PRODUCTION AND COMPARATIVE EVALUATION OF SOLID-STATE AND SUBMERGED FERMENTATION ON THE MICROBIAL, NUTRITIONAL AND SENSORY QUALITY OF SOY-MAIZE COOKIES.

BY

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GOU/U22/BTG/169

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TITLE PAGE

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DEPARTMENT OF BIOLOGICAL SCIENCES
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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR SCIENCE (B.Sc) DEGREE IN BIOTECHNOLOGY

SUPERVISOR: DR. C. A. OKAFOR AND MRS N.M UKWUEZE

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APPROVAL

This research titled **"PRODUCTION AND COMPARATIVE EVALUATION OF SOLID-STATE AND SUBMERGED FERMENTATION ON THE MICROBIAL, NUTRITIONAL AND SENSORY QUALITY OF SOY-MAIZE COOKIES"** has been assessed and approved by the Committee of Department of Biological Sciences and Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University Enugu Nigeria.

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CERTIFICATION

This is to certify that this research titled **"PRODUCTION AND COMPARATIVE EVALUATION OF SOLID-STATE AND SUBMERGED FERMENTATION ON THE MICROBIAL, NUTRITIONAL AND SENSORY QUALITY OF SOY-MAIZE COOKIES"** was carried out by **Egbunonu Fechukwu Chiamaka** under supervision of **Dr. C. A. Okafor and N.M Ukwueze** in the Department of Biological Sciences, Godfrey Okoye University Enugu, Nigeria.

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DEDICATION

This research work is dedicated to my family and to God almighty the author and finisher of our Faith whom by his grace foreknew, predestined and made this work a success and for seeing me through my academic years.

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ABSTRACT

Fermentation is an effective bioprocessing technique that enhances the nutritional quality, safety, shelf stability, and sensory characteristics of cereal-legume foods, thereby contributing to the development of affordable and nutrient-rich functional products. This study aimed to evaluate the effects of solid-state fermentation (SSF) and submerged fermentation (SMF) on the microbial, nutritional, and sensory quality of soy-maize composite flour and cookies. Soybean and maize grains were blended in a 30:70 ratio and subjected to SSF and SMF for 48 h, while an unfermented sample served as the control. The blends were processed into composite flour and subsequently used for cookie production. Physicochemical, microbial, proximate, energy value, and sensory analyses were conducted using standard methods. Results showed that fermentation significantly ($p < 0.05$) influenced the quality characteristics of the products. The pH decreased from 6.24 in the control to 6.12 and 4.45 in SSF and SMF samples, respectively, indicating active fermentation. Solid-state fermentation recorded the highest total viable count of 2.09×10^5 CFU/g and lactic acid bacteria count of 1.01×10^4 CFU/g, suggesting enhanced microbial activity. Proximate analysis revealed that SSF significantly improved protein content in composite flour to 14.81% and cookies to 8.25% compared with the control with 8.13% and 4.94%, respectively. Fermentation also increased ash, fat, fiber, and energy values while reducing moisture and carbohydrate contents. Sensory evaluation showed that cookies produced from SSF flour had higher consumer acceptability than those from SMF flour and were comparable to the control in most sensory attributes. The findings demonstrate that fermentation, particularly solid-state fermentation, enhances the nutritional quality and acceptability of soy-maize cookies. Solid-state fermentation therefore represents a promising approach for developing nutrient-dense cereal-legume snack products from locally available raw materials.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Fermentation remains one of the oldest and most effective food processing techniques used to improve the nutritional quality, safety, shelf stability, and sensory characteristics of food products. In recent years, there has been increasing interest in the application of fermentation technologies (green processing) in cereal-legume food systems due to their ability to enhance protein quality, mineral bioavailability, digestibility, and functional properties through microbial activity (Baruah *et al*. 2022). Soybean and maize are widely consumed in developing countries because of their nutritional and economic importance; however, their utilization is often limited by anti-nutritional factors, poor protein balance, and reduced sensory acceptability in some processed forms. Fermentation has been reported to significantly improve the nutritional composition and organoleptic qualities of cereal-based products by promoting the growth of beneficial microorganisms such as lactic acid bacteria and yeasts (Skrivan *et al*. 2023). Among the various fermentation techniques, solid-state fermentation and submerged fermentation are commonly employed due to their efficiency in modifying food substrates and enhancing product quality. Cookies produced from fermented composite flour may therefore serve as nutrient-enriched functional snack products with improved consumer acceptability.

Fermentation has been an old process and it is thousands of years old, it remains a popular processing option nowadays. It is explained by the fact that it prolongs the longetivity of foods and increases their nutritional value, in addition to enhancing human health, through the introduction of good bacteria (Anal 2019; Baruah *et al*. 2022). Food may also be preserved through ultra-processing, as well as E-numbers, however, these approaches have been linked with poor consequences on health outcomes (Asioli *et al*. 2017; Monteiro *et al*. 2019).

Natural-fermentation can frequently happen under uncontrolled circumstances, whereas controlled conditions are the current choice of fermentation to ensure good quality and safe handling products (Skrivan *et al*. 2023). For natural fermentation, they are done by the response of microorganisms inherent in raw materials or the environment. The method is common in traditional African dishes like ogiri, fufu, and iru where the microbial populations are not added and instead grow naturally under favorable environmental conditions (Holzapfel, 2018). Modern fermentation, on the contrary, involves the use of chosen or improved starter cultures (Skrivan *et al*. 2023). Carbohydrates are commonly used by microorganisms as a source of metabolic activity during the process of fermentation. Nevertheless, carbohydrates, proteins, and fats in most fermented foods and beverages are simultaneously altered by organisms and its catalyst (Assohoun *et al*. 2013; Hasan *et al*. 2014).

By bringing accurate methods of fermenting food products made of cereals and legumes to create composite flours, one enhances nutritional value and the overall palatability of the final products. Composite flour is a blend of various flours, such as wheat flour or other grains with those of beans, roots, oil-seeds. (Jenfa *et al.,* 2024).Attributes of flour blends determines on the farming circumstances in which they are cultivated. For example, millets can grow in dry climates whereas roots such as cassava and yams thrive in moist and tropical climates. Such crops are durable in case of little rainfall, unfertile soil, and unpredictable weather that ensures that flour supply is constant in areas where wheat cannot be cultivated. Composite flour has been utilized in the food industry in order to deal with food security, combat malnutrition, and enhance food variety in the world food supply. The present-day issue of the wheat supply in the global system due to climate-related crop failures, political issues, and supply-chain disruptions has demonstrated how fragile food systems relying on a single grain can become. These issues caused the prices of wheat to increase and accessibility to it became difficult in most of the nations in the low and middle-income categories. This adds to the necessity to incorporate local, climate-adaptable crops in composite flours to make food sources more varied (Amadeu *et al.,* 2024).

Composite mix plays an important role in ensuring the security of foods and , these flours depend on crops that are physically and environmentally diverse which exhibit resistance to drought, excellent nutrient utilization, and require minimal inputs. It is important to look at these things and determine whether they are sustainable and capable of changing with the environmental problems and remain nutritious. Most of the crops that are found in composite flour such as millets have resistance against droughts and they thrive well in arid regions. Their addition to flour mixtures enhances the consumption of local crops and maintains access to healthy food all year long (Twinomuhwezi *et al.,* 2020). In addition, flour blends are in line with the United Nations Sustainable -Development Goals such as inhibiting hunger strikes , health promotion , to ensure sustainability consumption , action on climate , and protecting land (Chaudhary *et al.,* 2024; Ehis-Eriakha *et al.,* 2025). But we must mention the disadvantages of the environment in order to remain objective when discussing sustainability. As an example, an increase in cassava will negatively impact forest cover and biodiversity, and thus, proper planning and farming techniques are required (Twinomuhwezi *et al.,* 2020).

Composite flour may also be used in the preparation of healthy food that is attractive to patients, working on chronic conditions. The addition of composite flour to food may reduce the effect and risk of serious diseases, this applies to recent diets (Chandra *et al.,* 2015; Alim *et al.,* 2024). Combining the sources of flour, makers have the opportunity to create new recipes that can satisfy any diet requirements and make people accept these products more. In such a manner, they satisfy the demand for healthier options and support the utilization of native crops in the preparation of foodstuffs (Noorfarahzilah *et al.,* 2014). Composite flour contributes to making food more nutritious, helpful, and cheaper. Harmonizing flours exploits the better qualities of each ingredient to profit in health, which no single type of flour can achieve by itself (Hasmadi *et al.,* 2020). Composite flours should be socially sustainable, which implies that they should be low-priced and made using local products. Enhancing access by ensuring recipes are affordable and local crops are used can empower local economies, and promote people to use them over time (Chandra *et al.,* 2015).

A confectionery product is a type of food or snack food like cookies that are eaten in large amounts in various countries where malnutrition of the body in terms of protein and calories may occur (Chinma *et al.,* 2012). They are nutritious snacks that are prepared using unsavory dough turned into a delicious and edible product through the use of heat in the oven (Ikuomola *et al* ., 2017).

Or rather has been the predominant ingredient in the manufacturing of pastries such as biscuits and other pastries, but manufactured as well using flours of legumes, roots, cereals, and tubers baked in pastries (Akinsanmi *et al.,* 2015). Soybeans (Glycine max) represent one of the most significant crops in the world with more than 350 million metric tons every year (Dilawari *et al.,* 2022). Soybean (Glycine max), is an edible legume with a high concentration of nutrients like protein, calcium, iron, fiber, magnesium, and minerals and vitamins.

One of the most vital crops or plants in Nigeria is maize (Zea mays), which is normally planted in large quantities across various nations in the world in a good ecosystem or environment. They also dominate the cheap and available food in other regions and countries such as Nigeria and typically as raw resources material allowing the production of affordable and available (staple foods) such as a variety of traditionally fermented foods such as maize pottage (pap), maize flour (masa harina), and other traditionally produced snacks. Maize mainly contains starch (60-75%), a low level of protein (9-12percent in comparison to legumes), and a source of most phytochemicals such as the phytosterols, phenolic compounds, and carotenoids. However, it is also very high in methionine and cysteine, maize kernel (typically the nutritious and wholesome portion of it) is a blend of nutrients, including vitamins such as C etc. (Adeola *et al* ., 2018). Soybean mixed with maize will make the cookie very nutritious. Research that has sought to increase the nutritional value of biscuits with the help of supplementation using legume flours has found that composite flours are able to boost protein weight in cookies without making these cookies unappealing in terms of taste, texture, and acceptability (Ayo *et al*.; Olaoye *et al.,*2020).

1.2 Statement of the problem

The continuous reliance on wheat flour in bakeries or industries has led to the limitation of the use or consumption of varieties of different products such as cereals and crops with time in other countries such as Nigeria. Mixed or blended flours have been proposed by various institutions to be used in making food or snacks such as cookies etc this concept enhanced the different usage of composite flours produced by tubers and roots such as (cassava, potatoes etc) legumes such as soybean, and crops such as maize etc among many other healthy sources of flour as well. The snack is affordable as the crops produced in our region are used (Abiola,2025).

The affordable snacks in Nigeria are not rich in nutrient and there is a necessity for better nutrient-rich and affordable snack products with better fermentation processes to produce high-quality products.

To identify the effect of various techniques of fermentation processes on the nutritional value of soymaize cookies such as establishing the optimal method of fermentation process or a method that optimizes the nutritional value and sensory characteristics of soymaize cookies.

Many research staff have engaged in a lot of work on using composite flours that are used during the manufacture of pastries such as cookies (Abiola,2025). The study is useful as it discusses nutrient content in cookies prepared out of the fermented soybean and maize flour mixture or blend.

1.3 Aim and Objectives

Aim

This is aimed at the production and comparative evaluation of solid-state and submerged fermentation on the microbial, nutritional, and sensory quality of soy-maize cookies.

The Objectives were to;

1. Produce soy-maize composite flour and cookies using solid-state and submerged fermentation methods.
2. Evaluate the physicochemical and microbial properties of fermented soy-maize flour produced by different fermentation techniques.
3. Determine the proximate composition of soy-maize composite flour and cookies produced from fermented samples.
4. Evaluate the sensory properties and overall acceptability of cookies produced from fermented soy-maize composite flour.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of soybean and maize

Dry seeds of legume plants and cereal grains which are called caryopses serve as fundamental food sources throughout the world because they provide essential energy together with protein and dietary fiber. The rich vitamin and mineral content together with health-boosting substances including polyphenols and carotenoids and other bioactive components results in various health advantages for people. The combination of cereal and pulse consumption leads to better protein content (Tamang *et al.,* 2016), which results in better health results (Agah *et al.,* 2017) because the body uses amino acids together with the flavonoids to improve health. The production of these crops supports sustainable agricultural systems which enhance human health while protecting natural ecosystems. The practice of rotating crops leads to better soil health through enhanced nutrient distribution and active microorganisms, which results in higher farm output (Brankatschk and Finkbeiner, 2015). The food sector uses these grains and pulses to produce a range of healthy products that meet consumer needs while providing budget-friendly choices from different cultural foods to support the market demand for healthy meal options. Cereals and legumes serve as essential elements for food security initiatives which aim to enhance environmental sustainability through their health benefits. Legumes contain essential nutrients which include high protein and fiber content while maintaining lower carbohydrate levels than cereals (Dhull, Kinabo, and Uebersax, 2022; Siddiq *et al.,* 2022).The production of legumes takes place through processes which maintain environmental sustainability, making them an economical protein option that costs less than animal protein sources (Uebersax *et al.,* 2022). The health advantages of legumes become available through their regular consumption according to scientific research (Didinger and Thompson, 2021). The value-added products which derive from their attributes will enable developed nations to increase their current per capita legume consumption beyond existing levels.

