 years	1.2	Mahesh et al. (2016)
Kuwait	2016-2016	Self-reported	17-30 years	12	Ali (2017)

Source: Zainal (2019)

2. Food allergy in Africa

Data on food allergy prevalence rates in Africa is limited. Approximately 10% of 14,000 patients of all ages referred to the only specialist allergy clinic in Harare, Zimbabwe, in the 5-year period from September 1997 to September 2002, were reportedly diagnosed with food allergies. Wood et al. (2002) conducted a study of 50 allergic patients in Zimbabwe for the presence of IgE antibodies to 20 food allergen extracts. Apple (24%), tomato (24%), soy (22%), crab (22%) and peanut (20%) were the most frequent detected food allergens.

Using skin prick tests, Lee (2017) reported 5% prevalence rate of food allergies in a cross-sectional study of 211 urban high school black children of Xhosa ethnicity in South Africa. In Ghana, a study of food allergy in 1,407 school children found 11% of children reporting adverse reactions to foods, and 5% of 1,431 children showed a positive skin pricking test reaction mostly directed against peanut and pineapple (Osborn et al., 2011). In another study, life prevalence of self-food allergy in Maputo, Mozambique was 19.1% with seafood (54.8%), meat (13.0%) and fruits and vegetables (13%) being the most frequently reported allergenic foods. (Gruptal e tal., 2019). Other exotic foods such as the mopane worm, a high protein delicacy, consumed in some individuals. (Okezie et al., 2010) also has similar characteristics.

More recently, Hossny et al. (2011) conducted a study of 100 students in Cairo (Egypt) diagnosed to have allergic reactions and found positive skin prick test with peanut extract in seven children (7%). Specific IgE results of these ranged from KUA/L. The 7 children sensitized to peanut had positive family history of allergic diseases. Six of the seven children consented to oral challenge studies and they were confirmed to have peanut allergy. Of the other children, 10 had confirmed allergy to other foods including egg allergy in 2, fish in 3, cow's milk in 2. Sesame in 1. Banana in 1, and prunes in 1. Nine of these children were not sensitized to peanut, however one of them was sensitized to both peanut and banana (Hossny et al., 2010). In their conclusion, the authors stated that peanut allergy in Egypt is underestimated and that sensitization rates may be even higher

than previously thought.

3. Prevention of food allergy

Allergy results when there is a breakdown in the normal 'tolerance' mechanisms, which leads to inappropriate and detrimental immune responses to normally harmless substances, including food allergens such as cow's milk protein, eggs, nuts, or shellfish (Gruptal et al., 2019)

Nutrition plays a key role in the development, maintenance, and optimal functioning of immune cells. Nutrients, such as zinc and vitamin D and nutritional factors, such as pre and probiotics, can influence the nature of an immune response and are important in ensuring appropriate functioning of the immune system.

The cornerstone of the nutritional management of food allergies is an individualized allergen avoidance management plan. Elimination diet should be supervised and monitored to a degree similar to that for drug treatment and the need for continued dietary elimination should be reviewed on a regular basis. However Cross-reactivity exists amongst various food items and as such, those with food allergies may also need to avoid related foods. For example, an individual who is allergic to shellfish, will most likely need to avoid the entire food group, due to high rates of cross reactivity (Abraham et al., 2018). Likewise, both cow's milk and goat's milk contain a similar protein structure, which forces the immune system to associate them with each other. Hence, those who are diagnosed with a cow's milk allergy, will 90% of the time also be allergic to goat's milk (Caffarelli et al., 2010). However, the issue is that whilst for some food allergic individuals the need to avoid related food groups is a prerequisite of safety, for others, inappropriate dietary restrictions are unnecessary and will definitively provoke a nutritional imbalance.

4. Conclusion

This review provides insights into the prevalence of food allergies, sources, prevalence and prevention among adolescents and adults in different countries. It was also concluded that food allergen elimination is a major means of treating food allergy reactions.

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Space Travel and Nutrition

Uzay Yolculuğu ve Beslenme

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Özet

Uzay besini, astronotlar tarafından kullanılmak amacıyla özel olarak formüle edilen ve üretilen yiyecekleri ifade eder. Uzay yolculuğu esnasında amaç sadece besin ihtiyacını karşılamak değildir, aynı zamanda astronotların vücutları üzerindeki olumsuz etkileri oldukça aza indirebilmek için yeterli ve dengeli miktarda beslenmeyi sağlamaktadır. Uzay yolculuğu sırasında astronotlar üstündeki oksidatif stresi, kemik ve kas kaybını önlemede beslenmenin rolü büyüktür. Dengeli beslenme için uzay ortamında besin tüketimiyle ilgili tüm riskleri önlemek ve uzay koşullarında besin ögelerini yeterli miktarda almak önemlidir. Yetersiz enerji alımı, enfeksiyonlara ve buna bağlı olarak hastalıklara karşı duyarlılığı arttırabilir. Aynı zamanda vücudun ağırlık kaybındaki artış, fiziksel performansta azalma ve artan yorgunluk ile ilişkili olabilir. Uzun süren uzay görevlerinde astronotların tüketmesi için besinler özel tasarımlar ve ambalajlarla hazırlanır. Astronotların tükettikleri besinler alışılmış olanlardan önemli ölçüde farklıdır. Bu besinler, temel olarak dünya mutfağına ait olsa da ambalaj tasarımı, mikro yerçekimi ortamında tüketilen besinleri içerdiği için standart besin ambalajlarından ayrılır. Besinlerin korunması ve paketlenmesinin kolay olması nedeniyle esnek ambalajlar kullanılır. Özetle, uzay yolculuğu gerek fizyolojik gerekse psikososyal durumu etkilemesi nedeniyle bireylerin ortam şartlarına bağlı olarak besin ögesi ihtiyaçları değişmektedir. Bu doğrultuda üretilen besinlerin uzay koşullarına dayanabilecek formda hazırlanması ve mürettebatın duyusal ve fizyolojik ihtiyaçlarının karşılanması göz önünde bulundurulmalıdır. Literatürde uzay besin teknolojisi alanında çok sayıda çalışma yapılmasına rağmen, bu besinlerin gereksinme düzeyini karşılama durumları, paketlenmesi ve tüketilebilir formları hakkında sınırlı sayıda çalışma bulunmaktadır. Bu bildirinin amacı, uzay yolculuğu sırasında ortaya çıkan fizyolojik değişiklikler, uzaydaki besin ihtiyacını ve uzay besinlerinin hazırlanmasını değerlendirmektir. Çalışma için geçmişten günümüze uzay beslenmesi ile ilgili yapılan bilimsel makale ve derlemeler incelenmiştir. Literatür taraması sonuçları derlenmiştir.

Anahtar Kelimeler: Beslenme, uzay besini, uzay yolculuğu.

Abstract

Space food refers to food specially formulated and produced for use by astronauts. During space travel, the aim is not only to meet the nutritional needs, but also to ensure adequate and balanced nutrition in order to minimise the negative effects on the astronauts' bodies. Nutrition plays a major role in preventing oxidative stress, bone and muscle loss on astronauts during space travel. For balanced nutrition, it is important to prevent all risks related to food consumption in the space environment and to take nutrients in sufficient amounts under space conditions. Inadequate energy intake may increase susceptibility to infections and consequently diseases. It may also be associated with increased body weight loss, decreased physical performance and increased fatigue. Foods are prepared with special designs and packaging for astronauts to consume during long space missions. The foods consumed by astronauts are different from the usual ones. Although these foods basically belong to the world cuisine, the packaging design differs from standard food packaging because it contains foods consumed in a microgravity environment. Flexible packaging is used because of the ease of preservation and packaging of the food. In summary, since space travel affects the physiological and psychosocial status, the food produced for space must be prepared in a form that can withstand space conditions and meet the needs of the crew. Although there are studies in the field of space food technology in the literature, there are limited number of studies on the requirements of these foods, packaging and consumable forms. The aim of this paper is to evaluate the physiological changes that occur during space travel, the nutritional needs in space and the preparation of space nutrients. For the study, scientific articles and reviews on space nutrition from past to present were examined. The results of the literature review were compiled.

Keywords: Nutrition, space food, space travel

1. Giriş

Uzay beslenmesi, mürettebatın uzayda sağlıklı bir şekilde kalması için yeterli ve dengeli miktarda besinleri tüketmesidir. Uzay besini ise astronotlar tarafından kullanılmak amacıyla özel olarak formüle edilen ve üretilen yiyecekleri ifade eder (Oluwafemi et al., 2018). Beslenmenin tüm insan keşiflerinde olduğu gibi uzay keşiflerinde de önemli bir rol oynadığı bilinmektedir. İnsanlar yıllardır uzayla meşgul olmaktadır ve olağanüstü başarılarla tamamlanmış birçok görev vardır. Bu görevleri yerine getirebilmek için astronotların besin ihtiyaçlarının giderilmesi oldukça önemlidir. Uzay yolculuğu esnasında amaç sadece besin ihtiyacını karşılamak değildir, aynı zamanda astronotların vücutları üzerindeki olumsuz etkileri oldukça aza indirebilmek için yeterli ve dengeli miktarda beslenmeyi sağlamaktadır. Uzay yolculuğu sırasında astronotlar üstündeki oksidatif stresi, radyasyona maruz kalmayı, kemik ve kas kaybını önlemede beslenmenin rolü büyüktür (Deveci ve Deveci, 2018). Sağlıklı ve besleyici bir diyetin uzay yolculuğu için en önemli faktörlerden birisi olduğu söylenebilir. Astronotların uzayda tüketeceği gıdalar dikkatli bir şekilde geliştirilmelidir. Örnek olarak, besinler sıfır yer çekiminde değişmeden kalacak şekilde ve uzun bir raf ömrüne sahip olarak üretilmelidir (Ay ve Özdemir, 2023). Uzay araştırmaları sayesinde yeni teknolojiler ve tekniklerle gıda muhafazası oldukça iyileşmiştir. Bu alandaki yeni gelişmeler uzayda kalma süresini uzatmaktadır. Ambalajlı ürünler paketlenmesi ve tüketilmesi basit olduğu için ambalaj malzemeleri uzay besinlerinin muhafazasında etkin bir rol oynar. Ek olarak, uzayda yer çekimi olmadığından astronotlar kalsiyum, nitrojen ve fosfor kaybeder. Kaybedilen bu minerallerin besin yoluyla geri alınması gerekmektedir. Dolayısıyla, uzay besinleri besleyici, hafif, kolay sindirilebilir, lezzetli, servisi hızlı, kolay temizlenebilir, kompakt fizyolojik uygunluğa sahip ve minimum hazırlıkla yenilebilir olmalıdır (Muslu, 2021). Bu bildirinin amacı, uzay yolculuğu sırasında ortaya çıkan fizyolojik değişiklikler, uzaydaki besin ihtiyacını ve uzay besinlerini değerlendirmektir.

2. Uzay Uçuşunun Sebep Olduğu Fizyolojik Değişiklikler

Astronotlar için birincil risk faktörü uzay uçuşu radyasyonudur. İyonlaştırıcı radyasyona maruz kalmak reaktif oksijen türlerinin üretiminde artış gösterir ve serbest oksijen radikalleri üretimiyle vücut savunması antioksidan kapasitesi arasında dengesizlik oluşturarak oksidatif strese sebep olur. Bununla birlikte mikro yer çekimi nedeniyle vücut sıvısı yukarıya doğru yani bacaklardan ve karından kalbe ve kafaya doğru dağılım göstermektedir. Bu durum kardiyovasküler sistem, vestibüler sistem ve iskelet-kas sisteminin yanı sıra oftalmik ve endokrin değişiklikleri de içeren çok sayıda sistem ve organlarda kondüsyon bozukluğuna sebep olur (Chaloulakou et al., 2022).

Üzerinde daha az çalışılmakta fakat temel faktörler arasında tecrit ve hapsetme de yer almaktadır. Sürekli olarak kapalı ve izole bir ortamda bulunmak sosyal gerginliklere, kaygı ve depresyona aynı zamanda uyku bozukluklarına sebep olabilir. Mürettebat görevi bırakma düşüncesine girseler bile uzay aracını terk edemezler fakat bu durum yüksek eğitimli olmalarından ötürü nadir görülen bir olaydır. Bu tip faktörler nörobilişsel değişikliklerden ve anormal seviyedeki stres hormonundan yorgunluğa, uyku bozukluklarına ve immünomodülatör değişikliklere kadar patofizyolojik belirti ve semptomları ortaya çıkarabilir (Chaloulakou et al., 2022; Muslu, 2021).

3. Astronotların Besin İhtiyaçları

Uzun süreli uzay görevlerinde, astronotların sağlığını korumak için beslenme büyük bir öneme sahiptir. Besinler, sadece temel besin ihtiyaçlarını karşılamakla kalmaz, aynı zamanda uzayda maruz kalınan zararlı etkilere karşı korunmada ve astronotların psikolojik iyilik hallerini sürdürmelerine yardımcı olur. Uzay beslenmesi, vücut sistemlerinin bakımı üzerinde kritik bir rol oynar, temel besinlerin sağlanmasının yanı sıra bağışıklık, endokrin ve kas-iskelet sistemleri gibi sistemleri destekler. Önceki uzay görevlerinden elde edilen sağlık verileri, gelecekteki görevler için uzay yiyeceklerinin tasarımında iyileştirmeler yapılmasına yol açmıştır. Bu, özellikle besin eksikliklerinin potansiyel sağlık sorunlarına yol açma riskini azaltmayı amaçlamaktadır (Muslu, 2021; Pandith et al., 2023).

