PHYSICAL, FUNCTIONAL, PASTING AND MICROBIAL PROPERTIES OF SORGHUM *(Sorghum bicolor* M.) OGI AS INFLUENCED BY FERMENTATION METHODS

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DEDICATION

This project work is dedicated to the pillar that holds my life, the one who makes all things possible in his appointed time- **JESUS CHRIST.**

And

To the memory of my son, **AYOMIDE EMMANUEL IBITOYE (JESUS BABY)**

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ABSTRACT

Ogi is a widely consumed food product but could possess low nutritional value when poorly processed. Fermentation had been found to improve its nutritional value, sensory properties and shelf life. However, information on fermentation of ogi using inocula from palm wine, burukutu and yoghurt is sparse. This study was designed to investigate the effect of processing and selected modified fermentation methods on quality attributes of sorghum ogi.

Based on preliminary studies, ogi was processed from unmalted, malted (germinated for 72 hours, dried at 48^{o}C for 24 hours) and milled sorghum using Spontaneous Fermentation (SF) method for between 24 and 72 hours. These samples were subjected to sensory evaluation using panelists. Ogi was also produced using sample with highest overall sensory score (control), and fermented with each of palm wine, *burukutu* and yoghurt inocula (0.08– 0.12 µL/500mL) as starter culture. Microorganism in SF samples, palm wine, *burukutu*, and yoghurt; Palm Wine Fermented Sorghum (PWFS), *Burukutu* Fermented Sorghum (BFS) and Yoghurt Fermented Sorghum (YFS) were cultured and isolated using ISO methods. Molecular characterisation of the isolates was done by polymerase chain reaction sequencing. Chemical, physical, functional and pasting properties, and *in-vitro* protein digestibility of samples were determined by AOAC methods. Sensory properties of the products were determined using panelists. Data were analysed using ANOVA at $\alpha_{0.05}$.

Ogi from unmalted-whole sorghum spontaneously fermented for 72 hours had lowest overall acceptability of 5.51, while 48 hours spontaneous fermented malted-milled sorghum had highest score of 7.34. *Lactobacillus plantarium, Bacillus subtilis,* and *Lactobacillus lactis* were dominant in SF samples. *Lactobacillus* spp. were present in palm wine, *burukutu*, and yoghurt. *Lactobacillus, Leuconostoc, Bacillus, Streptococcus* and *Saccharomyces* spp.; *Lactobacillus, Staphylococcus, Acetobacter, Weissella, Leuconostoc, Lactococcus, Bacillus* and *Saccharomyces* spp.; and *Streptococcus, Lactobacillus, Bacillus, Micrococcus* and *Pseudomonas* spp. were the isolated organisms in PWFS, BFS and YFS, respectively. The concentrations of the inocula significantly affected starter culture activities, with 0.1 µL/500 mL being appropriate. Carbohydrate, protein, fibre and ash contents of the samples varied significantly. The SF sample had least protein content of 5.7%, while YFS had highest protein of 10.3%. Potassium was the dominant mineral in all the samples. The YFS sample had lowest tannin (0.08±0.01%), phytate (0.08±0.01%) and oxalate (0.99±0.01%) contents. The L*, a* and b* colour values of samples ranged from 51.27 to 65.66, 10.08 to13.27 and 11.35 to 18.79, respectively. The loose and packed bulk density was 0.44-0.62 and 0.58-1.82 g/mL, respectively. Total titratable acidity ranged from 0.20 to 1.83 g/L and pH from 3.50 to 5.72. Spontaneously fermented samples had lowest pH. The swelling power and solubility of samples was 3.11-7.20 and 0.03-2.04%, respectively. Fermenting using starter culture significantly reduced peak viscosity of sorghum ogi with SF samples being highest (126.83±2.59 RVU). Introduction of starter culture significantly increased protein digestibility of sorghum ogi. The PWFS had highest overall acceptability score of 7.28.

Grain milling before fermentation and induced fermentation using palm wine, burukutu and yoghurt inocula improved the nutritional properties of sorghum ogi and were recommended for improving qualities of similar fermented food products.

Keywords: Sorghum ogi, Malted sorghum, Inocula-induced fermentation, Ogi qualities

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Sorghum is rated as one of the most important food crops in the world; it ranks behind barley, maize, rice and wheat. Sorghum is mostly used as food and fodder, but recently, it has gained interest as a biofuel crop due to its unique uses in the manufacturing of industrial bioproducts beverages, and non-gluten celiac products (Hariprasanna and Rakshit, 2016; Magdalena *et al*., 2020). In 2016, the world's sorghum production was 63.93 million metric tones, United State of America which is the world's largest producer, produced about 8.4million tones. Nigeria produced 6.94 million tonnes which is about 11% of the world's total production, this makes Nigeria the largest sorghum producer in Africa and the second largest in the world (USDA, 2017). Between 2019 and 2020, United States had a total production of about 8.6 million metric tons followed by Nigeria which produced about 6.7 million metric tons (Shahbandeh, 2020).

Majority of the impoverished in many nations consume sorghum as a primary source of calories, minerals, vitamins and proteins. It is not a favorite food among affluent people because of its poor nutritional value and poorer organoleptic features caused by the existence of antinutritional substances that combine with food components to create complexes and prevent certain minerals, such as iron, from being absorbed into the body (Elifatio *et al*., 2006). Sorghum's phytate and tannin content has been decreased through the use of a number of methods to enhance mineral availability and protein digestibility, they includes fermentation, cooking and malting (Abd El-Moneim *et al*.,2012). Malting entails the controlled germination of grains, which is followed by the use of heat to stop the natural process. The grain is then subjected to further heat to "kiln" it and give it the desired flavor and color. Development and activation of enzymes for the conversion of insoluble reserved foods is the primary goal of malting (Adeola, 2002). It also helps in reducing anti- nutrients and improving bioavailability and digestibility of foods.

Fermentation is the anaerobic oxidation of carbohydrates by yeasts and/or bacteria to produce intermediate substrates with the release of ethanol. Fermentation is a technique that results in the conversion of basic raw materials into a variety of value-added commodities using the phenomenon of the development of microbes and their operations on diverse substrates. Fermentation provides a natural way of eliminating undesirable componentsof foods, enhancing its nutritive value and digestibility as well as improves the organoleptic attributes, it is also a means of preserving foods (Anil, 2019). Grain fermentation provides optimum pH requirements for the release of minerals like calcium, iron, manganese and zinc and the enzymatic breakdown of phytate. As intricate microbes, Lactobacillus strains require nucleic acids, minerals, B-group vitamins, and fermentable carbohydrates to survive. As a result, grain fermentation can be an inexpensive means of creating a rich enviroment that fosters the creation of beneficial germs (Enujiugha and Adebanjo, 2017).

These helpful microorganisms known as probiotics are living organisms that, if given to a host in sufficient proportions, have a positive impact on its health (Danfeng *et al.,* 2012). They enhance the health of the host by enriching the gut microflora. Besides improving gut health, they can also have positive effects on a number of illnesses, such as lactose intolerance, allergies, liver disease, cancer, Helicobacter pylori infection, urinary tract infection, hyperlipidemia and decreasing blood cholesterol levels (Ejtahed *et al.,* 2011). Nutrition related benefits of these organisms include enhanced nutrient absorption and digestion, increased vitamin bioavailability, improved mineral absorption, and immune system activation and development (Ravinder *et al*., 2012). Although most of these organisms are dairy-based, they could also be incorporated into locally produced fermented cereal products like kunun zaki, burukutu and ogi, such local foods' worth may increase and their consumption might be encouraged if they were improved (Tersoo-Abiem *et al*., 2010). Low cost, retaining viability throughout processing and storage, and other desirable qualities encourage the use of Lactic Acid Bacteria (LAB). It performs a dual duty as a food fermentation agent and a possible source of health benefits (Danfeng *et al.,* 2012). Grains are often appropriate substrates for fermentation and the growth of these beneficial microorganisms.

Ogi is a naturally fermented food derived from wet milling grains like millet, corn, sorghum, or blends of various grains. (Odewole *et al.,* 2017). Moreover, the moist products can be converted into powder. For the purpose of enhancing its organoleptic and nutritional properties, it is occasionally possible to add other products such as soybeans, ginger and garlic during processing (Odewole *et al.,* 2017). Ogi is usually consumed by people of all ages and status; it is used as weaning diet for babies, a light food for the elderly and a choice for the sick (Olaniran *et al*., 2020). The typical processing steps for ogi include washing, steeping, grinding, sieving, fermenting and drying (Osungbaro, 2010). The nutritional qualities of the grains are impacted by the loss of components, such as protein and minerals, during processing (Wang *et al*., 2022). Efforts are currently being made to change how ogi is processed in order to increase its nutritional content, improve its keeping quality and possible bio-therapeutic qualities as a result of enriched microflora. The capacity to change the amount of starch in the grain such that there is no need for dilutions because it does not thicken, is what makes malting and fermentation procedures noteworthy. The suppression of pathogen growth by the fermentation process is among the additional advantages of fermentation.

1.2 Problem statement

Ogi is a fermented gruel from cereal grains, commonly consumed by both old and young. It is widely used weaning food for babies and suitable for lactating mothers, the aged, invalids and the convalescents. Ogi is considered to be a better option by those with low incomes, because it is comparatively cheap and easy to prepare. Sorghum is a cereal used second to maize in the production of *ogi*; however, it naturally has low nutritional value with poor protein and low protein digestibility which decreases further with cooking. The steps involved in the processing of *ogi* usually lead to loss of some nutrients especially protein. This results in *ogi* with lower nutritive values, this is disadvantageous to the consumers and will further aggravate the level of malnutrition. There is also the need to add value to sorghum based products as a result of its availability, poor organoleptic properties and abundance of antinutritional components In addition, proteins from animal sources (meat, milk and milk products) are expensive and could not be afforded by low income earners. Also, the processing of ogi usually takes a number of days.

1.3 Objectives

The main objective of this study is to investigate the effect of fermentation methods on the properties of sorghum ogi.

The specific objectives were to:

i. determine the synergestic effect of malting and sieving on the physical, chemical, sensory, digestibility and microbial properties of sorghum ogi
ii. modify and improve ogi processing methods by milling prior to fermentation
iii. produce ogi with improved quality by induced fermentation using inoculums from palmwine, burukutu and yoghurt.
iv. isolate and characterize microorganisms of importance during the fermentation processes.
v. determine the effect of modifying processing methods on the physical, chemical, pasting, sensory and microbial properties of the ogi

1.4 Justification of study

Nigeria is the second largest sorghum producer globally (Shahbandeh, 2020), there is therefore the need to diversify and add value to sorghum based products especially because of its low nutritional value and poor organoleptic properties. Grain-based products contain health-promoting microbes and potentially prebiotic fibre (Danfeng *et al.,* 2012). The utilisation of cereal based product as a vehicle for these useful organisms will combine the advantages of cereal and bacteria that promote health. Grain is a good medium for the growth of these beneficial organisms. Malting of the grains will help to boost the product's nutritional qualities as well as the improved processing methods which solve the problem of the loss of most essential nutrients during processing. A less dense *ogi* which requires no further dilutions because of its viscosity will be obtained, this will encourage the consumers to consume more of the product and therefore more of the nutrientswill be consumed.

Cereals' anti-nutritious components have been observed to be reduced by the biochemical process of fermentation, which also increases the digestibility of their carbohydrate and protein, maintains the balance of their amino acids, and increases their

nutritional value (Singh *et al*., 2012). Grain malting also improves protein digestibility and other protein quality characteristics in comparison to germination alone, the combination of germination and fermentation alters significant biochemical components, making both processes potentially useful for creating food products with better characteristics, nutritional value and mineral availability. The results will give a better product in terms of the organoleptic, physical, functional, pasting and microbial properties; more nutritious and digestible product will also be achieved.

Probiotics are beneficial microorganisms with nutritional advantages. Regular consumption of probiotic foods assists in creating a balanced population of helpful gut bacteria flora. These organisms are usually incorporated into fermented dairy products as a component of the fermentation process and are regarded as functional foods e.g. yoghurt, cheese and fermented milk .There is the need to introduce dairy free probiotic foods as they are cheaper, more readily available and suitable for vegetarians and lactose intolerant consumers.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sorghum (*Sorghum bicoIor M.*)

Sorghum is a large, coarse annual crop of the *Gramineae* (*Poaceae*) family that is somewhat similar to corn (but has the grain in a panicle rather than an ear) and is used for the same purposes. Sorghum is the fifth most significant grain (Prajapati *et al.,* 2018; Nyoni *et al*., 2020).

2.2 Origin and Variety of Sorghum

Sorghum is among the oldest crops cultivated in Asia, especially in China and India, and it is a native of the North Eastern quadrant of Africa. Sorghum has been brought to the United States and other nations because of its exceptional drought resistance (caused by its vast branching root system) and its capacity to survive hot weather better than maize (Getachew *et al*., 2016) It is a warm-season crop that can tolerate moisture and heat stress better than most other crops, but excessively high temperatures and prolonged droughts could lower output (Rajendra Prasad *et al*., 2021). From its introduction to the United States during colonial times, the crop has undergone numerous alterations as a result of naturally occurring genetic mutations and hybridization. Until relatively recently, the majority of important grain sorghums introduced into the country were late maturing. Many farmers found the crop to be appealing since it was possible to make hybrid grains sorghums with great yield potential and stems that were short enough to be harvested mechanically.

Sorghum, botanically known as *Sorghum vulgare* or *Sorghum bicolor (L)Moench,* includes grains such as sorghum, Sudan grass, broomcorn and tall sorghums that may be grown for forage, silage, or syrup (Verma *et al*.,2017). Common names of sorghum are; guinea corn, great millet, (West Africa), Kaffircorn (South Africa), milo, sorgo (United States), Kaoliang (China), dura (Sudan), Mitama (East Africa) and jola, jawa, cholam (India). Types of sorghum available include white durra, white kafir, brown durra, red

kafir, milo, shallu, Pink kafir, feterita and hegari. In commerce, milo, kafir corn and gyp corn are other terms sometimes used to identify the sorghum grain.

2.3 Ecology of sorghum plant

Sorghum needs a warm environment for healthy germination and development, and the ideal period for planting is when the soil has enough moisture. Germination requires a minimum temperature of between 7 and 10^{o}C. 80% of seeds germinate within 7 to 10 days of sowing at a temperature of 15^{o}C (Ajeigbe *et al*., 2020). The best time for planting is when the soil's temperature is about 15^{o}C and above and the soil is sufficiently moist. For optimum growth and development, a 27°C to 32°C temperature range is needed.. Very high temperatures lead to yield losses due to pollen loss. Sorghum thrives in a variety of soils, with the exception of flooded areas. The ideal soils for it are those with a clay-loam or loam texture and strong water retention capacity. even though it can thrive in unsuitable soil for rice and maize, such as poor, sandy soil. Sorghum thrives in soils between the pH range of 6 and 7.5 that are rich, well-drained, and have a moderate quantity of organic matter. The majority of nutrients are more readily absorbed by plant roots at this pH range (Ajeigbe *et al.*, 2020).

Sorghum needs 400 to 600 mm of water for a maximum yield, which should be evenly distributed throughout the growing season based on the crop's developmental stage and environmental requirements.

Compared to most other grain crops, sorghum is better suited to withstand drought. This drought resistance results from:

- Limited leaf area per plant restricts transpiration.
- The leaves fold up more effectively than those of maize in warm, dry conditions.
- The roots are particularly highly developed and finely branching, which makes them very efficient at absorbing water.
- The leaf's corky epidermis is covered in a layer of wax that guards the plant against desiccation.
- The stomata close quickly to reduce moisture loss

- The ability to remain in a nearly dormant stage during dry periods and resume growth as soon as conditions are favourable (Melissa–Panel *et al.*,2022; Justinus and Thinus, 2012).

2.4 Structure of sorghum grain

Sorghum has a similar grain structure to other grains. The basic structural components of the grain are the pericarp (outer covering), endosperm, embryo, and testa between the pericarp and endosperm. The endosperm, which can be corneous (vitreous) or floury, and the testa, which may include tannins, both affect the grain's nutritional value. The testa of some sorghum genotypes is intensely coloured. Sorghum kernels have a great deal of variation in terms of colour, shape, size, and other anatomical elements. The pericarp, the germ or embryo, and the endosperm are the three principal anatomical parts. The pericarp is entirely bonded to the endosperm in sorghum kernels of the caryopsis type (Abah *et al.,* 2020). The sorghum's kernel weight distribution kernel is pericarp 6%, endosperm 84% and germ 10%.

2.5 Scientific Classification of sorghum

Sorghum has about 30 species and its scientific classification is as follows;

Kingdom:	*Plantae*
Division:	*Magnoliophyta*
Class:	*Liliopsidas*
Order:	*Poales*
Family:	*Poaceae*
Genus:	*Sorghum*

Species: *sorghum almum, S. amplurn, S. angustum, S. arundinaceum, S. bicolor* (primarily cultivated species), *S. brachypodum, S. buibosum, S. burmduium, S. controversun, S. drummondii, S. ecarinatum, S. exstans, S. grande, S. halepeise, S. interjectum, S. intrans,*

Figure 2.1: Sorghum grain

S. laxifitorum, S. matarantense, S. miliaceum, S. nigrum, S. nitidum, S. plumosum, S. propinquum S. purpureosericeum1 S. sipodeum, S timorense, S. trianscladum, S versicolor S. vingatum, S. vulgare).(Magdalena, 2018, NWE, 2019, Oluwafemi, 2020*).*

The different types of sorghum are typically divided into four groups: sweet sorghums, also known as sorgos, which produce molasses and syrup from cane juice; broom corns, that also produce a fibre from the inflorescence that is used to make brooms; grass sorghums, which are used for pasture and hay; and grain sorghums.

2.6 Varieties and Cultivation of sorghum

Sorghum is available in a wide range of colours, such as red, brown, and white. Traditional species with open pollination, which rural farmers save seeds from for the following season's planting, typically yield less than the slowly evolving modern hybrids. To be cost-effective, hybrids must be grown with effective weed and insect control, the help of fertiliser, and excellent water management. Despite the poor yields of traditional cultivars, they are nonetheless viable in environments where maize production would be impractical or unprofitable. Typically, grain sorghum is grown in climates that are either too hot or too dry for maize to be successfully grown.

2.7 Production of sorghum

The top five global sorghum producers are Nigeria (7%), China (9%), Mexico (17%), India (21.5%) and the United States (25%) (FAO, 2020). They collectively account for 73% of the total global production (FAO, 2020). It is the next most significant grain to maize in sub-Saharan Africa and Nigeria is the largest sorghum producer in Africa (USDA, 2017, Shahbandeh, 2020). In 2022, United State of America is the world's leading sorghum producer followed by Nigeria, Mexico, Ethopia, India and Sudan (Figure 2.2) (Shahbandeh, 2023). There are a number of factors contributing to the declining trend in sorghum production, including unpredictable, irregular distribution of rainfall, declining soil fertility, ineffective production systems, biotic and abiotic stresses, and declining demand for sorghum. From 1980 to 2000, food demand in 90 developing countries increased by 2.9%, just slightly faster than the projected 2.8% rate of agricultural

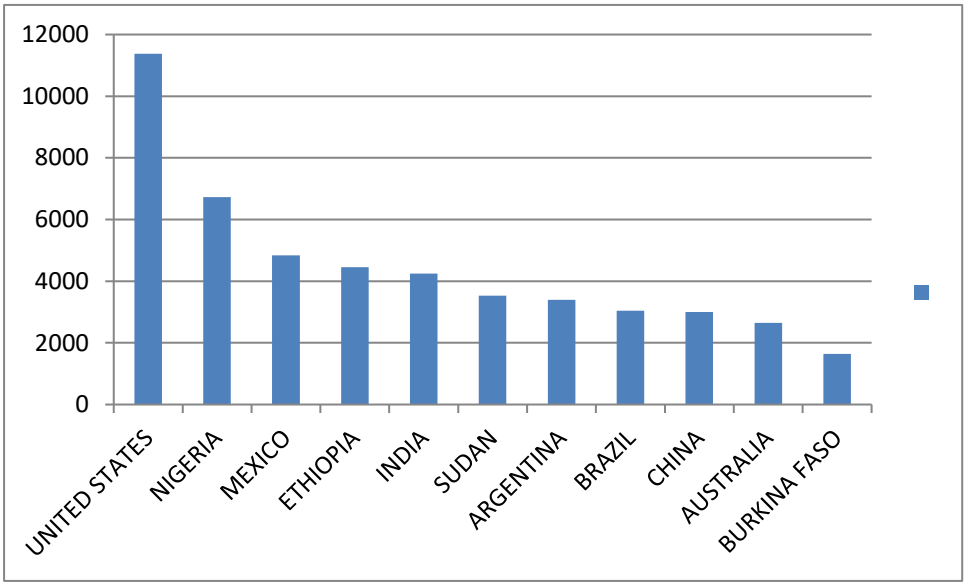

Figure 2.2: **Sorghum production worldwide in 2022 (in 1,000 metric tons)**
Source: (Shahbandeh, 2023).

production growth. However, the disparity was most obvious in Africa (demand 3.4%, production growth 2.6%), and it is anticipated that in the least developed nations, output growth will lag 25% behind that of demand growth.