Foods including soybean and maize are high in protein and can improve the quantity and quality of fermented foods like Agidi (Kikya, 2025). The use of soybean and maize mixture proposes a good strategy to the creation of nutrient rich products. Soybean belongs to the fabaceae family one of the most important group of plants in the world .Their seeds are protein rich containing essential amino acids and vitamins making them an important source of plant-based protein for both animal and human nutrition.However legumes also contain anti nutritional factors if not processed properly (Abdel-al, 2024). Maize is among the most extensively grown cereal crops worldwide, it is a vital contributor of energy, carbohydrates and dietary fiber for both people and animal, although they usually offer less proteins than legumes, cereals are grasses cultivated for their edible grain yields, Additionally it is used as a raw material for industrial products, animal feed and processed foods. Cookies are mostly made from white flour. All cookies relatively are alternations of one simple formula. Various research has been carried out on different fermented foods, also on different types of cereals the most common fermented products are made from maize, (Adinya, 2025). They are usually high in carbohydrate and low in protein. Most of the fermented maize products are highly deficient with nutrients such as lysine, also most nutrients are lost due to bad process methods from traditional fermentation (Adinya ,2025).

From previous research they were noticeable variations in the direct structure of cookies that were produced by different flour mixture. Mixed cereals with legumes that are protein rich is a highly recommended medium for reducing protein energy malnutrition, the family of legumes soybean contains up to 35-45% protein with an incredible and vital Amino compounds (Shirika *et al.,* 2015). Research of individuals who have worked with Maize and soybean to produce various meals suggest that each of them are rich in nutrients.

2.2 Soybean (*Glycine max*)

The annual herbaceous legume known as soybeans (Glycine max [L.] Merr.) is a member of the pea family Fabaceae. It was first domesticated in East Asia and is now grown all over the world because of its enormous nutritional and economic value. Soybeans, one of the most important oilseed crops in the world, offer high-quality protein and vegetable oil for animal feed, human consumption, and a variety of industrial uses (Sugiyama *et al.,* 2015; Modgil *et al.,* 2020).

2.2.1 Nutritional composition of soybean (Glycine max)

it is commonly known that soybean seeds are a high-protein legume crop, and current compositional studies regularly show that mature seeds have high protein levels. Soybean protein content varies by cultivar and growing conditions, but it is typically close to or slightly above 35% on a dry-weight basis. For instance, data from extensive surveys of soybean seed composition show that protein may account for between 30 and 42 percent of dry seed weight in a variety of varieties, highlighting the importance of soybeans as a source of protein for both human and animal nutrition (Meral *et al.,* 2024).

Fatty acid profile and fat content

Lipids, or fats, are also present in significant amounts in soybean seeds; they usually make up 18–22% of the dry seed weight. Polyunsaturated linoleic acid (C18:2), α-linolenic acid (C18:3), and monounsaturated oleic acid (C18:1) make up the majority of the unsaturated fatty acids in soybean oil's fatty acid profile, with smaller amounts of saturated fats like stearic and palmitic acids. Soybean oil is a valuable dietary source of essential fatty acids because of its composition (Meral *et al.,* 2024).

Minerals and vitamins

In addition to being high in macronutrients, soybeans also contain vital micronutrients. According to mineral profiling, soybean seeds have high concentrations of macro- and micro-elements that are vital to human health, including phosphorus (P), potassium (K), calcium (Ca), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu). Soybeans are known to contain B-complex vitamins and bioactive substances like vitamin E (tocopherols), which act as antioxidants, although research on vitamins in soybean seeds varies by genotype and environment (Meral *et al.,* 2024).

Figure 2.1 The Soybean pods (plant) and seeds

Source: Hussein Yahaya, 2021.

2.2.2 Anti-nutritional factors in soybean (*Glycine max*)

A number of naturally occurring substances known as anti-nutritional factors (ANFs) are found in soybeans (Glycine max). If not properly processed, these compounds can reduce the nutritional value of soy foods by interfering with digestion, metabolism, and nutrient absorption. Trypsin inhibitors, lectins, phytic acid, and oligosaccharides that cause flatulence, like raffinose and stachyose, are the main ANFs present in soybeans. When soybeans are eaten raw or improperly processed, these factors can also have negative health effects (Tripathi *et al.,* 2024; Padalkar, 2023).

Inhibitors of trypsin

Trypsin inhibitors are proteinaceous antinutrients that impede the digestion of proteins by binding to digestive enzymes like chymotrypsin and trypsin. The Kunitz trypsin inhibitor (KTI) and the Bowman-Birk inhibitor (BBI) are the two primary classes found in soybean seeds. These inhibitors decrease the efficiency of amino acid utilization and protein digestibility by forming stable complexes with digestive enzymes (Luo *et al.,* 2025).

Phytic acid

In soybeans, phytic acid, also known as phytate, is a substance that stores phosphorus. It forms complexes that are poorly absorbed in the gastrointestinal tract by chelating vital minerals like calcium (Ca), iron (Fe), zinc (Zn), and magnesium (Mg). As a result, minerals in animal and human diets have lower bioavailability (Padalkar, 2023).

Lectins

Lectins are proteins that bind carbohydrates and can adhere to the intestinal epithelium, changing the gut's integrity and ability to absorb nutrients. According to Tripathi *et al*. (2024), lectins, also referred to as agglutinins, are heat-labile proteins found in soybeans that can be rendered inactive through appropriate cooking or processing, but may still be active in unprocessed soy products.

Oligosaccharides causing flatulence

Raffinose and stachyose, two α-galactosides found in soybeans, cannot be broken down by human digestive enzymes. The large intestine receives these indigestible oligosaccharides, where bacterial fermentation generates gases that cause bloating, flatulence, and gastrointestinal distress (Padalkar, 20).

Health implications

High dietary levels of trypsin inhibitors can cause decreased growth performance, decreased protein digestibility, and increased secretion of pancreatic enzymes, which may result in pancreatic hypertrophy from the overproduction of digestive enzymes (Toomer, 2023). When diets are largely composed of unprocessed soybean products, high phytate consumption may lead to mineral deficiencies, particularly iron and zinc. But phytic acid also shows promise as an antioxidant, indicating that its function in health is multifaceted and situation-specific. Reduced nutrient absorption and gastrointestinal distress can result from untreated lectins. However, because of heat inactivation, properly cooked soybean foods like tofu and soy milk have very little lectin activity. These oligosaccharides may also function as prebiotics, promoting a healthy gut microbiota, despite being indigestible. However, if consumption surpasses tolerance, fermentation-linked gas production may result in discomfort.

2.2.3. Processing methods for soybean (Glycine max)

Conventional Processing Techniques

Soaking

One of the earliest and most straightforward techniques for preparing soybeans for consumption or additional processing is soaking. In order to soften the seed coats and start the partial hydration of proteins and carbohydrates, dried soybean seeds are submerged in water for extended periods of time (typically 8–24 hours) during the soaking process. By removing soluble compounds and preparing the seeds for further heat treatment or fermentation, soaking also aids in the reduction of some anti-nutritional factors (Agume *et al.,* 2017). In particular, it has been demonstrated that prolonged soaking in conjunction with roasting can change the protein and lipid levels in soybean flour while lowering the tannin and phytate content.

Boiling

Boiling or steaming food

Cooking requires chefs to use high-temperature heat methods which include boiling water at 100°C to cook food because this process makes food easier to digest while it breaks down seeds and eliminates heat-sensitive anti-nutritional substances which include lectins and trypsin inhibitors. Heat treatment improves protein digestibility while it decreases anti-nutritional substances but it also causes physical and chemical changes which result in the loss of heat-sensitive vitamins and the extraction of water-soluble nutrients into cooking water according to Thingujam *et al*. (2025). Research studies which examine the process of soaking soy-based products followed by cooking methods, have shown that this combination improves both protein digestibility and antioxidant capacity of fermented foods like hawaijar.

Roasting

The roasting process uses dry heat to treat soybeans, which leads to the breakdown of oligosaccharides that cause flatulence and the deactivation of trypsin inhibitors and the development of beneficial flavor changes. The roasting process of soybean flour decreases phytate and tannin content while it changes the material's physical and chemical properties, including viscosity and swelling capacity. The antinutrient content of roasted soybeans decreases when compared to raw seeds, while their protein digestibility shows improvement (Agume *et al.,* 2017).

Modern processing techniques

Industrial-level technologies are used in modern soybean processing to improve product safety, functional qualities, and nutritional quality:

Hydrothermal and thermal processes

Industrial processes use heat treatments through thermoplastic extrusion and hydrothermal treatment and high-temperature drying methods. The methods improve nutrient digestibility while eliminating anti-nutritional elements like phytates and trypsin inhibitors and enabling the creation of soy-based products which include defatted soy flour and textured vegetable proteins. The research demonstrates that pre-milling treatments combined with thermal processing achieve better results for protein digestibility improvement and anti-nutrient reduction than the results produced by roasting or soaking. The research shows that germination and roasting and hydrothermal processing methods enhance nutrient bioavailability by lowering trypsin inhibitor and phytic acid levels in processed soybean fractions (Sreechithra, 2025).

Germination and fermentation

Modern food science is developing better nutritional methods through its research on traditional processes which include fermentation and germination. The fermentation process which uses Bacillus subtilis microorganisms in kinema and natto products increases free amino acids and beneficial peptides while it decreases anti-nutrient content. The germination process activates enzymes which help proteins become better and minerals become more available to the body while it helps break down anti-nutritional compounds like phytates through enzymatic processes (Thingujam *et al.,* 2025).

Extrusion and high-pressure therapies

Industrial processes now use dielectrical heating and high hydrostatic pressure and extrusion cooking as vital operational improvements which both advanced industrial techniques have developed. The methods provide dual benefits because they establish safe conditions for storage while improving nutrient absorption and decreasing anti-nutritional elements. Extrusion serves as the standard method which manufacturers use to produce textured soy proteins and meal components that require precise functional characteristic control.

Processing's impact on nutritional quality

Soybean bioavailability and nutrient composition are significantly impacted by processing:

Protein Digestibility: The combination of heat treatment and fermentation processes improves protein digestibility because these methods destroy protein structures while eliminating anti-nutritional substances that include trypsin inhibitors which prevent protease enzymes from functioning properly (Sreechithra, 2025).

Vitamins and Bioactive Compounds: Fermentation processes enable the preservation and enhancement of bioactive phytochemicals which include isoflavones through the microbial transformation process. The application of extreme thermal treatment causes the partial degradation of heat-sensitive vitamins which includes vitamin E and carotenoids (Ogundana, 2025).

Functional Properties: Processing alters the water absorption and swelling capacity and viscosity of soybean flours while it affects their other functional properties which determine their taste and suitability for food production (Agume *et al.,* 2017).

Anti-nutritional Factors: Conventional techniques like soaking, cooking, and roasting greatly lower anti-nutrients like tannins, phytic acid, and trypsin inhibitors, increasing the bioavailability of minerals and proteins (Agume *et al.,* 2017).

2.3. Overview of maize (*Zea mays*) and its seeds

One of the most significant cereal crops in the world, maize (Zea mays L.) is used as a raw material for industry, animal feed, and staple foods. It belongs to the Poaceae family of grasses and is grown extensively on all continents except Antarctica because of its ability to adapt to a variety of agro-ecological zones and climates (FAO; Shiferaw *et al.,* cited in research on tropical maize, 2019). Because of its significant contribution to agricultural production and global caloric intake, maize is frequently referred to as the "queen of cereals"; in terms of global importance, it is only surpassed by rice and wheat (research review on tropical maize, 2019). According to botany, maize is a strong, annual grass that can grow to a height of 2-4 meters. It has a single stem, or culm, that is held up by a vast network of adventitious roots. According to the biological description of maize, the plant is monoecious, producing distinct male (tassel) and female (ear) inflorescences on the same individual, and its leaves are long and alternate along the stem. According to maize botanical descriptions, each female inflorescence matures into a cob (ear) that bears several kernels, or maize seeds, which are technically categorized as caryopses because the seed coat is fused with the fruit wall. The main edible and commercially valuable part of the plant is the kernels, or seeds, of maize. Because they are usually high in carbohydrates, especially starch, maize is an essential source of energy for both human diets and animal feed. Although their amino acid profile is restricted in essential amino acids like lysine and tryptophan (analysis of grain quality and nutritive properties), maize kernels do contain proteins (particularly the prolamin class known as zeins, which are major seed storage proteins), lipids, vitamins, and micronutrients.