4. Uzay Yemeği ve Gereksinmeleri

Dengeli beslenme için uzay ortamında besin tüketimiyle ilgili tüm riskleri önlemek ve uzay koşullarında makro ve mikro besin ögelerini yeterli miktarda almak önemlidir. Bu hedefe ulaşmak için gıda endüstrisi uzmanlarının iş birliğiyle fizyologlara, kimyagerlere ve malzeme bilimcilerine ihtiyaç vardır. Gelişen gıda trendleri doğrultusunda, araştırmacılar gıdaları zenginleştirmek için yeni yollar ve içerikler araştırmaktadır. Uzun süreli görevlerin planlanmasında, çoğu yiyeceğin uzay istasyonunda üretilmesi ve kapalı döngü yaşam destek sistemlerinin düzenlenmesi büyük önem taşır (Ay and Özdemir, 2023). Fakat mürettebattaki işleyişin önemli ölçüde psikolojik ve sosyal yönleri göz önüne alındığında, astronotlar dünyadan gelen besin stoklarını kullanmak istemeleri muhtemeldir. Uzay besini geliştiricilerinin amacı sadece uzay ekibinin psikolojik rahatlığını sağlamak değil, aynı zamanda fizyolojik riskleri de önlemek üzerine odaklanmalıdır. Bu, uzun süreli uzay görevlerinin başarıyla tamamlanabilmesi için kapsamlı bir beslenme stratejisinin benimsenmesini gerektirmektedir Bu doğrultuda üretilen uzay besinleri mürettebat üyelerinin ulusal, bölgesel ve kişisel kimlikleri ile uyumlu; aynı zamanda yeterli besin ögesi içeriğine sahip olmalıdır (Pandith et al., 2023; Tülüce ve Hızlısoy, 2022).

Uzay uçuşları sırasında, besin alımının genellikle düşük olduğu gözlemlenmiştir. Bu durum, vücut kütlesinde, kemik ve yağsız dokularda kayıp riskini artırabilir. Uzun süreli uçuşlarda %4-5'e varan vücut kütlesi kayıpları yaygındır ve bu, yetersiz beslenme ve enerji dengesizliğinden kaynaklanabilir. Altı aylık süren görevden sonra vücut ağırlığının uçuştan önceki seviyesinin birkaç kilogram altında olması normal kabul edilir (Pittia and Heer, 2022). Uzayda enerji alımını pozitifte sürdürmek kritik öneme sahiptir. Dünya şartlarındaki enerji alımının negatifliği vücutta dengelenebilir fakat uzay şartlarında dengesizlik oluşturabilir. Sürekli hale gelen enerji eksikliği, enfeksiyonlara ve buna bağlı olarak hastalıklara karşı duyarlılığı artırabilir. Aynı zamanda vücudun ağırlık kaybındaki artış, fiziksel performansta azalma ve artan yorgunluk ile ilişkili olabilir (Tang et al., 2021).

Uzay uçuşlarında mikro yerçekimi iskeletteki yükün boşalmasına neden olur. Bu durum, idrar içerisinden kalsiyumun atılım artışına ve böbrek taşı oluşum riskinde artışa yol açabilir. Uzay uçuşlarında vücudun kemik-mineral kayıp oranı oldukça yüksektir. Kemiğin yoğunluk kaybındaki bu artış ciddi durumlarda osteoporoz, beraberinde felce neden olabileceğini bildirilmiştir. Bu sebeplerden dolayı, kalsiyum ve kemiğin metabolizmaları, uzay yolculukları sırasında büyük bir endişe teşkil etmektedir (Venir et al., 2007). Kemik erimesindeki bu kayıpların önüne geçmek amacıyla çeşitli yöntemler kullanılmıştır. Uzay yolculuklarıyla alakalı bir araştırmada, yeterli D vitamini sağlanmasıyla beraber direnç egzersizlerinin, Uluslararası Uzay İstasyonu'ndaki astronotlarda kemik mineral kayıplarında azaltma sağlayabileceği belirtilmiştir (Ay ve Özdemir, 2023; Muslu, 2021).

Uzay uçuşlarında, erkekler ve kadınlar için önerilen günlük sodyum miktarı 1,5-2,3 gram arasında değişmektedir. Sodyum alımının yüksek olması, geceleri görüş sorunlarına ve idrarla alınan kalsiyumla bağlantılı böbrek taşında oluşum riskini artırabilir. Bu sorunları önlemek için uzay gıdalarında siyah tuz veya kaya tuzu kullanılabilir. Bu tuzlar, deniz tuzuna göre daha düşük sodyum içerir ve böylece astronotların sağlığını korumaya yardımcı olabilir. Bu şekilde, uzay seyahatleri sırasında beslenme stratejileri, astronotların vücutlarını en iyi şekilde destekleyerek uzun vadeli sağlık etkilerini azaltmaya yönelik önemli bir rol oynayabilir (Barone et al., 2020).

Uzay uçuşlarının ilk günlerinde hematolojik olarak değişiklikler meydana gelir, 10-14 gününde "uzay uçuşu anemisi" adı verilen bir fenomen gözlemlenir. Bu dönemde kırmızı kan hücresi kütlesinde %10-15 oranında bir kayıp meydana gelebilir. Kırmızı kan hücrelerindeki bu azalma, artan demir depolarını ve serum ferritin seviyelerini beraberinde getirir. Bu durum, transferrin reseptörlerinde azalma ve serum demirinde artışla birlikte görülür. Aşırı vücut demiri, oksidatif etkileri nedeniyle doku hasarına yol açabilir. Uzay görevlerinde kadın ve erkeklere günlük 8-10 mg diyet demir alımı önerilmektedir (Ay ve Özdemir, 2023; Muslu, 2021).

5. Uzay Yemeklerinin Üretilmesi ve Ambalajlanması

Uzun süren uzay görevlerinde astronotların tüketmesi için özenle planlanan çeşitli yiyecekler, özel tasarımlar ve ambalajlarla hazırlanır. Fakat, astronotların tükettikleri besinler alışılmış olanlardan önemli ölçüde farklıdır. Bu besinler, temel olarak dünya mutfağına ait olsa da ambalaj tasarımı, mikro yerçekimi ortamında tüketilen besinleri içerdiği için standart yiyecek ambalajlarından ayrılır. Bu nedenle besinlerin korunmasında ve paketlenmesinde esnek ambalajlar kullanılır. Esnek ambalajlar, kullanımı kolay olması ve bir çöp sıkıştırıcıya atıldığında az yer kaplaması nedeniyle uzay mekiği içinde tercih edilen paketleme türüdür (Cahill and Hardiman, 2020; Jiang et al., 2020; Tülüce ve Hızlısoy, 2022).

Şekil 1. Dondurarak kurutulmuş bir uzay besini (Jiang et al., 2020).

Özellikle sıvılar veya ekmek kırıntıları gibi maddeler, sıcak sıvıların mekik içinde istenmeyen durumlar yaratma potansiyeli nedeniyle özel bir şekilde muhafaza edilmelidir. Bu nedenle, içecekler genellikle toz halinde paketlenir ve özel tüplerle su eklenerek tüketilmeye hazır hale getirilir. Örnek bir uzay besin Şekil 1'de verilmiştir. Sandviçler için kullanılan unlu tortillalar gibi ürünler ve sıvı formdaki çeşniler (hardal, ketçap, mayonez), uzay yolculukları için özel tasarlanmış standart formlarda sunulmaktadır. Bununla birlikte, besinlerin raf ömürleri uzun olmalıdır. Tüketiminin kolay olması için ambalaj ve besin hafif olmalıdır. Nesneleri hareket ettirmek oldukça zor olduğundan yiyecekler kolay hazırlanabilir ve kolay atılabilir formda olmalıdır (Cahill and Hardiman, 2020; Jiang et al., 2020; Tülüce ve Hızlısoy, 2022).

6. Sonuç

Sonuç olarak, uzay yolculuğu mürettebatın gerek fizyolojik gerekse psikososyal durumunu etkileyen bir durumdur. Bu nedenle bireylerin ortam şartlarına bağlı olarak besin ögesi ihtiyaçları değişmektedir. Uzay ortamına ve mürettebatın fizyolojik süreçlerine uygun besinlerin hazırlanmasına yönelik çalışmalar yapılmaktadır. Bu doğrultuda üretilen besinlerin uzay koşullarına dayanabilecek formda hazırlanması ve mürettebatın duyusal ve fizyolojik ihtiyaçlarının karşılanması göz önünde bulundurulmalıdır.

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Investigation of Serum Superoxide Dismutase Levels in Mice with Disrupted Circadian Rhythms

Sirkadiyen Ritimleri Bozulmuş Farelerde Serum Süperoksit Dismutaz Düzeylerinin Araştırılması

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Özet

Giriş ve Amaç: Vücudun biyolojik saati olarak da bilinen sirkadiyen ritim, 24 saatte bir tekrarlanan doğal uyku-uyanıklık döngüsüdür. İnsan vücudunda önemli görevleri olan gastrointestinal sistem,sirkadiyen ritim ile yakından ilişkilidir. Sirkadiyen ritim bozulmasının inflamasyon parametrelerinin artışına sebep olduğu ayrıca vücutta oksidatif stresi tetikleyerek de süperoksit dismutaz gibi oksidatif stres belirteçlerinin seviyelerinin artışına sebep olduğu bilinmektedir. Süperoksit dismutaz hücrede serbest radikallere karşı temel savunma hattını oluşturur. Burdan yola çıkılarak çalışmamızda deney hayvanlarında aydınlık/karanlık döngüsünün iki haftada bir tersine çevrilmesiyle ön görülen sirkadiyen ritim bozulmasının süperoksit dismutaz seviyelerine etkisinin araştırılması amaçlanmıştır.

Yöntem: Çalışmada 16 adet 8 - 10 haftalık BALB/c cinsi erkek fare kullanılmıştır. Fareler kontrol (KON, n = 8) ve sirkadiyen ritimi bozulmuş (SRB, n = 8) olarak iki gruba ayrılmıştır. Deney süresince farelerin tamamı standart pelet yem ile beslenmiştir. KON grubundaki farelere 16 hafta boyunca herhangi bir müdahale yapılmamış ve standart çevre koşullarında barındırılmıştır. SRB grubundaki farelerin ise deney boyunca iki haftada bir aydınlık / karanlık döngüsü değiştirilmiştir. Deney sonunda farelerden kan alma işlemi yapılmış ve serum örnekleri elde edilmiştir. Süperoksit dismutaz konsantrasyonları üreticinin tavsiyelerine göre ticari olarak temin edilebilen enzim bağlantılı immünosorbent test kitleri kullanılarak tespit edilmiştir.

Bulgular: KON ve SRB gruplarının ortalama süperoksit dismutaz konsantrasyonları sırasıyla 23,7 ± 3,14 ng/mL ve 23,9 ± 3,61 ng/mL olarak belirlenmiştir. SRB grubunun süperoksit dismutaz konsantrasyonu daha yüksek olsa da bu artış istatistiksel olarak anlamlı düzeyde bulunmamıştır (p = 0,516).

Sonuç: Aydınlık/karanlık döngüsünün değiştirilmesi sonucu oluşan sirkadiyen ritim bozulmasının serum süperoksit dismutaz seviyelerindeki artışı istatiksel olarak anlamlı düzeyde bulunmamıştır.

Anahtar Kelimeler: Sirkadiyen ritim, gastrointestinal sistem, süperoksit dismutaz

Abstract

Introduction and Aim: Circadian rhythm, also known as the body's biological clock, is the natural sleep-wake cycle that repeats every 24 hours. The gastrointestinal system, which has important functions in the human body, is closely related to the circadian rhythm. It is known that circadian rhythm disruption causes an increase in inflammation parameters and also triggers oxidative stress in the body, leading to increased levels of oxidative stress markers such as superoxide dismutase. Superoxide dismutase forms the main line of defense against free radicals in the cell. Based on this, our study aimed to investigate the effect of circadian rhythm disruption, which is predicted by reversing the light/dark cycle every two weeks, on superoxide dismutase levels in experimental animals.

Method: Sixteen 8 - 10 week old BALB/c male mice were used in the study. Mice were divided into two groups as control (CON, n = 8) and circadian rhythm disrupted (SRD, n = 8). All mice were fed with standard pellet feed during the experiment. The mice in the KON group were not intervened for 16 weeks and were housed in standard environmental conditions. In the SRB group, the light/dark cycle was changed every two weeks throughout the experiment. At the end of the experiment, blood was obtained from the mice and serum samples were obtained. Superoxide dismutase concentrations were determined using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer's recommendations.

Result: The mean superoxide dismutase concentrations of the CON and SRB groups were 23.7 ± 3.14 ng/mL and 23.9 ± 3.61 ng/mL, respectively. Although the superoxide dismutase concentration of the SRB group was higher, this increase was not statistically significant (p = 0.516).

Conclusion: The increase in serum superoxide dismutase levels due to circadian rhythm disruption caused by changing the light/dark cycle was not statistically significant.