2.8 Consumption and Utilisation of sorghum

Since sorghum is primarily consumed in the countries where it is produced total consumption closely mirrors the global pattern of output. Sorghum is utilised for both human consumption and as animal feed, which are two separate uses. Africa and Asia is responsible for more than 95% of the world's sorghum consumption as food (Léder, 2004). Almost 75 percent of all use in Africa is for human consumption Many different varieties of sorghum are consumed as grains and in sorghum syrup or molasses. In regions where sorghum is grown as a subsistence crop, the main foods produced are thin and thick porridges, lactic and alcoholic beers and beverages, malted flour for brewing, fermented and unfermented breads, malted porridge mixes, and weaning foods.. Pearled sorghum, a maize substitute, has a limited but expanding market in South Africa and Kenya (Hassan *et al*., 2021). Dehulled sorghum has been suggested for use in newborn and toddler feeding regimens in India. Some red sorghum cultivars contains active amylase at dosages suited for particular brewing uses or the production of opaque beer where sorghum serves as both the adjunct and the malt supply.Sorghum forms an important starchy food both for feeding livestock and for human use. Almost all parts of the plant can find some form of application in the industry. Edible starches can be manufactured from starch endosperm of sorghum grain. In Nigeria, about 95% of sorghum grains are consumed as human food. Sorghum can be consumed in numerous forms such as flour or paste as *ojo* (thin porridge) as *tuwo* (thick porridge) or as deep fried snack. The local distilling and beverage industries also utilize sorghum for the production of native beer for example *pito, otika* and *burukutu*.

2.9 Processing of Sorghum

The three major components of cereals -the germ, the starch-containing endosperm, and the protective pericarp are partially separated and/or modified during processing. The majority of traditional processing methods require repetitive, tedious work and are nearly exclusively done by women. Decorticating, which often entails pounding subsequent to winnowing or occasionally sifting, malting, fermenting, roasting, flaking, and grinding are traditional techniques that are frequently utilised. These techniques typically require a lot of labour and provide poor quality products. If production was optimized and the demand for high-quality flour could be met,, sorghum would likely be utilised more frequently.

Sorghum and millet are generally not processed industrially in the same way as wheat and rice, which are generally regarded as being of much higher quality than sorghum and millet. Sorghum has good industrial processing potential, and efforts have been made in a number of nations to create more effective industrial processes. In certain African nations where it has lately been adopted, custom milling has had a considerable influence. In Nigeria, about 2.5 millions of sorghum grain which accounts for about80% of the total production is being processed in this manner (FAO, 1995)

The colour of the sorghum brown, white, or yellow is very important. for processing and to some extent for storage. External appearance is not an indication of cultivar type; Despite the fact that all three varieties can have seed coats that are white, yellow, brown, red, or purple, brown sorghum often has a darker colour than both yellow and white sorghums, brown sorghum presents the greatest challenges during processing due to the following reasons:

- The testa is almost the last layer to be eliminated as the pericarp is gradually peeled away from the outside.
- A brown sorghum that has recently been moist has a tendency for the endosperm to remain firmly attached, but the pericarp to separate directly above the testa. Brown sorghum is frequently quite soft, and mechanical impact tends to cause the endosperm to separate from the seed.

When roller milling brown sorghum, cutting the endosperm from the interior of the pericarp is the best method for separating the testa from the endosperm. However because some bitterness and some colour are not only permissible but frequently requested in beer, brown sorghums are typically only utilised in the brewing process.

2.10 Storage of sorghum grain

The goal of storage is to preserve the value of the grain for future use as much as possible.This entails either saving as much of the grain's food value as feasible for as long as possible or conserving the highest percentage of viable seeds to sow for the upcoming harvest. Many factors contribute to the loss of nutrients and viability, but pest damage from insects, birds, and rodents, as well as mould, are the primary causes of loss worldwide. Sorghum is typically kept in tiny quantities in conventional containers, frequently on the farm, in underdeveloped nations. Seldom do large amounts build up, and bulk storage is uncommon. Consumers and processors both store grain for a later use. Commercial dealers also keep it in storage for resale, in thedomestic market but seldom for export. The most significant physical factor that affect losses are grain moisture and storage temperature (Mrema *et al*., 2011).

The majority of activities that cause losses happens more quickly as the temperature rises. Moisture will migrate and collect in specific spots with even slight temperature changes, either close to the top of the container or in areas where it is cooler than the remainder. To provide insects as little room as possible to move around and breed, the grain should be packed as firmly as feasible. Sometimes sand is incorporated into the grain to further decrease the open space. White sorghum has more resistance to damage by insects during storage than brown sorghum, which has a tendency to be softer. Nonetheless, both in the field and during storage, they are significantly less vulnerable to fungal harm (Verma *et al.,* 2017).

2.11 Uses of sorghum

Sorghum is a significant food crop that has numerous uses for humans, some of its uses include:

2.11.1 Alcoholic Beverages: Sorghum is used in the manufacturing of alcoholic beverages from time immemorial; beverages like *burukutu* in the Western part of Nigeria, Sorghum beer in Europe, etc. In China, sorghum is the primary component used to make distilled beverages like maotai and kaoliang. (NWE, 2019).

2.11.2 Food: The grain is a mainstay or one of the basic foods consumed by rural and impoverished people in dry regions. It is also used to make sorghum molasses or sorghum syrup. The majority of types are heat and drought tolerant

2.11.3 .Fodder: Sorghum is utilized as animal feed. In the early growth phases of the plant, some varieties of sorghum may contain concentrations of hydrogen cyanide, hoordernine, and nitrate that are fatal to grazing animals. Stressed plants can also contain lethal levels of cyanide in later stages of growth. Because it does not build up static electricity and makes great wallboard for building homes as well as biodegradable packaging, sorghum straw (stem fibres) is also utilised in the manufacture of packaging for delicate electronic equipment (NWE, 2019).

2.12 Nutritional benefits of sorghum

Starch, protein, non-starch polysaccharide, and fat are the major constituents of sorghum grain. Sorghum's macromolecular composition is similar to wheat and corn (Miafo *et al*., 2022). Sorghum contains resistant starches that affect digestibility, especially for infants. The resistance is desirable in obese and diabetic nutrition. Linoleic acid makes up 49% of the sorghum fat, followed by oleic acid (31%), palmitic acid (14%), linolenic acid (2.7%), and stearic acid (2.1%) (Mahmut *et al.,* 2008). Sorghum protein's nutritional quality is poor as a result of protease resistant proteins in sorghum, however, there are variabilities in variety in terms of the proteins levels.Cooking sorghum may reduce its protein digestibility although fermentation may boost it.

2.13 Proximate Composition of Sorghum

Sorghum kernel consists of endosperm, germ and bran. A whole grain of sorghum contains; moisture, (11-14%); protein, (7.5-9.0%); fat, (2.7-3.5%); ash, (1.3-1.7%), carbohydrates (Reducing sugars: 1.8%; Starch, 74.1%; crude fibre: 2.6%, wax: 0.3%,

Pentosans 2.5%) (Abah *et al.*, 2020). The constituents of commercial sorghum grain has changed from what it used to be in the early period before the introduction and dominance of hybrids and the change has resulted in the decreases of protein content and a boost in starch content. The composition of the various kernel components also varies: the germ is rich in protein and fat but low in starch; the bran has medium starch content while the endosperm has higher starch content. Protein in sorghum is present in the endosperm, germ and bran but it is deficient in lysine, methionine and threonine (Abah *et al.*, 2020), Sorghum protein's quality is inferior to that of wheat and rice, but it is not of critical importance if suitably processed and blended with other foods. According to Eggleston *et al*., (2022), sorghums with protein levels higher than 9% is mostly composed of prolamine which is nutritionally insignificant.

2.14 Amino acids in sorghum grain

According to Abdel-Moneim *et al.* (2012), sorghum grains contain the following amount of amino acids (g/16g); depending on the varieties; Arginine (3.7-4.9), Histidine (2.0-2.5); Isoleucine (3.9-4.3), Leucine (13.3-14.1), Lysine (1.9-2.3), Cystine (1.7-2.2), Methionine (1.6-2.1), Phenylalanine (4.8-5.2), Threonine (3.0-3.5), Tryptophan (1.0-1.2), Tryrosine (3.8-4.2), Valine (5.3-5.5), Aspartic acid (7.0-7.5) Glutamic acid (20.1-21.1), Alanine (8.8-9.7), Glycine (3.0-3.3), Proline (8.0-9.7) and Serine (4.4-4.7).

2.15 Protein Quality of Sorghum

Prolamin proteins are the pepsin-indigestible protein found in sorghum and compared to other cooked cereal proteins, proteins in cooked sorghum is less digestible. Orlien *et al*., (2021) reported that when cooking sorghum with 2-mercaptoethanol, its protein digestibility increased when tested in vitro with pepsin or trypsin/chymotrypsin, to a level equivalent to that of other cereals.. Protein digestibility increased by 5% when maize was cooked with 2-mercaptoethanol compared to 25% for sorghum. Other reducing agents that improved sorghum’s protein digestibility include solutions of dithiothreitol, sodium bisulphate and L-cystein Prolamins in sorghum after cooking is considerably less soluble and pepsin digested than maize prolamins. Orliens *et al.,* (2021) reported an increase in sorghum's ability to digest proteins in vitro after fermentation with significant increase in the globulin plus albumin fractions during the first eight hours of fermentation.

According to Abdel-Moneim *et al.* (2012), crude protein level declined and free amino acids content increased in raw sorghum from 12.46 to10.62% and 0.66 to1.03mg/g respectively after 20 hours of soaking and 72 hours of germination, he described factors influencing sorghum protein digestibility especially when wet-cooked, as external factors/grain organisation structure, starch and non-starch polysaccharides, phytic acid, polyphenols, and endogenous factors and changes in protein secondary structure Depending upon variety or state of the sorghum grain i.e. whether it is whole grain, endosperm, protein body preparation, condensed tannin-free or tannic-rich, there might be multiple factors involved at once.. The authors found that protein cross-linking in sorghum protein is the largest factor in sorghum protein indigestibility.

2.16 Vitamins in Sorghum

Most sorghum varieties contain no vitamin A but contain mostly the B-vitamins such as niacin (51.4%), panthothenic acid (7.0%), Riboflavin (1.11%) Biotin (0.30%) and pyridoxine (6.4%). The distribution of these vitamins differs in the endosperm, germ and bran (Ratnavathi and Patil, 2013). Unlike the other grains, the endosperm of sorghum has a higher vitamin content followed by the germ and the bran has low vitamin content.

2.17 Minerals in Sorghum

Sorghum grains contain minerals such as calcium (0.01-0.02%), phosphorus (0.46-0.55%), Magnesium (0.13-0.23%), potassium (0.37-0.48%), sodium (0.01-0.02%), iron (40-66ppm), manganese (16-27ppm), copper (3-7ppm), Zinc (30-70ppm) and cobalt (0.5-1.0ppm) depending on the varieties. Sorghum also contains silicon, boron, lead, aluminum, molybdenum, nickel, chromium tin, titanium in varying amounts (Gerrano *et al.*, 2015 According to Raihanatu *et al.*, (2011), malting coupled with fermentation decreased the mineral composition of sorghum. They observed a decrease in magnesium from 4.80-2.12%, sodium from 3.93-3.01%, potassium, 12.63 to 3.17% and iron, 0.02-0.01%.

2.18 Anti Nutrients in raw Sorghum

As a result of antinutritional components such as protein crosslinkers, tannins, phytates, and trypsin inhibitors the usage of sorghum is subject to several restrictions. New techniques for processing sorghum have been investigated in an effort to stop those

limitations. It has been demonstrated that germination further increases nutritional content and decreases anti-nutrients. Moreover, sorghum flour's starch and protein digestion could be modified by fermentation with lactic acid bacteria, increasing the availability of those nutrients (Melisa *et al.*,2022).

2.18.1 Tanin in Sorghum

The presence and activity of antinutrients in sorghum can have some impact on its nutritional composition, condensed tannins and phytic acid, that are phenolic compounds are the main antinutrients present in sorghum. Both have a negative effect on the bioavailability of vital nutrients in the gastrointestinal tract, especially iron and zinc, In addition, the digestibility of sorghum protein is further decreased by tannins. Proteins and divalent cations can be bound by tannins and phytic acid. The human gastrointestinal enzymes responsible for the digestion of cereal can likewise be bound by the tannins. The antinutrients thus cause an imbalanced intake of vital nutrients and decrease metabolizable energy and amino acid availability. Because they can absorb metal ions, complex with macromolecules, and serve as antioxidants, tannins have different biological impacts on human and animal nutrition. Tannins influence the removal of trace elements by generating insoluble complexes in the digestive tract and decrease the digestibility of proteins, because tannins make minerals less bioavailable to the body, eating a lot of foods high in tannins, like sorghum, is linked to disorders caused by a lack of minerals, like iron deficiency anaemia.

Most sorghum varieties contain tannins which can be used as a basis for classifying sorghum into two classes; those containing negligible levels of tannins (low tannin sorghum) and those containing appreciable levels (high tannin sorghum). Tannins give sorghum grain its reddish to brown colour and act as nutritional inhibitors by combining with proteins to render them indigestible and inaccessible to the body.. Tannins in cooked sorghum products are associated with poor protein utilization. Tannins in both fresh and processed foods and products might cause browning or other pigmentation disorders. Tannins make food unpalatable by causing an astringent reaction in the mouth. Tannins can form complex compounds with proteins, precipitate them in the intestine and inhibit digestive enzymes like trypsin, amylase and micro-organisms and can also interfere with

dietary iron absorption. Raihanatu *et al*., (2011) reported 2.08-14.58% decrease in tannin and other alkaloids for sorghum as a result of synergetic effects of malting and fermentation.

2.18.2 Dhurrin in Sorghum

The majority of sorghum cultivars contain cyanogenic glycosides, though the amount varies depending on the cultivar, how they are dispersed throughout the environment and within the plant tissues. Acute cyanide poisoning is caused when dhurrin, the major cyanogenic glycoside, is degraded by glucosidase to create p-hydroxybenzaldehyde and prussic acid or hydrocyanic acid. The -glucosidase can come from plant matter or from a particular intestinal microflora of exposed humans or animals. When a plant is growing dhurrin is mostly found in the leaves and stem; it is absent (or only slightly present) in the stalks and caryopses and of mature plants. Dhurrin may consequently be a concern to animals that consume sorghum materials rather than to human. However in people with poor nutritional condition, long-term consumption of cyanide-containing foods is linked to chronic effects of cyanogenic glycosides ingestion . Cyanide poisoning can have severe effects, especially in weaker species. It reduces the amount of oxygen that is used by the tissues, which causes distress to the respiratory, neurological, circulatory, and digestive systems as well as, in rare circumstances, death.

2.18.3 Phytates in Sorghum

Phytic acid is present in nuts, legumes, grains, and oil seeds; it makes up to 90% of the overall phosphorous and up to 1.5% of the dry weight of the seed. Its primary roles in seeds include phosphate storage as an energy source and antioxidant action for the seed that is germination. Due to inadequate digestive tract degradative capacities and the pH level of the small intestine, phytic acid's phosphorus is largely not bioavailable to monogastric animals, humans inclusive Phytic acid, like tannins, has the capacity to chelate metal cations, primarily iron, digestive enzymes like amylase, pepsin and trypsin, as well as proteins, calcium, and zinc In populations where sorghum is the main source of food, how they form insoluble complexes with metals and proteins determines how readily available they are as nutrients and can cause deficiencies. (Boulibaly *et al*., 2011). Sorghum is a grain that is regularly used in the production of complementary foods for

infants in Africa. The consumption of phytates is directly correlated with the low iron and zinc levels of that are typically observed in weaned, preschool children after the age of six months. Moreover, phytic acid has the power to prevent the utilization of other crucial minerals such as calcium, magnesium, and zinc.

2.19 Effect of antinutrients in sorghum on human health

Similar to legumes and seed oils, sorghum's nutritional value is impacted by the presence of antinutrients. For instance, the three types of tannins—hydrolyzable (such as gallitannins and ellagitannins), condensed (such as proanthocyadinnins), and complex—are phenolic metabolites that are water-soluble. Condensed tannins can attach to protein components, precipitate them, and make them indigestible thereby limiting their availability. Tannis can bind to proteins as well as minerals and vitamins, making them inaccessible. Moreover, it has the capacity to inhibit the actions of hydrolytic enzymes like trypsin, glucoamylase, α-amylase, and lipase. (Hodges *et al*., and Cameron, 2021) The primary phosphate storage form is phytic acid, also known as phytate, which chelates protein cations and mineral cations to form insoluble precipitated complexes. As a result, trace mineral bioavailability is diminished, and protein digestibility is also impaired.(Afify *et al.,* 2012). Anti-metabolic proteins known as enzyme inhibitors including protease, trypsin, and chymotrypsin inhibitors, interfere with the digestion of proteins and prevent amino acids absorption. (Chhikara *et al*., 2018). When consumed and hydrolyzed, dhurrin, a cyanogenic glucoside mostly found in the shoot of the sorghum plant, releases hydrogen cyanide (HCN), which results in cyanide poisoning. Heart arrest, pulmonary failure, and central nervous system dysfunction are all brought on by HCN. Moreover, too many cyanide ions can quickly cause death by inactivating the cytochrome oxidase system, causing anoxia of the central nervous system. (Gleadow *et al.,* 2021).

2.20 Invitro Protein Digestibility

One of the indicators of the nutritional value of a food is the protein digestibility. In essence, protein quality relates to a protein's capacity to satisfy the necessary amino acid needs of humans. For proteins to be used by the body they must be broken down by the digestive tract's proteolytic enzymes in the stomach, pancreas, and intestines to produce tiny peptides then amino acids that may then be taken into the circulation and utilised by

the body. In vitro protein digestibility assays are frequently used because in vivo protein digestibility determination is expensive and is time-consuming, and because using humans in experiments raises ethical concerns. Fast in vitro assays, that utilise enzymes comparable to the ones present in the digestive system of man and are conducted under circumstances that mimic human physiological conditions, have been adapted to address these problems. A more objective way for measuring protein digestibility is to use in vitro enzymatic assays, which often include either treating the sample alone with proteolytic enzymes, or in combination. Protein solubility, amino nitrogen, or free amino acid changes are then used to evaluate protein digestibility. These procedures measure either the initial rate of hydrolysis, from which total digestibility can be calculated or measure maximum digestibility values. According to Raihanatu *et al.,* (2011) combined sprouting and fermentation increased protein digestibility to 93.78-95.68%.

2.21 Sorghum as main meal

Sorghum grain is primarily utilized as food and animal feed, and in the United States, its use as animal food accounts for about 90% of the total amount of sorghum produced. Sorghum is a crucial food grain and a major food for many people, both in urban and rural settings, in many African and Asian regions. Ukwuru *et al.,* (2018) reported that sorghum is eaten mostly in the northern part of Nigeria where it is used for the preparation of foods such as *tuwo dawa*, weaning food called *hatsi*, pap and alcoholic drinks. Sorghum grains can be malted and used for the industrial production of beer in Nigeria (Chaves-López, ,Rossi, Maggio, Paparella .and Serio, 2020; Dabija, Ciocan, Chetrariu, and Codin, 2021). Other food products from sorghum are dimped sugary sorghums in India; cooked high lysine grains in Ethiopia, the parched or roasted green grains in Maharashtra (Ratnavathi and Patil, 2013). Small corneous sorghum grains may be pearled and then boiled to give product resembling rice in India, Bangladesh, China, Ghana, Ethiopia, Kenya, Botswana, Nigeria and other parts of Africa (Ratnavathi and Patil 2013).

2.22 Fermented and Sour Products from Sorghum

According to Adegoke (1988), Lyumugabe *et al.,* (2012); Sawadogo-Lingani *et al.,* (2021), Dabija *et al.,* (2021) major fermented African foods and beverages are; bogobekoko, produced in Ghana from millet, sorghum and maize and fermented by

Lactobacillus spp and *yeast*; bouza, produced from wheat or sorghum, fermented by *Saccharomyces* Spp and lactic acid bacteria and produced in Mauritania and Nigeria, burukutu from Nigeria, mainly from sorghum and fermented by *Saccharomyces* spp, and lactic acid bacteria; enjara from Ethiopia, mainly from sorghum and fermented by *Lactobacillus* spp and *Candida* spp; kenkey in Ghana produced from sorghum , maize or millet, fermented by *Lactobacillus* and *yeast*; kisra from Ethiopia and Sudan produced from millet and sorghum by *Lactobacillus delbruecki, Saccharomyces, cerevisae* and *Lactobacillus sp*; kishk produced in Sudan and Ethiopia from sorghum and fermented by *Lactobacillus plantarum;* kunu-zaki from Nigeria, produced from sorghum or millet by lactic acid bacteria; merissa from Sudan, produced from sorghum by *Candida krusei; ogi* from Nigeria and Ethiopia, produced from millet, maize or sorghum by *L. plantarium*, *Strepococcus lactis*, *Saccharomyces cerevisae*, *Candida* spp*;* otita in Nigeria from sorghum by *Saccharomyces* spp and lactic acid bacteria; pito/kaffir from Nigeria, Ghana and Benin Republic from maize or sorghum and fermented by *Georichum candida* and *Lactobacillus* sppand uji from Sudan produced by fermenting sorghum or maize with *Candida krusei* and *S. cerevisae*.

2.23 *Ogi*

Ogi is a gruel produced from fermented corn, sorghum or millet. it is one of the major staple foods in West Africa and is used as a complementary food for babies, a light meal for the elderly and a choice for the sick (Olaniran *et al.*, 2020). It is described with different names in Nigeria such as *akamu, koko, eko, agidi* e.t.c. Bolaji, Adepoju, and Olalusi, (2015) explained how ogi is being prepared traditionally; soaking grains in water for one to three days, then wet milling and sifting to remove bran, hulls, and germ.. The filterate is fermented (for 1-2 days) to produce ogi, which is sour, white or brown starchy cake while the pomace is later used as animal feed. The degree of acidification of Ogi is influenced by the duration of the fermentation, which varies between 2 and 3 days. Ogi is usually sold as a moist cake packaged in transparent polythene bags or leaves. Prior to consumption, it is either heated and transformed into a hard gel called eko or agidi, or it is diluted to 8-10% solids and cooked to a slurry.