Figure 2.2. The Maize Corn and Plant

Source: Gardening Australia, 2022.

2.3.1. Nutritional Composition of Maize (Zea mays)

Content of Carbohydrates (70–75%)

Carbohydrates, mostly in the form of starch, which is the primary energy source in human diets, make up the majority of maize seeds. According to nutritional analyses, maize kernels have a dry-weight percentage of 71–75% carbohydrates, which is consistent with their status as a major energy-producing cereal staple. Because of its high carbohydrate content, maize is a significant source of calories for diets, especially in Latin America and sub-Saharan Africa, where it is a major cereal crop.

Protein content and amino acid profile

Maize contains lower protein content than legumes yet its seeds provide substantial protein that reaches between 8 and 12 percent across various plant varieties which grow in different environmental conditions. Maize protein content exists as prolamin fractions (zeins) which determine its functional characteristics. The protein contains insufficient essential amino acids because it lacks lysine and tryptophan which results in reduced complete protein quality. The breeding programs developed quality protein maize (QPM) to raise lysine and tryptophan levels which results in better nutritional content.

Vitamin

The body needs B vitamins which exist in maize kernels to support its energy metabolic functions and satisfy its energy production requirements. The different types of maize and their processing methods create varying amounts of vitamin B12 content. The nutritional composition of maize shows that it contains thiamine (B1) riboflavin (B2) niacin (B3) pantothenic acid (B5) and vitamin B6. The study found that maize grain contains approximately 0.39 milligrams of thiamine and 0.20 milligrams of riboflavin for every 100 grams of the grain. The B vitamins function as essential nutrients which support brain function and assist in processing carbohydrates.

Composition of minerals

Additionally, maize seeds contain vital minerals that are vital to human health. Significant amounts of phosphorus, magnesium, potassium, iron, and zinc are found in mature maize grain analyses. These elements support metabolic functions, bone health, and oxygen transport. For instance, it has been reported that phosphorus concentrations in maize kernels exceed 200 mg/100 g, and that iron and magnesium are also present in nutritionally significant amounts. Despite having a generally low calcium content, maize still contributes to its overall mineral profile.

2.3.2 Maize's (*Zea mays*) limitations

Deficiency in lysine and tryptophan

The essential amino acids tryptophan and lysine, which are necessary for human growth and protein synthesis, are naturally lacking in maize grain. When eaten by itself, conventional maize is a poor source of complete protein because its storage proteins, mainly zeins, do not contain enough of these amino acids. Protein-energy malnutrition in populations that primarily rely on maize as a staple food has been associated with deficiencies in lysine and tryptophan in diets based on maize (Nuss and Tanumihardjo, 2011; agricultural nutrition research, 2024). The risk of not getting enough of these vital amino acids rises when conventional maize is consumed without supplemental protein sources.

Poor protein quality

Due to an imbalanced amino acid profile, maize has poor protein quality. Even though maize has 7–13% protein by weight, the protein's nutritional value is low when compared to human amino acid requirements because zein proteins, which are low in tryptophan and lysine, predominate. On the other hand, while maize varieties designed to have higher levels of lysine and tryptophan (like Quality Protein Maize, QPM) exhibit better protein quality, they are still not as complete as animal proteins or balanced legume proteins alone (Maqbool *et al.,* 2022; FAO maize nutrition report, 2025).

Phytic acid

Phytic acid, also known as phytate, is an anti-nutrient found in maize that binds vital minerals like iron and zinc to form insoluble complexes that are poorly absorbed in the digestive system. Particularly in diets that are already deficient in a variety of food sources, high levels of phytic acid in maize and other cereals decrease mineral bioavailability and cause micronutrient deficiencies. According to research, lowering the amount of phytic acid in maize (for example, by breeding or processing it with low levels of phytic acid) greatly enhances mineral absorption, including zinc uptake in animal models (Wilcox *et al.,* recent studies on phytic acid mitigation, 2024).

2.3.3 Processing of maize (*Zea mays*)

Conventional approaches

Nixtamalization

The nixtamalization process involves soaking and cooking maize kernels with an alkaline solution which uses calcium hydroxide as its primary alkaline component. This method improves maize nutritional value because it increases niacin and mineral bioavailability while decreasing the presence of anti-nutrients which include phytic acid. The process enhances the taste and smell of maize flour while improving its functional characteristics which food products require to make tortillas and hominy (Gallego-Castillo *et al.,* 2021; Wikipedia, 2026).

The process of fermentation

Fermentation of maize flour uses traditional techniques which require yeasts and bacteria mainly *Lactobacillus* for their microbial fermentation process. The fermentation process improves protein digestibility because it reduces phytic acid and other anti-nutritional factors, which results in higher vitamin B content and better sensory properties of the food (Terefe *et al.,* 2021; Ilemobayo and Kone, 2024)..

Contemporary processing methods

Modern maize processing utilizes advanced techniques which include dry milling, wet milling, extrusion and fractionation to achieve efficient separation of starch, protein, fiber and oil components. The methods provide three advantages because they enhance functional properties and maintain nutritional value while enabling production of maize-based products which include starch and sweeteners and fortified flours (Bongjo *et al.,* 2025).

2.4. Protein complementation

Concept of protein complementation

Essential amino acids

Proteins contain necessary amino acids because the human body cannot create these amino acids. The FAO (2025) report states that the body requires these amino acids to produce proteins which enable both growth and tissue repair and support various metabolic functions.

Limiting amino acids in cereals and Legumes

Cereals like maize do not provide enough lysine and tryptophan for human consumption whereas soy and other legumes contain high levels of lysine yet lack the sulfur-containing amino acids methionine and cysteine. The combination of cereals and legumes creates a balanced protein source which contains all essential amino acids needed for human consumption (Ilemobayo and Kone, 2024; FAO, 2025).

Benefits of blending maize and soy

The use of soy in this formula provides essential amino acids which are missing from maize to enhance the protein quality. The study of cereal-legume combinations revealed that these dietary combinations produced better amino acid compositions which resulted in improved protein digestibility and more effective nutritional outcomes for communities that depend on maize (Ilemobayo and Kone, 2024).

2.4.1. Soy-maize blends' amino acid profile

Complementary impact

Blends of soy and maize greatly enhance the amino acid composition of food. Overall protein quality is improved by the complementary effect of soy's high lysine and tryptophan content and maize's low levels of these amino acids (Ilemobayo and Kone, 2024).

Enhanced biological value

A higher biological value (BV) and protein efficiency ratio (PER) result from the better balance of essential amino acids in soy-maize blends. This suggests that the body uses protein more effectively, promoting growth and development, particularly in young children and babies whose diets are primarily composed of maize (Ilemobayo and Kone, 2024).

Essential Amino Acids (%)	Proteins		
	Soybean [a]	Soybean-Maize [b]	Casein [c]
Histidine (His)	136.84	134.74	142.11
Isoleucine (Ile)	164.29	166.79	175.00
Leucine (Leu)	119.70	117.73	127.27
Lysine (Lys)	112.07	103.97	122.41
Threonine (Thr)	114.71	99.12	108.82
Valine (Val)	140.00	138.00	171.43
Tyrosine (Try)	118.18	138.18	127.27
Sulfur-containing amino acid			
Methionine (Met) + Cysteine (Cys)	104.00	98.40	105.60
Aromatic amino acid			
Phenylalanine (Phe) + Tyrosine (Tyr)	130.16	140.48	158.73

Table 2.1 Comparison of Pysiocochemical, Functional and nutritional properties between proteins of Soybean and a Novel Mixture of Soybean-maize.

Source: (Soria-Hernández et al., 2020).

2.5. Fermentation of foods overview

Fermentation serves as a method which allows microorganisms to decompose large organic compounds into their basic components. Yeast enzymes transform sugars and starches into alcoholic substances while they change proteins into peptide and amino acid forms. Food components undergo fermentation through microbial or enzymatic processes which produce beneficial biochemical transformations that cause major changes in the food. Fermentation naturally enhances the content of vitamins and essential amino acids while decreasing anti-nutrients and increasing protein content and improving food appearance and flavor and aroma. Fermentation reduces cooking energy requirements while it produces a safer final product according to Nkhata *et al.,* (2018) and Xiang *et al.,* (2019).

The complete life cycle of organisms from their initial growth to their final death sequence includes their entire biochemical processes of fermentation. Fermentation technology, which uses these organisms, enables industrial production of food and pharmaceuticals and alcoholic beverages. The method of industrial fermentation technology operates by following its main principle of creating ideal environments for organism growth. Growth requires essential raw materials that include carbon and nitrogen and salts and trace elements and vitamins according to Sharma *et al* 2020. The highest product yield requires control of both temperature and pH and oxygen concentration levels. The fermentation process requires continuous monitoring to protect organisms from toxic substances and harmful pathogens that could impact results. The increasing interest in environmental protection and renewable energy sources has created greater demand for obtaining fermentation products which include industrial chemicals and organic acids and feed and food additives. The increase in demand has resulted in manufacturers producing more items which include both high-value products and traditional low-volume pharmaceuticals because fermentation now competes with conventional methods for producing commodity chemicals. Companies need to enhance their operational efficiency while minimizing waste products in order to maintain their market competitiveness for manufacturing economical chemical products at high volumes. Scientific researchers currently focus on studying the biotechnological applications of agricultural waste materials that result from industrial production processes.

2.5.2. Group of microorganisms involved in food fermentation

Lactobacillus species, *Saccharomyces cerevisiae*, and *Aspergillus oryzae* are three most prevalent microbes that cause food fermentation. Lactic acid bacteria called *Lactobacillus* species are essential to the flavor development and preservation of fermented foods. These gram positive, non –spore forming bacteria produce lactic acid from sugars like glucose and lactose lowering food pH and preventing the growth of pathogenic or spoiling microorganisms, this acidification process adds flavor and texture (Zapasnik *et al.,* 2022). Another important microorganism is the yeast saccharomyces cerevisiae, which is primarily involved in alcoholic fermentation. This yeast converts sugars into ethanol and carbon dioxide, processes essentially in the production of alcoholic beverages such as beer and wine as well as bread making. Because of its efficiency and predictability, cerevisiae is considered one of the most economically important and well studied fermentation organisms (Maicas, 2020). *Aspergillus oryzae*, a filamentous fungus that is frequently utilized in traditional Asian fermentations is the third important microorganism. Numerous enzymes, including lipases, proteases, and amylases are secreted by this organism and convert complex proteins, fats and carbohydrates into simpler, more palatable substances. It improves food flavor and digestibility through enzymatic processes.(Tamang *et al.,* 2016).

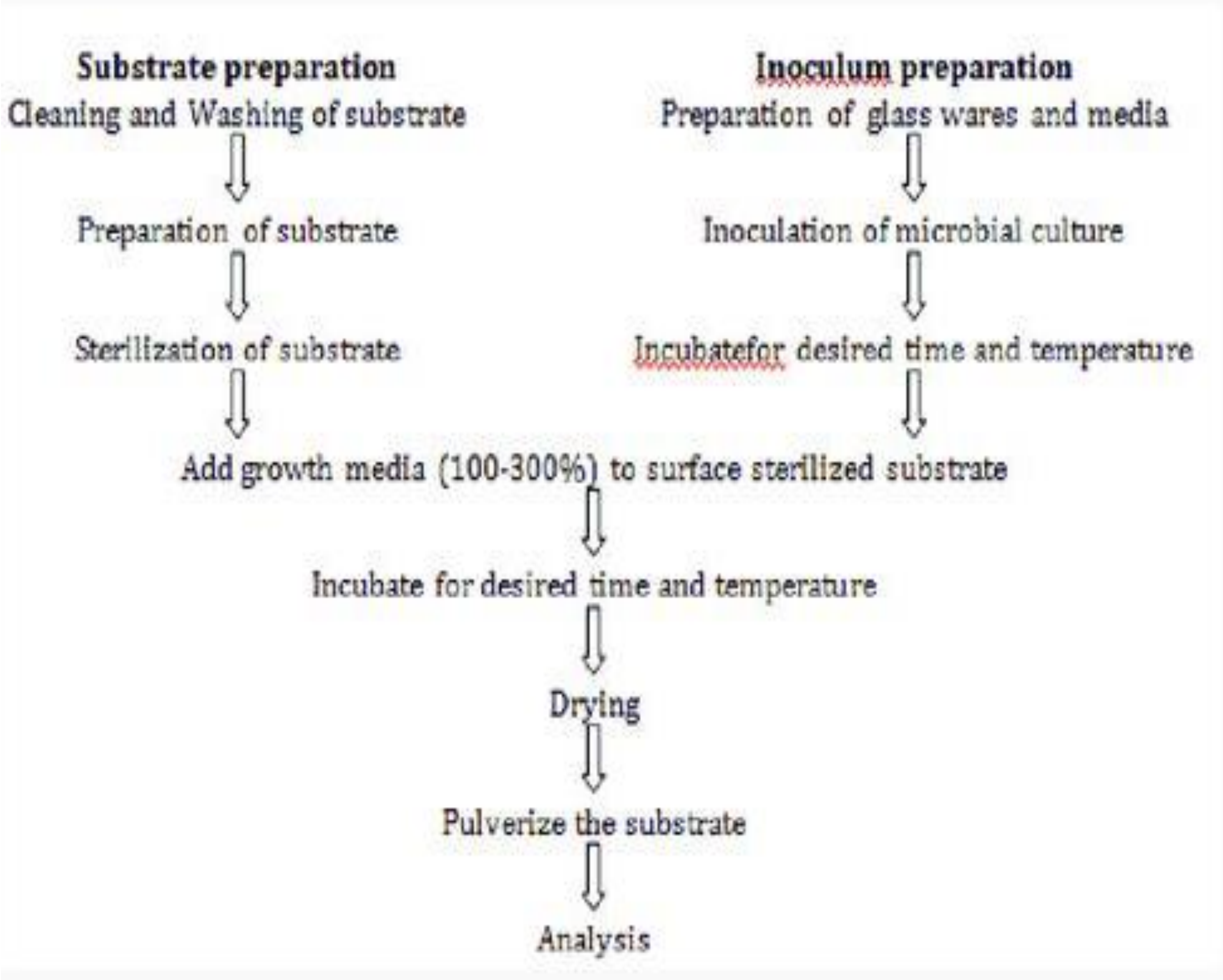

Figure 2.3. Flow Chart of fermentation process.