Keywords: Circadian rhythm, gastrointestinal tract, superoxide dismutase

1. Giriş

Sirkadiyen ritimler, neredeyse vücut hücrelerinin sayısı kadar saatten oluşan karmaşık bir içsel zamanlama sistemidir. Uyku-uyanıklık ritmi gibi çeşitli fizyolojik ve davranışsal süreçleri düzenlerler (Geoffray ve ark., 2017). Sirkadiyen ritim olarak da bilinen biyolojik ritimde bir takım bozulmalar meydana gelerek; stres bozukluğu, fizyolojik bozukluklar, davranışsal, duygusal, sosyal ve zihinsel bozuklukları meydana getiren bir olgu haline gelmektedir. Bu bozukluklar beraberinde obezite, insülin direnci, diyabet, kardiyovasküler hastalıklar ve psikolojik sorunları meydana getirmektedir (Uslu, 2021). Bu hastalıkların temelinde toplumda sıkça karşılaştığımız ekonomik kaygı, uyku düzensizliği, vardiyalı çalışma ve jet lag etkisi gibi nedenler sirkadiyen ritim sisteminin bozulmasında etkin rol oynamaktadır. Bahsedilen hastalıkları önlemek için vücutta etkin mekanizması bulunan ve sirkadiyen ritim ile yakından ilişkili olan gastrointestinal sistemindeki değişiklikleri incelenerek bu hastalıkların önüne geçilmesi hedeflenmektedir. İnsan gastrointestinal sisteminin çeşitli hastalıkların patogenezinde ve tedavisinde rol aldığı son yıllarda ortaya çıkmıştır. İnsan vücudunun sağlıklı bir yaşam sürdürebilmesi için sağlıklı bir gastrointestinal sisteme sahip olması gerekmektedir (Herrama ve ark., 2017). Sirkadiyen ritim bozulması, gastrointestinal sistemde bir takım olumsuz değişikliklere neden olmaktadır (Pehlivanlar ve ark, 2019). Bu değişikliklerle birlikte vücutta iltihaplanma olarak da bilinen inflamasyon, herkeste meydana gelen ve bağışıklık sisteminin vücudu çeşitli hastalık veya yaralanmalara karşı korumak amacıyla bir tepki oluşturur. Vücutta birçok iyileşme sürecinin temelinde inflamasyon yer almaktadır. Kronik inflamasyon zemininde gelişen hastalıklar arasında diyabet, romatizmal hastalıklar, inflamatuar bağırsak hastalığı, kalp damar hastalıkları, alerjiler, parkinson ve multiple skleroz gibi nörodejeneratif hastalıklar sayılmaktadır (Tanaka ve ark, 2014). Süperoksit dismutaz (SOD) hücrede serbest radikallere karşı temel savunma hattını oluşturur (Tanaka ve ark, 2014). Serbest radikaller özellikle mitokondriyal enerji üretim yoluyla sürekli olarak üretilir. Serbest radikallerin hücrede birikmesi oksidatif strese ve hücresel hasara neden olur (Erdoğan ve ark, 2022). Hücre reaktif oksijen türlerinde artışın nörodejeneratif, kardiyovasküler, diyabet ve böbrek hastalıkları gibi birçok hastalıkların patogenezinde rol oynadığı ifade edilmektedir. SOD'un hücresel hasarı önlemedeki rolü oldukça önemlidir (Aslankoç ve ark, 2019). Bu çalışmada, sirkadiyen ritmi bozulmuş farelerde serum SOD düzeylerindeki değişikliğin araştırılması hedeflenmiştir.

2. Yöntem

2.1. Deney Hayvanları ve Çalışma Düzeni

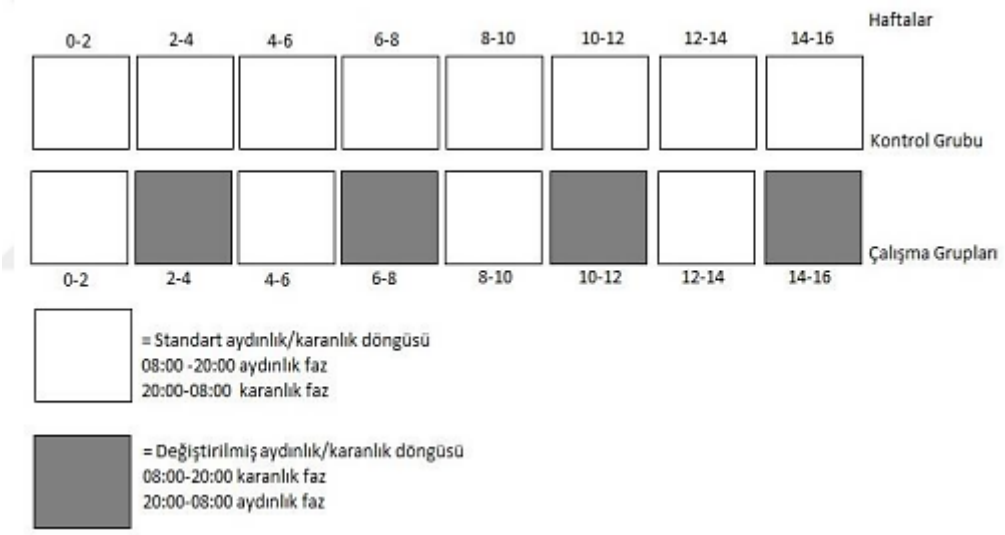
Bu çalışmada, 16 adet 8-10 haftalık BALB/c cinsi erkek fareler kullanılmıştır. Hayvanlara uygulanan tüm prosedürler Ondokuz Mayıs Üniversitesi Hayvan Deneyleri Etik Kurulu tarafından onaylanmıştır (Onay numarası: E68489742-604.01.03-97230, Tarih: 27.07.2021).Hayvanların refahı ve kullanımı için ARRIVE yönergeleri ve 3R kuralı (azaltma, yerine koyma ve iyileştirme) sıkı bir şekilde uygulanmıştır. Tüm hayvanlar kafeslere rastgele dağıtılmıştır ve herhangi bir işlemden önce kafesler rastgele seçilmiştir. Hayvanlar standart pelet diyetine ve normal musluk suyuna *ad libitum* erişimi olan paslanmaz çelik kapaklı ayrı polikarbonat kafeslerde barındırılmıştır. Çalışmanın başlangıcından sonlandırılmasına kadar olan süreçte hayvanlar her hafta 0.1 g'a duyarlı hassas terazi kullanılarak tartılmıştır. Adaptasyon periyodunun ardından hayvanlar kontrol grubu (KON) ve sirkadiyen ritimleri bozulmuş fareler (SRB) olarak rastgele iki gruba ayrılmıştır. Tüm gruplar standart diyetle toplam 16 hafta boyunca beslenmiştir.

- Kontrol grubu (KON) (n=8): 16 hafta boyunca herhangi bir müdahale yapılmayan grup.

- Sirkadiyen ritimleri bozulmuş fareler (SRB) (n=8): 16 hafta boyunca aydınlık karanlık döngüsü değiştirilerek sirkadiyen ritimleri bozulmuş grup.

2.2. Aydınlık/Karanlık Döngüsünün Değiştirilmesi

Kontrol grubu için 12 saatlik aydınlık/karanlık döngüsünde nem (55 ± %10) ve sıcaklık (22 ± 2 ◦C) sabit olacak şekilde çevresel koşullar ayarlanmıştır. Sirkadiyen ritimleri değiştirilecek olan grubun barındırılacağı oda çalışmaya başlamadan önce pencereleri siyah bantlarla ve perdelerle kapatılmıştır. Çalışma öncesinde odaya özel bir saat alınmış ve aydınlık-karanlık döngüsü deney süresi boyunca 15 günde bir değiştirilmiştir. Bu durumda 15 gün boyunca 12 saatlik aydınlık/karanlık döngüsünün ardından 15 gün boyunca 12 saatlik karanlık/aydınlık döngüsü uygulanmıştır. Aydınlık/karanlık döngüsünün değiştirilme protokolü Şekil 1'de gösterilmiştir.



Figür 1. Farelerin aydınlık/karanlık döngüsünün değiştirilme protokolü

2.3. Örneklerin Toplanması ve Biyokimyasal Analizler

Ötenazi işlemine başlamadan önce enjektabl (intraperitoneal) anestezikler kullanılmıştır ve farelerin derin anestezi altında kalması sağlanmıştır. Bacak çekme ve göz kapağı gibi refleksler tamamen kaybolduğunda kardiyak ponksiyon ile maksimum kan alma işlemi gerçekleştirilmiştir. Fareler daha sonra sakrifiye edilmiştir. Pıhtılaşmanın önlenmesi için kan örnekleri etilendiamintetraasetik asit (EDTA) eklenmiş tüplere konulmuştur. Örnekler 3000 rpm'de 10 dakika santrifüj edilerek serum elde edilmiştir. Serum örnekleri biyokimyasal analizlerin yapılacağı güne kadar -80°C dolapta muhafaza edilmiştir. SOD konsantrasyonları üreticinin tavsiyelerine göre ticari olarak temin edilebilen Mouse "Sandwich Enzyme-Linked Immune Sorbent Assay" (ELISA) kitleri kullanılarak serumda test edilmiştir. Biyokimyasal analiz sonuçları ELISA reader'da (Thermo Fisher Multiskan GO, Japan) (450 nm) okutularak değerlendirilmiştir.

2.4. İstatistiksel Analizler

Verilerin istatistiksel analizi SPSS programı (Versiyon 26.0) kullanılarak yapılmıştır. Veriler ortalama ± standart sapma olarak ifade edilmiştir. Sirkadiyen ritim değiştirilmesinin etkilerini değerlendirmek için "Bağım-

sız örneklemler t testi" kullanılmıştır. İstatistiksel olarak p<0,05 değeri anlamlı olarak kabul edilmiştir.

3. Bulgular

Çalışmada 16 adet erkek fare kontrol ve sirkadiyen ritimleri bozulmuş olarak iki gruba ayrılmıştır. İki grup da 16 hafta boyunca standart pelet diyet ile beslenmiştir. Yapılan biyokimyasal analizler ile farelerin serum SOD konsantrasyonları analiz edilmiştir. Buna göre KON ve SRB gruplarının ortalama SOD değerleri sırasıyla 23,7±3,14 ng/mL ve 23,9±3,61 ng/mL olarak belirlenmiştir. SRB grubunun SOD konsantrasyonu daha yüksek olsa da bu artış istatistiksel olarak anlamlı düzeyde bulunmamıştır (p=0,516).

Tablo 1. Deney gruplarının serum süperoksit dismutaz konsantrasyonlarının değerlendirilmesi

Parametre	KON (n=8) $\bar{X}$ ± SS Min-Max	SRB (n=8) $\bar{X}$ ± SS Min-Max	Toplam (n=8) $\bar{X}$ ± SS Min-Max	İstatistik	p
SOD (ng/mL)	23,7±3,14 (17,4-27,6)	23,9±3,61 (17,7-28,5)	23,8±3,27 (17,4-28,5)	0,444	0,516

KON: Kontrol grubu, SRB: Sirkadiyen ritimleri bozulan fareler, SOD: Süperoksit dismutaz, $\bar{X}$ ± SS: Standart sapma. Verilerin analizinde bağımsız örneklemler t testi kullanılmıştır.

4. Tartışma

Bu çalışma kapsamında kontrol grubunda ve aydınlık/karanlık döngüler değiştirilerek sirkadiyen ritimleri bozulmuş farelerde serum süperoksit dismutaz düzeyleri incelenmiştir. Sirkadiyen ritimler suprakiasmatik çekirdekte (SCN) tarafından kontrol edilen günlük ritimlerin tekrarlanması olayıdır (Akbay, 2020; Öney ve Balcı, 2021; Serrin ve Tek, 2019). Sirkadiyen ritmin başta ışık olmak üzere SOD, beslenme, sıcaklık, jet lag gibi parametrelere bağlı olduğu bilinmektedir (Öney ve Balcı, 2021). Hemen hemen tüm organizmalarda, fizyolojik ve davranışsal süreçler, sirkadiyen bir saat tarafından kontrol edilen yaklaşık 24 saatlik ritimler sergiler. Bu ritimler, çoğu organizmada temel biyolojik sistemlerdir. Memelilerde, ana saat hipotalamusun ventral ucundaki SCN'de bulunur. SCN, periferik saati gündüz/gece döngüleriyle senkronize etmek için çıkış ritimlerini yönlendiren sinirsel ve humoral sinyalleri kontrol eder. Işık ve sıcaklık gibi çevresel zaman ipuçlarının, saatin ritmini sürekli olarak sıfırlamada rol oynadığı bilinmektedir. Sirkadiyen ritimdeki bozulmalar prevelansı gün geçtikçe artan obezite, Tip2 DM gibi metabolik bozukluklar yanı sıra pek çok hastalığa da zemin hazırladığı bilinmektedir. Son yıllarda yapılan çalışmalarda modern yaşam, teknoloji ve sanayileşmenin hayatımıza girmesiyle birlikte günümüzde pek çok bireyde sirkadiyen ritim bozukluğunun meydana geldiği gösterilmektedir (Öney ve Balcı, 2021; Serrin ve Tek, 2019). Sirkadiyen sistem günlük olarak çevremizin gece-gündüz döngüsüyle senkronize olur. Bu ritmin bozulması, örneğin serbest radikallerin aşırı üretimiyle oluşan ve hücresel bileşenlerin oksidatif hasarına yol açan birçok hastalığın ortaya çıkmasını ve gelişmesine neden olabilir (Budkowska ve ark., 2022).