Lactobacillus plantarium, which is a lactic acid bacterium, the aerobic bacteria *Corynebarterium* and *Acetobacter,* the yeasts *Candida mycoderma, Sacchromyces cerevisae* and *Rhodotorula,* and moulds *Cephalosporium, fusarium, Aspergillus* and *Pennicillium* are the primary microorganisms that ferment ogi and improve its nutritional quality., according to microbiological and nutritional studies. The primary organisms in the fermentation that produced the lactic acid was *L. plantarum; S. cereviseae and Candida mycoderma* helped to create flavour while *Corynebacterium* converted corn starch to organic acids (Anumudu *et al.,* 2018). According to Makinwa *et al*., (2019), Significant nutrient losses occur throughout the processing processes of soaking, milling, and sifting since the majority of the cereal grains protein is found in the germ and testa, that are separated out.

2.23.1 Consumption of *Ogi*

According to Olawale and Festus, (2012), low earners ogi is a better alternative as baby food for people with low income, when preparing ogi for meals, a roughly 20-minute cooking step replaces the normal 24-48 hour soaking of corn/sorghum before milling. Ogi is primarily consumed on its own and can be supplemented as a weaning diet using legumes. Ogi has been commonly associated with kwashiorkor in infants (Omah *et al.,* 2021) and this has prompted numerous researchers to try to fortify it with plant protein sources to increase its nutritional value with the aid of plant protein sources like melon, okra, cowpea, soybean and animal protein sources. Ogi is a fermented traditional food but has been modernised and processed industrially (Ukwuru *et al.,* 2018).

2.23.2 Physical Properties of *Ogi*

Grain type, variety, milling method, particle size, soaking and fermentation times and others affect the texture of Ogi porridges. The fermentation period affects the swelling properties (thickening) of ogi. There was a corresponding decrease in viscosities after fermentation of sorghum flour samples, it has been observed that in terms of paste viscosities and consistencies, a two-day fermentation of corn is ideal for ogi production. Ogi porridge was found to demonstrate poor stability, a tendency to gel, and inconsistent consistency after a four-day fermentation of maize.

2.24 Malting

Malting is the controlled germination of cereals, which is then followed by the application of heat to stop this natural process. The grain is then subjected to additional heat to "kiln" it and give it the desired flavor and color. It is a method used to treat cereal grains where the grains are soaked in water, germinated, then promptly halted from germinating by being dried out or heated with hot air before the plant grows. It is done by germinating the seed until the rootlets are about 2cm long. The objective of malting is to activate alpha and beta amylase naturally present in the endosperm. Alpha amylase hydrolyses the starch, while beta amylase converts the hydrolyzed starch to maltose and other fermentable sugars.

The primary goals of malting are the development and activation of enzymes for the conversion of insoluble reserve nutrients. The activities of these enzymes are terminated by the drying (kilning) of the young plant so that the endosperms are not depleted fully by the embryo's respiration and growth (Fayera, 2021). Essentially, the malting process aims at converting the grains into malt that is high in vitamins and enzymes. These enzymes are necessary to transform the starches present in the grain into sugars like monosaccharides (glucose, fructose, etc.) and disaccharides (sucrose, etc). The proteins found in the grain are being degraded by the enzymes produced, such as proteases, into forms that yeast can use.

Sorghum is malted and used in Africa to make lactic or alcoholic fermented drinks. It is used in the manufacture of beer, whisky, malt vinegar and other spirits. Most of the complex sugars and carbohydrates needed to give beer its particular flavour and alcohol are provided by sorghum malt. Malt is becoming increasingly popular in food processing as a flavouring agent and a nutrient-dense ingredient that is used in the preparation of baby food and/or weaning foods. (Taylor and Duodu, 2019).

In contemporary brewering, barley is the predominant grain for malting because of certain characteristic features such as its possession of high diastatic power, its low protein content, low molecular weight particles, ease of germination and the presence of husks which facilitate filtration process. Malting of other grains like rye, oats, sorghum, and maize wheat have also been done. Wheat and rye malt, are well known to have diastatic

power comparable in amount with that of barley, but are mainly used in baking industry to furnish amylases for degrading starch to sugar, they are not used for brewing because they confer a characteristic flavour. Oat has a very low diastatic power (β-amylase despite its high content of α-amylase which is comparable to that of barley. The malting of maize and rice is not too successful due to the inferior diastatic power relative to barley malt and competition with other local uses like foodstuff and animal feeds. So far Sorghum appears to be the most suitable alternative to barley malt based on cost utilisation and production levels (Ogunyemi, 2011).

2.25 Toxicity in sorghum malt

Malting is all about germinating and allowing grain to sprout. The grain is typically soaked for 16 to 24 hours, allowing it to absorb enough moisture for germination and the development of sprouts. However, sorghum rootlets and sprouts that have germinated contain significant levels of dhurrin, a cyanogenic glucoside that, when hydrolyzed, creates a toxic substance also known as cyanide, hydrocyanic acid (HCN), prussic acid, and other names . Hence, neither humans nor animals should consume the young shoots and rootless of germinated sorghum, or their extracts, unless extremely little amounts are consumed, e.g. (when the germinated grain is used just as a source of enzymes). The processing that followed the removal of the shoots and roots lowered the HCN concentration by more than 90%, and it appears that the germination circumstances promoted fungi growth, resulting in high fungal counts in sorghum malt. While bacteria and yeasts are also present in the sorghum malt microflora, the possible mycotoxigenicity of the mould pollutants has been the main cause for concern.

2.26 Sorghum Malt

Sorghum is malted and used in Africa to make a variety of meals, primarily fermented foods like gowe and beverages like traditional beers (dolo, pito, chibuku, biu-biu, choukoutou) and non-alcoholic drinks (rum, oodo, otika) or as a component of weaning foods. About 200,000 tonnes of sorghum are malted commercially yearly in Southern Africa, where it is mostly used to make sorghum beer. Production of sorghum malt, which is used to make beverages and baby food, is a traditional practise in West Africa. (Hounbougan, 2010).

2.27 Malting Process

Malting is a process that involves steeping, germination, and kilning (Hounbougan,2010).

2.27.1 Steeping

The goal of steeping is to achieve a suitable moisture content in the grain, which is typically between 44% and 46%. Depending on the specifications of the final malt, regular steeping regimens range from 12 to 72 hours. Aeration and CO_2 extraction are typically done during steeping.

2.27.2 Germination

The grain is transferred to a jar for germination after steeping. The aerospires (new growths) that sprout from the ends of the kernels and extend up to the rear of the grain during germination grow between 3/4 and the whole length of the kernel without actually sprouting. Depending on the intended purpose, germination can be stopped at any time and typically takes 3 to 7 days. During germination, there are two main changes that take place. First, enzymes are created that break down the cell walls, which are composed of gummy materials that are insoluble in water and release the starch. Second, new enzymes that degrade proteins are created.

Other enzymes are being created to transform the starch into fermentable sugars at a later stage of brewing, if necessary. These alterations are referred to as "modifications," and they can be managed by controlling grain moisture content, processing temperature, and time (Murphy, 2010). The amount of tannins and total phenol content decreases during germination. The amount of vitamins A, B, C, and E also rises noticeably. The protein efficiency index rises from 1.5 to 1.7, and the relative nutritional value of sprouted soghum increases from 54.6 to 63%. Furthermore, flavour is synthesised into the malt during germination, giving it its distinctive flavour and colour (Anthonia, 2014).

2.27.3 Kilning

Usually, a kiln or drying technique is used to end the germination process. To make the malt stable and secure for storage, it is dried. In order to avoid destroying the early enzymes created during germination, large air flows and relatively low temperature inputs

must be used for the first 24 hours of the kilning process. After 24 hours, the temperature can be raised again for colour development. The malts are traditionally sun dried to stop germination.

2.28 Industrial Scale Malting

The process of industrial malting involves malting cereals after storage for 4-6 weeks when harvested to dry the grains, grains are soaked (steeped) at room temperature for about 36 hours and aerated during this period. The grains are drained of their steeping water, which is then spread out on the malting floor at a thickness of 20 to 22 cm.. It is sprayed with water and incubated at temperature of 10-14^{o}C. After 5-6 days, the growth of embryo is terminated by kilning for 10-114 hours at 45-55^{o}C or for 4 hours at 80-84^{o}C after which the light coloured malt is obtained. The malt is allowed to dry to a moisture content of about 12-13% to preserve the enzymes and to store before use. It is later milled, using roller mills to split open the endosperm.

2.29 Malting Conditions

The conditions required for a successful malting are as follows:

2.29.1 Steeping

Steeping is an essential step in producing high-quality malt since it's the first stage of the malting process. Grain swells by one third and absorbs water during steeping. The grain in steep builds up CO_2 and heats up as respiration increases gradually at first and later more quickly. In the soaking phase, the grain is alternately soaked in water for short bursts of time (known as underwater periods) and has water drained from it (referred to as air rest periods). This is necessary for germination efficiency to be promoted and maintained. To have a good product during subsequent malting, the water content of the grains must be increased to around 42% to 46% during the steeping process the temperature within the range of 15°C to 20°C. (Luis and Manuella, 2013)

2.29.2 Germination

The process of germination is typified by the development of the grain embryo, which is reflected in the growth of the rootlets and lengthening of the shoot (aerospire), as well as the concurrent alteration of the endosperm's contents (Luis and Manuella, 2013). The goal

of malting is to achieve the necessary modification for a specific type of malt while minimizing weight loss due to the activity of the embryo. By adjusting the grain's temperature and moisture content, one may control the rate of germination and the severity of modification. Typically, the soaked grain is kept moist and ventilated for 4 to 6 days. The germination temperature of the grain is maintained between 14°C and 20°C. (Luis and Manuella, 2013).

2.29.3 Kilning

The kiln has the features of a standard industrial drying procedure. that takes place in two different phases. The green malt is first rapidly dried,having its moisture content reduced from about 44% to 12% This drying stage is known as withering or the free drying phase.. The second drying phase, where the malt is dried from 12% to 4%, takes place in a significantly slower procedure known as the falling rate. or drop velocity phase. After the drying process has been completed, the oven's temperature can be elevated for an hour or two, before cooling to a temperature suitable for unloading and keeping. Kilning removes unfavourable odours, certain hydrolytic and other enzymes are destroyed, either fully or partially, and creates flavour and colour in the finished product in addition to drying the malt, which prevents future development and alteration. In addition, malt is kilned to create a crumbly, easy-to-grind, stable product that has a long shelf life and is easily de-rooted (Luis and Manuella, 2013).

2.30 Protein Modification in Malted Sorghum

Protein alteration in some sorghum cultivars was evaluated by Okolo *et al*., (2010) using steeping time, moisture content, and germination times. The grains underwent an 8-day germination process after being soaked for 45 hours utilising 6-hour wet and 3-hour dry cycles, the moisture content and the effect it has on protein modification was monitored during the process.. Optimal moisture contents of 37-43% and alternative values of 32-35% were found. The author reported that optimal levels of free alpha-amino nitrogen, total non-protein, occurred by day 5 of germination in all sorghum varieties. The author also reported the effects of adding 200 – 400 ppm $CaCl_2$ steeping water on carbohydrate modification of sorghum, this increased progressively as the as the levels of $CaCl_2$

increases and it ranged between 56.9 to 85.4% . The treatment reduced excessive root development and enormous malting losses during malting.

2.31 Grain Modification indices in Malting

Uvere *et al.,* (2014) established that changes in the resistance to fracture of malted sorghum grains using a hardness tester is an effective method for monitoring grains modification. He reported sorghum grains hardness decreasing progressively from 134.35N and 137.29N in red and white sorghum respectively to 76.98N and 69.14N in white and red sorghum using a hand-held tester. According to the authors, the method is simple, fast and has a good malting modification index , it was established that the malting loss as an effective index of malting. One hundred malted kilned grains were counted and the roots and shots removed from the dried material. The difference in weight of 100 kernels of unmalted grains and 100 kernels of malted grains (minus roots and shoots) as a percentage unmalted grains is expressed as malting loss. The germination energy is the percentage the of grain that will germinate under the test condition, while the germination capacity is the percentage of viable grains in the sample (potential germination). The usual method of determination is by AOAC methods. Alpha-amylase determination is another index of modification. Several methods are being used for this determination including Wohlegmuth method, where malt extracts are made to act on a standard dextrin solution, and the reaction carried to a given colour end point with iodine.

2.32 Fermentation

The process of anaerobic oxidation of carbohydrates using yeasts, bacteria, or a combination of both, to produce organic acid, ethanol, etc. as intermediate substrates with the emission of carbon dioxide is called fermentation. Fermentation is described as a process that converts raw materials into a a range of products with enhanced value by taking advantage of the phenomenon of microorganism growth and activities on the materials. This implies that understanding microbes is necessary to comprehend the fermentation process.

The term fermented foods refers to foods or beverages that have undergone controlled microbial growth and enzymatic transformation, These are foods that have undergone

desired biochemical changes as a result of exposure to microorganisms or enzymes, which result in a noticeable change in the food (Eirini *et al*., 2019,).

The fermentation process depends on the actions of bacteria to create substances capable of inhibiting the growth and survivall of undesirable microflora in foods and enable the synthesis of desired end products ike diacetyl, acetaldehyde, and vitamins. One of the oldest and most significant food processing techniques used globally today is fermentation. Food fermentation is a common practise in Ghana and the majority of African nations as part of traditional food preparation and preservation.

In general, fermented foods make up a sizeable portion of many people's fundamental diets and are also viewed as a way to add diversity to the consumption of staples like cassava, maize, and fish. A natural method of reducing the amount of material to be delivered, eliminating undesired components, improving the food's nutritional value, and improving its look is through fermentation (Ranjana *et al.,* 2020). Fermentation usually softens the texture of foods and change their chemical makeup or composition, requiring less energy to prepare and preserve (Sadat *et al.,* 2017). Moreover, it is a way to make raw materials more palatable and improve their sensory quality, leading to the preference of some meals in their fermented form , moreover, it also improves the, texture, flavor, aroma and overall shelf life of the end product. Traditionally, food fermentation in Africa has been spontaneous, making it difficult to control, be unpredictable in terms of quality of product and duration, undesirable by-products or commodities with a short shelf life, and occasional unsafe infections from pathogens. The microorganisms that cause this kind of fermentation may be contaminants or they may exist naturally on the raw materials.

The majority of items, including grains, legumes, root crops, fish, and meat, spontaneously ferment creating a vast range oof foods eaten across the continent. Substrates like corn, sorghum, and millet are fermented to develop a range of food products, including kenkey, banku, mahewu uji, injera, and koko in ways that vary by region. Fermented foods makes up of almost one-third of the world's diet (Osungbaro *et al.,* 2010). In view of the fact that indigenous fermented foods are widely consumed in Africa, a number of studies has been conducted on them. Although grains are lacking in

some nutrients, like essential amino acids, according to (Wang *et al*., 2014.), The most effective and economical way to increase the nutritional content, functional properties and sensory attributes of foods may be through fermentation. African fermented foods can be grouped as follows:

- Fermented non-alcoholic starchy foods e.g. *Ogi, gari.*
- Alcoholic beverages, such as Burukutu, kaffir beer, and palm wine.
- Fermented plant proteins, such as ogiri and iru.
- Fermented animal proteins, such as Nono and Momoni.

2.33 Natural Fermentation Process

Several raw materials used in fermentation—typically those that haven't been heated—contain both desired and related bacteria. The incubation parameters are altered to promote the desirable types' rapid growth and the related forms' (often undesirable) slower growth. As a result of the related flora's metabolism, a product produced by natural fermentation could have a flavour that consumers find appealing. It is challenging to create a product with consistent qualities over time, as the inherent microflora in the raw materials may differ. In addition, there is a high likelihood of product failure due to the growth of unwanted plants and foodborne illnesses from the pathogens

2.34 Microorganism Isolated during Fermentation

The fermentation processes involve some microorganisms. They are:

2.34.1 Yeast

There are 1,500 species of yeast, which are eukaryotic organisms categorised as members of the kingdom Fungi and this number is estimated as only1% of all yeast species. A few species reproduce through binary fission, but the bulk do so asexually through budding. Whereas The majority of moulds possess pseudohyphae, which are a network of connected budding cells. Certain yeast-forming species can grow into multicellular organisms. *Saccharomyces cerevisiae* is the commonest yeast species observed, and it is primarily responsible for three types of locally produced fermented products: fermented milk, non-alcoholic starchy meals, and alcoholic beverages.

Saccharomyces cerevisiae's functions are primarily related to the production of alcoholic and other aroma compounds, but they also include stimulating lactic acid bacteria, enhancing nutritional value, producing tissue-degrading enzymes, inhibiting undesirable microorganisms, and improving nutritional value. The commonest yeast found in fermented fruit and vegetable-based beverages and foods is *Saccharomyces cerevisiae*. All strains ferment glucose, and many also ferment sugars found in plants, including maltose, sucrose, and raffinose but they cannot ferment lactose, an animal sugar. *S. cerevisiae* is used in baking and in fermenting alcoholic beverages for centuries.

Yeast has been reported in the fermentation of numerous varieties of native fermented foods and drinks from Africa. They serve a potential role in traditional African fermented meals and drinks. Yeast-rich fermented sorghum smells better than yeast-light bread. The importance of yeast and moulds for the fermentation of corn dough to make Kenkey in Ghana have also been reported, also reiterated the importance of yeasts in maize and sorghum fermentation for the production of ogi. Different strains of yeast belonging to 8 genera were identified from *pito* brewed in Ghana; they include *Schizosaccharomyces pombe, Saccharomyces cerevisiae, Kluvyeromyces africanu, Torulaspora delbrueckii, Kloeckera apiculata, Hansenula anomala,* and *Candida tropicalis. Saccharomyces cerevisiae* and *S. chavelieri was isolated* among the prevalent organisms in the fermentation of *ogi.*

2.34.2 Lactic Acid Bacteria (LAB)

Lactic acid bacteria (LAB) are a heterogeneous family of microorganisms capable of fermenting a wide variety of nutrients. LAB are Gram-positive, nonspore forming rods or cocci; Majority of them are aerotolerant anaerobes and are catalase and oxidase negative because they lack cytochromes and porphyrins. The two main types of lactic acid fermentation occur in lactic acid producing bacteria. Homofermenters, the fermentation of glucose can almost exclusively produce lactate as a single product. Heterofermenters convert glucose to lactate, ethanol/acetate and carbon dioxide in nearly equimolar amounts. The capacity of heterofermenters to generate carbon dioxide under conditions containing glucose allows for an easy distinction between them and homofermenters. LAB

are mostly mesophilic, however, some of them can thrive at refrigeration temperature (4 °C) and up to 45 °C. They prefer a pH between 4.0 and 4.5, however certain strains can survive and thrive at pH levels as high as 9.0 or as low as 3.2.

Generally, the LAB contains cocci (Aerococcus Pediococcus, Leuconostoc, Streptococcus, Lactococcus, Enterococcus Weissella), Tetragenococcus, Vagococcus and Oenococcus) and rods (Lactobacillus, Carnobacterium).Occasionally, Bifidobacterium, Microbacterium and Propionibacterium are linked to the LAB; however they are not part of the LAB. They are found everywhere and are able to thrive on meals that are rich in accessible carbohydrates, minerals, vitamins, and amino acids but low in oxygen. The LAB group contains microorganisms that vary with regard to temperature of growth, heat resistance, nutrient requirements, water activity needed for growth, products produced, etc. LAB is typically distinguished by its microscopic appearance, catalase test, carbohydrate fermentation, homo- or hetero-glucose fermentation, and requirement for nutritionally complex media. LAB have the ability to produce probiotics.

2.35 Bacteriocins production by Lactic Acid Bacteria (LAB)

Lactic acid bacteria have a significant antagonistic effect on a variety of food-contaminating microorganisms as a result of synthesis of bacteriocins, diacetyl, organic acids, hydrogen peroxide, and other inhibitory enzymes. Bacteriocins are proteinaceous antimicrobials with a narrower spectrum of activity compared to antibiotics. They are secreted by some bacteria, which are thus adapted to compete with other microorganisms growing in the same medium. A range of distinct bacteriocins are produced by numerous LAB found in many fermented and unfermented foods (Saeed *et al.,* 2014.). LAB bacteriocins are regarded as natural biopreservatives as they are believed to be broken down by the proteases of the digestive tract. Since they can stop the growth of numerous bacteria that cause food spoilage and harmful bacteria like *Clostridium botulinum*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus*, bacteriocins generated by LAB are of tremendous interest as natural preservatives in the food fermentation industry (Nasrollahzadeh *et al*., 2022). The bye products of fermentation for example lactic acid and bacteriocins by microorganisms in the fermentation substrates, make them safe for consumption. The probiotic bacteria isolated from maize and sorghum ogi were

Lactobacillus plantarum, Lactobacillus fermentum, and Saccharomyces cerevisiae. After the first day of steeping, some microorganisms like *Clostridium perfiringes, Staphylococcus aureus, Corynebaterium* sp, *Aspergillus niger Rhizopus stolonifer,* and *Penicillium* sp, were isolated; however, after 48 and 72 hours of steeping, they were no longer isolated.