Source: Nutritional Food Science International Journal Value Addition of Grains using Solid State Fermentation (Eyarkai *et al.,* 2018).

2.5.3. Benefits of fermentation

The research work by Hasan *et al.,* (2014) proves that processed foods which undergo fermentation have better nutritional value than their original unprocessed versions. The process of fermentation which involves microorganisms creates three different methods through which microorganisms enhance the nutritional content of fermented foods. The microorganisms which exist in the environment function as both catabolic and anabolic processes that decompose complex materials and produce vitamin compounds together with other essential substances for growth. The human body can obtain nutrients from plant materials because these substances together with their cellular components can be broken down through digestion. The phenomenon especially affects single seeds and grains. The milling procedure has created physical breaks in the cellulosic and hemicellulosic components that protect the endosperm which contains proteins and carbohydrates that the body can digest. The method of crude milling enables people in less developed areas to obtain nutritional value from plant materials, but this method fails to extract all the nutritional benefits from the plant materials. The human body cannot digest certain nutrients which remain bound to food even after cooking. The problem at hand finds a solution through specific bacteria together with molds and yeasts which have the ability to break down both the cell walls of products and their inedible protective layers through two different methods of physical and chemical destruction (Hasan *et al.,* 2014). Fermented foods provide multiple benefits which include the following

1. Fermented foods remain safe to eat for longer periods than their original counterparts.
2. The organoleptic properties of the cheese improve beyond what its raw milk substrate can provide to its taste.
3. The process of garri production removes toxic components from cassava while the fermentation process eliminates flatulence-inducing substances found in soybeans.

4. The presence of fermenting microorganisms leads to better nutritional properties in food. The yeast present in bread together with the yeast and lactic acid bacteria in garri produce nutritional benefits for the dish.
5. The fermentation process enables faster cooking of food products. The West African dish Ogi which uses fermented maize together with soybean products represent this cooking method.
6. Fermented products show increased antioxidant capacity which researchers measure through in vitro testing. The antioxidant properties of fermented milk and yogurt exceed those of regular milk because milk protein proteolysis results in bioactive peptides from protein breakdown, especially from α-casein and α-lactalbumin and β-lactoglobulin proteins (Melini *et al.,* 2019; Sanlier *et al.,* 2019).

2.6 Fermentation methods and techniques

Fermentation is said to be a biochemical process where microorganisms alter sugars to basic molecules (Steinkraus, 2018). This organic process is one long established practice of food conservations increasing its longevity, digestive quality, sensory quality and Nutrient content. The essentiality of fermentative process on food products not only controls food deterioration but also improves sensory attributes and increases bioavailability of nutrients (Campbell-platt, 2017). Fermentation clearly improves protein value by improving crude and bioavailable protein levels, partially due to microbiological protein biosynthesis and the targeted utilization of carbohydrates, this assists in raising the protein proportion. There are various types of fermentation techniques and they are widely classified into spontaneous and controlled fermentation. In spontaneous fermentation, the method occurs via the activity of microorganism inherently contained in unprocessed material or our environment (sooresh *et al.,* 2023). The result of the natural fermentation is usually unpredictable because of the microorganism population is influenced by surrounding conditions and sanitation standards.

Controlled fermentation conversely entails intentional use of selected microbial cultures to obtain consistent fermentation outcomes. This method is frequently used in commercial food production where product consistency of food safety are essential (Tamang *et al.,* 2020). In the pursuit of biotechnological processing solid state fermentation is prominent as an adjustable and sustainable method with broad applications in multiple sectors, compared with submerged fermentation SSF requires growth of microorganisms on solid supports devoid of moisture or free water. SSF is the close association between microbial community and solid substances which acts as site for microbial growth and nutrient supplier (Peng, 2024). Submerged fermentation is another technique whereby microorganisms are developed in a nutrient solution or liquid substrate that comprises of all essential nutrients needed for their development and biochemical activity (David, 2023). Submerged fermentation is commonly applied technique in biotechnological bioprocessing sector for the generation of assorted biological products, this technique generates maximal production of specified bio product, accurate management over cultivation parameters and industrial scalability (David, 2023).

Fermentation in batch culture using starter cultures

The simplest method is batch fermentation, in which the substrates, nutrient and starter culture are all added at the beginning of the process and left to ferment without further feeding until it is finished . it is less prone to contamination, it is widely used in industrial biotechnology and food production . Recent research indicates that batch fermentation is still a common technique for lactic acid and food fermentation because it is easy to use and produces reliable yield when using starter culture (Rahman *et al.,* 2025).

Fed batch fermentation

This is a semi continuous process where the culture stays in the same vessel while nutrients are added gradually. By preventing substrate inhibition and improving control over the growth and metabolism of microbes in the starter culture, this method can boost biomass density and product yield. In the production of food and pharmaceuticals, this method is usually employed, particularly when starter cultures are used to sustain active growth phases (Sahin, 2023). research of individuals who have worked with Maize and soybean to produce various meals suggest that each of them are rich in nutrients.

Table 2.2: Comparison between solid-state fermentation and liquid fermentation.

Factors for Comparison	**Solid-State Fermentation (SSF)**	**Submerged Fermentation (SmF)**
Definition	Fermentation process where microorganisms grow on solid substrates with minimal or no free water.	Fermentation process where microorganisms grow in a liquid nutrient medium.
Microbial Environment	Microorganisms interact directly with the solid substrate; moisture is bound within the matrix.	Microorganisms are suspended or dispersed in a liquid medium.
Nutrient and Metabolite Transfer	Diffusion-driven through the solid matrix; slower and dependent on substrate porosity and moisture.	Occurs via convection and diffusion in liquid; faster mass transfer.
Water Availability	Very low (no free water present).	High (ample free water present).
Control Parameters	Requires careful control of moisture, pH, temperature, and aeration to optimize diffusion and growth.	Easier to monitor and control parameters like pH, temperature, dissolved oxygen, and nutrient concentration.
Suitable Microorganisms	Filamentous fungi, actinomycetes, and some bacteria that thrive in low-moisture environments.	Bacteria, yeasts, and fungi adapted to liquid environments.
Main Products	Enzymes, organic acids, antibiotics, bioactive compounds, fermented foods (e.g., tempeh, koji, fermented vegetables).	Antibiotics, enzymes, organic acids, biofuels, and other bioproducts.
Applications	Food, feed, pharmaceuticals, bioremediation, biofuel production, enzyme and probiotic production.	Industrial biotechnology, pharmaceuticals, enzyme and biofuel production, organic acid synthesis.
Advantages	Low water use and energy demand Utilizes agro-industrial residues High product concentration Eco-friendly and cost-effective.	High productivity and yield Easier process control and automation Suitable for large-scale bioreactors.
Disadvantages	Difficult to monitor and control parameters Scale-up is challenging Uneven nutrient distribution.	High equipment and operational costs Higher contamination risk Requires significant water and energy.
Industrial Example	Tempeh, koji, enzyme production using fungi, waste bioconversion.	Production of antibiotics (e.g., penicillin), citric acid, ethanol, and industrial enzymes.

Source. Adapted from Manan and Webb, 2017.

2.7. Effect of fermentation on the nutritional properties of soy-maize

Digestibility and protein quality through microbial proteolysis activity fermentation improves the quality of proteins in soy maize composites. Protein digestibility is improved by the hydrolysis of complex proteins into peptides and free amino acids by lactic acid bacteria and yeast enzymes. Due to partial protein denaturation and enzymatic breakdown during fermentation, studies on fermented cereal legumes blends have demonstrated increased in vitro protein digestibility (Adebo *et al.,* 2018; and medina meza, 2020). Fermentation lowers trypsin inhibitors, which typically restrict protein utilization in soybean products, soybean based fermented flours are especially advantageous. Diminishment of Anti-nutritional elements*, the* reduction of anti-nutritional element like phytates, tannins and polyphenols is one of fermentations most important nutritional advantages. Fermented cereal legumes blends had significantly lower phytate content which improved mineral accessibility. Because phytate compounds are naturally present in both maize and soybean, this effect is particularly significant in soy maize (Adebo *et al.,* 2018).

2.7.2. Effect on carbohydrate

Breakdown of complex carbohydrates

Fermentation promotes microbial hydrolysis of complex carbohydrates (starches, non-digestible polysaccharides) into simple sugars. This increases digestibility and provides substrates for further fermentation, improving energy availability and reducing gastrointestinal discomfort (Long *et al.,* 2022).

Manufacturing of Simple Sugars

During fermentation, simple sugars like glucose and maltose are released by microbial enzymatic activity. These sugars improve the sensory and nutritional qualities of fermented foods by increasing their fermentability, sweetness, and texture (Adebo and Njobeh, 2022).

Impact on Dietary Fiber

By changing some insoluble fibers into soluble, more fermentable forms, fermentation can change dietary fiber and enhance mineral absorption and gut health (Tamang *et al.,* 2020).

2.7.3. Effect on vitamins

Impact on vitamins

B-Vitamin Synthesis (B1, B2, B12, Folate)

Specific microorganisms produce B-vitamins through the process of fermentation. Lactic acid bacteria and yeasts enhance riboflavin and folate and cobalamin levels which results in increased vitamin content of cereals and legumes according to LeBlanc *et al.,* (2013) and Rossi *et al.,* (2017).

Vitamin content variations during fermentation

Microbial metabolism during fermentation results in both positive and negative changes to specific vitamins which include folate and B2. The vitamin bioavailability of fermented foods tends to be higher than that of non-fermented foods (Brody 2020).

2.7.4 Effect on minerals

Impact on minerals

Bioavailability of minerals

By reducing phytate content through phytase activity, fermentation increases calcium, zinc, and iron bioavailability (Tamang *et al.,* 2020).

Effects of chelation

Mineral absorption is further enhanced by organic acids (lactic and acetic) generated during fermentation, which lessen mineral chelation by anti-nutrients (Hurrell and Egli, 2010).

2.7.5. Diminishment of anti-nutritional elements

Trypsin inhibitors: They increase the digestibility of proteins by partially inactivating them during fermentation (Terefe *et al.,* 2021).

Tannins and Flatulence Factors: Fermentation improves flavor and lessens gastrointestinal distress by reducing tannins and oligosaccharides (raffinose, stachyose) (Ravindran and Sivakumar, 2019).

2.7.6. pH changes during fermentation

Lactic acid bacteria (LAB) during fermentation process their carbohydrate substrates to create lactic acid and various organic acids. The accumulation of lactic acid creates an acidic environment which decreases food pH levels and prevents the development of harmful microorganisms and spoilage (Tamang *et al.,* 2020; Adebo and Njobeh, 2022). The process of acidification establishes essential stable conditions which protect the safety of fermented products.

The connection between microbial activity and pH

The pH values decrease throughout the fermentation process which establishes optimal conditions for beneficial microorganisms that include lactic acid bacteria (LAB) and certain yeasts that thrive in acidic environments while it stops harmful bacteria from growing. The relationship between pH and microbial activity is significant because proper acidification improves vitamin and mineral bioavailability and increases fermentation speed and nutrient enzymatic breakdown efficiency (Pandey *et al.,* 2021; Long *et al.,* 2022).