SOD enzimi, serbest radikallerin neden olduğu oksidatif strese karşı hücrelerin savunmasında rol oynayan önemli bir faktördür. Bu enzim, tüm aerobik organizmalarda serbest oksijen radikallerine (ROS) karşı ilk sa-

vunma hattı olarak kabul edilir (Suliman ve ark., 2005; Clair ve ark., 2009). Ancak SOD, oksijenin ayrışmasının hücreler için toksik olan H_2O_2 üretimine neden olması nedeniyle pro-oksidatif olarak da hareket edebilir. Tehlikeli H_2O_2'yi uzaklaştırmak için , antioksidan sistemlerin varlığı gereklidir (Gebicka ve ark., 2017). Antioksidan enzimler, serbest radikallerin fizyolojik ve fizyolojik olmayan üretiminin neden olduğu oksidatif strese karşı savaşmak için herhangi bir organizmanın savunma sisteminin temel taşıdır. Dokuların oksidatif durumunun, hormonlar ve sitokinler gibi diğer faktörlerle birlikte bu enzimleri düzenlediği bilinmektedir (Martin ve ark., 2009). Yapılan çalışmalarda en önemli antioksidan enzimlerden biri, süperoksit radikallerinin hidrojen peroksite dismutasyonunu katalize eden SOD'dur. Bu nedenle sirkadiyen ritim bozulması durumda hücrede serbest radikallere karşı temel savunma hattı olan SOD düzeyleri incelenmiştir. SRB grubunun serum SOD konsantrasyonu kontrol grubuna kıyasla daha yüksek olduğu tespit edilmiştir, bu artış istatiksel olarak anlamlı bulunmamıştır (p=0,516). Çalışmamızdan elde edilen veriler önceki çalışmalarla örtüşmektedir (Budkowska ve ark., 2022; Martin ve ark., 2009). Son yıllarda yapılan çalışmalarda aydınlık/karanlık döngüsü değiştirilerek sirkadiyen ritimleri bozulan deney hayvanlarında serum SOD seviyelerine dair literatürde yeterli veriye ulaşılamamıştır. Bununla birlikte Budkowska ve ark. (2022) yaptığı sağlıklı bireylerin farklı zaman dilimlerinde kan örnekleri alınarak SOD ve diğer oksidatif stres enzimlerinin aktiviteleri ölçülmüştür. Bu araştırmada, SOD aktivitesinin özellikle gece saatlerinde (saat 02.00 civarında) en yüksek seviyelere ulaştığı gözlemlenmiştir. Bu durum, uyku sırasında vücudun oksidatif dengeyi koruma mekanizmalarının daha aktif hale geldiğini ve oksidatif stresin azaltıldığını göstermektedir (Budkowska ve ark., 2022). Başka bir çalışmada ise sirkadiyen ritim bozukluklarının SOD seviyeleri üzerindeki etkisi incelenmiştir. Araştırma, sirkadiyen ritimdeki bozulmaların SOD aktivitesini olumsuz yönde etkileyebileceğini ve bu durumun oksidatif stres seviyelerini artırarak çeşitli kronik hastalık risklerini artırabileceğini ortaya koymuştur (Smith ve ark., 2020). Bu araştırmayı destekler nitelikte olan başka bir çalışmada ise sirkadiyen ritim bozulması durumunda, SOD gibi antioksidan enzimlerin aktivitelerinde azalmalar gözlemlenmiştir. Bu azalma, hücresel düzeyde daha fazla oksidatif hasara yol açabilir ve bu durum, nörodejeneretif hastalıklar, kanser ve kardiyovasküler hastalıklar gibi çeşitli sağlık sorunlarına zemin hazırlayabilir (Little ve ark., 2020). Diğer bir çalışmada ise özellikle gece uykusunun bozulmasının SOD aktivitesinde düşüşe yol açtığını ve bunun da artan oksidatif stres ve hücresel hasar riski oluşturduğunu göstermektedir. Bu bulgular, sirkadiyen ritmin korunmasının oksidatif stresin yönetilmesinde kritik bir rol oynadığını vurgulamaktadır (Li ve ark., 2021). Yine başka bir araştırmada ise sirkadiyen ritimdeki düzensizliklerin SOD seviyelerini azaltarak oksidatif stresin artmasına neden olabileceğini ve bu durumun uzun vadede hücresel hasar ve hastalık riskini artırabileceğini göstermektedir (Thompson ve ark., 2018). Bu nedenle, çalışmamızda serum SOD düzeylerini incelenmiş olması literatüre farklılık kazandıran bir yaklaşımdır.

5. Sonuç

Günümüzde modern yaşamın meydana getirdiği ve tüm yaş gruplarını etkileyen beslenme bozuklukları kaynaklı; obezite, insülin direnci, diyabet, kardiyovasküler hastalıklar, psikolojik sorunlar gibi birçok hastalık ulusal ve uluslararası alanda prevalansı gün geçtikçe artan ve tedavisi zorlaşan sağlık sorunlarıdır. Tedavisinin küresel açıdan büyük önem taşıdığı bu hastalıklar; sirkadiyen ritim bozulması ile yakından ilişkilidir, bahsedilen hastalıkların etki mekanizmalarının her yönden değerlendirilmesi oldukça önemlidir. Sirkadiyen ritim bozulmasında SOD seviyesi gibi belirteçlerin tespit edilmesi, sirkadiyen ritim bozulması ile ilgili hastalıkların önlenmesi bakımından yol gösterici olabilir. Bu kapsamda çalışmamızda elden edilen verilerin daha kapsamlı çalışmalarla desteklenmesinin yararlı olabileceği düşünülmektedir.

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Investigation of Serum Serotonin Levels in Mice with Disrupted Circadian Rhythms

Sirkadiyen Ritimleri Bozulmuş Farelerde Serum Serotonin Düzeylerinin Araştırılması

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Özet

Giriş ve Amaç: Sirkadiyen ritim, biyolojik yapının 24 saatlik döngüsünde oluşan davranışsal ve fizyolojik salınımlarıdır. Sirkadiyen ritimlerde oluşan olumsuz değişiklikler; uyku bozuklukları, obezite, diyabet, psikolojik durum bozuklukları ve kardiyovasküler bozukluklar gibi çeşitli sağlık sorunlarıyla ilişkilendirilmektedir. Aynı zamanda, sirkadiyen ritim gastrointestinal sistemde ile önemli ölçüde ilişkilidir. Bu bağlantıyı sağlayan parametreler arasında serotonin yer almaktadır. Serotonin, psikolojiyi ve fizyolojiyi önemli ölçüde etkileyen filogenetik olarak eski bir biyojenik amindir. Bu çalışmada, deney hayvanlarında aydınlık / karanlık döngüsünün iki haftada bir tersine çevrilmesiyle ön görülen sirkadiyen ritim bozulmasının serotonin seviyelerine etkisinin araştırılması amaçlanmıştır.

Yöntem: Çalışmada 16 adet 8 - 10 haftalık BALB/c cinsi erkek fare kullanılmıştır. Fareler kontrol (KON, n=8) ve sirkadiyen ritimi bozulmuş (SRB, n = 8) olarak iki gruba ayrılmıştır. Deney süresince farelerin tamamı standart pelet yem ile beslenmiştir. KON grubundaki farelere 16 hafta boyunca herhangi bir müdahale yapılmamış ve standart çevre koşullarında barındırılmıştır. SRB grubundaki farelerin ise deney boyunca iki haftada bir aydınlık / karanlık döngüsü değiştirilmiştir. Deney sonunda farelerden kan alma işlemi yapılmış ve serum örnekleri elde edilmiştir. Serotonin konsantrasyonları üreticinin tavsiyelerine göre ticari olarak temin edilebilen enzim bağlantılı immünosorbent test kitleri kullanılarak tespit edilmiştir.

Bulgular: KON ve SRB gruplarının ortalama serum serotonin konsantrasyonları sırasıyla 19,1 ± 6,03 pg/mL ve 17,1 ± 2,85 pg/mL olarak belirlenmiştir. SRB grubunun serotonin konsantrasyonundaki azalma istatistiksel olarak anlamlı bulunmuştur (p = 0,033).

Sonuç: Aydınlık / karanlık döngüsünün değiştirilmesi sonucu oluşan sirkadiyen ritim bozulmasının serum serotonin seviyelerinde azalmaya neden olduğu sonucuna varılmıştır. Sirkadiyen ritimleri bozulmuş farelerde serum serotonin seviyelerinin azalması, serotonin sisteminin açıklanmasına yönelik yapılan çalışmalara yön verici olabilir.

Anahtar Kelimeler: Gastrointestinal sistem, serotonin, sirkadiyen ritim

Abstract

Introduction and Aim: Circadian rhythm is the behavioral and physiological oscillations of the biological structure that occur in a 24-hour cycle. Adverse changes in circadian rhythms are associated with various health problems such as sleep disorders, obesity, diabetes, psychological disorders and cardiovascular disorders. At the same time, circadian rhythm is significantly associated with the gastrointestinal system. Serotonin is among the parameters that provide this connection. Serotonin is a phylogenetically well-known biogenic amine that significantly affects psychology and physiology. In this study, we aimed to investigate the effect of circadian rhythm disruption, which is predicted by reversing the light/dark cycle every two weeks, on serotonin levels in experimental animals.

Method: Sixteen 8 - 10 week old BALB/c male mice were used in the study. Mice were divided into two groups as control (CON, n = 8) and circadian rhythm disrupted (SRD, n=8). All mice were fed with standard pellet feed during the experiment. The mice in the KON group were not intervened for 16 weeks and were housed in standard environmental conditions. In the SRB group, the light/dark cycle was changed every two weeks throughout the experiment. At the end of the experiment, blood was obtained from the mice and serum samples were obtained. Serotonin concentrations were determined using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer's recommendations.

Result: The mean serum serotonin concentrations of KON and SRB groups were determined as 19.1 ± 6.03 pg/mL and 17.1 ± 2.85 pg/mL, respectively. The decrease in serotonin concentration in the SRB group was statistically significant (p=0.033).

Conclusion: It was concluded that the circadian rhythm disruption caused by changing the light/dark cycle caused a decrease in serum serotonin levels. The decrease in serum serotonin levels in mice with disrupted circadian rhythms may be instructive for studies to explain the serotonin system.

Keywords: Circadian rhythm, gastrointestinal tract, serotonin

1. Giriş

Sirkadiyen sistem, metabolik olaylardan ve biyolojik süreçlerden (uyku / uyanıklık programları, vücut ısısı, hormon salınımı, nörotransmiter değişiklikleri, fiziksel enerji yoğunluğunu, yeme-içme alışkanlıklarını ve psikolojik davranışları) oluşan 24 saatlik bir döngüdür (Telias and Wilcox, 2019). Günümüzde değişen yaşam şartlarıyla birlikte (vardiyalı çalışma şartları, değişen sosyal yaşantı, jet lag ve uyku eksikliği) bireylerde sirkadiyen ritim bozukluğu oluşmasına yol açmaktadır. Sirkadiyen ritimlerde oluşan olumsuz değişiklikler; uyku bozuklukları, obezite, diyabet, psikolojik durum bozuklukları ve kardiyovasküler bozukluklar gibi çeşitli sağlık sorunlarıyla ilişkilendirilmiştir (Huang ve ark., 2011). Bu sağlık sorunlarının önüne geçmek için ise sirkadiyen ritim bozukluklarının olumsuz etkilediği parametre ise gastrointestinal sistemdir (GİS). 1970'lerin başından beri gastrointestinal sistemin işlevinin sirkadiyen ritimleri etkilediği bulunmuştur. Artan kanıtlar, sirkadiyen ritim bozulmanın bir dizi gastrointestinal patolojisine yol açabileceğini, bariyer fonksiyonunu bozabileceğini göstermiştir (Bishehsari ve ark., 2021). Gastrointestinal sistem, çeşitli uzunluk ve zaman ölçeklerinde koordine edilmiş bir dizi karmaşık işleve sahiptir. Bu mekanizmanın bozulması ise birçok fizyolojik ve psikolojik hastalıklara yol açmaktadır. Gastrointestinal sistem ile etkileşiminde dikkat çeken parametrelerden biri de vücudumuzda psikolojik ve biyolojik düzenleyici bir hormon olan serotonindir (Güzel ve Mirowska-Güzel, 2022). Serotonin (5-HT) beyindeki ana nörotransmiterlerdendir. Serotonin psikolojiyi ve fizyolojiyi önemli ölçüde modüle eder. Psikolojik sorunların (depresyon, anksiyete, yeme-içme problemleri vb.) tedavisinde kullanılan en yaygın nörotransmiterdir. Memeli konakçı serotoninin %90'ından fazlası bağırsakta bulunmaktadır. Serotonin üretiminin bağırsakta en yüksek olduğu yer, enterokromafin (EC) hücreleridir. Bağırsakta serotonin salgılanması diyetten etkilenmekte ve bağırsak hareketi, iştah, uyku, fizyolojik ve bilişsel işlevleri düzenlemektedir. Psikolojik sorunların (depresyon, anksiyete, yeme-içme problemleri vb.) tedavisinde kullanılan en yaygın nörotransmiterdir. 5-HT yoluyla ilgili olarak, ruh hali, motor kontrolü, sirkadiyen ritmin düzenlenmesi ve gastrointestinal sistem düzenleme gibi çeşitli biyolojik süreçleri içeren hayati işlevler de üstlenir (Daut and Fonken, 2019). Genel olarak, 5-HT düzeylerinin artmasının ve reseptörleri ile yollarındaki bozulmaların da bu hastalıkla yaygın olarak ilişkili olduğu bilinmektedir. Bu derlemede üretiminin % 90'ından fazlası bağırsakta olan serotonin hormonunun bu bozulan sistemden etkileneceği düşünülmekte ve bu konunun güncel olup literatürde eksikliği görülmüştür. Bu bilgiler ışığında çalışmamızda sirkadiyen ritmin olumsuz etkilendiği durumlarda serotonin düzeyleri üzerine etkilerinin belirlenmesi hedeflenmiştir.