There are various bacteriocins produced by LAB which have been identified and characterized (Saeed *et al.,* 2014), A bacteriocin called nisin, which is produced by particular strains of Lactococcus lactis and effective against a variety of gram-positive bacteria, is an example of one of them. Currently, this bacteriocin is the only one that is commercially available and commercialised and has been exhaustively researched.This bacteriocin has been used to prevent wine and beer from spoilage due to undesired LABs. Several lactobacilli species create bacteriocins with distinctly diverse inhibitory spectra.; the majority inhibit closely related gram-positive bacteria and other *lactobacilli* while others are active against a wide range of gram-positive or gram-negative bacteria.

Bacteriocins are currently receiving more attention as natural food preservatives, and there is significant evidence that these antimicrobials may be crucial to wine fermentation. In order to use bacteriocins as food preservatives, one of two methods can be used: either the bacteriocin is added as an additive to the food, or the bacteriocinogenic strain is used as a starter culture and/or a protective culture. So, from a marketing point of view, an affordable technology for mass production of cultures with high concentrations of viable bacteriocin producers in a format appropriate for product applications is greatly desired

2.36 Characteristics of African Food Fermentation

Natural fermentation because of its simplicity and the fact that it is inexpensive is a popular means of traditional processing and preservation of local food stuff for human consumption. The fermentation of African foods is natural in most cases, and so the control over the process is often limited; virtually in all cases epiphytic bacteria and yeasts are the fermenting organisms. In natural fermentation of foods there is microbial succession during fermentation; the reasons are; the manufacturing process, the water used for soaking, and the raw material's surface microorganisms, the environment in which

processing occurs, and the microflora that the local processor harbours. Ranjana *et al.*, (2020) worked on the main bacteria that were isolated from fufu and these are *Streptococcus faecalis Lactobacillus fermentum,, B. polymyxa, Coliforms, Bacillus subtilis, Lactobacillus brevis* and *Saccharomyces cerevisae*.

In West Africa, foods are fermented mainly by solid state fermentation where all the fermenting materials are solids suspended in water which is either left to stand or stirred, or alternatively, the liquid medium is circulated through the solid placed in columns.. The shortcomings of traditional methods of fermentation are that in some instances the desired product cannot be obtained by solid substrate fermentation, it is mixed with other undesirable products or it is produced in poor yields,

2.37 Benefits of fermentation in African traditional Food Processing

The benefits of fermentation in African traditional food processing inclue:

2.37.1 Simplicity and low cost

The traditional fermentation techniques are very simple and rely on locally available material. Sophisticated expensive equipments that require specialized training for operation and maintenance are not necessary.

2.37.2 Means of food Preservation

Majority of African fermented foods rely on lactic acid bacteria as fermenting organisms. After fermentation, the foods become acidic and most spoilage and pathogenic microorganisms cannot thrive or even survive the condition (Mani, 2018; Sanjana and Jastin, 2019).

2.37.3 Improvement in Flavour and texture of fermented Foods

Many fermented foods such as *garri, ogi* etc. develop a desirable sour taste as a result of fermentation. *Dawadawa* is valued as a condiment because of the flavor it impacts.

2.37.4 Detoxification

Many plant foods contain naturally occurring nutritional stress factors and other toxicants (e.g. locust bean, soya bean, sorghum and cassava) which are present naturally in them or due to microbial contamination or other factors. Traditional fermentation reduces the level of some of these toxicants (Tadele, 2015).

2.37.5 Removal of anti-nutritients

In some healthy foods, there may be high concentrations of unfavourable substances including phytic acid, trypsin inhibitors, flatulence causes, and lectins. Trypsin inhibitors and phytic acid, which can also bind minerals, both prevent proper digestion by binding digestive enzymes.reducing bioavailahility. Fermentation helps in reducing these substances occurring naturally in food products (Ranjana *et al*., 2020).

2.37.6 Conversion of otherwise inedible plant items to edible foods

Conversion of otherwise inedible plant items to edible foods is effected by fermentation. Without fermentation such plant items as African locust bean will not be edible; they become edible by significant hydrolysis of their digestible compound by micro-organisms and by the elimination of nutrients stress factors. (Hassan *et al.*,- 2013).

2.37.7 Improvement of Nutritional Value

The improvement in nutritional value through fermentation is achieved by increasing protein digestibility through hydrolysis of protein to amino acid; conversion of oligosaccharide e.g. raffinose starchyose and verbasiose to simple sugars as in the production of *tempeh* from soya beans. This also reduces the problem of flatulence associated with legumes such as cowpea and soya beans and increase the nutrient level especially B vitamins as a result of microbial synthesis as in *dawadawa* preparation (Elaine and Danilo, 2012).

2.37.8 Improved digestibility

Some enzymes, including cellulases, which are not synthesised by humans, are found in microorganisms. Cellulose is hydrolyzed by microbial cellulases into sugars that are easily absorbed by people. In addition, pectinases soften the texture of food and release sugars for digestion. When compared to non-fermented foods, fermented foods are frequently simpler to digest (Hassani *et al*., 2014). Weaning foods fermented with lactic acid are developed to improve the food's safety and digestibility. The infant cannot physically absorb enough energy to meet its high demands due to the nature of starchy gruels and the infant's small stomach capacity. By making the cereal acidic as a result of lactic acid fermentation, starch is hydrolyzed into short chains of glucose and dextrose., this lowers

the viscosity and raises the energy density of the porridge (Msheliza *et al.,* 2018; Adebayo-Oyetoro *et al*., 2017).

2.38 Bio-therapeutic and Prophylactic benefits of lactic acid fermented foods

Yogurt and other lactic acid-fermented foods like ogi and kunu are excellent sources of lactic acid bacteria (probiotics). According to some reports, probiotics may be helpful in the treatment and prevention of a number of diseases, including gastrointestinal conditions like diarrhoea and dysentery. Probiotics can also be used to treat metabolic problems such as lactose intolerance, sucrose intolerance, and maltase deficiencies. They have anticarcinogenic properties and immune system modulation. They also control hypercholesterolemia (David and Famurewa, 2010).

2.39 Probiotics

Probiotics are defined as non-pathogenic living organisms that, if given to a host in adequate proportions, confers a health benefit To maintain a healthy balance of helpful germs in the gut flora, regular ingestion of foods containing probiotic microorganisms is essential. When ingested, they enhance or restore the intestinal flora, which enhances health., the gut flora is very crucial in the upkeep of human health (Miriam *et al*., 2012; Moha *et al.,*, 2015. mmad *et al.,* 2015). Probiotic organisms improve the Gastro-Intestinal Tract (GIT), generate vitamins, and enhance the availability of vitamins to the human host Probiotics are frequently utilised in therapeutic settings throughout many nations, and consumers can frequently purchase them with or without a prescription. (Wilkins and Sequoia, 2017; Deshpande *et al*., 2018.). Probiotics are usually derived from dairy products or from healthy human gut flora, primarily from *Bifidobacterium* or *Lactobacillus* species. The major probiotic groups are *Lactobacillus, Lactococcus, Pediococcus, Bifidobacterium, Streptococcus Enterococcus, Bacillus* and yeasts strains that are often utilized. Some species of probiotic organisms are as highlighted in Table 2.1 (Bayane *et al*., (2010); Miriam *et al.,* (2012), Adel and Sari, (2017).

Foods, medications, and dietary supplements are just a few of the various products that might incorporate probiotics into the mix. In addition to acting as catalysts for food fermentation, lactic acid bacteria, such as Lactobacillus species, may also confer health

benefits (Francisco *et al.,* 2011)**.** Fermented dairy products, other fermented foods, and foods that have been supplemented with probiotics all contain live probiotic microorganisms. Probiotics are typically found in a variety of products, including food, infant formula, nutritional supplements, and medical devices (Bermúdez-Brito *et al.,* 2012).

2.39.1 Nutritional and health benefits of probiotics

Much emphasis is recently being laid on the health promoting benefits of food rather than just its nutritional advantage with respect to provision of nutrients and prevention of nutritional deficiencies This has given rise to functional foods that will improve consumers' overall health. the following are noteworthy among the primary effects of probiotics at the gastrointestinal level, Immunomodulation, pathogen defence, gut microbiota balance and restoration, and intestinal barrier integrity maintenance. Probiotics are a type of living and beneficial microorganisms present in the digestive tract of humans and rodents, and also naturally found in fermented dairy products. They have shown a high therapeutic potential treatments for a range of illnesses, primarily gastrointestinal ones (such as necrotizing enterocolitis, antibiotic-associated diarrhoea, ulcerative colitis, irritable bowel syndrome, functional gastrointestinal disorders, and acute infectious diarrhoea), but also extra-intestinal ones like hepatic encephalopathy (Wilkins and Sequoia, 2017; Draper *et al*., 2017) *Species of Lactobacillus* and *Bifidobacterium* resides naturally reside in the human GIT and are believed to be essential to preserving human health. (Kato *et al*., 2017).

Table 2.1: Some species of microorganisms utilized as probiotics

Probiotic species	Group of Microorganisms
Lactobacillus bulgaricus, Lactobacillus acidophilus Lactobacillus fermentum,, Lactobacillus casei, Lactobacillus lactis, Lactobacillus paracasei, *L. rhamnosus, Lactobacillus plantarum,, L. delbrueckii subsp. bulgaricus, L. brevis,* *L. johnsonii, Lactobacillus salivarius, Lactobacillus kefi*	Lactic acid producing bacteria
Enterococcus faecalis, Enterococcus faecium, Esherichia coli *Streptococcus thermophiles, Propinobacterium*	Non lactic acid producing bacteria
Sacchromyces boulardii	Nonpathogenic yeast
Lactobacillus, Leuconostoc, Streptococcus, Lactococcus lactis subsp. Lactis Bifidobacterium, Pediococcus, Propionibacterium, Enterococcus, Enterococcus durans, , Bacillus, Bacillus coagulans, Saccharomyces cerevisiae, Bacillus subtilis, Candida pintolopesii, A. oryzae, Aspergillus niger, B. cereus Bacillus lichenformis, var. toyoi, B. clausii, B. coagulans, B. laterosporus, B. pumilus, B.racemilacticus, Streptococcusthermophiles	Non spore forming

Source: Bayane *et al.*, (2010), Miriam *et al.*, (2012), Adel and Sari, (2017).

The physiological actions of the gut microbiota are therefore thought to be strengthened at the intestinal level by the oral administration of probiotics. In the gut, probiotics battle with pathogens for nutrients and other growth factors as well as adhesion and colonisation, creating antimicrobial chemicals and lowering pH (through the formation of lactic acid) (Kim *et al*., 2017) and bind to gram-negative bacteria to stop the growth of harmful microorganisms (Wilkins and Sequoia, 2017; Bermúdez-Brito *et al.,* 2012.).

Probiotics can be useful in the treatment of constipation, reduce lactose intolerance and protect the host from intestinal infections among other health benefits. the prevention of vaginal and urinary tract infections; the anticarcinogenic impact the anticholesterolaemic benefits, the nutritional synthesis and bioavailability and the immunostimulatory effects . Other potential health benefits of consuming probiotic include Improving gut health by regulating the microbiota, promoting immune system growth and function, synthesising and improving nutrient bioavailability, reducing the symptoms of lactose intolerance and the risk of some illnesses (Nagpal *et al.,* 2011). Probiotics have mostly been used in clinical settings for the treatment and prevention of gut infections and ailments. Modulation of an imbalanced native microbiota serves as a basis for probiotic therapy, as variations in the gut microbiota have been associated with higher risk of certain diseases.

Probiotics have some antibacterial effects as well, and the intestinal microbiota is very complex. and competitive, therefore to introduce a new organism is difficult except the organism can produce substances that inhibits the growth of existing organism which confer its competitive advantage, this enables probiotics to be established in the intestinal tract because they are able to eliminate competitors (Ravinder *et al.,* 2012). Probiotic organisms also produce citric and acids hippuric in addition to acetic and lactic acids; lactic acid bacteria also generate hydrogen peroxide, diacetyl, and bacteriocin as antibacterial compounds. These inhibitors generate an environment that is unfavourable to food borne pathogens and organisms that cause deterioration.

2.39.2 Application of probiotics

As a result of an increase in knowledge about functional foods there are developments of foods that aside nutrition possesses some health benefits. Consumer interest in functional foods, notably those containing probiotics, has recently surged. Some health benefits of probiotics in commercial foods have been asserted. Therefore industries concentrate on developing new probiotic health products and using probiotics in a variety of food-related applications. (Danfeng *et al*., 2012). Owing to both their intrinsic characteristics and the fact that they are mostly kept at cold temperatures, milk and dairy products are a good vehicle of probiotic strains. They are found in commercial dairy products, such as cheese, fresh and sour milk, etc. As milk products provide an enabling environment for the development and viability of probiotic organisms, they are crucial in the delivery of these organisms to humans.

Yogurt is one of the traditional supply of probiotics due to its intrinsic properties. It is made with a culture of *L. delbrueckii subsp. bulgaricus and Streptococcus salivarius subsp. thermophilus bacteria* and is renowned for its nutritional content and health advantages. There is a need for non-dairy based probiotic food vehicles in order to create variety and add value to food products, to reduce cost of probiotic food products and to serve as alternatives to those who are lactose intolerant and the vegetarians. A highly intriguing area of research is how probiotics, which are frequently included in meals as a part of the fermentation process, might affect host immunity when combined with fermented African foods and beverages like Kunnu and Ogi.

2.39.3 Cereal-based probiotic products

Cereals are an excellent substrate for the proliferation of probiotic strains and can also serve as prebiotics because the cereal matrix contains non-digestible fibre components (Salovaara *and Ganzle* 2011). The production of novel functional foods that integrate the benefits of grains with microbes that promote health is a challenge. Lactic fermentation is required for many products made from cereal, frequently in combination with yeast or moulds. Several fermented grain products have been developed and probiotic microorganisms have been discovered to be present in traditional fermented grain

foods. (Danfeng, 2012). Charalampopoulos *et al*., (2012) experimented with different types of cereals to establish the primary factors to be taken into consideration in the production of probiotic microorganisms as: the features of cereal grains, how they are processed, how the substrate is made, how productive and viable the starter culture is, how stable the probiotic organisms are during storage, how the finished product smells and tastes, and the quantity of nutrients in it.. Oat is frequently utilised in research on cereals that have been fermented by probiotic bacteria. Yosa is a snack meal produced from oat bran custard cooked and fermented with LAB and Bifidobacteria.. The texture and taste is comparable to yoghurt but it is totally free of dairy, it has a low fat content, lactose-free and has beta-glucan and is suitable for vegetarians. Yosa is regarded as a nutritious food because it contains oat fibre and probiotic LAB, which combines the actions of beta-glucan to lower cholesterol and LAB to maintain and enhance the consumer's gut flora balance (Danfeng, 2012).

2.40 Use of starter cultures in fermentation processes

A starter culture is a preparation that has numerous different types of microorganisms in it. that can be employed to speed up the fermentation process and usually results in a higher-quality output. Spontaneous fermentations are usually the consequence of competition between different microorganisms; with the strains that are more suited and have the fastest growth rate dominating at different stages of the process. Most processors develop pure starter cultures to optimize fermentation processes to ensure the production of safe fermented products with consistent taste, texture and extended shelf life (Holzapfel, 2002). Manufacturing processes are hastened using starter cultures due to reduction in pH and increment in Total Titratable Acidity (TTA), resulting in cost savings for the producer. Most of the starter cultures are natural isolates of beneficial microorganisms usually found in substrates (Holzapfel, 2002).

Enhanced sensory qualities (taste, texture, aroma, appearance, consistency), increased inhibition or removal of foodborne pathogenic organisms, and increased shelf life and reduction in preparation requirements (shorter cook times and lower energy consumption) are all potential benefits of using starter cultures for fermentation The nutritional value of fermented products made with starter culture could be improved through the removal of

anti-nutritional factors, enhanced protein digestion, increased micronutrient bioavailability, and biological enhancement, such as through the production of proteins, vitamins, and essential amino acids. Elimination of hazardous constituents such as linamarin in cassava, as well as possible degradation of mycotoxins, could lead to increased toxicological safety. Indeed, probiotic properties may develop, contributing to an improvement in overall well-being and health (Sornyotha *et al*., 2010; Ahaotu *et al.*, 2011; Umeh and Odibo, 2014).

While natural food fermentations may be linked to many of the desirable characteristics mentioned above, the degree and quality of these fermentations are unpredictable and uncontrollable. Pure cultures obtained from naturally fermented foods have shown that different strains, even within the same species, have different metabolic activities Single or mixed cultures of preferred strains of microorganisms having the defined characteristics that help produce the desired product can be utilized as starter cultures. In food industries, a diverse variety of microorganisms have been utilised as starter cultures, and many more are being explored to see if they may be adopted as well. The utilisation of well-defined starter cultures with complimentary physiological and metabolic features has a significant impact on the consistent quality, safety and acceptance of a fermented product like ogi. However, it appears that employing a single strain to produce a food with a diverse range of organoleptic properties is too restricted.

2.41 Growth and harvest of microbial culture

Microbial cultures are usually grown in liquid medium (broth) containing essential nutrients at optimal concentrations. Thereafter, the cells are separated by centrifugation and subsequently subjected to Deoxyribonucleic Acid (DNA) isolation.

2.42 Isolation of deoxyribonucleic acid (DNA)

Deoxyribonucleic Acid (DNA) is an intricate nucleic acid that includes the genetic code, which contains the instructions for the formation and operation of all known living things, beside some viruses. The genetic information is carried by genes, which are DNA segments. Transcription convert DNA to RNA, used as template for protein synthesis. The method of obtaining DNA in its purest form from a cell is known as DNA

isolation. Purified DNA is obtained from other biological components such as proteins, lipids and RNA during, the extraction process. Bones, saliva, tissues, sperm, blood, hair, nails, feces, shed feathers, urine, bacteria, epithelial cells and plants are among the nucleated cells from which DNA can be extracted.

Proteins, lipids, polysaccharides, and other components present during DNA preparation can compromise DNA quality, which might interfere with DNA analysis procedures. Sample size, sample freshness and the biochemical composition of the cells from which DNA is obtained must all be considered when developing extraction methods to reliably purify DNA from a wide range of sources. The isolation process must be modified according to the size of the sample. The extraction procedure is affected by the sample's freshness. Depending on the biochemical composition of the donor cells, several extraction techniques are used. In a cell extract of bacteria, for example, the principal biological components detected are protein, DNA and RNA. As a result, pure DNA can be obtained by phenol extraction or protease treatment followed by RNA separation using ribonuclease.

2.43 The Principles of DNA Isolation

Isolating DNA typically starts with the disintegration of cells or tissues, which breaks protein structures and enables nucleic acids to depart the nucleus. This is commonly done in a hydrolysis solution containing essential chemicals such as a buffer (Tris at pH 8) and a salt (sodium chloride) that helps to neutralise the negative charges on the DNA through precipitation and allowing the molecules to bind together. A metal chelator, such as Ethylene Diamine Tetraacetic Acid (EDTA), that sequesters to the divalent metal ions needed for nuclease activity and hence inhibits it. A detergent that destroys the cell membrane and nuclear envelope, causing the cells to break open and release their DNA, commonly Cetyl Trimethyl Ammonium Bromide (CTAB) or sodium dodecyl sulphate (SDS). Histone proteins are still tightly bound to DNA. Proteinase K (a serine protease) is a known DNA extraction enzyme that hydrolyzes histone proteins and releases DNA, causing cell collapse and membrane breakdown.

2.44 Polymerase Chain Reaction

The Polymerase Chain Reaction (PCR) is one of the technologies that has affected many areas of our daily life as well as the scientific community. It is a fast, cheap, and simple method for copying particular DNA segments from very small amounts of starting DNA material, even when the original DNA is not of good quality. It does not always necessitate the use of hazardous chemicals or radioisotopes.

2.45 Working Principle of Polymerase Chain Reaction

It is a chain reaction, as the name suggests, in which a small amount of the target DNA area is selected and used as a pattern for the primers that begins the reaction. A DNA molecule is used to make two copies, four copies, eight copies, etc. Polymerases are responsible for this continuous duplication, these are enzymes that can connect individual building blocks of DNA into long strands of molecules. Nucleotides, which are DNA building blocks consisting of the four bases adenine (A), thymine (T), cytosine (C) and guanine (G), are required by polymerases. A primer, which is a little piece of DNA to which the basic components are bonded, is also required, a longer DNA molecule as well to serve as a model for creating the new strand. The enzymes will synthesize exact clones of the templates if these three elements are provided.

2.46 Limitation in Polymerase Chain Reaction

Excess salts such as guanidinium, NaCl and KCl, ionic detergents such as sarkosyl, sodium deoxycholate, and SDS, and alcohols such as isopropanol, ethanol, and phenol can reduce PCR performance. These PCR inhibitors usually affect amplification by interfering with the DNA polymerase or interacting with the DNA. Polyphenolic compounds found in impure preparations (such as melanin and humic acid) can alsobind Mg^{2+} and impede PCR. High-quality, inhibitor-free DNA is needed to succeed with qPCR, DNA ligation/cloning, sequencing, arrays, and other techniques.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials Used in the Study

Red sorghum variety was obtained from a local farmer in Iwo and was identified at the Department of Crop Production, Bowen University, Iwo. Palm wine was obtained from Shekinah Farms, Iwo and freshly laboratory brewed *burukutu* was prepared at Bowen University food processing laboratory. All reagents used are of analytical grade and food standard.