Impact on food safety and shelf life

Lactic acid and other organic acids create an acidic environment that inhibits harmful bacteria like Salmonella and Escherichia coli, improving food safety. Additionally, a lower pH prolongs the shelf life of fermented foods by preventing microbial spoiling and enabling longer periods of storage without refrigeration (Tamang *et al.,* 2020; Steinkraus, 2002).

2.8.1. Effect of fermentation on the sensory properties of cereal-legume based fermented foods like soy-maize

Cereal grains serve as a vital source of nutrition for people globally, offering proteins, carbohydrates, vitamins, minerals, and dietary fiber. The sensory qualities of these products do not reach the same level of excellence which animal-based products provide (Blandino *et al.,* 2023). Fermentation serves as an essential process which enhances the texture and flavor and scent of cereal-based food and beverage products (Blandino *et al.,* 2023). Research shows that volatile compounds which form during fermentation process create complex flavor combinations found in finished products. The presence of diacetyl and acetic acid and butyric acid creates appealing aromas which improve the attractiveness of fermented cereal-based products. Microorganisms work with malt enzymes during fermentation to transform proteins into amino acids which get converted into flavor-enhancing aldehydes (Mugula *et al.,* 2023). The fermentation process enhances these products through starter cultures which provide enzymes that develop fresh and better-tasting flavors for the final product (Senanayake *et al.,* 2023). Enzymes initiate the process of nutrient breakdown into smaller components during the early stages of the fermentation process (Smit *et al.,* 2020).The fermentation process begins with the transformation of proteins into peptides which then break down into amino acids and fatty acids and glucose and maltose and galactose to create the sensory attributes that emerge during fermentation (Bintsis 2018). Starter cultures use simple compounds to create various organic compounds through fermentation which include organic acids and alcohols and esters and ketones to develop the distinct flavor and aroma profile of the finished product (Liu *et al.,* 2020).

2.8.2 Effect of fermentation on the sensory attributes of soy-maize.

2.8.3. Aroma changes

Development of flavor and Aroma by producing organic acids, esters, alcohols and other volatile compounds, fermentation has a substantial impact on the development of flavor. These metabolites lessen the unwanted beany flavor connected to soy flour and contribute to complex and appealing flavor profiles. (Adebo and medina –meza, 2020).

2.8.4. Texture modification

Dough rheology and the texture of baked goods are altered by the acidification and enzymatic activity that occur during fermentation .Cookies made with fermented composite flours have better mouth feel, less hardness and increased crispness. Research on cookies made from fermented cereals has demonstrated that fermentation increases speed ratio and decreases breakage both which have a favorable impact on consumer perception(oluwa *et al.,*2021).

2.8.5. Color and appearance changes

Through enzymatic browning reactions and pigment modification, fermentation can also affect color of soy maize cookies. Excessive fermentation may produce darker products because of increased maillard reactions during baking, but mild fermentation improves visual appeal by encouraging uniform browning for acceptable appearance, controlled fermentation is therefore necessary(ojha *et al.,*2021).

2.8.6. General acceptability of fermented food

The general acceptability of fermented foods is greatly influenced by consumer preferences. Flavor, aroma, texture, and appearance are examples of sensory attributes that significantly influence liking and purchase intention. According to a systematic review of fermented food products and beverages, one of the most significant factors influencing consumer preference and acceptance is intrinsic product attributes, primarily sensory qualities (García-Barón *et al.,* 2025). As an illustration of how preferences differ by product and population, some consumers find sour or traditional fermented flavors less acceptable, while others prefer sweet and milky characteristics in fermented yoghurt-type drinks (Onyenekwe *et al.,* 2015; Silva *et al.,* 2023).

Cultural aspects

Cultural backgrounds have a major impact on how people view and accept fermented foods. Cross-cultural studies show different patterns of product acceptance because various regions have different ways of consuming the same fermented products. African consumers show a strong preference for the sweet and milky taste of fermented cereal products which include Akpan, while European consumers prefer products that have less sourness. Cultural expectations drive both sensory preferences and total product acceptance. (Lee *et al.,* 2020) conducted a research which studied fermented sauces from three countries which included Korea and the United Arab Emirates and the United States, discovering that people had specific taste preferences for spicy and sweet flavors. Cultural taste norms have a strong influence on consumer acceptance, which the researchers found to be a fundamental factor in their study.

Previous sensory studies on fermented products

Prior Sensory Research on Fermented Foods

Researchers have conducted multiple sensory studies to examine how people accept fermented foods and which types of fermented foods they prefer, including Doenjang, which is a fermented soybean paste. The sensory analysis results showed that different consumer groups had different levels of acceptance for Doenjang. People preferred the samples that contained a sweeter taste together with higher umami content. Researchers used cluster analysis to identify different consumer groups who had distinct preferences, and they found that sensory attributes were the main factors which determined product acceptance (Lee and Cho, 2011).

Soybean-Maize fermented pastes: A sensory assessment of fermented pastes made from soybean and soybean-maize blends revealed that most consumers favored naturally fermented products over lactic acid bacteria-fermented ones, demonstrating how fermentation type affects sensory acceptance (Food Sci Nutr study). [1]

Artisanal kefir: Studies on artisanal milk kefir revealed that consumers generally enjoyed the product and that it retained acceptable sensory qualities (particularly texture and flavor) over storage time, suggesting good overall acceptability (de Oliveira *et al.,* 2025).

2.9 Cookies as a food product

2.9.1. Definition and classification of cookies

Usually consumed as snacks or desserts, cookies are little, sweet baked goods made from flour, sugar, fat, and other ingredients. According to science, cookies differ from breads and cakes in that they are low-moisture cereal-based foods that experience minimal gluten development. For texture and shape during baking, their doughs depend less on gluten structure and more on the functionality of ingredients and the aeration provided by leaveners (Jukić *et al.,* 2022). Cookies can be divided into types like drop cookies, rolled cookies, pressed cookies, and bar cookies based on their texture and method of production. Bar cookies are baked in a pan and then cut after baking, rolled cookies are formed and cut from sheeted dough, pressed cookies are extruded or molded, and drop cookies are portioned from a viscous dough (Jukić *et al.,* 2022).

2.9.2. Nutritious importance

Cookies, which have different nutritional values, use their ingredients to provide energy from both fats and carbohydrates. When traditional wheat-based cookies receive an upgrade through legume or whole grain flour, their protein content and fiber content increase while their blood sugar response decreases and their entire nutritional value improves. Wheat-based cookies typically contain low fiber content but they have high levels of sugars and fats according to research by Suo *et al*, (2025).

Ingredients in cookies and how they work

Cookies depend on cookies to produce cookies through their different ingredient functions which include flour sugar fat and leavening agents. The components of the mixture work together to create the cookies' texture and structure and flavor and complete product quality according to research by Jukić *et al.,* (2022).

Flour

Serves as the main ingredient in cookies because it creates their fundamental structure from wheat flour. The protein and starch content of the ingredient determines how the dough will be handled and the final product will turn out in terms of texture. Cookie dough requires flour for body creation, whereas bread dough needs strong gluten development. The baking process starts proteins turning solid and starch transforming into a gel form, which enables the dough to establish its intended shape and structure according to Jukić *et al.,* (2022).

Sugar (Texture, Sweetness)

Sugar exists in cookie recipes as sucrose which provides sweetness and flavor while determining how cookies will spread and develop their texture. The substance participates in browning through both caramelization and Maillard reaction processes. The baking process causes sugar to dissolve which creates moisture distribution throughout the product while determining whether the product will become crisp or chewy. Cookie surface patterns and internal cell structures change according to variations in concentration (Jukić *et al.,* 2022).

Fats (Flavor, Tenderness)

Cookies obtain their tenderness and richness through fats (Flavor, Tenderness) which come from butter margarine or oil. Fat enhances flavor while creating a pleasant mouthfeel because it completely covers flour particles which prevents gluten development. Fat affects cookie spread and texture through its function of lubricating the dough matrix which allows air to enter during the creaming process when sugar is mixed with it (as discussed in ASBe sources).

Leavening agents

The mixing and baking processes require agents like baking powder and baking soda which produce carbon dioxide gas through their chemical reactions. The process produces carbon dioxide gas which results in decreased dough weight and increased dough volume. The desired cookie texture requires a specific amount of leavening agents because it controls both dough thickness and consistency while preventing too much gluten formation (Bakerpedia, 2026).

Chemical characteristics (pH, Moisture)

Cookies' texture and shelf life are impacted by their moisture content. Better shelf life is indicated by composite flour cookies, which usually have moisture levels within safe bounds (<10%). Proximate profiles are also affected by composition variations, with composite samples frequently showing higher ash and fiber content.

Sensory characteristics

For composite cookies, sensory evaluation taste, texture, appearance, and overall acceptability is essential. Although higher substitution may result in lower scores due to changes in flavor, texture, and color, numerous studies show that cookies with modest levels of composite flours (e.g., up to 30%) achieve sensory scores comparable to wheat controls (PMC cookies; ResearchGate composite flour study). According to Aljobair (2022), properly formulated composite cookies can satisfy consumer preferences. For example, cookies made from sorghum and millet composite flours had sensory scores above the acceptability threshold.

Figure 2.4 Flow Chart for preparation of cookies.

Source: Studies on development of high protein cookies (Kulkarini *et al.,* 2018).

Figure 2.5 Simple Vanilla Cookie

Source: Erik Clarkson 2022.

2.10. Knowledge gaps and challenges on the use of composite flours

The rising demand for composite flours comes from their ability to deliver better nutritional value while decreasing the need for standard wheat-based products. The industrial sector faces multiple obstacles that prevent experts from using their technology because they need to meet essential nutritional standards and maintain product sensory characteristics and their supply chain requirements. Many alternative flours present their main challenge because they contain little to no gluten content which serves as the essential component that provides dough with its necessary elasticity and structural integrity and gas holding capacity. The absence of this component leads to bread production which results in smaller loaves that contain a solid and heavy center together with an unattractive mouthfeel. The solution to this problem uses xanthan gum and guar gum as two gluten alternatives which fulfill the same functional roles as gluten does. The international shipping system causes two main problems for these additives because it creates cost variations and makes product availability unpredictable which decreases their practical value. The ingredient sovereignty control process needs further investigation to determine if it can maintain cost-effective operations throughout the extended period. The dependency on imported hydrocolloids creates a conflict with the objective of achieving reduced international trade dependency. Researching local functional alternatives to cassava and rice and sweet potato starches and natural gums and plant extracts that contain mucilage can provide functional benefits while supporting local ingredient development. The alternatives can absorb water and change their viscosity through mechanisms that resemble commercial hydrocolloids which operate within the boundaries of local farming capabilities (Compaore-Sereme *et al.,* 2023).

The effective functioning of composite flours requires people to learn about their mechanisms because these flours create critical questions which investigate nutrient absorption into the bloodstream. The combination of grains with legumes and tubers results in increased protein and fiber content, yet phytates and tannins and enzyme inhibitors create obstacles which impede mineral absorption and protein digestion. The assessment of protein concentration reveals no connection between enhanced nutritional value and protein content because proteins require a particular level of digestibility and amino acid availability (Amadeu *et al.,* 2024).

People find it challenging to accept composite flour because its flavor differs from the taste of wheat-based products. People experience taste differences based on their cultural background and their dietary preferences and the cooking practices they follow to prepare food. Some consumers showed reduced interest in bread after bakers used cassava flour at levels between 30-40% because this ingredient altered the bread's color and taste and texture. People in regions where cassava serves as a main agricultural product will show higher acceptance of cassava because it functions as a fundamental food item. People require multiple factors to determine how different sensory experiences will develop across various population groups, which requires product development and testing to occur in specific geographical locations. The local people will better accept the recipe changes which include local flavors and local cooking methods (Ohimain *et al.,* 2014). The high moisture content of composite flours together with their unsaturated lipid content results in faster spoilage development. The choice of correct packaging needs to be done. Multilayer packaging provides superior freshness protection for this type of flour compared to both LDPE and kraft paper (Romani *et al.,* 2015). The implementation of better packaging solutions leads to higher expenses for small businesses, which requires them to evaluate their financial advantages against their operational expenses (Romani *et al.,* 2015).

Non-wheat flours create unique physicochemical characteristics which lead to different method needs for recipe development and processing. The three factors which determine how millet-based composite pasta behaves during production and how it tastes to consumers are the ratio of flour to hydrocolloids and the amount of water and the length of thermal processing time.

The process of ingredient optimization needs to establish functional requirements while observing operational constraints. The economic aspects together with supply chain problems create obstacles for business expansion. The process of obtaining sufficient alternative flours faces difficulties because of seasonal fluctuations and raw material quality variations and unreliable supply sources according to Aderinola and his colleagues 2015. The requirement for functional additives or enzymes to ensure proper functionality may negate the cost benefits of using local ingredient alternatives according to Compaore-Sereme *et al.,* (2023).