2. Yöntem

2.1. Deney Hayvanları ve Çalışma Düzeni

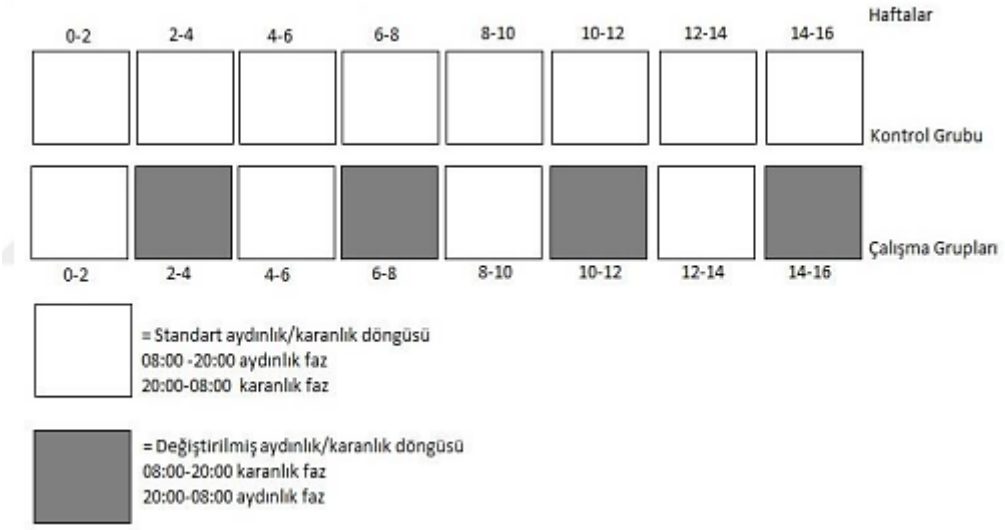
Bu çalışmada, 16 adet 8-10 haftalık BALB/c cinsi erkek fareler kullanılmıştır. Hayvanlara uygulanan tüm prosedürler Ondokuz Mayıs Üniversitesi Hayvan Deneyleri Etik Kurulu tarafından onaylanmıştır (Onay numarası: E68489742-604.01.03-97230, Tarih: 27.07.2021). Hayvanların refahı ve kullanımı için ARRIVE yönergeleri ve 3R kuralı (azaltma, yerine koyma ve iyileştirme) sıkı bir şekilde uygulanmıştır. Tüm hayvanlar kafeslere rastgele dağıtılmıştır ve herhangi bir işlemden önce kafesler rastgele seçilmiştir. Hayvanlar standart pelet diyetine ve normal musluk suyuna *ad libitum* erişimi olan paslanmaz çelik kapaklı ayrı polikarbonat kafeslerde barındırılmıştır. Çalışmanın başlangıcından sonlandırılmasına kadar olan süreçte hayvanlar her hafta 0.1 g'a duyarlı hassas terazi kullanılarak tartılmıştır. Adaptasyon periyodunun ardından hayvanlar kontrol grubu (KON) ve sirkadiyen ritimleri bozulmuş fareler (SRB) olarak rastgele iki gruba ayrılmıştır. Tüm gruplar standart diyetle toplam 16 hafta boyunca beslenmiştir.

• Kontrol grubu (KON) (n=8): 16 hafta boyunca herhangi bir müdahale yapılmayan grup.

• Sirkadiyen ritimleri bozulmuş fareler (SRB) (n=8): 16 hafta boyunca aydınlık karanlık döngüsü değiştirilerek sirkadiyen ritimleri bozulmuş grup.

2.2. Aydınlık/Karanlık Döngüsünün Değiştirilmesi

Kontrol grubu için 12 saatlik aydınlık/karanlık döngüsünde nem (55 ± %10) ve sıcaklık (22 ± 2 ◦C) sabit olacak şekilde çevresel koşullar ayarlanmıştır. Sirkadiyen ritimleri değiştirilecek olan grubun barındırılacağı oda çalışmaya başlamadan önce pencereleri siyah bantlarla ve perdelerle kapatılmıştır. Çalışma öncesinde odaya özel bir saat alınmış ve aydınlık-karanlık döngüsü deney süresi boyunca 15 günde bir değiştirilmiştir. Bu durumda 15 gün boyunca 12 saatlik aydınlık/karanlık döngüsünün ardından 15 gün boyunca 12 saatlik karanlık/aydınlık döngüsü uygulanmıştır. Aydınlık/karanlık döngüsünün değiştirilme protokolü Şekil 1'de gösterilmiştir.



Figür 1. Farelerin aydınlık/karanlık döngüsünün değiştirilme protokolü

2.3. Örneklerin Toplanması ve Biyokimyasal Analizler

Ötenazi işlemine başlamadan önce enjektabl (intraperitoneal) anestezikler kullanılmıştır ve farelerin derin anestezi altında kalması sağlanmıştır. Bacak çekme ve göz kapağı gibi refleksler tamamen kaybolduğunda kardiyak ponksiyon ile maksimum kan alma işlemi gerçekleştirilmiştir. Fareler daha sonra sakrifiye edilmiştir. Pıhtılaşmanın önlenmesi için kan örnekleri etilendiamin tetraasetik asit (EDTA) eklenmiş tüplere konulmuştur. Örnekler 3000 rpm'de 10 dakika santrifüj edilerek serum elde edilmiştir. Serum örnekleri biyokimyasal analizlerin yapılacağı güne kadar -80°C dolapta muhafaza edilmiştir. Serotonin konsantrasyonları üreticinin tavsiyelerine göre ticari olarak temin edilebilen mouse "Sandwich Enzyme-Linked Immune Sorbent Assay" (ELISA) kitleri kullanılarak serumda test edilmiştir. Biyokimyasal analiz sonuçları ELISA reader'da (Thermo Fisher Multiskan GO, Japan) (450 nm) okutularak değerlendirilmiştir.

2.4. İstatistiksel Analizler

Verilerin istatistiksel analizi SPSS programı (Versiyon 26.0) kullanılarak yapılmıştır. Veriler ortalama ± standart sapma olarak ifade edilmiştir. Sirkadiyen ritim değiştirilmesinin etkilerini değerlendirmek için "Bağım-

sız örneklemler t testi" kullanılmıştır. İstatistiksel olarak p<0,05 değeri anlamlı olarak kabul edilmiştir.

3. Bulgular

Çalışmada 16 adet erkek fare kontrol ve sirkadiyen ritimleri bozulmuş olarak iki gruba ayrılmıştır. İki grup da 16 hafta boyunca standart pelet diyet ile beslenmiştir. Yapılan biyokimyasal analizler ile farelerin serum serotonin konsantrasyonları analiz edilmiştir. Buna göre KON ve SRB gruplarının ortalama serotonin değerleri sırasıyla 19,1±6,03 pg/mL ve 17,1±2,85 pg/mL olarak belirlenmiştir. SRB grubundaki serotonin konsantrasyonundaki bu azalma istatistiksel olarak anlamlı bulunmuştur (p=0,033).

Tablo 1. Deney gruplarının serum serotonin konsantrasyonlarının değerlendirilmesi

Parametre	**KON (n=8) X̄ ± SS Min-Max**	**SRB (n=8) X̄ ± SS Min-Max**	**Toplam (n=8) X̄ ± SS Min-Max**	İstatistik	**p**
Serotonin (pg/mL)	19,1±6,03 (12,1-29,1)	17,1±2,85 (12,9-22,4)	18,1±4,68 (12,1-29,1)	5,602	0,033

KON: Kontrol grubu, SRB: Sirkadiyen ritimleri bozulan fareler, X̄ ± SS: Standart sapma. Verilerin analizinde bağımsız örneklemler t testi kullanılmıştır.

4. Tartışma

Bu çalışmada kontrol grubu ve sirkadiyen ritmi bozulmuş grupta serotonin düzeyleri incelenmiştir. Serum örneklerinde serotonin konsantrasyonu bu gruplarda ticari olarak sağlanan olan kitler ile sandviç ELISA tekniği kullanılarak belirlenmiştir. Yapılan çalışmada sirkadiyen ritmi bozulmuş grubun serotonin konsantrasyonundaki azalma istatistiksel olarak anlamlı bulunmuştur.

Sirkadiyen ritim canlının uyku-uyanıklık durumundan etkilenen biyolojik bir döngüdür (Telias and Wilcox, 2019). Sirkadiyen sistemin düzenleyicisi, uyku-uyanıklık döngüsünün özelliklerini senkronize eden hipotalamus içindeki suprakiasmatik çekirdektir (SCN). Sirkadiyen bozukluklar ise uyku-uyanıklık düzenlerinin istenen uyku saatiyle örtüşmemesi durumunda ortaya çıkar (Gentry ve ark., 2021). Sirkadiyen sistem düzensizliği obezite, depresif bozukluk, uyku bozuklukları, diyabet ve kalp hastalıkları (Alim ve Ayten, 2019) gibi birçok hastalığa yol açmaktadır (Huang ve ark., 2011). Son yıllarda yapılan çalışmalarda değişen yaşam şartları ise bireylerin sirkadiyen sistem bozulmasına yol açmaya yatkın olduğu gösterilmiştir (Öney ve Balcı, 2021).

Bu bilgilerden yola çıkarak deney hayvanlarında sirkadiyen ritmi bozulmuş modeller kullanılmıştır. Bu biyolojik döngünün bozulması ise gastrointestinal sistem sorunlarına yol açmaktadır. GİS'te ise serotonin hormonunun büyük bir kısmı üretilmektedir (Güzel ve Mirowska-Güzel, 2022). Bu bilgiler ışığında ise sirkadiyen ritmi bozulmuş haliyle gastrointestinal sistemde sorunlar olmuş deney hayvanlarında serotonin seviyelerinde anlamlı bir değişiklik olacağını düşünmekteyiz ve bulgularımız yapılan çalışmaları desteklemektedir.

Serotonin seviyelerinin düşmesi ise birçok psikolojik sorunlara yol açmaktadır. Yapılan çalışmalarda sirkadiyen sistemdeki kesintilerin ruh halini nasıl değiştirdiğine tam olarak açıklık getirememişlerdir. Bununla bir-

likte, serotonin sistem modülasyonunun sirkadiyen sistemin depresyona karşı duyarlılığı düzenlediği olası yollardan biri olduğuna dair kanıt sağlanmıştır (Daut and Fonken, 2019). Çalışmamızdan elde edilen sonuçlar bu yaklaşımla örtüşmekte ve sirkadiyen sistem bozulmalarının ne gibi sorunlara yol açabileceği açıklamaktadır. Literatüre bu kapsamda katkı sağlanmıştır.

5. Sonuç

Günümüz yaşantısının oluşturduğu sorunlar biyolojik döngünün bozulmasına yol açmaktadır. Bu döngünün bozulmasıyla diyabet, obezite, depresif bozukluklar gibi birçok sorun oluşmaktadır. Bu yüzden sirkadiyen ritim bozukluklarının mekanizmalarını incelemek büyük önem taşımaktadır. Yaptığımız çalışmada sirkadiyen ritim bozulmasının ise serotonin düzeyleri üzerinde etkili olduğu bulunmuştur. Serotonin düzeylerinin anlamlı olarak değişmesi yeni çalışmalara yön verici olabilir. Bu çalışmamızda elde ettiğimiz yeni bilgiler diğer çalışmalar için yararlı olacağı düşünülmektedir.

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The Effect of Different Cooking Methods on K1 Vitamin Content in Broccoli

Brokoli Sebzesinde Farklı Pişirme Yöntemlerinin K1 Vitamini İçeriğine Etkisi

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Özet

Giriş ve Amaç: K vitamini, kanın pıhtılaşmasında önemli bir rol oynamaktadır. K1 ve K2 vitaminleri, K vitamininin besinlerde bulunan doğal formlarıdır. K1 vitamini (filokinon) yeşil yapraklı sebzelerin klorofil yapısında bulunur ve temel alım kaynağı diyettir. Bununla birlikte, yüksek K vitamini içeren besinlerin diyetle alımının antikoagülan ilaçların etkisini azaltabileceği bilinmektedir. Bu araştırmada, farklı pişirme yöntemlerinin K vitamini yönünden zengin brokoli (Brassica oleracea var. italica) içerisindeki K1 vitamini düzeyine etkisinin incelenmesi ve elde edilen sonuçlar doğrultusunda kardiyovasküler hastalığı bulunan ve antikoagülan ilaç kullanan bireylerin beslenmesine katkı sağlamak amaçlanmıştır.

Yöntem: Araştırma, Aralık 2023 tarihinde Marmara Üniversitesi Beslenme ve Diyetetik Bölümü bünyesinde gerçekleştirilmiştir. Brokoli numuneleri; haşlama, fırında ve buharda pişirme yöntemi ile hazırlanmıştır. Her bir numune 200 gram olacak şekilde hazırlanmıştır. Numuneler pişirme sonrası soğutulmuş ve vakumlanarak paketlenmiştir. Numunelerin K1 vitamini analizi, TÜBİTAK Marmara Araştırma Merkezi'nde HPLC yöntemi ile yapılmıştır.

Bulgular: Çiğ brokoli 245 µg/100g K1 vitamini içerirken, haşlanmış brokoli 230 µg/100g, buharda pişirilmiş brokoli 247 µg/100g ve fırında pişirilmiş brokoli 262 µg/100g K1 vitamini içermektedir. Haşlama yöntemi, brokolideki K1 vitamini düzeyini düşürürken, buharda pişirme ve fırında pişirme yöntemlerinde brokoli içerisinde bulunan K1 vitamini düzeyi korunmuştur.

Sonuç: Brokolinin pişirme yöntemine göre K1 vitamini düzeyinde değişiklikler görülmüştür. Kardiyovasküler hastalığı olan ve antikoagülan ilaç kullanan bireylerin beslenmesinde, besin kısıtlaması yapılmadan K vitamini alımını azaltmak için K vitamini bakımından zengin sebzeleri haşlama yöntemi ile pişirmek düşünülebilir. Ancak, herhangi bir pişirme yönteminin önerilmesi için daha fazla çalışmaya ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Brokoli, K Vitamini, pişirme yöntemleri, filokinon düzeyi, HPLC analizi,antikoagülan

Abstract

Introduction and Aim: Vitamin K is a crucial compound that plays a role in blood clotting. K1 and K2 are the natural forms of vitamin K found in foods, with K1 (phylloquinone) being present in the chlorophyll structure of leafy green vegetables and primarily obtained through diet. However, it is known that the dietary intake of foods high in vitamin K can reduce the efficacy of anticoagulant medications. This research aims to examine the effect of different cooking methods on the K1 vitamin levels in broccoli (Brassica oleracea var. italica), which is rich in vitamin K, and to contribute to the dietary recommendations for individuals with cardiovascular disease who are using anticoagulant drugs.