3.2 Malting of Sorghum Grains

This was carried out as described by Adeola, (2002) with some modifications (figure 3.1); samples were sorted to remove foreign matters and broken kernels; they were then washed thoroughly to remove impurities and were weighed. The cleaned grains were steeped in water (1:2 w/v sorghum: water) at ambient temperature for 12hours, the water used for steeping was changed at 1hour interval. The grains were then germinated in an improvised germinating chamber for 72hrs Prior to germination, the germinating chamber was washed with 0.1% sodium hypochlorite to prevent fungal contamination of the grains. The green malt was kilned at 48^{o}C for 24 hours and culm was then removed from the germinated grains and packaged for further processing.

3.3 Preparation of Ground Sorghum Samples (flour)

Sorghum grains (unmalted and malted) were sorted, cleaned to get rid of chaff, impurities and broken kernels. The cleaned grains were then weighed and milled into a fine granules. The milled sorghum samples were thereafter packaged and stored for further analysis (figure 3.2) Adeola, (2002).

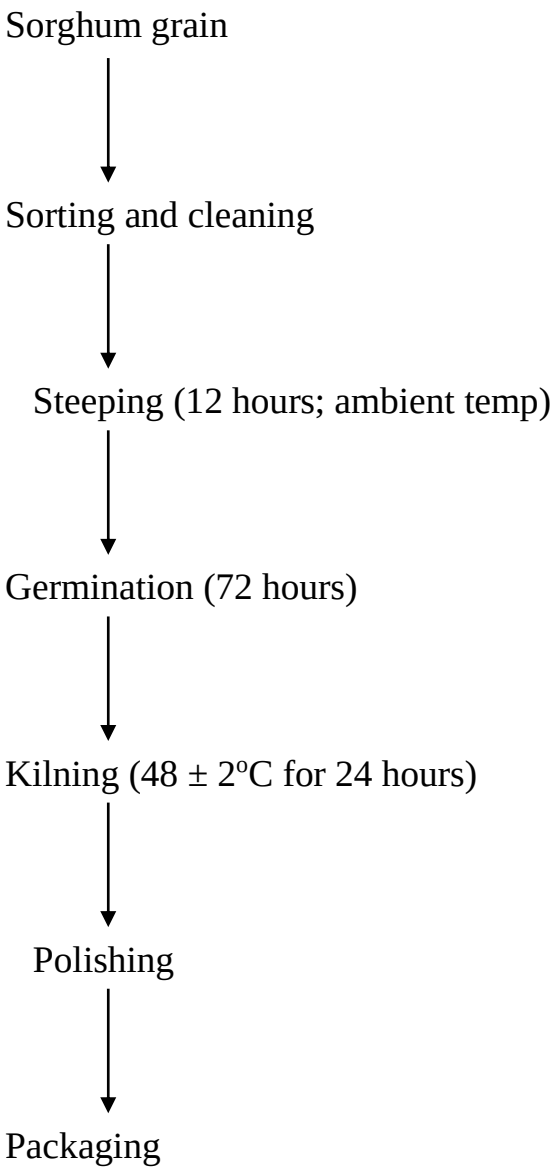

Figure. 3.1: Sorghum malting process
Source: (Adeola, 2002).

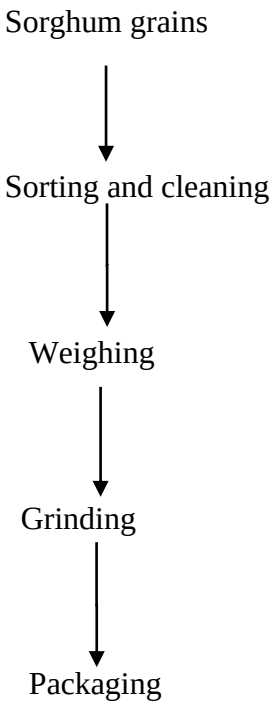

Fig. 3.2: Production of ground sorghum

Adeola, (2002)

3.4 Production of *ogi* from malted and unmalted sorghum grains by conventional wet milling method.

Ogi was processed using malted and unmalted sorghum grains using the conventional wet milling process reported by Adelekan and Oyewole ,(2010) with some modifications.
Cleaned sorghum grains were soaked in distilled water and soaked for 72 hours at room temperature. The grains were rinsed after being removed from the steep water with copious amount of distilled water, then the kernels were wet milled.

The slurry was then split into two pieces after grinding; one part was not sieved and the other part was sieved with a fine mesh sieve (500µm aperture size) to seperate the shafts. Slurries were allowed to ferment and sediment for 24hours, 48hours and 72hours at room temperature (souring). The acidifying water was removed by decanting, and the ogi slurry that resulted was poured in a muslin cloth and drained to eliminate extra water, leaving the semi-moist ogi cake, that was dried at 45°C for 12 hours to give a dry ogi cake that was later ground into fine powderr (Agrico model 2A, New Delhi, India). A 24hour, 48hour, and 72hour fermented, malted and unmalted; sieved and unsieved dry *ogi* powder was thereafter obtained. The samples were well packaged and labeled for further analysis (Figure 3.3).

3.5 Analysis of *ogi* produced by conventional wet milling method

Proximate, mineral, antinutrient, functional, pasting, invitro protein digestibility and sensory analysis of the ogi were carried out as follows:

3.5.1 Proximate composition

Proximate composition of the ogi samples was done as described below

3.5.1.1 Moisture Content

The sample's moisture content was calculated gravimetrically by drying the sample in an oven at 105°C (Memmer, UF 55). The result was expressed as percent moisture loss (wet basis) (AOAC, 2022). Approximately 2 g of *Og*i powder (W) was weighed (Ohaus PA214) in petri dishes with known weights M0), after that, it was air dried for 4 hours at 105 °C. (Memmer, UF 55). The material was weighed after cooling in a dessicator (Ohaus

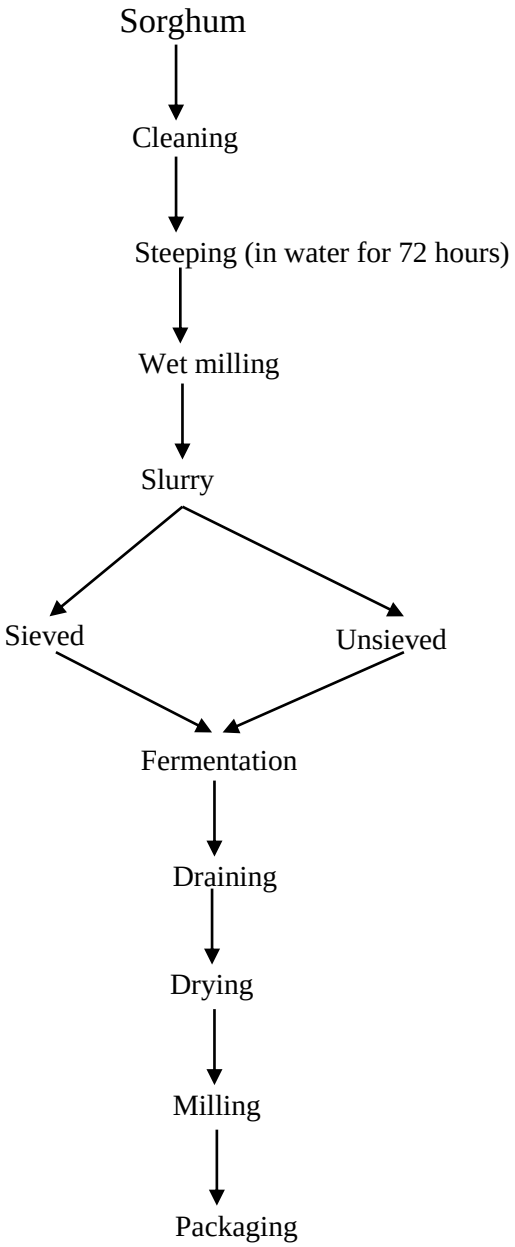

Fig 3.3: Production of *ogi* powder
Source: (Adelekan and Oyewole, 2010).

PA214) (M1). The sample was dried again and reweighed to obtain a constant weight . The moisture content (wet basis) was calculated as:

$$\text{Moisture Content (\%)} = \frac{(W)-(M_1-M_0)}{W} X\ 100 \qquad \ldots \qquad \text{Equation 3.1}$$

3.5.1.2 Crude Fat

Diethyl ether extraction was used to determine the fat content using a soxhlet extractor (AOAC, 2022). The extraction flask was washed with petroleum ether, dried in an oven and weighed after cooling in the desiccator. Approximately 2g of the material were weighed into extraction thimbles, which were then put into extraction flasks of predetermined weight... Extraction was done for 6 hours using diethyl ether. After extraction was complete, the solvent was recovered and the remaining fat was oven dried for 1hr at 110^{o}C to remove traces of diethyl ether. The flask and its contents were weighed after cooling in the desiccators. The weight of the extracted fat was expressed as a percentage of the sample.

$$\text{\% crude fat} = \frac{Weightoffatinsample\ (g)}{Weightofsample\ (g)} \times 100 \qquad \text{..... Equation 3.2}$$

3.5.1.3 Crude Protein

Micro Kjeldhal method was used in determining the nitrogen content (AOAC, 2010). The sample (1g) was weighed into Kjeldhal digestion flask and two Kjeldhal catalyst tablet was added to it. After adding 12 mL of concentrated H_2SO_4, the mixture was digested until a clear solution was obtained.The digest was cooled and washed with 50mL distilled water. 20 mL of the digest were transferred into the kjedahl apparatus' neutralisation and distillation chamber (Kjeltec 2100). where it was neutralized and distilled with 50mL of 40% W/v NaOH for about five minutes; the distillate was then titrated with two drops of indicator using standardised 0.1N HCl. A blank titration was done and the % protein content was estimated as

$$\text{\% Kjedahl Nitrogen} = \frac{(Vs - Vb)\ \times C\ \times 1.4007}{W} \qquad \text{Equation …3.3}$$

$$\text{\% Crude Protein } = \text{\% Kjedahl Nitrogen } \times 6.25 \qquad \text{Equation ... 3.4}$$

Where, Vs = titre value, W = Weight of sample (grams), Vb = titre value of the blank determination, C = Concentration of acid (0.1N HCl),

3.5.1.4 Crude Fibre

Two grams (2g) of the sample was weighed into a fibre flask and 150mL of 1.25% H_2S0_4 was added. The mixture was refluxed on a heating mantle for 30 minutes. A fibre sieve cloth was used to screen the heated mixture. secured with an elastic band. The residue was then rinsed with distilled water to ensure that all the filterate was washed off the residue. The residue was gently transferred to the fibre flask containing 200mL of 1.25% NaOHThis mixture was refluxed for 30 minutes using a heating mantle. The mixture was then filtered, and the residual particles were dried for 2 hours at 120°C. The clay fibre was scrapped into a crucible of known weight reweighed. The pre weighed crucible was placed in the furnace set at 550^{o}C for 3 hours. The crucible was cooled in the desiccator, weighed and the percentage fibre was calculated (AOAC, 2022).

3.5.1.5 Ash content

Two grams (2g) of the sample was measured into pre-weighed crucibles. The samples were pre- ashed over a low flame and was later moved to a muffle furnace preset at 550^{o}C for 5 hours. The crucibles were cooled in a dessicator and weighed. Percentage ash was calculated from weight differences (AOAC, 2022).

3.5.1.6 Carbohydrate

The difference in sum of other composition was recorded as total carbohydrate.

3.5.2 Mineral Analyses

One gram (1g) of the sample was digested with 4mL 60% perchloric acid, 25mL conc HNO_3 and 2mL conc H_2SO_4 in a fume cupboard. After allowing the mixture to cool, the volume was brought up to 100 mL using distilled water. Na and K were determined by flame photometry while Ca, Mg, Mn, Zn and Fe were determined using atomic absorption spectrophotometry (AOAC, 2022).

3.5.3 Antinutrients

The antinutrients analysed are Oxalate, Tanin and phytate as follows

3.5.3.1 Oxalate

High Performance Liquid Chromatography was used to evaluate the oxalate content of the sorghum ogi samples, as described by Bhatia *et al.* (2016). Column 18 (C-18) of the

instrument was used, and methanol: 0.1% TrifluoroAcetic Acid in water (40: 60, v/v) as the mobile phase. Two grams of sample to be evaluated was dissolved in 20 mL of 6 N HCl, which was then allowed to stand for 1 hour. This was passed through a filter paper of Whatman No 2, and the pH of the filtrate checked and adjusted to pH 6 with dilute NaOH solution. Distilled water was used to make up to the mark of 50 mL, and the solution was refrigerated to keep at low temperature till the solution was read with the HighPerformance Liquid Chromatography machine (Agilent 1200 infinity series).

The sample solution (20 µg) was injected, using rheodyne injector at a flow rate of 1.0 mL/min and wavelength of analysis, 254 nm. The oxalate was resolved at 1.47 min with good retention parameters under the above chromatographic conditions. The peak area of the oxalate was recorded for each sample by software, after that of a standard solution of oxalic acid has been recorded. The oxalate content was calculated by equation 3.5

$$\text{Oxalate content (mg/100g)} = \frac{(PA\ of\ sample) \times C \times \text{V}}{PA\ of\ standard \times 10 \times \text{W}} \qquad \ldots.3.5$$

Where; P/A = Peak Area, C = Concentration of standard (100 ppm), V = total extract volume, W = weight of the sample

3.5.3.2 Tannin

Dried, finely ground sample (200mg) was extracted with 10mL of methanol in capped rotating test tubes with constant shaking in a Gallenkamp flask shaker. The tubes were centrifuged for 10 minutes at 6000rpm. Tannin content was determined using vanillin hydrochloric acid method. 5mL vanillin reagent (50:50 mixture of 1% vanillin and 8% concentrated HCl in methanol) prepared freshly was poured to 1mL aliquot of the sample. Also, 5mL 4% concentrated HCl in methanol was added to another aliquot (1mL blank) at 1 minute interval. Tubes and contents were left in water bath at 30^{o}C for 20 minutes, the absorbance was then read at 500nm in a spectrophotometer (spectronic 20) (AOAC, 2022)

3.5.3.3 Phytate

50mL of 3% Trichloroacetic Acid (TCA) was added to one gramme (1g) of finely ground sample, and mechanically shaken for 30 minutes.. The mixture was centrifuged at 6000rpm for 30 minutes Phytic acid in a 10 mL portion of the supernatant was washed Using 4mL of $FeCl_3$ that contained 0.20 percent $FeCl_3$ in TCA. The precipitate consisting of ferric phytate was transformed to $Fe(OH)_3$ using 3mL 1.5N NaOH. (1971). The $Fe(OH)_3$ was made to dissolved in 40mL of hot 3.2N HNO_3. Absorbance was read at

480nm in spectronic 20 spectrophotometer. Iron content was determined using $Fe(NO_3)_3$ standard curve. Using iron measurements, phytate phosphorus was estimated using the assumption of a 4:6 iron: phosphorus molecular ratio. The phytic acid content was calculated assuming it contained 28.20% phosphorus (AOAC, 2022).

3.5.4 Functional properties

The functional properties of the samples were evaluated as described below

3.5.4.1 Loosed and packed bulk densities, Hausner ratio and Carr index

Bulk densities were determined by putting an accurately weighed 20g of the samples into a measuring cylinder noting the initial volume; the cylinder was tapped severally until there was no considerable volume change. Loosed bulk density, packed bulk density, Hausner ratio and Carr index were thereafter determined (AOAC,2022).

3.5.4.2 Swelling power and solubility

The method cited by Zakpaa, Al-Hassan, and Adubofour, (2010) was modified for the evaluation of swelling power and solubility index. The method involved weighing 0.5 gram of the starch sample into a centrifuge tube with cap that have been previously weighed, this was followed by the addition of 10 mL distilled water and then proper mixing. The samples in the centrifuge tubes were subjected to heating at 85 ℃ with constant shaking for 30 min inside a water bath. After the heating period, the tubes were brought out and the temperature allowed to lower-down, and then centrifuging at 2,200 rpm for 15 minutes. The supernatant was drained into pre-weighed moisture cans and then heated at 105 ℃ to constant weight, after which the weight was noted, as well as the weight of the residue left in the centrifuge tubes. The swelling power and percentage solubility were estimated (eqn 3.6 and 3.7)

$$\text{Swelling power} = \frac{\text{weight of sediment}}{\text{Weight of sample} - \text{weight of solubles}} \quad \text{Equation . .. 3.6}$$

$$\%\ \text{Solubility index} = \frac{\text{weight of solubles}}{\text{Weight of sample}} \times 100 \quad \text{Equation … 3.7}$$

3.5.5 Pasting properties of sorghum *ogi*

The pasting properties of the sorghum ogi samples were evaluated with the aid of a Rapid Visco Analyzer (RVA4500) which was connected to a PC running ThermoCline for

Windows (TCW) version 3 software (Perten Instruments of Australia, 2015).The parameters that were evaluated include: final viscosity, set back viscosity, pasting temperature, peak time, break down viscosity, trough viscosity also known as holding strength and peak viscosity, from the pasting profile. Based on the sample's moisture content, the analyser's sample calculator calculated the weight of the sample that would be required for the analysis. This could be calculated manually as shown in eqn. 3.8. To make the starch suspension, the required sample weight and volume of water were weighed, totaling 28.0 g of slurry in the RVA canister. This was mounted in a central manner with the paddle in the canister unto the RVA machine, followed by pressing the instrument's motor toweronce initiated. The progress of the pasting activities, showing the pasting profile was monitored and viewed on the monitor of the connected computer, till the end of each experimental run.

RVA sample weight (S) = $\frac{A \times 100}{100 - M}$ Equation 3.8

Volume of distilled water (V) = 28 – S

Where A = 3 g

S = Calculated sample weight for RVA

M = Moisture content of the sample

V = Volume of water

3.5.6 Invitro protein digestibility

The invitro protein digestibility was carried out as described by AOAC (2022). Protein was digested using Porcine pepsin (EC.4.23.1, 1, 200 units per milligram of protein, sigma chemical co.st Louis MO). Two hundred (200) mg of flour was suspended in 2mL of water and stirred in a boiling water bath for 20 minutes to prepare the cooked sampleSamples were suspended in 35 mL of 0.1 M phosphate buffer, 1.5 g of pepsin per litre (pH 2.0), for protein digestion. They were then heated to 37^{o}C in a water bath while shaking for 2 hours. Digestion of Pepsin was halted by adding 2mL of 2M NaOH. The mixture was then centrifuged at 3500rpm for 20minutes. The residue was rinsed in 15mL of buffer and recentrifuged after the supernatant was discarded. The residue’s nitrogen content was evaluated using the micro-kjeldahl method (AOAC, 2022). The amount of soluble nitrogen was reported as invitro digestibility by subtracting indigestible nitrogen from total nitrogen. Determinations were done in triplicate.

3.6 Sensory analysis

Sensory evaluation of *ogi* produced was carried out using individuals who were familiar with the product. It was conducted in a quiet and well- lit sensory evaluation room. A nine-point hedonic scale, from 1 (extremely like) to 9 (extremely dislike) was given to the panellists to evaluate the products and assess them according to their degree of likeness. The assessed parameters include colour, smoothness, taste, aroma, texture, and overall acceptability.

3.7 Fermentation studies

The fermentation studies of the samples was carried out as described below

3.7.1 pH Determination

The method of AOAC (2022) was employed to determine the pH. pH of *ogi* powder was measured by direct reading with the aid of a digital pH meter (Mettler Toledo, Model MP220, Switzerland). About 5 g of *ogi* powder was dissolved in 50 mL distilled water and stirred using a glass rod. pH was measured after 10 minutes

3.7.2 Total titratable acidity

Four drops of phenolphthalein indicator was added to about 10mLof the sample in a 250mL conical flask This was titrated to a distinct faint pink point.using standard 0.1N NaOH Total titratable acidity was expressed as lactic acid (g/100mL) as described by (Agarry *et al*., 2010).

3.7.3 Characterisation and identification of microorganisms

The fermenting organisms were characterized and identified as follows:

3.7.3.1 Sample collection

During the soaking stage, the content of the soaking vessel was thoroughly mixed after which sample of the soak water (1mL) was aseptically taken for analysis at 24 hour intervals for 3days. At the souring stage, *ogi* (1g) was aseptically transferred to saline solution (9mL) and mixed thoroughly. The pour plate method was used to serially dilute the samples and was plated under aseptic conditions.

3.7.3.2 Isolation of organisms

The dilutions (1mL) was plated in prepared duplicate plates in standard media. Nutrient Agar, MacConkey Agar and Mannitol Salt Agar and then incubated at 35ºC for 24hours, De- Man Rogosa Sharpa kept in an anaerobic jar for 72hours. Distinct colonies were picked from the agar media and were repeated on new agar plates until pure cultures were achieved.The pure cultures were put on agar slants and stored at 4ºC.

3.7.3.3 Characterisation and identification of isolates

The isolates were characterized and identified using standard morphological and biochemical tests. Taxonomical identification was according to the criteria given in (Bergey and Holt, 2000).

3.7.3.3.1 Gram's reaction

The cellular morphologies of the bacteria isolates were carried out by observing under microscope after carrying out the Gram's staining procedure. The organism was lightly suspended in sterile distilled water using an inoculating loop, and a Bunsen flame was used to prepare it on a clean glass slide. The slide after cooling was placed on a staining rack and flooded with two drops of aqueous solution of Crystal violet (BDH). The slide was left for one minute then was washed off gently under running water. Lugol's iodine solution (i.e. the mordant) was flooded on the slide and left for one minute, then rinsed with water. 70% ethanol (decolourizer) was run over the film for 20seconds and was immediately washed off with water. The slide was then flooded with 0.5 aqueous solution of safranin (counterstain), allowed to stay for one minute before washing off under running water. An oil immersion drop was placed on the film and the slide was allowed to air dry before being inspected under a microscope with 100 oil immersion lenses. (AOAC 2022).