Justification of current study

This research is justified based on the fact that there's a burning need for improved highly nutrient packed food options with numerous health benefits. This research could therefore be a solution to this problem by providing a healthier food product (nutrient dense cookies), created under safer and less nutrient stripped versions. This could in the nearest future replace already market existing products which are not precisely made with the health of consumers at heart.

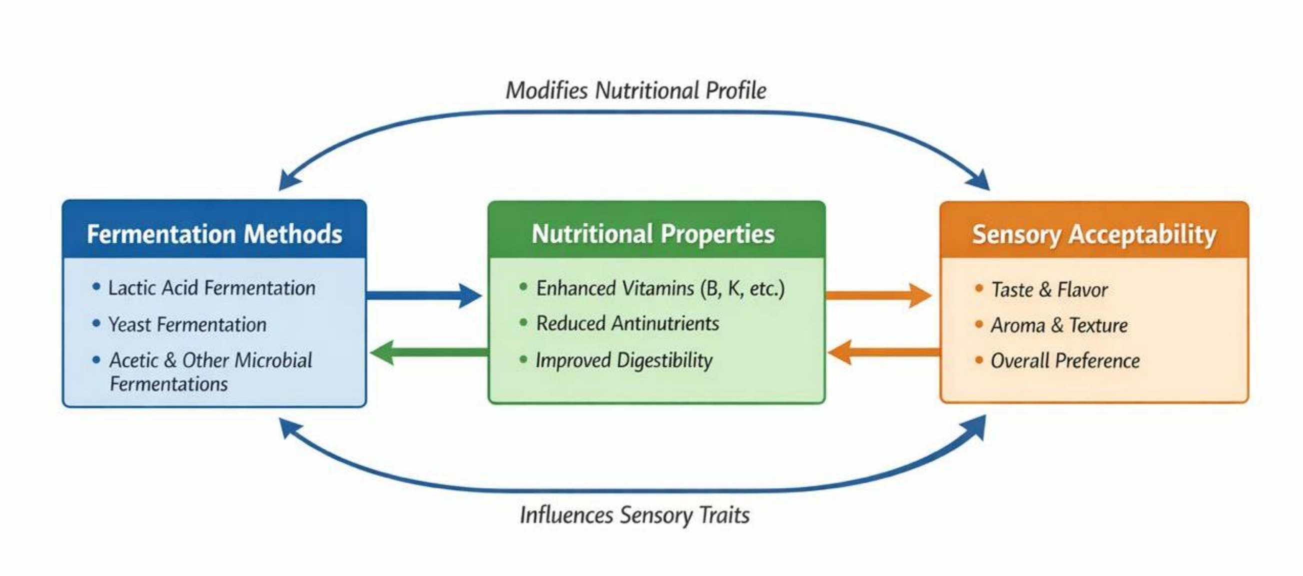

Figure 2.6 Conceptual framework showing relationships between fermentation methods, nutritional properties, and sensory acceptability

Source: User generated image via AI Algorithm

CHAPTER THREE

MATERIAL AND METHODS

3.1 Materials

Soybeans (*Glycine max*) and maize grains (*Zea mays*) samples were sourced from Ogbette main market, Enugu, Nigeria. Additional cookie ingredients include wheat flour, sugar, margarine, baking powder, salt, and vanilla essence, all sourced from a local supplier to ensure accessibility and relevance for small-scale production. All samples were taken to Godfrey Okoye University for analysis. The following reagents and equipment were utilized; distilled water, peptone water for microbial dilutions, Nutrient Agar for total viable counts, Potato Dextrose Agar for yeast and mold enumeration, and various reagents required for proximate analysis as specified in AOAC methods (AOAC, 2019). Basic equipment includes fermentation trays, hot air oven, baking oven, hammer mill, pH meter, weighing balance, muffle furnace, Soxhlet apparatus, Kjeldahl apparatus, incubator, autoclave and Petri dishes. These align with standard laboratory setups for fermentation and baked product studies, as reported in similar research on composite flour products (Adeyeye, 2017).

3.2 Experimental design

The study adopted a Completely Randomized Design (CRD) with three treatments: T1 (control - unfermented soy-maize blend), T2 (solid-state fermented soy-maize blend), and T3 (submerged fermented soy-maize blend). All analyses were conducted in triplicate to ensure reliability and statistical validity, consistent with approaches in fermentation studies on cereal-legume blends (Adebo *et al.,* 2022).

3.3 Sample preparation

3.3.1 Sorting and cleaning

Soybeans and maize grains were manually sorted to eliminate damaged seeds, dirt, and foreign materials, then thoroughly washed with clean water and drained. Aimed at minimizing contaminants and ensuring uniform fermentation, as emphasized in methodologies for fermented composite flours (Awolu *et al.,* 2017).

3.3.2 Blending ratio

The cleaned soybeans and maize were mixed in a 30:70 ratio (by weight), selected for complementarity in amino acid profiles and nutritional enhancement in cereal-legume combinations (Adebo *et al.,* 2022). This blend served as the base for all treatments.

3.4 Fermentation processes

3.4.1 Control sample (T1 - Unfermented)

The cleaned soy-maize blend was dried at 60°C for 6-8 hours, milled into flour using a hammer mill, sieved through a 0.5 mm mesh, and stored in airtight containers. This preserved the baseline properties without microbial intervention.

3.4.2 Solid-state fermentation (T2)

The blend was coarsely ground, adjusted to 50% moisture with distilled water at a 2:1 (blend: water) ratio, mixed evenly, and spread in thin layers (2-3 cm depth) in sterile trays. Trays were covered with sterile muslin cloth and fermented at room temperature (28-30°C) for 48 hours, with periodic turning every 12 hours for uniformity. Post-fermentation, the material was dried at 60°C, milled, sieved, and stored. Solid-State Fermentation (SSF) was chosen for its efficiency with agro-industrial substrates and its ability to enhance nutrient content in plant-based foods (Olukomaiya *et al.,* 2020; Yegin, 2025).

3.4.3 Submerged fermentation (T3)

Whole grains were placed in sterile containers with distilled water at a 1:3 (grains: water) ratio, covered with muslin cloth, and fermented at 28-30°C for 48 hours, with the water changed every 24 hours to reduce off-odours. After fermentation, grains were drained, rinsed, dried at 60°C, milled, sieved, and stored. Submerged fermentation (SMF) represents traditional African processes for making pap (Adebo *et al.,* 2022).

3.5 Cookie production

3.5.1 Cookie formulation

The formulation per batch was as follows: Fermented/unfermented soy-maize flour: 250g, Sugar:125g , Margarine: 125g, Bicarbonate of soda 3.5 g,, Vanilla essence: 2 ml, Egg 20 g.This recipe adapts composite flour approaches for nutritional enrichment (Adeyeye, 2017; Awolu *et al.,* 2017).

3.5.2 Cookie preparation procedure

Margarine and sugar were creamed until light and fluffy, vanilla essence was added, and dry ingredients (flours, baking powder, salt) were mixed separately before gradual incorporation. Water was added to form a stiff dough, which was rolled to 0.5 cm thickness, cut into shapes, were cooled on wire racks and packaged in airtight containers for analysis, following standard baking protocols (Adeyeye, 2017).

3.6 Determination of flour yield

This is to determine the final flour yield to be worked with, the formula is Weight of flour obtained divided by weight of samples used x 100.

3.6 Microbial analysis

The preparation of the agar were followed according to manufacturers guide ; PDA, MRS, NA

PDA- 2.34g in 60ml of saline water for 3 plates

MRS- 4.05 in 60ml of saline water for 3 plates

NA- 2.82 in 60 ml of saline water for 3 plates

3.6.1 Sample preparation

Microbial culture and isolation from the samples

Sterile peptone water was used for the dilution of the fermented water samples to obtain serially diluted concentrations (up to 10^6) for plating the samples on sterile semi solid agar plates. For the first test tube, one mL of the sample water was added into 9mL of peptone water and was homogenised properly before being transferred serailly in sequence up to the 6th test tube and was discarded effectively. For the inoculation on the agar plates, 0.1mL of 3 dilution concentrations (10^2, 10^3) were transferred unto the surface of the agar plates.

3.6.2 Lactic acid bacteria count

For the enumeration of lactic acid bacteria present in the fermented the fermented liquid samples, the MRS agar was used. The MRS agar was prepared according to manufacturer's instructions by sterilising in an autoclave for 15 minutes at 121°c and was allowed to cool down to about 45°C. Utilising the pour-plate method, 0.1mL of the sample were transferred into sterile petri dishes, followed by about 20mL of the cooled sterilised MRS agar, and was allowed to gel completely before being inverted and transferred into an anaerobic incubator at 37-41°C for intervals respectively. Colonies were enumerated as CFU/mL and their equivalent was log10 CFU/mL. (Hoxha *et al*., 2023; Sarvari *et al*., **2014).**

3.6.2 Total viable count (TVC)

The pour plate method used Nutrient Agar, with incubation at 37°C for 24-48 hours. Colonies were counted as CFU/g. (Hoxha *et al*., 2023; Sarvari *et al*., **2014).**

3.6.3 Yeast and mold count

The spread plate method on Potato Dextrose Agar involved incubation at 25°C for 3-5 days. Counts were expressed as CFU/g using the formula: CFU/g = (colonies × dilution factor) / sample weight. These methods ensure assessment of microbial safety and fermentation efficacy (AOAC, 2019).

3.7 Physicochemical analysis

3.7.1 pH Determination

A 10 g sample was blended with 100 ml of distilled water, filtered, and the pH was measured using a calibrated meter in triplicate.

3.7.2 Proximate analysis

Proximate analysis were all analyzed using methods by AOAC (2019), as follows;

3.7.1 Moisture content determination

Sample (5 g) was measured into a crucible that is dried and its mass known. Then all placed into the oven at 105 ^{o}C for 3 hours, after which, the samples were removed from the oven and immediately placed in a desiccator, allowed to cool, reweighed and noted. Then, returned to the oven drier for another 1 hour, cooled and reweighed. This procedure was repeated till a constant mass was reached. The mass difference of the initial and final drying was used to calculate the moisture content.

3.7.2 Determination of crude protein (Kjeldahl Method)

Each sample (0.2 g) was measured into a Kjeldahl digestion flask and 5 g of anhydrous sodium sulphate, 1 g of hydrated copper sulphate and a pinch of selenium dioxide added. Five (5) ml of concentrated sulphuric acid was introduced and the flask with its content placed on a heating digester of a Kjeldahl unit at a slanted position of about 45^{o} and heated/digested till the aqueous black solution changes to light green or is very clear. After cooling to room temperature the content of the flask was quantitatively transferred to a volumetric flask of 100 ml. The digestion flask, properly rinsed with distilled water into the digester/volumetric flask and its content increased 100 ml mark with distilled water. The same steps as above were carried out for a blank sample without the digested test sample.

Ten (10 mL) millilitres of the volumetric flask content (diluted digest) was transferred into distillation flask with the help of a pipette. Also 10 mL of boric acid (2%) measured into a 50 mL beaker then 2 drops of indicator (methyl red) added to it forming a brown colouration. The delivery tube tip of the apparatus was ensured to go below the boric acid solution's surface. After which, 40% sodium hydroxide solution (10 mL) measured into a distillation flask, placed on the distillation unit of the apparatus and distillation commenced. This continued till the solution of boric acid changed from brown to blue. Then the changed solution titrated over 0.1

N hydrochloric acid until a noticeable colour change to pale pink / peach from blue reached. Titre value recorded then protein content calculated as

Calculation:

$$\%N = \frac{Vs - Vb \times Na \times 0.014 \times 100 \times DF}{W} \quad \text{Equation 3.1}$$

Where:

V_s = Acid volume (ml) required to titrate sample; V_b = Acid volume (ml) required to titrate the blank; N_a = Acid normality (0.01); W = Sample weight (g); DF = Dilution factor [i.e (digest vol. + diluent vol.)/ digest vol.] = 10; %Crude protein = 6.25 * %N

3.7.3 Ash content determination

Sample (5g) was placed into a dried crucible of known weight, then the weight of the sample and crucible together recorded as W_1. It was then placed in a pre-heated muffle for 3 h at 550°C. Thereafter, the sample was transferred to a desiccator to cool, and the weight was noted (W_2). The difference in weight ($W_1 - W_2$) was used to calculate the percentage ash.

3.7.4 Crude fibre determination

The digestion method was employed for this analysis; 2 g of each sample was placed in a conical flask and digested with 1.25% H2S04 solution (200 ml) for 30 minutes, boiling under reflux. The digest was cooled to room temperature before filtration. Buchner funnel sealed with muslin cloth, and held with elastic band firmly to prevent silage or escape of debris. The residue after filtration was rinsed thrice using hot distilled water, then transferred to a conical flask and boiled in 200 ml of 1.25% NaOH solution for 30 minutes under reflux again. Thereafter, the digest was allowed to cool, filtered as above and rinsed three times with hot distilled water.