Method: The research was conducted in December 2023 within the Department of Nutrition and Dietetics at Marmara University. Broccoli samples were prepared using boiling, baking, and steaming methods. Each sample was prepared to weigh 200 grams. After each cooking method, the samples were cooled, vacuum-sealed, and sent to the TÜBİTAK Marmara Research Center for K1 vitamin analysis, which was conducted using the HPLC method.

Results: Raw broccoli contains 245 µg/100g of K1 vitamin, while boiled broccoli contains 230 µg/100g, steamed broccoli contains 247 µg/100g, and baked broccoli contains 262 µg/100g of K1 vitamin. The boiling method decreased the K1 vitamin levels in broccoli, whereas steaming and baking methods preserved the K1 vitamin levels.

Conclusion: Changes in K1 vitamin levels were observed depending on the cooking method used for broccoli. For individuals with cardiovascular disease who are on anticoagulant medication, boiling vitamin K-rich vegetables could be considered as a method to reduce vitamin K intake without restricting the diet. However, more reliable data is needed before recommending any specific cooking method.

Keywords: Broccoli, Vitamin K, cooking methods, pPhylloquinone levels, HPLC analysis, anticoagulant.

1. Giriş ve amaç

K vitamini, karaciğerde sentezlenen protrombinin yapımında görev almakta ve kanın pıhtılaşmasını sağlamaktadır (Baysal, 2019). Doğada K vitamini, K1 vitamini (filokinon) ve K2 vitamini (menakinon) olmak üzere iki farklı biçimde bulunur. K1 vitamini (filokinon) yeşil yapraklı sebzelerin kloroplast yapısında doğal olarak yer almakta ve diyetle alınan K vitamininin ana kaynağını oluşturmaktadır.

Yüksek K vitamini içeren besinlerin vücutta antikoagülan mekanizmayı azaltıcı etkiye neden olabileceği düşünülmektedir (Göz, 2006). K1 vitamininin (filokinon) diyetle 1 - 10 mg/gün alınmasının antikoagülan ilaçların etkisini değiştirdiği belirlenmiştir. K vitamini içeren besinlerin, diyetle alımının tamamen kesilmesi mümkün ve sürdürülebilir olmadığından, yüksek doz K1 ve K2 vitamini içeren besinlerin günlük olarak tüketilen miktarlarının azaltılmasının faydalı olabileceği düşünülmektedir (Taze Köksal ve Avşar, 2015). Franco ve ark. (2004) tarafından yapılan çalışmada, oral antikoagülan ilaç kullanan hastalarda protrombin zamanı (PT) değerindeki dalgalanmaların temel sebebinin diyetle alınan K vitamini miktarındaki değişimler olduğu ortaya konulmuştur (Franco ve ark., 2004). Diyetle alınan K vitamininin ana şekli olan filokinon (K1) içeriği bitkilerdeki kloroplast miktarı ile doğru orantılı olarak artış göstermektedir. Ortamın sıcaklık, ışık, oksijen, pH ve su seviyesi ile depolama koşulları sebzelerde K1 vitamini kayıplarına neden olabilmektedir. Bununla birlikte pişirmenin de K1 vitamini düzeyine etki edebileceği düşünülmektedir (Xu ve ark., 2020). Booth ve ark. (1999) tarafından yapılan bir çalışmada sebzelerin dondurulması, kaynatılması, buharda veya mikrodalgada pişirilmesinin K1 miktarını değiştirmediği saptanmıştır (Booth ve Centurelli, 1999). Bununla birlikte, K vitamini bakımından zengin çiğ ve pişmiş besinlerin K1 ve K2 düzeylerinin HPLC yöntemi ile analiz edildiği bir çalışmada haşlama yönteminin besinlerde bulunan K1 ve K2 vitamini düzeylerinde azalttığı belirlenmiştir (Meral, 2019).

Ispanak, brokoli, brüksel lahanası, kuşkonmaz, lahana, karnabahar, bezelye, fasulye, yeşil çay, zeytin, zeytinyağı, soya tohumu, yumurta, tahıllar ve süt ürünleri K vitamini yönünden zengin besinlerdir (Demirtas ve ark., 2010). Brokoli (*Brassica oleracea* var. *italica*), dünyanın her yerinde tüketilen, Brassicaceae familyasına ait bitki türüdür. Brokolinin farklı alt türleri bulunmakla birlikte en sık tüketilen türü yeşil çiçekli ve kalın saplı *Calabresa*'dır. Tek yıllık bir bitki olan brokoli (*Brassica oleracea* var. *italica*), içerdiği besin ögeleri, polifenoller, flavonoidler, karotenoidler, sülforafan ve glukozinolatlar gibi biyoaktif bileşikler nedeniyle popüler hale gelmiştir (Nagraj ve ark., 2020). Brokoli (*Brassica oleracea* var. *italica*), vitamin ve mineraller bakımından zengin sebzelerden biridir (Nagraj ve ark., 2020). Brokoli (*Brassica oleracea* var. *italica*), flavonoid içeriği sayesinde LDL kolesterolü dengelemekte ve kardiyovasküler hastalıkların gelişimini engellemektedir. Ayrıca, içerdiği biyoaktif bileşenler sayesinde bağışıklığı desteklemektedir (Moreno ve ark., 2006; Nagraj ve ark., 2020). Yapılan bir çalışmada brokoli (*Brassica oleracea* var. *italica*), buharda pişirme, haşlama, mikrodalgada pişirme, tavada kızartma, karıştırarak kızartma ve ardından haşlama gibi çeşitli pişirme yöntemleri kullanılarak pişirilmiş; buharda pişirme yöntemi hariç tüm pişirme yöntemlerinin K1 vitamininde azalmaya yol açtığı görülmüştür (Nagraj ve ark., 2020).

Farklı pişirme yöntemlerinin sebzelerde bulunan filokinon düzeyinde değişikliğe neden olmadığını ifade eden çalışmaların yanı sıra haşlama yöntemi pişirilmiş sebzeler karşılaştırıldığında K1 miktarında kayıpların olduğunu gösteren çalışmalar da mevcuttur (Booth ve Centurelli 1999; Meral, 2019). Buna karşın çiğ ve pişmiş besinlerde bulunan K vitamini miktarının tayini için daha fazla çalışmaya gereksinim duyulmaktadır (Lee ve ark., 2017).

Bu çalışmada haşlama, fırın ve buharda pişirme yöntemlerinin; K1 vitamini (filokinon) yönünden zengin brokoli (*Brassica oleracea* var. *italica*) sebzesinin içerisinde bulunan K1 vitamini (filokinon) düzeyine etki edip etmediğinin incelenmesi amaçlanmıştır.

2. Yöntem

2.1. Numunelerin Hazırlanması:

Numuneler Marmara Üniversitesi Sağlık Bilimleri Fakültesi Beslenme ve Diyetetik Bölümü'nün laboratuvarında hazırlanmıştır. K1 vitamini (fillokinon) analizi Türkiye Bilimsel ve Teknik Araştırma Kurumu (TÜBİTAK) Marmara Araştırma Merkezi'nde yapılmıştır. Araştırma için gerekli olan brokoli (*Brassica oleracea* var. *italica*) sebzesi İstanbul'daki yerel bir marketten temin edilmiştir. Yıkanan brokoli (*Brassica oleracea* var. *italica*) sebzeleri çiğ numune için 200 gr, pişirilecek numuneler için 300 gr olacak şekilde hassas terazi ile ölçülerek hazırlanmıştır. Farklı pişirme yöntemleri ile pişirilen numuneler soğutulduktan sonra ev tipi vakum makinesi kullanılarak paketlenmiştir.

2.2. Fırında Pişirme:

Fırınlama işlemi için ev tipi sıcak hava fırını kullanılmıştır. Çiçeklerine ayrılan brokoli (*Brassica oleracea* var. *italica*), fırının metal tepsisi üzerine yerleştirilen silikon pişirme matı üzerine tek tabaka halinde yayılarak 180º C'de 30 dakika pişirilmiştir.

2.3. Buhar Yöntemi ile Pişirme:

Küçük parçalar haline getirilen brokoli (*Brassica oleracea* var. *italica*), tencere içinde kaynayan suda ve buharda pişirme aparatı ile 5 dk boyunca üstü kapalı olacak şekilde pişirilmiştir.

2.4. Haşlama Yöntemi ile Pişirme:

Küçük parçalar haline getirilen brokoli (*Brassica oleracea* var. *italica*), tencerede kaynayan suya daldırılmış; üstü açık şekilde 5 dk pişirilmiştir.

2.5. Numunelerin Soğutulması:

Tüm numuneler kaplarda soğumaya bırakılmıştır.

2.6. K1 Vitamini Analizi:

K1 analizi, HPLC yöntemi ile yapılmıştır. Mobil fazda; 1000 mL'lik mezür içerisine, 100 mL diklorometan ve 900 mL metanol eklenmiş ve üzerine 5 mL çinko klorid asetat çözeltisi ilave edilerek çözeltinin homojen bir şekilde karışması sağlanmıştır. Hazırlanan çözelti 0,22 µm filtre yardımı ile süzülmüş; dedektör, floresans ayarına getirilmiştir. Eksitasyon 243 nm dalga boyu, 430 nm emisyon ve 20 µl enjeksiyon hacminde; akış hızı 1 mL/dakika olacak şekilde 10 dakika süre ile analiz edilmiştir.

3. Bulgular

Tablo 1. Pişirme yöntemine göre brokoli (*Brassica oleracea* var. *italica*) sebzesinde analiz edilen k1 vitamini miktarı

Numune (100 g)	K1 Vitamini Miktarı (µg)
Çiğ Brokoli	245 µg
Buharda Pişirilmiş Brokoli	247 µg
Haşlanmış Brokoli	230 µg
Fırında Pişirilmiş Brokoli	262 µg

Numunelerin K1 vitamin miktarları Tablo 1'de gösterilmiştir. Numunelerin 100 gramında analiz edilen K1 vitamin miktarları 230 – 262 µg arasında değişmektedir. En düşük K1 vitamin miktarı haşlanmış brokoli numunesine (230 µg) ait bulunurken, en yüksek K1 vitamin miktarı fırında pişirilmiş brokoli numunesine (262 µg) ait bulunmuştur.

4. Tartışma

Bu araştırmada, farklı pişirme yöntemlerinin brokoli (*Brassica oleracea* var. *italica*) sebzesinde bulunan K1 vitamini (filokinon) düzeyine etkisi incelenmiştir. Elde edilen sonuçlar, farklı pişirme yöntemlerinin K1 vitamini içeriği üzerinde belirgin farklılıklara neden olabileceğini göstermiştir.

Çalışmada, fırında pişirmenin, brokolideki (*Brassica oleracea* var. *italica*) K1 vitamini düzeyini diğer pişirme yöntemlerine kıyasla daha fazla artırdığı saptanmıştır. Bu sonuç, Booth ve Centurelli (1999) tarafından yapılan çalışmada ifade edilen, pişirme yöntemlerinin filokinon oranlarını değiştirmediği bulgusu ile çelişmektedir. Ancak, Meral (2019) tarafından yapılan çalışmada, haşlama yönteminin K1 vitamini kaybına neden olduğu belirtilmiş olup, bu çalışmanın bulguları ile uyum göstermektedir.

Bildirilen günlük K vitamini alımı uluslararası düzeyde farklılık göstermekle birlikte ortalama 76 ila 217 µg arasında değişmektedir. Diyetle alınan K vitamini ile pıhtılaşma arasında bir etkileşim olduğunu bildiren çalışmalar, pıhtılaşma üzerinde bir etkinin yalnızca yüksek miktarda vitamin alımında (yani >150 µg/gün) tespit edilebileceğini göstermiştir; bu da yaklaşık 106 g/gün pişmiş brokoliye karşılık gelmektedir. Ancak bu eşiğin sağlıklı bireyler veya antikoagülan tedavisine başlayan hastalar için güvenli aralığı oluşturduğuna dair yeterli kanıt bulunamamıştır. (Violi ve ark., 2016)

Fırınlama işlemi sırasında, sıcak havanın filokinon içeriğini koruma veya artırma potansiyeline sahip olduğu düşünülmekte; bununla birlikte, buharda pişirmenin K1 vitamini düzeyini önemli ölçüde etkilemediği belirlenmiştir. Yapılan çalışmalarda buharda pişirmenin besin öğesi kayıplarını en aza indiren yöntem olduğu tespit edilmiştir. (Moreno ve ark., 2006; Nagraj ve ark., 2020)

Haşlama yönteminin, brokolideki (*Brassica oleracea* var. *italica*) K1 vitamini düzeyinde en fazla kayba yol açan yöntem olduğu saptanmıştır. Bu durumun, haşlama sırasında vitaminin suya geçişi ile açıklanabileceği düşünülmüştür (Meral, 2019).

Son yıllarda brokoli (*Brassica oleracea* var. *italica*) ve diğer yeşil sebzelerin pişirilmesi sırasında K vitamini korunumu üzerine çalışmalar artmıştır (Soares ve ark., 2017; Rana ve ark., 2021; Kim ve ark., 2023) Farklı pişirme yöntemlerinin gıdaların besin bileşimi üzerinde önemli etkileri olduğu gösterilmiştir. (Lee ve ark., 2017)

Buharda pişirme brokoli (*Brassica oleracea* var. *italica*) ve diğer sebzelerdeki K vitamini ve diğer besin ögelerinin korunmasında en etkili yöntemlerden biri olarak kabul edilmektedir. Haşlama ve benzeri yöntemlerin ise besinlerin pişirme suyuyla temas ederek besin ögesi kaybına neden olabileceği belirtilmektedir. Buharda pişirmenin K vitaminlerini daha yüksek oranda koruduğunu ortaya konmuştur (Kim ve ark, 2023).