3.7.3.3.2 Indole test

One milliliter of peptone water and 1g of sodium chloride were prepared and dissolved in 200mL of distilled water. 7mL of the medium was poured into a clean test tube and sterilized at 121ºC at 1.02 kg^{cm-2} for 15minutes. The test organism was innoculated into the medium, and then it was incubated at 35ºC for 48hours. An uninoculated indole

medium served as control; after 48hours0.5mL of Kovac's solution was added to the test tube, which was then gently shaken. A reddish colour in the alcohol layer indicates a positive reaction (AOAC 2022).

3.7.3.3.3 Sugar fermentation test

The medium used was 1.0% peptone water, 0.1% sodium chloride, 0.01% phenol red indicator. Sugar liquid was prepared by adding the particular sugar (Mannitol, Glucose, and Lactose). 5mL of the indicator-sugar broth was poured into test tubes. Durham tubes were placed inverted in each test tube leaving no air space in the Durham tubes. Cotton wool was used to cover the test tubes and autoclaved for 15 minutes at 121^{o}C at 1.02 kg^{cm-2} to sterilize them. Using a sterile inoculating loop, the test organism was inoculated into the sterile medium, and incubated. at 35ºC for 48-72hours which served as a control. Fermentation of the sugar present was indicated by a colour change in the medium from red to yellow as a result of acid production while production of gas was indicated by an air space in the inverted Durham tube. Gas production is dependent on acid production, or in other words, no gas can be produced without acid production, but acid may be produced without gas being produced (AOAC 2022).

3.7.3.3.4 Methyl red test

The medium used was peptone water and dextrose (0.5%w/v). The mixture was prepared and sterilized in test tubes at 121ºC at 1.02kgcm^2 for 15minutes. The medium was inoculated with the test organism in the test tube and incubated at 37ºC for 48-72hours. One of the test tubes containing the medium was uninoculated to serve as a control. About 5 drops of methyl red indicator was added, stirred and read immediately. A red colour indicates a positive reaction i.e. acid produced and yellow colour indicates a negative reaction (AOAC 2022).

3.7.3.3.5 Voges-Proskauer test

The medium used was peptone water, KH_2PO_4 and dextrose 0.5%. The mixture of peptone water was prepared and sterilized in test tubes at 121ºC at 1.02kgcm^2 for 15minutes. The medium in the test tube was inoculated with the test organism and incubated at 37ºC for 48-72hours. An uninoculated vogues-proskauer medium served as control. 0.5mL of 6% α-naphthol was added to the inoculated tubes followed by 0.5mL of Potassium hydroxide

(KOH) and mixed well. The development of a pink colour in the medium within 30 minutes shows a positive reaction while a yellow colour implies a negative reaction (AOAC 2022).

3.7.3.3.6 Citrate utilisation test

Koser's citrate medium was used for this test. 24-28g of the agar was dissolved in 100mL distilled water. 5mL was poured into clean test tubes and sterilized at 121ºC for 15minutes. A loop of the isolates was inoculated at 35ºC for 48-72 hours. One of the test tubes containing the medium was left uninoculated to serve as a control. Positive results were indicated by the medium's colour changing from green to blue (AOAC 2022).

3.7.3.3.7 Hydrolysis of starch

The medium used was 1% starch powder and nutrient agar. 7g of nutrient agar and 2.5g starch powder was added and the mixture was sterilized at 121ºC for 15miuntes. 20 mL of melted starch agar at 45°C was poured aseptically into sterile Petri dishes and allowed to solidify. The inoculated medium was incubated invertly at 35ºC for 48hours. Gram iodine was used to flood the surface of the medium. . Starch hydrolysis was visible as a clear zone surrounding the streak line while unhydrolyzed starch formed a blue-black surface when combined with iodine (AOAC 2022).

3.8 Production of *ogi* using a modified method

Ogi was produced from both malted and unmalted sorghum by a modified method which involves milling the grains before fermentation. The method described by Ilelaboye and Pikuda, (2013) with some modifications was used. Malted and unmalted sorghum grains were cleaned, weighed, milled (Agrico model 2A, New Delhi, India), steeped and was allowed to ferment for 48 hours based on preliminary experiments. (Figure 3.4).

3.9 Preparation of inocula for fermentation of sorghum ogi

The selected starter culture to be used were harvested by adding aseptically 10mL of sterile peptone water to each slant, The result of the suspension was adjusted using a spectrophotometer with sterile peptone water to give a concentration of 10^6-10^7 cfu/mL and used subsequently as inocula. MRS and Nutrient Agar were the growth media

used for slants of *Lactobacillus plantarium* and *Leuconostoc mesenteroides* and other lactic acid bacteria isolated from this research work, 10 mL of sterile peptone water was added to a slant maintained for 18-24 hours, followed by aseptically scraping the agar surface with vigorous shaking. From the resulting suspension, 0.08µL/500mL – 0.12 µL/500mL each of lactic acid bacteria from burukutu, palmwine and yorghut were added aseptically to the samples and was allowed to ferment for 48hrs.

3.10 Extraction of DNA using CTAB method

Bacterial isolates were cultured in an Eppendorf tube overnight and was centrifuged at 14,000rpm for 2 minutes. The supernatant was thereafter removed and 600µL of 2X CTAB buffer added to the pellets, cultured at 65^{o}C for 20 minutes and subsequently cooled to room temperature following removal from the incubator. Chloroform was added to it and the tube gently mixed in an inverted position severally.

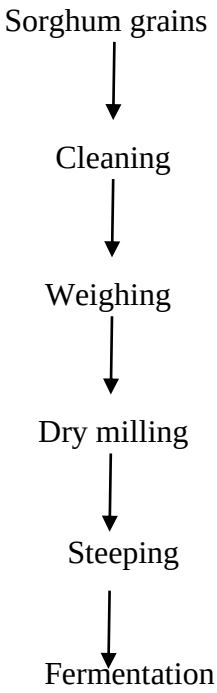

Figure 3.4: Production of *ogi* using a modified method
(Ilelaboye and Pikuda, 2013)

The sample was then centrifuged for 15 minutes at 14,000rpm. To precipitate the DNA, the supernatant was put into a fresh Eppendorf tube and the same volume of cold isopropanol was added. Prior to being spun for 10 minutes at 14,00 rpm, the material was frozen for an hour. After washing the pellets in 70% ethanol and discarding the supernatant, they were left to air-dry on a bench for 30 minutes The pellet was resuspended in 100 mL of sterile distilled water and the DNA concentrations were determined using a spectrophotometer as 260 nm and 280 nm while genomic purity of the samples were evaluated. All the DNA samples had a genomic purity of between 1.8 and 2.0 (Wawrik *et al.,* 2005).

3.11 DNA Electrophoresis

The DNA's integrity and purity were evaluated using agarose gel electrophoresis. through size separation on 1.0% agarose gels. Preparation of agarose gels involved dissolving and boiling 1.0 g agarose in 100 mL 0.5X TBE buffer solution. After allowing the gels to cool to around 45°C for 10 µL of 5 mg/mL. After correctly mixing with ethidium bromide, the mixture was put into an electrophoresis chamber equipped with combs. After the gel has solidified, 3µL of DNA was added to 5µL of sterile distilled water and 2µL of 6X loading dye and poured into the well. Electrophoresis was carried out for 2 hours at 80V. UV light source was used to visualize and photograph the integrity of the DNA. (Frank *et al.,* 2008)

3.12 PCR analysis using I6S primer

A universal primer for bacteria known as 16S was employed for the Polymerase Chain Reaction (PCR) analysis. The PCR mix comprise of 1µL of 10X buffer, 0.4 µL of 50 Mm $MgCl_2$, 0.5 µL of 2.5mM dNTPs, 0.5 µL of 5mM forward primer, 0.5 µL of 5mM reverse primer, 0.05µL of 5 units/ µL Taq with2 µL of template DNA and 5.05 µL of distilled water to make up 10 µL reaction mix. The PCR profile used was first denatured for 3 minutes at 94°C., followed by 30 cycles at 94°C for 60 seconds, 56° C for 60 seconds, 72°C for 120 seconds and the final extensions and pressure of 72°C for 5 minutes (Wawrik *et al.,* 2005).

3.13 Purification of PCR products

The amplicon was further purified using 2M Sodium Acetate wash methods prior to sequencing. One microlitre (1µL) of 2 M Sodium Acetate (pH 5.2) was added to around 10 µL of PCR product, followed by 20 µL of absolute ethanol. The combination was held at -20°C for 1 hour then spun for 10 minutes at 10,000 rpm before being washed with 70% ethanol. The sample was then aired dried before being redissolved in 5 µL of deionized water and kept at 4° C for genotyping (Wawrik *et al.,* 2005).

3.14 PCR for sequencing

The reaction primer used was forward I6S. The PCR mix consisted of 0.5µl of BigDye Terminator Mix,1µl of 5X sequencing buffer, 1µl of M13 forward primer, 6.5µl of distilled water and 1µl of the PCR product making a total of 10µl. The genotyping PCR template was a rapid template , involves 25 rounds of a rapid thermal ramp to 96^{o}C for 10 seconds after an initial rapid thermal ramp to 96^{o}C for 1 minute. Rapid heat ramp to 50^{0}C for 5 seconds and Rapid thermal ramp to 60^{0}C for 4 minutes, then followed by Rapid thermal ramp to 4^{0}C (Wawrik *et al.,* 2005, Frank *et al.*, 2008).

3.15 Purification of PCR sequencing products

Prior to sequencing, the PCR sequence product was cleaned using 2M Sodium Acetate wash techniques. 1µl 2M Sodium Acetate (pH 5.2) was added to 10µl of the PCR product followed by 20µl of Absolute Ethanol; the mixture was held at -20^{0}C for 1hr, spun at 10,000rpm for 10 minutes, then washed with 70% Ethanol and air-dried before being redissolved in 5µl of sterile distilled water and kept at 4^{0}C for sequencing running (Wawrik *et al.,* 2005).

3.16 Preparation of sample for Gene Sequencer (ABI 3130xl machine)

Gene sequencing was done using ABI 3130xl machine. A mixture of 9 L of Hi Di Formamide with 1 L of purified sequence, making a total of 10 L, was used as the cocktail mix The samples were fed into the machine and the data in form of A, C, T, and G were generated. The strains of the microorganisms (bacteria and fungi) were identified by blasting of the DNA sequencing results against sequence in the GeneBank database (Wawrik *et al.,* 2005).

3.17 Fermentation studies of *ogi* produced by induced fermentation

This was carried out as described in section 3.7

3.18 Analyses on ogi produced by induced fermentation

This was done as described in section 3.5

3.19 Colour determination by analytical method

Hunter colorimeter (Konica Minolta CR 410 chromameter) by the aid of the Commission Internationale de l'Eclairage (CIE) tristimulus was used for the evaluation. This evaluated the L*-signifying lightness, a*- red-green axis and b*-the yellow-blue axisas the colour parameters of the sorghum ogi samples. Standardization of the equipment was carried out using a white tile according to Lui-ping *et al*. (2005). The parameters evaluated were L*, a* and b* axis of the CIE scale. Where, *L**(lightness) axis showed 0 is black and 100 is white; *a**(red-green) - positive values indicates red while negative values are green and 0 is neutral; *b**(yellow-blue) axis - positive values indicates yellow, while negative values are blue and 0 is neutral.

3.20 Sensory analysis

Sensory evaluation of *ogi* produced was carried out using individuals who were familiar with the product. It was conducted in a quiet and well- lit sensory evaluation room. A nine-point hedonic scale, from 1 (extremely like) to 9 (extremely dislike) was used by the panellists to evaluate the products and assess according to their degree of likeness .The assessed parameters include colour, smoothness, taste, aroma, texture, and overall acceptability.

3.21 Statistical analysis

All the experiments were done in triplicate and averages of values were determined. The data were analysed using SPSS version 20 statistical software (2004). Analyses of variance were carried out. Differences ($p < 0.05$) between variables were found.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Proximate composition

The proximate composition of unfermented, malted and fermented sorghum are as shown in Table 4.1 Protein content of unmalted and modified grains ranged between 5.70 ± 0.36 and 9.55± 0.12%. Malted sorghum with fermentation period of 48hours and with bran not sieved from the grains have the highest protein content of 9.55±0.12%; next to it is the sample containing malted sorghum fermented for 72hours without sieving which has protein level of 9.04 ±0.18%. From the result, malting and fermentation causes an increase in the protein content; the protein content of both sieved and unsieved malted sorghum were higher significantly ($p<0.05$) than that of the samples of unsprouted grains, which may be a result of the grains' stored nitrogen being released to help sprouting. This might also happen due to the production of enzymes or a modification of the compositionl spurred by the breakdown of other components. Oluwalana (2014) also observed a notable rise in the protein content of sprouted sweet yellow and white corn compared to those that had not. Ijarotimi and Keshinro (2011) also observed a considerable rise in protein content after sprouting.. Sieving of ogi and other products of modified grains reduced the protein level because protein in sorghum is concentrated in the bran. Fermentation for 48 hours is the optimum as fermentation beyond this period has adverse effects on protein contents. These results are in agreement with those of Osman *et al*., 2022.

The fat contents of raw and modified sorghum grains ranged from 2.31 ±0.04% (malted sorghum with fermentation period of 48hours and sieving) to 3.79± 0.11% (unmalted grains with 24 hours fermentation and sieving). From the results, malting and fermentation

Table 4.1: Proximate compositions (%) of unmalted, malted, sieved and unsieved sorghum *ogi*

SAMPLE	PROTEIN	FAT	FIBRE	MOISTURE	ASH	CARBOHYDRATE
U	7.33 ± 0.53^{g}	3.62 ± 0.01^{b}	3.05 ± 0.07^{a}	9.44 ± 0.20^{o}	1.74 ± 0.01^{b}	74.47 ± 0.60h
M	8.72 ± 0.30^{bc}	3.53 ± 0.04^{c}	2.90 ± 0.06^{b}	11.16 ± 0.06^{f}	1.47 ± 0.07^{c}	72.22 ± 0.34^{k}
U2N	7.19 ± 0.33^{gh}	3.44 ± 0.02^{d}	2.88 ± 0.08^{b}	10.82 ± 0.06^{g}	0.74 ± 0.01^{efg}	74.94 ± 0.41^{h}
U2S	6.44 ± 0.14^{h}	3.79 ± 0.11^{a}	2.29 ± 0.03^{hij}	9.41 ± 0.17^{o}	0.72 ± 0.15^{fg}	77.35 ± 0.35^{b}
M2N	8.18 ± 0.3^{de}	3.26 ± 0.04^{e}	2.71 ± 0.07^{c}	10.35 ± 0.12^{ij}	0.52 ± 0.03^{hi}	74.98 ± 0.32^{h}
M2S	7.71 ± 0.19^{f}	3.51 ± 0.03^{c}	2.14 ± 0.10^{kl}	10.69 ± 0.05^{gh}	0.27 ± 0.01^{jkl}	75.67 ± 0.29^{g}
U4N	7.60 ± 0.22^{fg}	2.61 ± 0.02^{h}	2.62 ± 0.03^{d}	10.05 ± 0.03^{kl}	0.51 ± 0.06^{hi}	76.00 ± 0.30^{de}
U4S	6.76 ± 0.12^{h}	2.53 ± 0.02^{i}	2.14 ± 0.08^{kl}	13.44 ± 0.27^{b}	0.62 ± 0.02^{gh}	74.51 ± 0.42^{h}
M4N	9.55 ± 0.12^{a}	2.56 ± 0.02^{hi}	2.37 ± 0.02^{fgh}	10.22 ± 0.01^{jk}	0.41 ± 0.21^{ij}	74.89 ± 0.20^{h}
M4S	8.46 ± 0.17^{cde}	2.31 ± 0.04^{mn}	2.15 ± 0.04^{kl}	13.04 ± 0.14^{c}	0.26 ± 0.06^{jkl}	73.78 ± 0.21^{i}
U7N	7.22 ± 0.12^{gh}	3.04 ± 0.03^{f}	2.33 ± 0.04^{hi}	9.68 ± 0.04^{n}	0.93 ± 0.14^{d}	76.80 ± 0.11^{cde}
U7S	5.70 ± 0.36^{i}	2.93 ± 0.04^{g}	2.03 ± 0.03^{m}	9.73 ± 0.06^{mn}	0.26 ± 0.08^{jkl}	79.36 ± 0.32^{a}
M7N	9.04 ± 0.18^{b}	2.96 ± 0.02^{g}	2.19 ± 0.03^{jk}	9.94 ± 0.01^{lm}	0.25 ± 0.08^{jkl}	75.61 ± 0.09^{g}
M7S	8.27 ± 0.17^{de}	2.95 ± 0.05^{g}	2.03 ± 0.03^{m}	10.48 ± 0.01^{hi}	0.42 ± 0.91^{ij}	75.85 ± 0.18^{fg}

Means with the same superscript along the columns are not significantly different from each other ($p<0.05$)

U- unmalted; M- malted; S-sieved, N-not sieved; 2=24hours fermentation; 4=48hours fermentation; 7=72hours fermentation

reduced the fat content of sorghum *ogi* this is probably because fat is used up during the biochemical reactions that takes place during fermentation and malting. Sieving does not significantly affect the fat contents of raw and modified grains. Malting increased significantly the moisture contents of unsieved malted grains but reduced that of sieved grains. The findings agree with the results of Modu *et al.,* (2013) and Onyango *et al.,* (2013) who also agreed that variety and environment of growth could affect various parameters.

The highest level of fibre was recorded in unmalted sorghum (3.05± 0.07%) while the lowest (2.03 ± 0.03%) was recorded for both sieved and unsieved malted sorghum with 72 hours fermentation period. The values obtained for modified sorghum grains were lower than those obtain by Modu *et al.,* (2013) and the differences could be due to varieties.

The percentage ash content was higher in raw sorghum (1.74± 0.01%) and relatively stable and lower in the modified samples. The result is similar with the findings of Onyango *et al.,* (2013). The carbohydrate contents of the samples were fairly stable (72.22 ±0.34 to 79.36 ±0.32%) since the biochemical reactions in malting and fermentation involves the conversion of one form of carbohydrate to others (Onyango *et al.,* 2013).

4.2 Mineral Composition

Table 4.2 presented the mineral composition of unmalted and malted sorghum ogi samples. The Magnesium, calcium, iron, zinc potassium, sodium and manganese contents were evaluated. From the results, samples that were not sieved contained higher level of minerals in each case and this may be due to higher level of bran in them. The magnesium content was in the range 55.13± 0.03- 91.54±0.01; Calcium: 80.54±0.45; Iron:16.17±0.04-88.96±0.00; Zinc: 1.91±0.00-20.22±0.00; P0tassium: 50.67±0.15 -145.33±0.07; Sodium: 31.86±0.09-236.54±0.06 and Manganese: 1.72±0.00 - 12.94±0.04 When compared to unsprouted grains, sprouted grains have higher mineral contents. This was in line with previous reports of Oluwalana (2014) who evaluated the effect of sprouting on proximate, mineral and functional properties of white and yellow sweet maize and observed an increase in mineral composition with malting. The favourable nutritional changes may

Table 4.2: Mineral contents (ppm) of unmalted, malted, sieved and unsieved sorghum *ogi*

SAMPLE	Mg	Ca	Fe	Zn	K	Na	Mn
U	88.61 ± 0.08^{b}	111.27 ± 0.07^{h}	35.26 ± 0.00^{h}	20.22 ± 0.00^{a}	81.23 ± 0.14^{b}	150.49 ± 0.05^{b}	4.72 ± 0.02^{h}
M	91.54 ± 0.01^{a}	80.54 ± 0.45^{n}	16.17 ± 0.04^{m}	14.89 ± 0.01^{c}	73.43 ± 0.09^{d}	150.56 ± 0.09^{b}	6.28 ± 0.00^{g}
U2N	68.02 ± 1.71^{f}	163.27 ± 0.06^{e}	27.74 ± 0.00^{j}	12.48 ± 0.02^{d}	58.79 ± 0.08^{i}	43.21 ± 0.19^{j}	9.74 ± 0.00^{c}
U2S	64.76 ± 0.01^{j}	103.60 ± 0.07^{l}	64.37 ± 0.00^{b}	11.02 ± 0.00^{g}	56.30 ± 0.07^{k}	111.86 ± 0.07^{d}	11.58 ± 0.00^{b}
M2N	67.36 ± 0.08^{g}	229.63 ± 0.40^{a}	88.96 ± 0.00^{a}	9.81 ± 0.02^{i}	68.54 ± 0.33^{e}	107.47 ± 0.08^{f}	3.19 ± 0.00^{i}
M2S	62.55 ± 0.02l	178.63 ± 2.33^{c}	41.42 ± 0.00^{g}	1.91 ± 0.00^{m}	73.43 ± 0.01^{d}	134.46 ± 0.15^{c}	1.73 ± 0.00^{k}
U4N	68.95 ± 0.06^{e}	118.42 ± 0.16^{g}	24.42 ± 0.00^{k}	12.29 ± 0.01^{e}	65.15 ± 0.16^{f}	31.86 ± 0.09^{k}	8.91 ± 0.00^{e}
U4S	65.22 ± 0.02^{i}	103.96 ± 0.05^{k}	33.02 ± 0.00^{i}	11.65 ± 0.01^{f}	60.64 ± 0.56^{h}	56.02 ± 0.16^{h}	9.16 ± 0.00^{d}
M4N	65.09 ± 0.03^{i}	190.85 ± 0.03^{b}	16.79 ± 0.00^{l}	2.32 ± 0.00^{l}	50.67 ± 0.15^{m}	134.48 ± 0.18^{c}	1.73 ± 0.00^{k}
M4S	63.20 ± 0.02^{k}	174.91 ± 0.02^{d}	43.74 ± 0.00^{f}	2.63 ± 0.00^{k}	145.33 ± 0.07^{a}	96.86 ± 0.13^{g}	3.01 ± 0.00^{j}
U7N	71.22 ± 0.04^{d}	107.27 ± 0.22^{i}	32.94 ± 0.01^{i}	15.33 ± 0.00^{b}	55.62 ± 0.54^{l}	107.7 ± 0.18^{e}	12.94 ± 0.04^{a}
U7S	67.12 ± 0.02^{h}	82.96 ± 0.06^{m}	51.44 ± 0.00^{c}	10.33 ± 0.02^{h}	57.76 ± 0.23^{j}	51.7 ± 0.12^{i}	7.98 ± 0.00^{f}
M7N	73.39 ± 0.02^{c}	161.09 ± 0.06^{f}	46.40 ± 2.31^{e}	9.97 ± 0.00^{i}	78.18 ± 0.08^{c}	236.54 ± 0.0^{a}	1.72 ± 0.00^{k}
M7S	55.13 ± 0.03^{m}	106.98 ± 0.06^{j}	49.92 ± 0.00^{d}	7.30 ± 0.00^{j}	61.13 ± 0.14^{g}	43.29 ± 0.32^{j}	1.72 ± 0.00^{k}

Means with the same superscript along the columns are not significantly different from each other ($p<0.05$)

U- unmalted; M- malted; S-sieved, N-not sieved; 2=24hours fermentation; 4=48hours fermentation; 7=72hours fermentation

result from the breakdown of complex molecules into simpler forms, and the rise in calcium may be caused by a decrease in phytic acid while sprouting. Some of the mineral loss may be due to their possible washing out into the steep water during the germination phase (Tatsjieu, 2004).