Lastly, this residue was placed in a dry-cleaned, porcelain dish of known weight and oven dried at 85°C to a constant known mass. Placed a muffle furnace at 550°C for 4 hours to ash, removed into a desiccator, cooled, and the new weight was noted. The weight difference before and after the muffle furnace was calculated and reported as crude fibre.

3.7.5 Determination of fat content

Each sample (2 g) was weighed into a filter paper, properly wrapped and reweighed. Their weight noted. It was carefully descended into the thimble, fixed to a flask that had a solvent (hexane) in it. The Soxhlet extraction unit containing the round-bottom flask was heated slowly under reflux for 3 hours. The wrapped filter paper with the de-fatted (spent) sample was lifted from the extractor flask, and the refluxed solvent (hexane) was recovered by distillation. The spent sample with filter paper dried at 85 ^{o}C, cooled in a desiccator and weighed. The difference in mass was calculated as crude fat.

3.7.6 Determination of carbohydrate

The carbohydrate content of the samples was determined by difference. This determination option of carbohydrate was upheld on the basis that the quantity of vitamins and minerals in foods are negligible. Percentage values of ash, fiber, moisture, fat, and protein contents are usually summed up and subtracted from 100 %; the difference is taken as carbohydrate content in that sample.

3.8 Determination of sensory evaluation

Thirty semi-trained panelists (aged 18-45 years, e.g., students/staff) were selected, briefed, and provided informed consent. Coded samples (random 3-digit numbers) were presented at room temperature on white plates, with water for palate cleansing and random order to reduce bias. A 9-point hedonic scale (1 = dislike extremely, 9 = like extremely) assessed color, aroma, taste, texture, crispiness, and overall acceptability, as described by (Adeyeye, 2017; Awolu *et al.,* 2017).

3.9 Data analysis

Analyses were in triplicate, with results as mean ± standard deviation. One-way ANOVA determined significant differences ($p \leq 0.05$), followed by Duncan's Multiple Range Test for mean separation using SPSS version 25.0. Results were presented in tables and graphs.

CHAPTER FOUR

RESULTS

4.1. Processing yield and pH properties of fermented soy-maize samples

The results of the analysis are presented below. Comparison of the yields from the different fermentation methods and the control samples, as well as the pH values, is presented in Table 1. The processing yield and pH of fermented soy-maize samples varied significantly ($p < 0.05$) across fermentation methods. The control sample (T1) recorded the highest final flour weight (852 ± 1.25 g) and yield (85.2 ± 0.9%), followed by submerged fermentation (T3) with 786 ± 2.50 g and 78.6 ± 2.0%, while solid-state fermentation (T2) produced the lowest flour weight (771 ± 3.50 g) and yield (77.1 ± 1.6%). In terms of pH, submerged fermentation showed the greatest reduction, with a significantly lower pH (4.45 ± 0.03), indicating stronger acidification than the control (6.24 ± 0.02) and solid-state fermentation (6.12 ± 0.02). The decrease in yield and pH following fermentation suggests active microbial metabolism and biochemical modifications during the fermentation process.

Table 4.1. Processing Yield and pH Properties of Fermented Soy-Maize Samples

Sample	Initial Grain Weight (g)	Final Flour Weight (g)	Yield (%)	pH
Control (T1)	1000 ± 0.0[a]	852 ± 1.25[a]	85.2 ± 0.9[a]	6.24 ± 0.02[a]
Solid-state fermentation (T2)	1000 ± 0.0[a]	771 ± 3.50[c]	77.1 ± 1.6[c]	6.12 ± 0.02[b]
Submerged fermentation (T3)	1000 ± 0.0[a]	786 ± 2.50[b]	78.6 ± 2.0[b]	4.45 ± 0.03[c]

Values are mean ± SD (n = 3). Different superscripts within the same column indicate a significant difference ($p < 0.05$).

4.2 Microbial counts of fermented soy-maize samples at 48 h fermentation (CFU/g)

The microbial counts of fermented soy-maize samples at 48 h fermentation showed significant variations between the fermentation methods. Solid-state fermentation (T2) recorded the highest total viable count (2.09×10^5 CFU/g), indicating greater microbial activity compared to submerged fermentation (T3), which had a total viable count of 9.72×10^4 CFU/g. Similarly, the total coliform count was higher in SSF than in SMF although they were too little to count The total lactic acid bacteria count was also highest in T2 (1.01×10^4 CFU/g), suggesting enhanced fermentative and potential probiotic activity under solid-state conditions, whereas T3 recorded 6.25×10^3 CFU/g. In addition, total fungi count was greater in T2 (9.81×10^3 CFU/g) than in T3 (5.59×10^3 CFU/g). The control sample showed negligible microbial growth ($<1.0 \times 10^2$ CFU/g) across all microbial groups. Overall, solid-state fermentation promoted higher microbial proliferation and fermentation activity than submerged fermentation.

Table 4.2. Microbial Counts of Fermented Soy-Maize Samples at 48 h Fermentation (CFU/g)

Sample	Total Viable Count	Total Coliform Count	Total Lactic Acid Bacteria Count	Total Fungi Count
Control (T1)	$1.0x10^2 \pm 0.05^a$	TFTC	$1.0x10^2 \pm 0.50^a$	$1.0x10^2 \pm 0.05^a$
Solid-state fermentation (T2)	$2.09x10^5 \pm 0.27^c$	TFTC	$1.01x10^4 \pm 0.25^c$	$9.81x10^3 \pm 0.11^b$
Submerged fermentation (T3)	$9.72x10^4 \pm 0.50^b$	TFTC	$6.25x10^3 \pm 0.05^b$	$5.59x10^3 \pm 0.28^b$

Values are mean ± SD (n = 3). Different superscripts within the same column indicate a significant difference ($p < 0.05$).

Figure 4.1; Figure 4.1shows the baked cookies

4.3. Sensory evaluation of soy-maize cookies

The sensory evaluation of soy-maize cookies revealed significant differences ($p < 0.05$) among the samples in terms of colour, aroma, taste, texture, crispiness, and overall acceptability as presented in Figure 4.1. The control sample (T1) recorded the highest scores for colour (8.2), taste (8.0), texture (7.3), and overall acceptability (7.1), indicating superior sensory quality compared to the fermented samples. Solid-state fermented cookies (T2) showed relatively high sensory ratings, particularly for crispiness (6.2), and maintained acceptable scores for aroma (6.5), taste (7.0), and overall acceptability (6.8), which were comparable to the control sample. In contrast, submerged fermented cookies (T3) received significantly lower scores across most sensory parameters, especially aroma (2.5), taste (2.8), texture (3.8), and overall acceptability (3.7), suggesting lower consumer preference. Overall, solid-state fermentation produced cookies with better sensory properties and consumer acceptability than submerged fermentation.

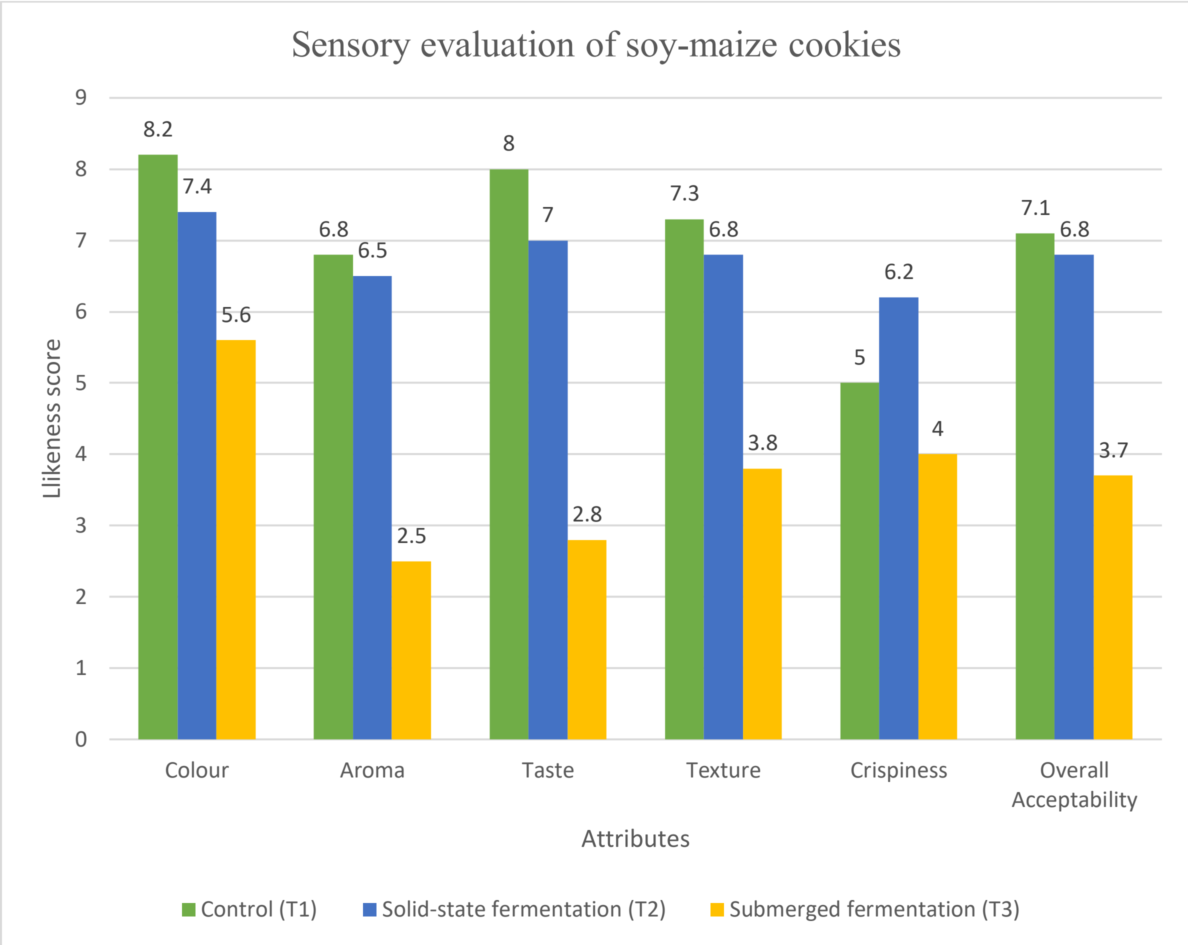

Figure 4.2: Sensory evaluation of soy-maize cookies

4.4 Proximate composition of fermented soy-maize composite flour and cookies samples

The proximate composition of fermented soy-maize composite flour and cookies showed significant variations ($p < 0.05$) among the fermentation methods (Table 3). Solid-state fermented composite flour (T2) recorded the highest protein (14.81%), ash (2.24%), fat (8.15%), and fiber (1.86%) contents, while the control flour (T1) had the highest moisture (9.78%) and carbohydrate (73.28%) contents. Similarly, solid-state fermented cookies (T5) exhibited the highest protein (8.25%), fat (17.48%) contents and energy value (457.40 kcal/100 g). Whereas the control cookies (T4) showed the highest moisture (21.78%), fiber (1.40%), and carbohydrate (74.87%) contents. Submerged fermented samples (T3 and T6) showed intermediate proximate values between the control and solid-state fermented samples. Overall, fermentation, particularly solid-state fermentation, improved the protein, ash, fat, and fiber contents of both flour and cookies while reducing moisture and carbohydrate levels.

Table 4.3 Proximate Composition of Fermented Soy-Maize Composite Flour and Cookies Samples

Sample	Protein (%)	Moisture (%)	Ash (%)	Fat (%)	Fibre (%)	Carbohydrate (%)	Energy Value
Control (T1)	8.13 ± 0.10[c]	9.78 ± 0.20[a]	1.66 ± 0.02[c]	5.75 ± 0.08[c]	1.40 ± 0.03[c]	73.28 ± 0.45[a]	379.39± 1.25[c]
Solid-state fermentation (T2)	14.81 ± 0.15[a]	3.70 ± 0.10[c]	2.24 ± 0.03[a]	8.15 ± 0.10[a]	1.86 ± 0.04[a]	69.24 ± 0.40[c]	406.47± 1.42[a]
Submerged fermentation (T3)	13.38 ± 0.12[b]	5.43 ± 0.12[b]	1.70 ± 0.02[b]	7.24 ± 0.09[b]	1.45 ± 0.03[b]	70.80 ± 0.42[b]	399.08 ± 1.30[b]
Cookies							
Control (T4)	4.94 ± 0.08[c]	21.78 ± 0.35[a]	1.63 ± 0.02[a]	14.38 ± 0.15[b]	1.40 ± 0.03[a]	74.87 ± 0.50[a]	447.30 ± 1.85[b]
Solid-state fermentation (T5)	8.25 ± 0.10[a]	4.85 ± 0.10[c]	1.63 ± 0.02[a]	17.48 ± 0.18[a]	1.32 ± 0.02[b]	66.47 ± 0.45[c]	457.40 ± 1.90[a]
Submerged fermentation (T6)	7.63 ± 0.09[b]	5.80 ± 0.12[b]	0.29 ± 0.01[b]	12.19 ± 0.12[c]	0.34 ± 0.01[c]	73.75 ± 0.48[b]	431.83 ± 1.76[c]

CHAPTER FIVE

DISCUSSION

5.1 Discussion

The result from this research showed that fermentation methods significantly impacted the nutritional, microbial and sensory properties of soy-maize blend cookies flour and cookies. Both fermentation methods substantially improved the quality attributes of the soy-maize cookies compared to those of the unfermented control sample.