Ayrıca, brokolide (*Brassica oleracea* var. *italica*) bulunan özellikle glukozinolatlar ve C vitamini gibi diğer yararlı bileşiklerin, farklı pişirme yöntemlerine göre konsantrasyonlarının değiştiği gösterilmiştir. Haşlama gibi yöntemler önemli kayıplara neden olabilirken, mikrodalgada pişirme ve buharda pişirme yöntemleri bu bileşikleri korumakta ve sebzenin sağlık üzerindeki faydalarına katkı sağlamaktadır (Soares ve ark, 2017).

Bu çalışma ile sebzelerin besin ögesi değerini korumak için doğru pişirme yönteminin seçilmesinin önemi ortaya konmuştur. K vitamini korunumunu en üst düzeye çıkarmayı hedefleyen bireyler için buharda pişirme en etkili seçenek olarak öne çıkmakta ve sağlık açısından avantajlar sunmaktadır (Lee ve ark, 2017). Bununla birlikte, farklı pişirme yöntemlerinin brokolideki (*Brassica oleracea* var. *italica*) K1 vitamini içeriğini etkilediği ortaya konmuş ve elde edilen verilerin, günlük beslenmenin planlamasında dikkate alınması gerektiği belirlenmiştir.

Franco ve ark. (2004) tarafından belirtilen, diyetle alınan K vitamini miktarındaki değişimlerin ilaç etkilerini bozabileceği göz önüne alındığında, özellikle fırında pişirilen brokolinin (*Brassica oleracea* var. *italica*) tüketimi konusunda dikkatli olunması gerektiği ve haşlama esnasında oluşan maksimum K vitamini kaybı göz önünde bulundurulabilir (Franco ve ark, 2004).

5. Sonuç

Haşlama, buharda ve fırında pişirme teknikleri brokolide (*Brassica oleracea* var. *italica*) bulunan K1 vitamini düzeyinde değişikliklere neden olmuştur. Kardiyovasküler hastalığı olan ve antikoagülan ilaç kullanan bireylerin beslenmesinde K vitamini kısıtlaması için sebzeleri haşlama yöntemi ile pişirilmesi önerilebilir. Bu araştırmanın sınırlılıkları arasında, kısıtlı numune sayısı ve sadece üç pişirme yönteminin incelenmesi bulunmaktadır. Bununla birlikte daha fazla pişirme yönteminin dahil edildiği ve K vitamini düzeylerinin karşılaştırıldığı daha kapsamlı araştırmalara ihtiyaç duyulmaktadır.

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3rd International Symposium on Traditional Foods and Sustainable Nutrition				
October 3-4, 2024				
	3.10.2024			
10.30-11.00	**Opening Speeches** *Dr. Başak ÖNCEL, Symposium Co-Chair* *Prof. Dr. Bahar TANER, Editor of JFNG* *Prof. Dr. Ömer ARIÖZ, Rector of Toros University*			
11.00-11.30	**MAIN ROOM** **Prof. Dr. Ferruh ERDOĞDU** **Sustainable Food Processing for Industry x.0**			
11.30-13.00	**LUNCH BREAK**			
	SESSION 1			
13.00-13.30	***ROOM 1*** *Keynote Speaker* **Prof. Dr. Sc. Ines BANJARI** **The thin line between social disparity and obesity in children**			
	ORAL PRESENTATIONS			
	ROOM 1			
	Achieving Sustainable Equity in Food and Nutrition			
	Code	**Session chair: Prof. Dr. Gülden PEKCAN**		
13.30-13.45	**F133**	Food, Nutrition and Food Sustainability: Ethically Where to Start? *Tatiana Silva, Bruno Sousa*		
13.45-14.00	**F105**	Food Based Interventions and Diet Diversification for Food and Nutritional Security *Srilatha Vasanthu, Pedda Nagri Reddy*		
14.00-14.15	**F113**	Food Irradiation for Food Security: Trends in Malaysia and Southeast Asia Countries *Nur Sumirah Mohd Dom, Nor Khaizura MahmudAb Rashid, Zainah Adam*		
14.15-14.30	**F111**	Comparative Assessment of Some Selected Bread Brands in Ondo State, Nigeria For Public Acceptability *Adebayo Ibidapo Nathaniel, Adedipe Abioye*		
14.30-15.00	**BREAK**			
	SESSION 2			
15.00-15.30	***ROOM 1*** *Keynote Speaker* **Dr. Sanije Zejnelhoxha** **Polycyclic Aromatic Hydrocarbons (PAHs) in cooked meat: public concern and mitigation strategies**			
	ROOM 1		**ROOM 2**	
	ORAL PRESENTATIONS			
	Recent Evidence in Clinical Nutrition		**Functional Foods for Health**	
	Code	**Session Chair: Asst. Prof. Özlem ÇETİNER**	**Code**	**Session Chair: Asst. Prof. Tuğçe Nur BALCI**
15.30-15.45	**N116**	Relationship Between FAD Diets and Nutritional Well-being: A KAP Study Among Young Adults of Maharashtra, India *Pooja Panchal, Parth Tailor, Alkama Mulla*	**N123**	Functional Foods for Healthy Aging and Disease Management *Muhammad Aqib, Maryam Arshad, Madiha Rafique, Amara Arif*
15.45-16.00	**N105**	The Effect of the Dietary Approaches to Stop Hypertension (DASH) Diet on Sleep, Mental Health, and Hormonal Changes: A Randomized Clinical Trial in Women with Type 2 Diabetes *Elnaz Daneshzad*	**N114**	Investigation of pumpkin seeds for enhancing physical performance in gym trainees Mubarra Saeed, Rushba Irfan, Madiha Rafique, Huma Ambreen, Aysha Saleem
16.00-16.15	**N133**	Prevalence of Obesity, Metabolic Risk Factors, and Unhealthy Lifestyle Behaviors Among University Staff: A Cross-sectional Study *Noor Salihah Zakaria*, Lee Yi Chen, Aslina Nasir	**N113**	Soybean as a sustainable solution to combat protein energy malnutrition: a systematic review Madiha Rafique, Muhammad Aqib, Mubarra Saeed, Muhammad Naeem
16.15-16.30	**N124**	Effect of Nutritional Knowledge of Mothers on School-aged Children Health *Abeer M. Aljaadi, Mai A. Ghabashi, Abrar M. Babteen*	**N115**	Assessing the nutritional profile of chickpea-based nutriment to improve iron intake among infants *Rushba Irfan, Beenish Israr, Mubarra Saeed*
16.30-16.45	**N101**	Food Allergies Among Adolescents and Adults: A Review *Adekunle Ayodeji Folorunso*	**N109**	Dose-dependent effect of tart cherry on blood pressure and selected inflammation biomarkers: A GRADE-assessed systematic review and meta-analysis of randomized controlled trials *Seyedeh Tayebeh Rahideh, Mostafa Norouzzadeh, Minoo Hasan Rashedi, Hossein Shahinfar*

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11.30-13.00	LUNCH BREAK			
	SESSION 1			
13.00-13.30	*ROOM 3* *Keynote Speaker* **Asst. Prof. PhD Ozbekova Zhyldyzai** **Physicochemical properties of various animal fat mixtures**			
	ORAL PRESENTATIONS			
	ROOM 3		ROOM 4	
	Microbiology in Food Systems		Exploring Bioactives and Functional Ingredients in Food Products	
	Code	Session chair: Dr. Hira Singh	Code	Session chair: Assoc. Prof. Fabio Chinnici
13.30-13.45	F118	Effectiveness Of Different Types of Sanitizer On The Co-Aggregation Of Staphylococcus Aureus And Escherichia Coli On Cutting Board *Pawitra Ramu, Nor Ainy Mahyudin, Nur Amira binti Rosli*	F102	Valorizing A Typical Italian Extra Virgin Olive Oil: Bioactive Compounds and Sensory Attributes *Enrico Casadei, Enrico Valli, Alessandra Bendini, Sara Barbieri, Rosalba Tucci, Mario Guida, Ferioli Federico, Tullia Gallina Toschi*
13.45-14.00	F119	Transfer Rate of Antibiotic-Resistant Escherichia Coli Isolates from High-Density Polyethylene Cutting Board Surface to Fresh-Cut Fruits *Nur Amira binti Rosli, Nur E'zzatya Nawal Jauza Mohd Azurin, Nor Ainy Mahyudin*	F104	Impact Of Acha Flour and Starch on The Microbiological, Physicochemical and Sensory Properties of Soymilk *Adekunbi Adetola Malomo, James Kenneth Chukwundum Okeke, Adedoyin Fikayo Bosrotsi*
14.00-14.15	F120	Aptamer-based Biosensor for Rapid Detection of Escherichia coli O157:H7 in Water *Noordiana Nordin*	F114	Chitosan As a Tool for The Production Of Sulfite-Free Wines: A Focus On Its Antioxidant Properties *Fabio Chinnici*
14.15-14.30	F130	Anti-Sallmonela Activity of Thymol-Based Deep Eutectic Solvents *Teodora Kukrić, Boris Popović*	F126	Characterization of Soursop Fruit Tablets: A Study on Physicochemical, Antioxidant Properties, and Sensory Attributes *Mannur Ismail Shaik, Engku Alya Najiha, Norizah Mhd Sarbon*
14.30-14.45	F131	Natural Plant Compounds as Effective Antifungal Agents Against Botrytis Cinerea in Postharvest Disease Management *Teodora Kukrić, Boris Popović*	F140	In vitro Anti-inflammatory Activity of Green Extracts from Plant Species Belonging to Lamiaceae Family *Ruzica Ždero Pavlović, Tatjana Jurić, Boris Popović*
14.45-15.15	BREAK			
	SESSION 2			
	ROOM 3		ROOM 4	
	ORAL PRESENTATIONS			
	Advancements in Functional Flours & Bakery Products: Nutritional and Sensory Insights-1		Advancements in Functional Flours & Bakery Products: Nutritional and Sensory Insights-2	
	Code	Session Chair: Adekunle Ayodeji Folorunso	Code	Session Chair: Mustafa Kadir Esen
15.15-15.30	F122	Functional Properties, Vitamin Content and Sensory Attributes of Instant Swallow Meal from Pre-Gelatinized Composite Flours *Adaora Ngozi Nwosu, Ernest Eguono Emojorho, E. Nwamaka Aniagor*	F117	Production of Functional Muffins Using Natural Ingredients (Okra, Mango Leaves, and Ribes Rubrum) *Sara Gashtasbi, Zahra Emamdjomeh*
15.30-15.45	F138	Proximate Composition and Functional Properties of Wheat (Triticum aestivum) And Fermented Bambara Groundnut (Vigna Subterranea verdc) Flour Blends for Production of Acceptable Cookies *Chetachi Maryann Eze*	F141	Antioxidant Composition of Breakfast Cereals from Acha (*Digitaria Exilis*), Pigeon Pea (*Cajanus Cajan*) And Oyster Mushroom (*Pleurotus Ostreatus*) Flour. *Rita Ogodo Nwankwegu*
15.45-16.00	F101	Quality Improvement of Cookies Produced Using Wheat and Abelmoscus Caillei Flour Blends *Adekunle Ayodeji Folorunso*	F127	Enhancing Protein Quality with Acha, Pigeon Pea, Oyster Mushroom Breakfast Cereals: Benefits for Diabetic Patients *Rita Ogodo Nwankwegu, Ifeoma Elizabeth Mbaeyi-Nwaoha*
16.00-16.15	F124	Nutritional And Sensory Attributes of Cookies Made from A Blend of Plantain (Musa Paradisiaca) Flour, Snot Apple (Azanza Garckeana), and Ginger (Zingiber Officinale) Powder *Abioye Adedipe, Adegunwa, M.O, Alamu, E.O, Ogundiran, V.E, Ayoola, T.E, Ogungbesan, B.O*	F128	Enrichment Of Wheat-Acha Flour Based Cookies with Elm Oyster Mushroom (Hypsizygus Ulmarius) Flour *Oluwadamilola Ogunsade, Adedayo Olubunmi Adeboye, Oluseye Oladapo Abiona, Akinsola Albert Famuwagun*
16.15-16.30	F137	Physicochemical Composition and Microbiological Studies of Stored Kunu-Zaki, Produced from Millet (Pennisetum Glaucum)/Acha (Digitaria Exilis) And Sesame Sesamum Indicum L.) Blends *Precious Garba*	F115	Effect Of Transglutaminase and Cellulase on The Technological Properties of Gluten-Free Brown Rice Steamed Cake *Nor Afizah Mustapha, Ayesha Azli*