4.3 Antinutrients

There were significant reductions of tannin, phytate and oxalate with germination and fermentation of raw sorghum (Table 4.3) and this aligns with the findings of Modu *et al.,* (2013). The tannin content reduced from 2.32 in raw sorghum to 0.11 after malting and fermentation for 72 hours. This was also noticed in phytate and oxalate contents of the samples which reduced from 0.92 to 0.17 and 1.82 to 0.86 respectively. Tannins have anti nutritional properties which depend on their chemical structure and dosage. The antinutrients are more concentrated inside the pericarp of sorghum grains and this accounts for the lower polyphenol contents of sieved than unsieved samples.

A reduction in tannin was accompanied by an increase in protein digestibility. The anti-nutrients in sorghum ogi were significantly reduced by malting, indicating that some modifications occurred during the malting process, this is supported by Ogbonna *et al.,* 2012) who reported that lentil germination significantly lowered phytate concentration compared to soaking and cooking. Leaching during steeping reduced some of the anti-nutrients as evidenced by a change in the steep water's colour. Endogenous enzymes, such as phytases activated during sorghum grain germination, may have had a significant impact on phytate. It is known that Phytases convert phytate to intermediate forms of inositol and inorganic phosphorus.

4.4 Loose bulk density, packed bulk density, Hausner ratio and Carr index of raw and malted samples of sorghum *ogi*

Table 4.4 showed the loose bulk density, packed bulk density, hausner ratio and carr index of raw and modified grains. The loose bulk densities were from 0.48 and 0.62 for unmalted and malted sorghum respectively. From the results,unsieved malted sample with 48 hours fermentation time had the lowest value of 0.34 ± 0.01g/cm3. The packed bulk density ranged from 3.32g/ml for malted sorghum to 1.12g/ml for unmalted sorghum fermented for 24 hours and sieved. This is in agreement with the findings of Ocheme *et al.,* (2015) that denser flour is produced with inceasing bulk density.This suggests that raw sorghum grains are denser than

Table 4.3 Anti nutrients present in unmalted, malted, sieved and unsieved sorghum *ogi*

Sample	Phytate (mg/kg)	Tannin (mg/kg)	Oxalate (mg/kg)
U	0.92 ± 0.22^{a}	2.32 ± 0.05^{a}	1.82 ± 0.02^{b}
M	0.63 ± 0.3^{b}	1.65 ± 0.04^{b}	1.97 ± 0.01^{a}
U2N	0.30 ± 0.01^{e}	0.96 ± 0.00^{d}	1.64 ± 0.00^{e}
U2S	0.27 ± 0.01^{f}	0.88 ± 0.01^{e}	1.54 ± 00^{f}
M2N	0.27 ± 0.02^{f}	0.54 ± 0.00^{g}	1.76 ± 0.01^{c}
M2S	0.12 ± 0.02^{mn}	0.36 ± 0.00^{h}	1.72 ± 0.01^{d}
U4N	0.22 ± 0.02^{g}	0.29 ± 0.00^{j}	1.03 ± 0.01^{m}
U4S	0.18 ± 0.01^{h}	0.22 ± 0.00^{k}	0.97 ± 0.01^{o}
M4N	0.16 ± 0.02^{hij}	0.19 ± 0.01^{l}	1.37 ± 0.00^{h}
M4S	0.18 ± 0.01^{h}	0.12 ± 0.00^{m}	1.29 ± 0.00^{j}
U7N	0.18 ± 0.01^{h}	0.23 ± 0.00^{k}	0.73 ± 0.00^{q}
U7S	0.16 ± 0.02^{hijkl}	0.22 ± 0.00^{k}	0.65 ± 0.01^{r}
M7N	0.18 ± 0.02^{h}	0.13 ± 0.00^{m}	1.00 ± 0.00^{mn}
M7S	0.17 ± 0.01^{h}	0.11 ± 0.00^{mn}	0.86 ± 0.33^{p}

Means with the same superscript along the columns are not significantly different from each other ($p<0.05$)

U- unmalted; M- malted; S-sieved, N-not sieved; 2=24hours fermentation; 4=48hours fermentation; 7= 72hours fermentation

Table 4.4: Loose bulk density, packed bulk density, Hausner ratio and Carr index of unmalted, malted, sieved and unsieved sorghum *ogi*

Sample	LBD (gcm^{-3})	PBD (gcm^{-3})	HR (gcm^{-3})	CI (gcm^{-3})
U	0.48 ± 0.01^{c}	1.69 ± 0.02^{e}	0.41 ± 0.03^{i}	1.56 ± 0.06^{jkl}
M	0.62 ± 0.01^{a}	3.32 ± 0.02^{a}	0.85 ± 0.04^{a}	5.53 ± 0.15^{a}
U2N	0.44 ± 0.02^{fghi}	1.33 ± 0.03^{h}	0.67 ± 0.02^{j}	3.00 ± 0.02^{o}
U2S	0.38 ± 0.01^{m}	1.12 ± 0.17^{ij}	0.62 ± 0.02^{k}	2.68 ± 0.03^{p}
M2N	0.40 ± 0.01^{lm}	1.82 ± 0.02^{d}	0.78 ± 0.04^{cde}	4.56 ± 0.07^{de}
M2S	0.43 ± 0.01^{hijk}	1.55 ± 0.02^{fg}	0.73 ± 0.02^{hi}	3.67 ± 0.14^{ij}
U4N	0.45 ± 0.01^{efgh}	1.56 ± 0.02^{efg}	0.73 ± 0.02^{ghi}	3.39 ± 0.01^{klmn}
U4S	0.42 ± 0.01^{ijkl}	1.44 ± 0.01^{gh}	0.71 ± 0.02^{ij}	3.43 ± 0.01^{klmn}
M4N	0.34 ± 0.01^{n}	1.19 ± 0.01^{i}	0.72 ± 0.01^{i}	3.42 ± 0.02^{klmn}
M4S	0.48 ± 0.01^{cd}	1.82 ± 0.03^{d}	0.76 ± 0.02^{defgh}	3.90 ± 0.02^{h}
U7N	0.40 ± 0.02^{lm}	2.15 ± 0.17^{c}	0.81 ± 0.02^{bc}	5.27 ± 0.31^{b}
U7S	0.45 ± 0.01^{efg}	1.67 ± 0.05^{ef}	0.74 ± 0.02^{fghi}	3.69 ± 0.02^{ij}
M7N	0.38 ± 0.01^{m}	1.67 ± 0.02^{ef}	0.77 ± 0.02^{defg}	4.35 ± 0.02^{fg}
M7S	0.43 ± 0.02^{ghij}	1.82 ± 0.04^{d}	0.77 ± 0.01^{def}	4.27 ± 0.02^{g}

Means with the same superscript along the columns are not significantly different from each other ($p<0.05$)

U- unmalted; M- malted; S-sieved, N-not sieved; 2=24hours fermentation; 4=48hours fermentation; 7=72hours fermentation

malted and fermented grain due to malting losses and biochemical reaction that took place during malting and fermentation (Raihemattu *et al* 2011).

Bulk density of the sample indicates how compact the matrices are and is useful in determining the packaging requirement of foods. Carr index measured the relative significance of inter-particle interaction. It is used in foods and pharmaceutics as a measure of flowability of a powder. A Carr index above 25 indicates poor flowability, whereas one below 15 indicates good flowability. The Hausiner ratio is also used to determine flowability of a powder or granules. A Hausener ratio more than 1.25 is a measure of poor flowability. From these results the samples had a Carrr index of between 1.56 and 5.53 and a Hausner ratio of between 0.41 and 0.85 which are indications of good flowability.

4.5 Swelling power and solubility index

Table 4.5 showed that unmalted grains fermented for 72 hours exhibited the highest swelling power of $7.20 \pm 0.02\%$ and the malted raw grains had the lowest value of 3.11±0.01%. From the result, fermented sorghum grains have better swelling power than the raw sorghum; this is in line with the values, reported by Singh *et al.,* (2017). Swelling power which is a measure of the water holding capacity of starch is a function of the amylose to amylopectin ratio and this is used to differentiate different types of starches; the swelling power increases with fermentation time (Bolaji *et al.,* 2014). For the fermented grains, unmalted sorghum fermented for 72 hours and unsieved had the highest value of solubility (1.64%) while the unmalted grains that was fermented for 24 hours without sieving had the least value of 0.03 ± 0.02.

Solubility is an index of reconstitution and processing, the higher the solubility, the better the Ogi will reconstitute in water (Bolaji *et al.,* 2014). The solubility increases with increased period of fermentation, this is because when starch degrades, simpler molecules which are easier to dissolve in water are produced with increased period of fermentation (Dedin *et al.,* 2020). From the results, malting increased the solubility and this is probably because parts of the starch had been converted to more soluble sugar (Raihanatu *et al* 2011).

Table 4.5 Swelling power and solubility index of unmalted, malted, sieved and unsieved sorghum *ogi*

Sample	Swelling power (%)	Solubility index (%)
U	4.50±0.02^{m}	2.04±0.02^{d}
M	3.11±0.01^{o}	1.20±0.01^{h}
U2N	4.42±0.02^{n}	0.03±0.02^{r}
U2S	5.13±0.02^{j}	1.09±0.02^{i}
M2N	4.71±0.26^{l}	0.76±0.02^{j}
M2S	5.47±0.01^{i}	0.19±0.01^{p}
U4N	5.45±0.02^{i}	0.77±0.02^{j}
U4S	5.50±0.01^{hi}	1.37±0.02^{f}
M4N	5.09±0.01^{jk}	0.70±0.01^{l}
M4S	5.44±0.02^{i}	0.37±0.02^{o}
U7N	7.20±0.02^{a}	1.64±0.02^{e}
U7S	7.09±0.01^{b}	0.43±0.02^{n}
M7N	4.51±0.02^{m}	0.70±0.02^{kl}
M7S	6.08±0.02	0.12±0.02^{q}

Means with the same superscript along the columns are not significantly different from each other ($p<0.05$)

U- unmalted; M- malted; S-sieved, N-not sieved; 2=24hours fermentation; 4=48hours fermentation; 7= 72hours fermentation

4.6 Pasting properties

The pasting profile of samples of sorghum *ogi* is as represented in Table 4.6. The final viscosity of samples ranged from 1.83±0.17 to 492.38±10.88 RVU. Unmalted and unsieved grains had the highest value of 492.38±10.88 RVU followed closely by malted and unsieved sample with the value of 337.08±0.17RVU. Malted sieved and fermented sorghum ogi samples and unmalted, sieved and fermented sorghum ogi samples had values of 323.75±16 and 304.46.±3.79 respectively. Malting converts starch to sugar while fermentation tends to increase the thickness of the slurry, by concentrating the total solid.

The breakdown viscosity of sorghum ogi ranged between 4.50±0.08 and 76.08±1.25 RVU in malted sorghum and in unmalted, fermented and sieved samples respectively. The degree of granule or paste stability disintegration during cooking is a measure of the breakdown viscosity (Newport Scientific, 1998). The paste from malted sorghum has unstable paste regarding its viscosity during cooking.

The values of setback viscosity of the samples ranged from 0.42±0.00 to 454.94±17.33RVU. Set back viscosity is a measure of reordering or retrogradation of starch molecules after cooling. Reduced digestibility is indicated by higher setback values, while lower setback as a paste cools indicates less tendency to retrograde (Shandu *et al.*, 2007, Zubair and Oshundahunsi, 2016). In this study, malted sorghum with setback viscosity of 0.42±0.00RVU has the lowest tendency for retrogradation, while unmalted, fermented and unsieved sample has the highest tendency for retrogradation.

The values for peak time ranged from 3.73minutes in malted sorghum and 6.17 minutes unmalted, fermented and unsieved samples. Peak time is an indicator of the cooking time. Pasting temperature of the pastes was in the range of 79.93^{o}C and 86.40^{o}C. The pasting temperature is the lowest temperature required to cook a specific food sample. (Sandhu *et al.*, 2007).

Table 4.6 Pasting properties of unmalted, malted, sieved and unsieved sorghum *ogi*

Sample	Peak viscosity (RVU)	Holding strength (RVU)	Breakdown viscosity (RVU)	Final viscosity (RVU)	Set back viscosity (RVU)	Peaktime (min)	Pasting temp (^{0}C)
U	110.92±0.75^{f}	85.00±0.92^{d}	25.92±1.67^{de}	235.21±4.38^{g}	150.21±3.46^{de}	5.27±0.70^{e}	83.58±0.48^{a}
M	5.92±0.25^{g}	1.42±0.17^{e}	4.50±0.08^{f}	1.83±0.0.17^{i}	0.42±0.00^{f}	3.73±0.00^{h}	Error
UFN	180.71±2.46^{d}	151.17±0.67^{b}	39.39±17.34^{bc}	492.38±10.88^{a}	454.94±17.33^{a}	6.17±0.33^{a}	82.73±0.43^{a}
UFS	240.86±0.79^{a}	164.79±0.46^{ab}	76.08±1.25^{a}	304.46±3.79^{e}	139.67±3.33^{de}	5.43±0.03^{d}	80.63±0.0.03^{a}
MFN	140.33±0.33^{e}	117.17±0.67^{c}	23.17±0.33^{e}	337.08±0.17^{d}	219.92±0.83^{bcd}	5.87±0.00^{b}	86.40±0.10a
MFS	204.33±5.67^{bc}	159.83±2.92^{ab}	44.50±0.2.75^{b}	323.75±16.67^{d}	163.92±13.75^{cde}	5.27±0.20^{e}	80.73±0.83^{a}

Means with the same superscript along the columns are not significantly different from each other
U–Unmalted, M–Malted, F-Fermented, N-Notsieved, S–Sieved

4.7 In vitro Protein Digestibility (IVPD) of raw and modified sorghum *ogi*

Table 4.7 shows the values of invitro protein digestibility of sorghum samples. The unmalted sorghum had the least value of 46.78 $\pm$49 while the range of values for ogi samples were (66.55$\pm$ 1.42 – 90.58 $\pm$0.76%) for unmalted and malted sorghum. From the results, the values for protein digestibility in malted sorghum were higher than in unmalted samples. Malting and sieving increased protein digestibility in all the samples. The results obtained in this research is in agreement with the results of Modu *et al.*, (2013). The grain's absence of outer layers improved the sorghum protein digestibility as evident in sieved samples, this may be due to the fact that lipids in the germ cover the grain's protein constituents, making them less accessible to proteases.

Due to decreased protease accessibility, proteins bonded to pericarp fragments produced during milling may be less digestible. The increased digestibility of sorghum protein after the pericarp was removed further raises the possibility that polyphenols are involved, Protein digestibility of high tannin sorghum is negatively affected by condensed polyphenol. Plant polyphenols are oxidised to produce oxidising agents such as quinnines and very reactive peroxides, these peroxides may oxidise protein amino acid residues and subsequently polymerize them, which may reduce the protein digestibility. Fermentation improved protein digestibility, cooking also reduces protein digestibility of sorghum grains although this is not so with other cereals; malting however negates the impact of cooking on protein digestibility.

Table 4.7: Invitro protein digestibility of unmalted, malted, sieved and unsieved sorghum *ogi*

Sample	IVPD (%)
U	46.78±10.49^{k}
M	66.70±1.25^{i}
U2N	66.55±1.42^{i}
U2S	69.06±1.17^{hi}
M2N	71.54±1.16^{gh}
M2S	71.57±1.11^{gh}
U4N	81.87±1.38^{f}
U4S	82.33±1.01^{f}
M4N	83.68±.51^{ef}
M4S	85.01±0.82^{def}
U7N	87.54±1.48^{cde}
U7S	88.39±1.10^{bcd}
M7N	90.38±0.85^{abc}
M7S	90.58±0.76^{abc}

Means with the same superscript along the columns are not significantly different from each other

U- unmalted; M- malted; S-sieved, N-not sieved; 2=24hours fermentation; 4=48hours fermentation; 7=72hours fermentation

4.8 Sensory evaluation of *ogi* produced from modified and unmodified sorghum grains

Table 4.8 shows the mean scores for quality attributes of *ogi* gruel produced from sorghum samples. The most preferred sample was the gruel from malted sorghum fermented for 48 hours and sieved; followed by the one fermented for 48 hours but not sieved with no significant difference at $P<0.05$. There were no significant differences among samples fermented for 24 hours and 48 hours in terms of taste and aroma, but there was a significant difference from the samples fermented for 72 hours. There was no significant difference among the samples score in terms of mouthfeel. Malting improved the colour of the samples.

4.9 Fermentation studies of unmalted and malted sorghum *ogi*

Tables 4.9 and 4.10 shows the organisms isolated during the fermentation of *ogi* (from unmalted and malted sorghum) and morphological tests carried out on isolated organisms respectively. The water used for steeping raw and malted sorghum contained *Staphylococcus spp*. And the organisms disappeared after 72hours because of the pH values of the soaking water. *Klebsiella pneumonia* isolated from water used for steeping the sorghum samples persisted for 48hours of secondary fermentation. *Lactobacillus plantarium, Bacillus subtilis* and *Lactobacillus lactis* were isolated from the samples upon the completion of fermentation, In addition to this, *Citrobacter treundii* and *Enterobacter asburiae* were isolated from malted sorghum sample. Adegoke and Babalola (1988) , Achi andAkubo, (2000).reported that *Staphylococcus aureus* and *Bacillus subtilis* were isolated from the mashing water for maize and *Lactobacillus spp* from the end product. The results of this study agreed with those of Achi andAkubo, (2000).

While fermentation of Ogi is inherently exothermic and occurs under natural conditions, after a brief period of initial aerobic fermentation, anaerobic conditions usually prevail. Following that, volatiles, flavours, and acids start to show up in the finished products.which are possibly a result of the microorganisms' metabolic processes.