Physicochemical Analysis indicated a decrease in pH after the fermentation process. The lowest pH value was obtained by the submerged fermentation (4.45) the control had a higher pH value (6.24).

The microbial analysis showed no observable growth in the control; the fermented sample showed a detectable amount of microbes. The solid –state fermentation process showed higher mold count, yeast count (TVC) compared to that of the submerged fermentation process. The excess liquid in submerged fermentation reduced the microbial movement than that of the solid state fermentation. The solid state fermentation showed more microbial count due to that of increased surface area and oxygen availability (Olukomaiya *et al*., 2020; Yegin, 2025). During fermentation, the presence of microorganisms plays a crucial role in the biochemical properties, therefore improving the sensory and nutritional properties of soy-maize flour and cookies (Marco *et al*., 2021; Adebo *et al*., 2022).

The sensory evaluation indicated that the solid state and control cookies had better acceptability in their sensory properties to that of the submerged cookie. Although nutritional properties improved, the increased water content and anaerobic conditions during fermentation led to a more acidic flavor, which reduced consumers' interest in submerged cookies (Blandino *et al*., 2023; García-Barón *et al*., 2025). Solid state cookies showed more consumer acceptability in color, aroma, taste, texture and crispiness.

The result from the proximate analysis showed significant increase of protein content in both composite flours and cookies. The solid state flour had the highest protein content value (14.81%) while the control recorded the lowest (8.13%). The increase was recorded due to microbial protein synthesis during fermentation and there was observable reduction of carbohydrate, the ash content also improved after fermentation this showed mineral availability in the fermented snacks, the moisture content decreased after fermentation especially that of the solid state fermented sample, the reduction of the moisture content is crucial because it enhances shelf life, the fibre and fat content increased slightly after the fermentation improving the nutritional property ,the reduction of carbohydrate shows that microorganisms used carbohydrates as a source of energy during fermentation (Long *et al*., 2022; Adebo and Njobeh, 2022).. This study shows that fermentation improved nutritional quality, especially solid-state fermentation, while maintaining sensory acceptability.

The study shows that fermentation significantly influenced the microbial, nutritional, and sensory properties of soy-maize composite flour and cookies, with solid-state fermentation resulting in greater improvements than submerged fermentation. Fermented samples exhibited higher protein, fat, fiber, and ash content, and a higher energy value, while moisture content decreased, suggesting improved nutritional quality and shelf stability. Microbial analysis revealed active fermentation processes characterized by increased viable microbial and lactic acid bacteria counts, particularly in solid-state fermentation. Sensory evaluation further showed that cookies produced from solid-state fermented flour were more acceptable in terms of colour, taste, texture, and overall acceptability than submerged fermented samples. Overall, the findings indicate that solid-state fermentation is a promising technique for improving the nutritional value, microbial quality, and consumer acceptability of soy-maize cookies and may contribute to the development of functional cereal-legume snack products.

5.2 Conclusion

The result from this study shows that fermentation improved nutritional composition of the soy-maize snacks compared to the control cookies, the pH values reduced after fermentation, fermentation increased the microbial activity, protein, fat, ash and fibre components. The solid-state fermentation process produced the best result in improving protein content and maintaining high sensory acceptability. The solid-state fermentation was the most suitable fermentation method for the production of improved nutritional and acceptable soy-maize cookies. The use of fermented soy-maize cookies can make provision for improved quality of bakery products and utilization of locally sourced crops, which are healthier than many highly processed snacks in the market today.

5.3 Recommendation

1) Further research and studies can be carried out for the identification of the microorganism responsible for the improved nutrition after fermentation.
2) Controlled fermentation methods can be studied to ensure improved quality output and consistency.
3) The solid-state fermentation method should be encouraged and adopted in producing soy-maize flour and cookies for several reasons, because it produces better nutritional and sensory properties.
4) Fermented soy-maize cookies can be used as affordable and high-protein-content snacks for students, children and households with low income.
5) Health agencies can create public awareness on the nutritional benefits of fermented legume-cereal snacks.

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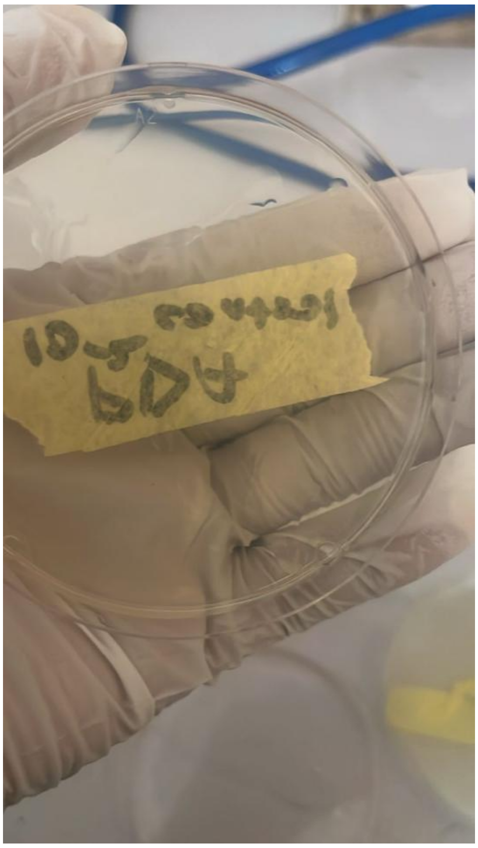
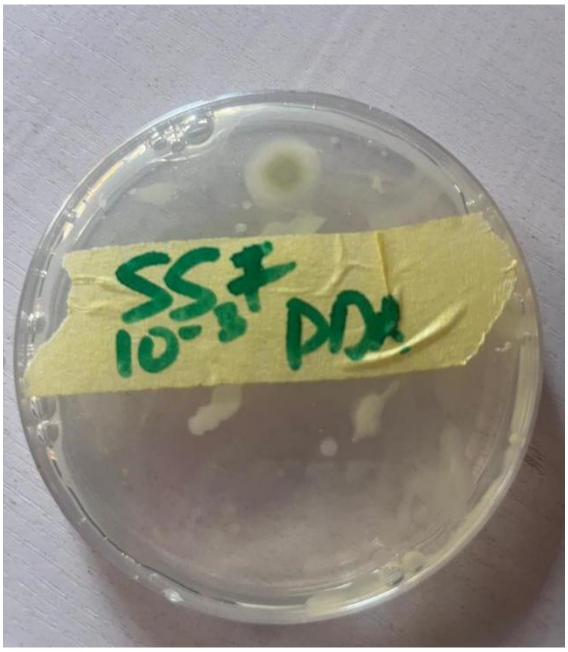
APPENDICES

APPENDIX 1: Solid State and Submerged Fermentation Process



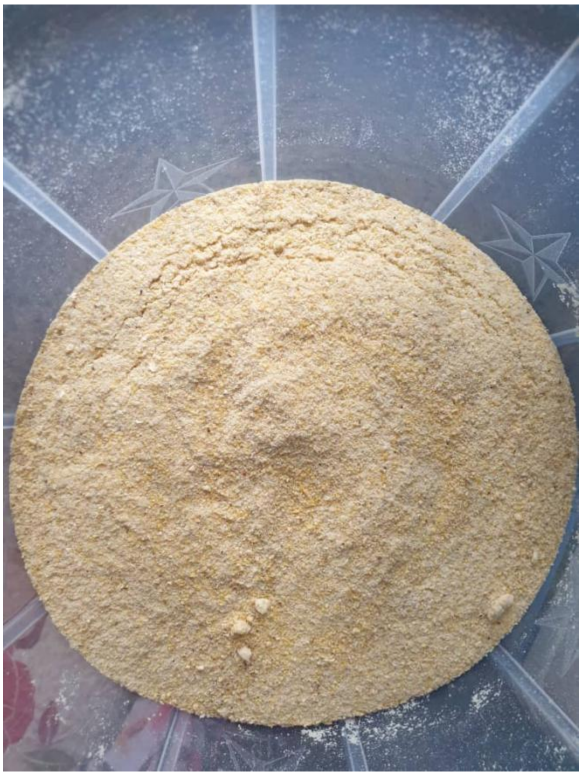
APPENDIX 2: No Growth on PDA Plate After the Culturing of Unfermented Soymaize Sample



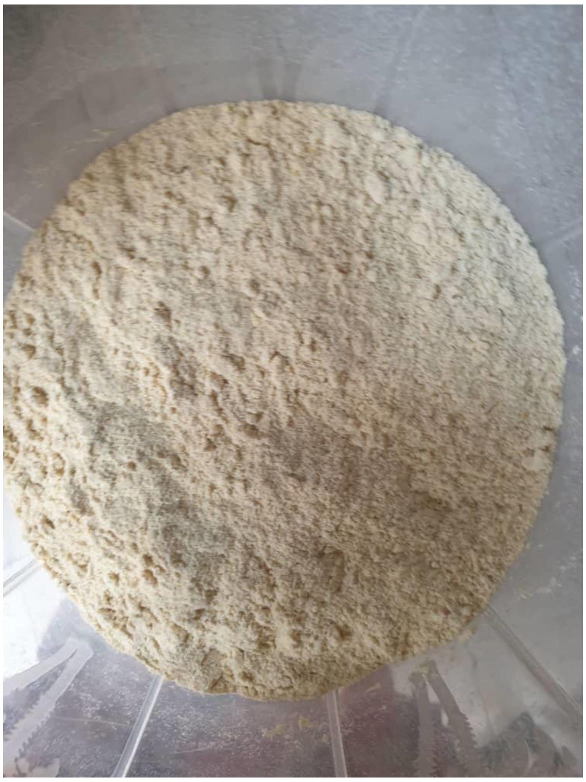
APPENDIX 3: Growth of yeast/mold on PDA after culturing the solid state fermented sample

APPENDIX 4: Growth of yeast/mold on PDA after culturing the submerged fermented sample

APPENDIX 5: Solid state fermented composite flour

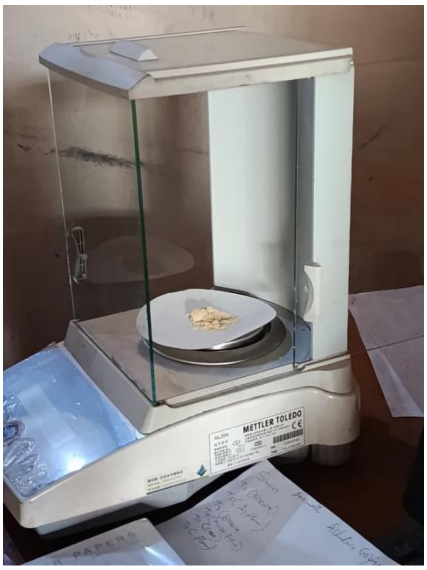


APPENDIX 6: Control sample (unfermented composite flour)



APPENDIX 7: Submerged fermented flour

APENDIX 8: Proximate analysis of Composite flour samples and cookies



APPENDIX 9: Proximate analysis of Composite flour samples and cookies

APPENDIX 10: Proximate analysis of Composite flour samples and cookies

APPENDIX 11: Proximate analysis of Composite flour samples and cookies

APPENDIX 12: Cookies produced from composite flours

APPENDIX 13:

Sensory Evaluation of Milled Composite Flour

Sensory Evaluation Questionnaire
Department of Biological Sciences,
Godfrey Okoye University,
Enugu.
Date…………………………

HEDONIC RATING TEST FOR SENSORY EVALUATION

Instruction: Please taste the coded samples for the following quality attribute and check how much you like or dislike it. Use the appropriate scale by checking the point that best describes your feeling about the sample.

Using score 9 to 1, rate each of the coded samples as follows:
9 – Like extremely
8 – Like very much
7 – Like much
6 – Like
5 – Neither like nor dislike
4 – Dislike slightly
3 – Dislike much
2 – Dislike very much
1 – Dislike extremely

Quality attributes evaluated					
Sample	Aroma	Colour	Taste	Texture	Crispiness

Comment freely on any other observation and compare samples with displayed products ……………………………………………………………………………………
…………………………………………………………………………………………
………………………………………………………………………………………

Just tick:
Age bracket (years) 15-25….; 26-35…..; 36-45……; 46-55…; above 56…….