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	SESSION 3				
10.00-10.30	ROOM 1 Keynote Speaker Asst. Prof. Agniezska PIEKARA Upcycling towards new ingredients and sustainable food options				
	ROOM 1		ROOM 2		
	ORAL PRESENTATIONS				
	New Horizons in Nutritional Science-1 (Turkish session)		New Horizons in Nutritional Science-2 (Turkish session)		
	Code	Session Chair: Assoc. Prof. Betül GÜLŞEN	Code	Session Chair: Asst. Prof. Eda PARLAK	
10.30-10.45	N107	Farklı Diyet Modellerinin Bağırsak Mikrobiyotası Üzerine Etkisi Cansu Balkan, Emre Batuhan Kenger	N106	Toplu Beslenme Kuruluşlarında Çalışan Personelde Sürdürülebilirlik Bilincinin Değerlendirilmesi Elif Güner, Sıla Söylemez, Sefa Merve Aydın, Dilan Yıldırım, Sevde Neslişah Çambel	
10.45-11.00	N132	Probiyotik, Prebiyotik ve Psikobiyotiklerin Nörogelişimsel Hastalıklar Üzerindeki Etkileri: Otizm Spektrum Bozukluğu Üzerine İnceleme Ayşe Gökçe Alp, Elif Ayça Alp	N111	Sporcu Beslenmesinde Sürdürülebilir Diyetler Ahmet Serhat Afşar, Muttalip Ayar	
11.00-11.15	N131	Bitki Bazlı Gıdaların Sürdürülebilirliği: Çevreci Besin Zenginleştirme Yaklaşımları Ayşe Gökçe Alp	N112	Bilişsel Fonksiyonda Antioksidanların Rolü Ahmet Serhat Afşar, Vahibe Uluçay Kestane	
11.15-11.30	N126	Sirkadiyen Ritimleri Bozulmuş Farelerde Serum Süperoksit Dismutaz Düzeylerinin Araştırılması Elif Nur Tok, Mehtap Ünlü Söğüt, Sevtap Kabalı	N120	Sağlık Bilimleri Fakültesi Öğrencilerinin Sürdürülebilir Beslenme Davranışları ve Besin Tüketim Sıklıkları İlişkisi Beyza Gülsüm Uğurlu, Ayşe Betül Demirbaş	
11.30-11.45	N127	Sirkadiyen Ritimleri Bozulmuş Farelerde Serum Serotonin Düzeylerinin Araştırılması Elvan Kaya, Mehtap Ünlü Söğüt, Sevtap Kabalı	N121	Üniversite Öğrencilerinde Besin Neofobisi, Yeme Davranışı ve Diyet Kalitesi Arasındaki İlişkinin İncelenmesi Sinem Cengiz, Sinem İnal, Zeynep Çankaya, Şeyma Arslan, Esra Tansu Sarıyer, Gonca Yıldırım	
11.45-12.00	N122	Bazı Popüler Diyetlerin Protein Kalitesi, Antioksidan ve İnflamatuar Değerlerinin İncelenmesi Hatice Merve Bayram, Arda Öztürkcan	N129	Brokoli Sebzesinde Farklı Pişirme Yöntemlerinin K1 Vitamini İçeriğine Etkisi Zehra Margot Çelik, Simay Kundakçı, Beyza Turgut, Hilal Aksoy, Şerife Köse	
12.00-12.15	N108	Sporcular için Sürdürülebilir Beslenme Hande Seven Avuk, Esengül Özkan	N110	Uzay Yolculuğu ve beslenme Sevtap Kabalı	
12.15-13.00	BREAK				
	SESSION 5				
13.00-13.30	ROOM 1 Keynote Speaker Assoc.Prof. Tetiana STEPANOVA Modern advances in technological aspects of fruit and berry jelly production				
	ORAL PRESENTATIONS				
	ROOM 1		ROOM 2		
	Cultural Heritage and Sustainablity (Turkish session)		Food Quality, Safety, and Sensory Insights (Turkish session)		
	Code	Session Chair: Assoc. Prof. Sancar BULUT	Code	Session Chair: Prof. Dr. Yüksel ÖZDEMİR	
13.30-13.45	F147	Geleneksel Develi Gaceri Bulguru ve Unu Sancar Bulut	N128	Çöven Otu (Gypsophila Bicolor) Kökünün Suyu ve Farklı Bitkisel Sütler Kullanılarak Vegan Dondurma Geliştirilmesi Simay Kundakçı, Güleren Sabuncular, Elanur Bal, İrem Gül Arslan	
13.45-14.00	F148	Geleneksel Develi Gaceri Ekmeği Sancar Bulut	G101	Siyah ve Beyaz Nohut, Mercimek ve Pirinç Unlarından Yapılan Glutensiz Kek Ve Kurabiyelerin Duyusal Olarak Karşılaştırılması Zeliha Duyar, Kerem İlaslan, Zehra Dilistan Shipman	
14.00-14.15	G108	Gastronomide Alternatif Protein Kaynağı Olan Alglerin Sürdürülebilirlik Açısından Değerlendirilmesi Ayşe Nur Elmaskaya	F145	Sensory Evaluations on Consumer Acceptance of Fish Gelatin in Food Products Cemile Buse Çopur, Edibe Seda Erten	
14.15-14.30	G103	Malakan Peyniri: Tarihi Kökler ve Üretim Sanatı Aleyna Mutlu, Ahmet Emirmustafaoğlu	F149	Microbiological Safety Assessment Of Food Wastes: Their Potential Use in Functional Foods Aysegul Kirmizigul Peker, İlkin Şengün	
14.30-14.45	G107	Kırklareli Ürünlü Köyü Sütlü Kahve Festivalinin Kültürel Miras Açısından Değerlendirilmesi Mehmet Selman Bayındır	F112	Saponinlerin Kromatografik ve Spektrofotometrik Yöntemlerle Kantifikasyonu Emine Nakilcioğlu, Gizem Tiryaki	
14.45-15.00	F146	Türkiye'nin geleneksel bir tahıl ürünü: Kars Kavılca Bulguru Asya Çetinkaya, Güven Gülbaz	F150	Geleneksel Yolla Üretilmiş Turşuların Probiyotik Potansiyelinin İncelenmesi Özlem Yalçınçıray	
15.00-15.15	G102	Türkiye'de Şarap Turizminin Sürdürülebilirliği; Mevcut Durum ve Geleceğe Yönelik Öneriler Bahar Bayındır	F134	Kırmızı mercimekten patlatılmış yenilikçi ürün üretimi üzerine araştırma Caner Çelikkol, Mustafa Bayram	
15.15-15.30	BREAK				
15.30-16.00	ROOM 1 Keynote Speaker Assoc. Prof. Seden Doğan Gastronomy 4.0				
	Closing Session				

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	SESSION 3			
10.00-10.30	ROOM 3 *Keynote Speaker* **Prof. Dr. Mustafa BAYRAM** **Gıda İçin "Yeni" Bir Gelecek (A "New" Future for Food)**			
	ROOM 3		ROOM 4	
	ORAL PRESENTATIONS			
	Food Heritage, Cultural Gastronomy, and Sustainable Practices		Modern Approaches in Food Processing & Agrifood System	
	Code	Session Chair: Prof. Dr. Bahar TANER	Code	Session Chair: Asst. Prof. Çağla ÖZBEK
10.30-10.45	F107	Refugee Gastronomy Culture Interactions *Nur İncetahtacı*	F129	Application of Transglutaminase Enzyme as A Substitute of Phosphates in Meat Processing Technology *Vilma Gurazi, Xhuljana Sula, Kapllan Sulaj, Suela Lulollari*
10.45-11.00	G105	Consumer Knowledge, Perception of Food Imagery, and Acceptance of Food Heritage in State of Kelantan, Malaysia *Rahijan Abdul Wahab, Nasha Alyssa Ab Ghani*	F143	Green Extraction Techniques & Characterization for Recovery of Bioactives from Fruit & Vegetable Industry Wastes *Sudarshan Ramanathan, Sumit Sudhir Pathak*
11.00-11.15	G109	Cultural Gastronomy and Sustainable Tourism Development: The Role of Organic Rose Cultivation in Enhancing Community-Based Tourism in Pha Nam Thieng Village, Khon Kaen, Thailand *Donruetai Kovathanakul*	F123	Evaluation of Protein Extraction Procedures for Gel-Based Proteomic Studies of *Caulerpa lentillifera* *Fisal Ahmad, Azwan Awang*
11.15-11.30	G106	Exploring the Influence of Social Media Marketing Activities on Customer Satisfaction at Mid-Scale Restaurants in Penang, Malaysia *Aziz Bin Yusof, Teow Jin Zhe, Asma' Binti Ali*	F109	Enzymatic Extraction of Total Sugars from Olive Leaves *Maja Dent, Jelena Buha*
11.30.11.45	G110	From Standardized Commodity to Assetization. A Sociohistorical Approach to The Transformation of Olive Oil in Greece *Vasiliki Karantzavelou, Stathis Arapostathis*	F106	Evaluation of Carotenoid Stability During Bio Fortified Cassava Fermentation and Associated Bacterial Community *Lateefah Oyafajo, Lateef Sanni, Taofik Shittu, Sarafadeen Kareem, Wasiu Awoyale, Harun Aremu, Omotayo Oyedara, Luqman Azeez*
11.45-12.00	G104	Development and Characterization of Indian Traditional Low Alcoholic Beverage *Roji Waghmare, Prem Mishra*	F121	Nutritional Profiles of Fermented Defatted Soybean Meal Using Staphylococcus Succinus *Leony Tham Yew Seng*
12.00-12.15	F142	Traditional Alcoholic Beverages of Himachal Pradesh, India *Ashwani Kumar*	F132	*Newbouldia Laevis* and *Icacina Trichantha* Leaves Influenced Chemical and Microbiological Quality of Fermented Melon Condiments *Oladeji Oluwatoyin Ajoke, Clement Olusola Ogidi, Akinde Folake Ruth, Okunowo Omowumi A.*
12.15-12.30	G111	High World Happiness Record of Finland Seven Consecutive Times: Could Commitment to Sustainable Development Goals be the Reason behind this Great Success? *Bahar Taner*	F103	Studies on the Technologies Involved in Street Food Vending in Osun State, Nigeria *Titilayo Olubunmi Olaposi, Sunday Soladoye Asa*
12.30-13.00	BREAK			
	SESSION 5			
	ORAL PRESENTATIONS			
	ROOM 3		ROOM 4	
	Food Technologies for Animal-Based Production: Impacts on Sustainability		Advancing Food Science to Sustain Traditional Practices	
	Code	Session Chair: Wendy Wee	Code	Session Chair: Prof. Ifeoma Elizabeth Mbaeyi-Nwaoha
13.00-13.15	N117	The potential of kaffir lime, *Citrus hystrix DC,* leaf as feed additive for aquaculture uses *Yeu Hooi Kon*	N133	Effects of Fruit Stage on Nutritional Value of Guava *Kuldeep Kamboj, Gagandeep Kaur, Kirandeep Kaur Kang, Naresh Kumar Arora, Jaswinder Singh Brar*
13.15-13.30	N118	Potential of using novel *Staphylococcus succinus MF 116251* fermented soybean meal (FSBM) as fish meal replacement in African catfish (*Clarias gariepinus*) feed formulation *Zakaria Muhammad Khairulanam*	F144	Biochemical Studies of Carrot for Its Nutritional and Antioxidant Properties *Navjot Sharma, Shilpa Gupta, Usha Nara, Harshneet Singh Sran*
13.30-13.45	N119	The potential of *Etlingera elatior* (Jack) bud flower powder as feed additive in African catfish, *Clarias gariepinus* farming *Liew Vui Kien*	N130	Compositional Analysis of Palak W.R.T. Its Nutritional and Anti-Nutritional Attributes *Jashandeep Kaur, Shilpa Gupta, Hira Singh*
13.45-14.00	F125	The potential of potato as starch source in juvenile African catfish (*Clarias gariepinus*) feed formulation *Mohd Shaiful Azman Abdul Rahim, Lee Seong Wei, Kon Yeu Hooi, Martina Irwan Khoo, Mohamad Nor Azra, Wendy Wee*	F151	Quality Evaluation of Herbal Yoghurt produced using Utazi (*Gongronemalatifolium*) and Uziza (*Piper guineense*) Leaf Extract *Ifeoma Elizabeth Mbaeyi-Nwaoha, Ohaeri Favour Mmesoma, Ngozi Chioma Okoronkwo, Onyeaka Helen*
14.00-14.15	F135	The potential of Curcuma longa L. leaf as feed additive in African catfish (*Clarias gariepinus*) farming *Wendy Wee, Kon Yeu Hoo, Martina Irwan Khoo, Mohamad Nor Azra, Lee Seong Wei*	F152	Effect Of Addition of Diced African Bush Mango (*Irvingia Gabonensis*) Pulp in Formulated Spoonable Yoghurt *Ifeoma Elizabeth Mbaeyi-Nwaoha, Ngozi Esther Abosi, Ngozi Chioma Okoronkwo, Onyeaka Helen*
14.15-14.30	F136	The potential of black fungus, Auricularia auricula, as a feed additive in African catfish (*Clarias gariepinus*) farming *Alvin Amos Adrian Susin, Lee Seong Wei, Albaris B Tahiluddin, Liew Vui Kien, Wendy Wee*	F153	Microbial, Functional and Sensory Properties of Herbal Yoghurt Formulated with Broccoli (*Brassica oleracea var. italica*) and Garden Egg Leaf (*Solanum aethiopicum*) Extract *Ifeoma Elizabeth Mbaeyi-Nwaoha, Onyeka Rita Ohaegbulem, Ngozi Chioma Okoronkwo, Onyeaka Helen*
14.30-14.45	F116	Development Village Chicken Production in Malaysia *Aida Zakaria, Mamat Hamidi Kamalludin*	F139	Quality assessment and nutritional characterization of traditional Indian products from processed non-conventional legumes *Prashant Sahni, Savita Sharma*
14.45-15.00	F154	The potential of ginger, Zingiber officinale Rosc, leaf powder as feed additive in African catfish farming *Lee Seong Wei, Zulhisyam Abdul Kari, Muhammad Anamul Kabir, Martina Irwan Khoo, Mohamad Nor Azra, Wendy Wee*	F110	Evaluation Of Eleven Accessions of Groundnut *(Arachis Hypogaea L.)* In Nsukka Derived Savannah Agro-Ecology of Nigeria *Uchenna Noble Ukwu, Nathaniel Dauda, Solomon Oluwaseyi Adewuyi*
15.00-15.30	BREAK			
15.30-16.00	ROOM 1 *Keynote Speaker* **Assoc. Prof. Seden Doğan** **Gastronomy 4.0**			
	Closing Session			