Table 4.8: Sensory evaluation of unmalted, malted, sieved and unsieved sorghum *ogi*

Sample	Colour	Aroma	Taste	mouth feel	Overall acc.
U	4.62^{c}	4.23^{d}	4.02^{d}	5.12^{b}	4.27^{c}
M	5.34^{bc}	5.46^{c}	5.97^{c}	5.23^{b}	5.46^{b}
U2N	5.32^{bc}	5.69^{ab}	6.51^{ab}	5.44^{b}	6.01^{ab}
U2S	5.44^{bc}	5.77^{ab}	6.62^{ab}	5.72^{a}	6.24^{ab}
M2N	5.56^{bc}	5.97^{ab}	6.84^{a}	5.52^{ab}	6.64^{a}
M2S	5.67^{bc}	6.03^{ab}	6.87^{a}	6.01^{a}	6.81^{a}
U4N	6.87^{a}	6.82^{a}	6.91^{a}	5.48^{ab}	6.72^{a}
U4S	6.98^{a}	6.88^{a}	7.01^{a}	5.81^{a}	6.97^{a}
M4N	7.01^{a}	7.22^{a}	7.31^{a}	5.99^{a}	7.05^{a}
M4S	7.34^{a}	7.46^{a}	7.66^{a}	6.12^{a}	7.34^{a}
U7N	5.43^{bc}	5.41^{c}	5.22^{c}	5.43^{ab}	5.51^{b}
U7S	5.48^{bc}	5.43^{c}	5.47^{c}	5.61^{a}	5.60^{ab}
M7N	5.76^{b}	5.58^{ab}	5.43^{c}	5.54^{ab}	5.67^{ab}
M7S	5.82^{b}	5.62^{ab}	5.71^{c}	5.79^{a}	5.81^{ab}

Means with the same superscript along the columns are not significantly different from each other

U- unmalted; M- malted; S-sieved, N-not sieved; 2=24hours fermentation; 4=48hours fermentation; 7= 72hours fermentation

Table 4.9: Frequency of distribution of isolates in unmalted and malted sorghum *ogi*

S/N	ORGANISM	WATER	UNMALTED				MALTED			
			12HRS	24HRS	48HRS	72HRS	12HRS	48HRS	24HRS	72HRS
1	*Micrococcus sp*	+	-	-	-	-	-	-	-	-
2	*Klebsiella pneumonia*	+	+	-	+	-	-	-	+	-
3	*Escherichia coli*	+	-	-	-	-	-	-	-	-
4	*Bacillus flexi*	-	+	-	-	-	-	-	-	-
5	*Lactococcus lactis*	-	+	-	-	+	+	-	-	-
6	*Bacillus megaterium*	-	+	-	-	+	-	-	-	-
7	*Staphylococcus aureus*	+	-	-	-	-	-	-	-	+
8	*Streptococcus species*	-	-	+	-	+	-	+	-	+
9	*Lactobacillus plantarum*	-	-	+	-	+	-	+	-	-
10	Bacillus subtilis	-	-	+	-	+	-	-	-	-
11	*Enterobacternimipressuralis*	-	-	-	-	+	-	-	-	-
12	*Citrobacterfreundii*	-	-	-	-	-	+	+	+	-
13	*Enterobacterasburiae*	-	-	-	-	-	+	-	+	-

KEY:+:Positive -: Negative

Table 4.10 Morphological and biochemical characterisation of isolates from sorghum ogi

GRAMS REACTION	SHAPE	MANNITOL	LACTOSE	GLUCOSE	MR	VP	INDOLE	CITRATE	STARCH	LIKELY ORGANISM
+	Cocci	A	AG	AG	-	-	+	+	-	*Micrococcus varians*
-	Rod	AG	AG	AG	-	+	-	+	+	*Klebsiellapneumoniae*
-	Rod	AG	AG	AG	+	-	+	+	+	*Escherichia coli*
+	Rod	AG	AG	AG	+	-	-	+	+	*Bacillus megaterium*
+	Cocci	AG	AG	AG	+	-	-	-	-	*Staphylococcus aureus*
-	Rod	AG	AG	AG	-	+	-	+	+	*Klebsiella pneumonia*
+	Rod	AG	AG	AG	+	-	-	+	+	*Bacillus flexus*
+	Cocci	AG	AG	AG	-	-	-	+	-	*Lactococcuslactis*
+	Cocci	AG	-	AG	+	-	-	+	+	*Streptococcus species*
+	Rod	AG	AG	-	-	-	-	+	-	*Lactobacillus plantarum*
+	Rod	AG	AG	AG	+	+	+	+	+	*Bacillus subtilis*
-	Rod	AG	AG	AG	-	+	-	+	-	*Klebsiella pneumonia*
+	Cocci	AG	-	AG	+	-	-	+	+	*Streptococcus species*
+	Cocci	AG	AG	AG	+	+	-	+	-	*Enterobacternimipressuralis*
+	Rod	AG	AG	AG	+	-	-	+	+	*Bacillus megaterium*
+	Rod	A	A	AG	-	-	-	+	-	*Citrobcterfreundii*
-	Rod	AG	AG	AG	+	-	-	+	-	*Enterobacterasburiae*
+	Cocci	AG	AG	AG	-	-	-	+	-	*Lactococcuslactis*
+	Cocci	AG	-	AG	+	-	-	+	+	*Streptococcus species*
+	Rod	AG	AG	-	-	-	-	+	-	*Lactobacillus plantarum*
-	Rod	AG	AG	AG	-	+	-	+	-	*Klebsiella pneumonia*
+	Rod	A	A	AG	-	-	-	+	-	*Citrobcterfreundii*
-	Rod	AG	AG	AG	+	-	-	+	-	*Enterobacterasburiae*
+	Cocci	AG	-	AG	+	-	-	+	+	*Streptococcus species*
+	Cocci	AG	AG	AG	+	-	-	-	-	*Staphylococcus aureus*

Key; A- acid production, G-gas production, + - positive, - - negative

Forty eight hours of secondary fermentation (souring) was taken as optimum period for fermentation of raw and malted sorghum. In this study, it was discovered that fermentation of malted grains was faster than unmalted grains; this may be due to the fact that most of the grain has been transformed into fermentable sugar making the fermentation to be faster.

4.10 pH and total titratable acidity of samples fermented with inoculums at different concentrations

pH and total titratable acidity of the samples fermented with inoculums are as shown in Tables 4.11 and 4.12 respectively. The pH values ranged from 3.51in sample inoculated with 0.1µL/500mL inoculums from burukutu to 4.22 in sample inoculated with 0.09 µL/500mL inoculums from palm wine while the total titratable acidity ranged from 0.23 gL^{-1} in sample inoculated with 0.08 µL/500mL inoculum from burukutu to 0.96 gL^{-1} in sample inoculated with 0.1µL/500mL inoculum from palm wine. The total titratable acidity of the samples decreases with increasing pH. 0.1µL/500mL was taken as the most appropriate concentrations with pH range of 3.51-3.80 and total titratable acidity range of 0.89 -0.98; these values fall within the optimum range for ogi production (*Bolaji et al.*, 2011).

Table 4.11: pH of samples fermented with inoculums at different concentrations (µL/500mL)

Samples	0.08	0.09	0.10	0.11	0.12
USF	4.65±0.02^{d}	4.73±0.01^{e}	4.01±0.01^{c}	4.42±0.01^{d}	4.36±0.22^{e}
SF	4.12±0.08^{b}	4.11±0.10^{b}	3.92±0.01^{b}	4.01±0.05^{bc}	4.06±0.03^{c}
UBFS	4.51±0.05^{d}	4.99±0.01^{ef}	3.84±0.02^{b}	3.99±0.03^{b}	3.92±0.06^{b}
BFS	4.13±0.05^{b}	4.10±0.01^{b}	3.51±0.03^{a}	3.75±0. 02^{a}	3.83±0.10^{a}
UPWFS	4.46±0.04^{cd}	4.52±0.02^{d}	3.96±0.04^{b}	3.92±0.04^{b}	4.03±0.01^{c}
PWFS	4.17±0.02^{bc}	4.22±0.14^{c}	3.60±0.05^{ab}	3.84±0.01^{a}	3.98±0.09^{b}
UYFS	4.33±0.01^{c}	4.03±0.11^{a}	3.98±0.10^{b}	4.10±0.06^{bc}	4.22±0.01^{d}
YFS	4.01±0.09^{a}	4.00±0.03^{a}	3.80±0.13^{b}	3.99±0.09^{b}	4.01±0.05^{c}

Values represent mean of three values ± standard deviation
Means with the same superscript along the columns are not significantly different from each other.

USF – Unmalted Sorghum Fermented Spontaneously
SF – Malted Sorghum Fermented spontaneously
UBFS – Unmalted Sorghum Fermented with burukutu
BFS – Malted Sorghum Fermented with burukutu
UPWFS – Unmalted Sorghum Fermented with palmwine
PWFS – Malted Sorghum fermented with palmwine
UYFS – Unmalted Sorghum fermented with yoghurt
YFS – Malted Sorghum fermented with yoghurt

Table 4.12 : Total titratable acidity (g/L) of samples fermented with inoculums at different concentrations (µL/500mL)

Samples	0.08	0.09	0.10	0.11	0.12
USF	0.12±0.01^{a}	0.11±0.03^{a}	0.68±0.03^{b}	0.22±0.01^{a}	0.25±0.03^{a}
SF	0.18±0.06^{b}	0.18±0.10^{b}	0.98±0.01^{cd}	0.24±0.03^{a}	0.23±0.0^{a}
UBFS	0.19±0.03^{b}	0.20±0.00^{b}	0.55±0.00^{a}	0.23±0.03^{a}	0.22±0.03^{a}
BFS	0.26±0.04^{c}	0.26±0.02^{c}	0.89±0.04^{c}	0.27±0. 02^{ab}	0.26±0.09^{a}
UPWFS	0.19±0.02^{b}	0.18±0.03^{b}	0.67±0.01^{b}	0.24±0.04^{a}	0.75±0.01^{b}
PWFS	0.24±0.01^{c}	0.24±0.11^{c}	0.96±0.06^{cd}	0.25±0.01^{a}	0.84±0.08^{c}
UYFS	0.18±0.01^{b}	0.20±0.14^{b}	0.64±0.10^{ab}	0.21±0.05^{a}	0.22±0.03^{a}
YFS	0.25±0.08^{c}	0.25±0.03^{c}	0.94±0.09^{cd}	0.26±0.07^{ab}	0.24±0.05^{a}

Values represent mean of three values ± standard deviation
Means with the same superscript along the columns are not significantly different from each other.

USF – Unmalted Sorghum Fermented Spontaneously
SF – Malted Sorghum Fermented spontaneously
UBFS – Unmalted Sorghum Fermented with burukutu
BFS – Malted Sorghum Fermented with burukutu
UPWFS – Unmalted Sorghum Fermented with palmwine
PWFS – Malted Sorghum fermented with palmwine
UYFS – Unmalted Sorghum fermented with yoghurt
YFS – Malted Sorghum fermented with yoghurt

4.11 The isolation and purification of DNA

Tables 4.13- 4.15 shows the organism isolated. One of the most frequent techniques used in molecular biology nowadays is the separation and purification of DNA from cells, which reflects a move from cell biology to molecular biology. Previously, strain level microbial identification of newly discovered microorganisms has been difficult. The techniques that has been used in isolating and identifying bacteria and fungi from its natural habitat and culturing in the laboratory involving further immunochemical or biochemical characterisation was the only method of characterizing microorganisms. From today's perspective, all of these methods are imprecise and inconclusive.

Polymerase Chain Reaction (PCR) amplification conducted on the DNA samples facilitated geometric multiplication of the DNA fragments in billion folds. ITs primers used in this work align with specific nucleotide sequences with the aid of the enzyme DNA polymerase at appropriate temperature in a PCR machine to make several copies of the DNA. The DNA polymerase then synthesises the final section of the DNA strand after the primers bind to the DNA fragments. The amount of DNA as analysed by gel electrophoresis increased significantly following PCR amplification.. The PCR products were purified to remove impurities such as proteins, Ribonucleic acid. Agarose gel electrophoresis employed the principle of electric field to separate the DNA fragments according to its size such that the smaller the DNA size, the faster it moved across the semi-permeable gel matrix.

TABLE 4.13: OCCURRENCE OF ORGANISMS IN BURUKUTU AT INTERVALS

ORGANISM	12HOURS	24 HOURS	36 HOURS	48 HOURS
L. brevis		+	+	+
B.subtilis			+	+
L fermentum		+	+	+
S. lactis				+
S. cerevisae		+	+	+
L planetarium		+	+	+
L.mesenteroides			+	+
L. lactus			+	+
S.epidermis	+		+	+
A.Orientalis				
EP. aerogenosa	+	+	+	+
W. cibaria	+	+		
L. delbrueckii				
M. lactus			+	
K. pneumonia				
B.megatarium				
B. cereus				
Enterococcus sp				+
M. luteus				

TABLE 4.14: OCCURRENCE OF ORGANISMS IN PALMWINE AT INTERVALS

ORGANISM	12 HOURS	24 HOURS	36 HOURS	48 HOURS
L. brevis				
B.subtilis			+	+
L fermentum		+	+	+
S. lactis				
S. cerevisae		+	+	+
S. aureus		+	+	+
C tropicalis				+
Micrococcus sp			+	+
L planetarium			+	+
L.mesenteroides			+	+
L. lactus				
S.epidermis				
A.Orientalis			+	+
P. aerogenosa				
B.anthracis	+			+
S. subtilis				+
M. luteus	+			+

TABLE 4.15 OCCURRENCE OF ORGANISMS IN YOGHURT AT INTERVALS

ORGANISMS	12 HOURS	24 HOURS	36 HOURS	48 HOURS
L. brevis				
B.subtilis				
L fermentum	+	+	+	+
S. lactis				
L planetarium	+	+	+	+
L.mesenteroides				
L. lactus				
S.epidermis				
A.Orientalis				
P. aerogenosa				
W. cibaria				
L. delbrueckii	+	+	+	+
L. licheniformis	+	+	+	+
L. rhaminosu	+	+	+	+
L. bulgaricus	+	+	+	+
S. thermophillus	+	+	+	+

4.12 Total Titratable Acidity (TTA) and pH

Total titratable acidity and pH Values are presented in Tables 4.16 and 4.17 respectively. As fermentation period increases, the total titratable acidity of the sorghum ogi samples increased, and the pH also dropped. The values of total titratable acidity which ranged between 0.20 -1.83 and 3.51- 5.72 respectively follows the trend of the results obtained by (Mbajiuka *et al.,* 2010) and Kolawole *et a.,l* (2013). Malting increased the total titratable acidity of the samples and this led to a corresponding decrease in pH.

The generation of organic acids led to an increase in the total titratable acidity and a decrease in pH, which promoted yeast growth and invertase activity which encourages souring of the samples, The hydrolysis of some complex organic compounds may also be the cause of the souring. the reduction in pH suggests longer shelf-life of the samples. Fermentation to a pH of 4 and below is desirable for products intended for children's complementary foods. This pH helps to preserve them during storage due to the high levels of acidity in which many microorganisms cannot tolerate (Nakneah *et al.,* 2010). This decrease in pH turns the fermented mixture sour and also improves the keeping quality because lower pH has an impact on microbial suppression.

Samples of sorghum fermented with palmwine had the highest value of 1.83 for total titratable acidity and lowest value of 3.5 for pH while the samples fermented spontaneously had the lowest for titratable acidity and highest for pH which were 0.30 and 4.38 respectively at the end of fermentation, thus induced fermentation encouraged the souring of the ogi samples and in the long run gave the product a longer shelf life as observed later in this work. Lactic Acid Bacteria (LAB), which can produce organic acids and subsequently lower the pH of the digestate, dominated a population of microorganisms that emerged during fermentation (Omemu, 2011). The pH of a food material determines whether microorganisms can thrive in such a product (Sadler and Murphy, 2010) while the TTA of a food product measures the extent to which it can alter the taste of that product (Sadler and Murphy, 2010).

Table 4.16: Total titratable acidity values of sorghum *ogi* produced by induced fermentation methods

Samples	0 hour	12 hours	24 hours	36 hours	48hours
USF	0.21±0.01^{c}	0.23±0.04^{d}	0.24±0.14^{e}	0.26±0.01^{f}	0.30±0.01^{d}
SF	0.26±0.02^{c}	0.28±0.02^{d}	0.36±0.02^{d}	0.31±0.02^{f}	0.39±0.02^{d}
UBFS	0.20±0.11^{c}	0.63±0.10^{bc}	0.73±0.09^{bc}	0.78±0.09^{d}	0.94±0.11^{c}
BFS	0.25±0.11^{c}	0.71±0.10^{b}	0.84±0.09^{b}	1.02±0.01^{b}	1.43±0.09^{b}
UPWFS	0.21±0.00^{c}	0.70±0.01^{b}	0.81±0.o8^{b}	0.95±0.01^{c}	1.06±0.03^{c}
PWFS	0.96±0.01^{a}	0.98±0.003^{a}	1.04±0.01^{a}	1.56±0.02^{a}	1.83±0.09^{a}
UYFS	0.20±0.10^{c}	0.56±0.01^{c}	0.62±0.02^{c}	0.68±0.03^{e}	0.74±0.43^{cd}
YFS	0.41±0.01^{b}	0.63±0.02^{bc}	0.72±0.02^{bc}	1.03±0.01^{b}	1.22±0.02^{bc}

Values represent mean of three values ± standard deviation
Means with the same superscript along the columns are not significantly different from each other.

USF – Unmalted Sorghum Fermented Spontaneously
SF – Malted Sorghum Fermented spontaneously
UBFS – Unmalted Sorghum Fermented with burukutu
BFS – Malted Sorghum Fermented with burukutu
UPWFS – Unmalted Sorghum Fermented with palmwine
PWFS – Malted Sorghum fermented with palmwine
UYFS – Unmalted Sorghum fermented with yoghurt
YFS – Malted Sorghum fermented with yoghurt

Table 4.17: pH of sorghum *ogi* produced by induced fermentation methods

Samples	0 hour	12 hours	24 hours	36 hours	48 hours
USF	5.65±0.01^{a}	5.63±0.01^{a}	4.96±0.01^{c}	4.42±0.01^{a}	4.38±0.22^{a}
SF	5.53±0.09^{b}	5.55±0.11^{a}	4.82±0.01^{d}	4.31±0.06^{b}	4.26±0.02^{ab}
UBFS	5.72±0.01^{a}	5.23±0.01^{b}	5.23±0.00^{a}	4.32±0.01^{b}	3.90±0.06^{bc}
BFS	5.68±0.04^{a}	5.01±0.01^{c}	5.01±0.04^{b}	4.04±0. 02^{d}	3.80±0.10^{cd}
UPWFS	4.62±0.02^{d}	4.02±0.02^{e}	4.06±0.02^{f}	3.92±0.04^{e}	3.67±0.01^{de}
PWFS	4.46±0.03^{e}	4.03±0.10^{e}	3.99±0.05^{g}	3.64±0.02^{g}	3.5±0.10^{e}
UYFS	4.83±0.01^{c}	4.31±0.11^{d}	4.20±0.10^{e}	4.1±0.06^{c}	4.01±0.01^{b}
YFS	4.36±0.08^{f}	4.02±0.02^{e}	4.06±0.13^{f}	3.80±0.07^{f}	3.90±0.05^{bcd}

Values represent mean of three values ± standard deviation
Means with the same superscript along the columns are not significantly different from each other.

USF – Unmalted Sorghum Fermented Spontaneously
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BFS – Malted Sorghum Fermented with burukutu
UPWFS – Unmalted Sorghum Fermented with palmwine
PWFS – Malted Sorghum fermented with palmwine
UYFS – Unmalted Sorghum fermented with yoghurt
YFS – Malted Sorghum fermented with yoghurt

4.13 Fermentation profile of dry milled sorghum ogi

Different microorganisms were obtained from *ogi* samples, morphological and biochemical properties were used to identify and classify them. The distribution of the strains observed during fermentation and their biochemical and morphological characters are as shown in Tables 4.18 and 4.19 for the different cultures. *Lactobacillus, Bacillus, Leuconostoc, Streptococcus* and *Saccharomyces* species were the predominant probiotics present in *ogi* produced by induced fermentation. Probiotics help in the production of digestive enzymes, vitamins and also stimulate the immune system Their presence also helped to improve the nutraceutical and functionality of *ogi* as compared to the ones allowed to ferment spontaneously (Chandrasekhar *et al.*, 2012).

Fermentation process was faster with inoculated substrate and with samples that were dry-milled before fermentation with formation of bubbles after 12 hours of fermentation. It has been observed that fermentation was faster while fermenting *burukutu* with induced microorganisms. Fermentation was also very fast in malted samples and this could be attributed to the fact that the grains have been converted to fermentable sugars. *Lactobacillus plantarium* and *Leuconostoc mesenteroides* were the prevalent lactic acid bacteria found in *ogi* fermented with palm wine, *Gluconobacter* and *Acetobacter* genera were the dominant acetic acid bacteria which were found after 36 hrs of fermentation, this is attributed to possible substantial alcohol levels at this stage of fermentation. *Lactobacillus plantarium, Corynebacterium and mycoderma* contributed to the flavour of the products, samples that were steeped prior to dry milling had a stronger aroma. Ouoba *et al.,(*2012) and Santiago-Urbina *et al.,* (2013) stated that lactic acid bacteria and acetic acid bacteria apart from being responsible for the aroma also contributed to the suppression of harmful bacteria and acidification. *Lactobacillus Staphylococcus, Streptococcus, Bacillus and Saccharomyces* were isolated from the substrates fermented by *burukutu* inoculum.

Sap of the palm tree inoculated into the fermenting substrate which was initially sweet (Naknean *et al.,* 2010; Santiago-Urbina *et al.,* 2013) is a fertile medium for the development of a wide variety of microorganisms,

TABLE 4.18: OCCURENCE OF ORGANISMS IN OGI AT 48 HOURS FERMENTATION

ORGANISMS	USF	SF	UBFS	BFS	UPWFS	PWFS	UYFS	YFS
L. brevis	+	+		+				
B.subtilis	+	+	+	+	+	+		
L fermentum	+	+	+	+	+	+	+	+
S. lactis	+	+		+				
S. cerevisae	+	+	+	+	+	+		
S. aureus		+	+	+	+	+		+
C tropicalis		+						
Micrococcus sp		+						
L planetarium	+		+	+	+	+	+	+
L.mesenteroides	+		+	+	+	+		
L. lactus	+		+	+				
S.epidermis		+	+	+				+
A.Orientalis			+	+				
P. aerogenosa			+	+				
W. cibaria			+	+				
L. delbrueckii							+	+
L. licheniformis		+					+	+
L. rhaminosu							+	+
L. bulgaricus							+	+
S. thermophillus							+	+
M. lactus			+	+	+	+		
B.anthracis					+	+		
S. subtilis			+		+	+		
B.megatarium		+						
B. cereus	+	+			+	+		
Enterococcus sp	+							
Corynebacterium						+	+	

USF – Unmalted Sorghum Fermented Spontaneously
SF – Malted Sorghum Fermented spontaneously
UBFS – Unmalted Sorghum Fermented with burukutu
BFS – Malted Sorghum Fermented with burukutu
UPWFS – Unmalted Sorghum Fermented with palmwine
PWFS – Malted Sorghum fermented with palmwine
UYFS – Unmalted Sorghum fermented with yoghurt
YFS – Malted Sorghum fermented with yoghurt

TABLE 4.19: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISATION OF ORGANISMS

Gram	Motility	Oxidase	Catalase	citrate	starch hydrolysis	M.R	V.P	Indole	Glucose	lactose	Fructose	Sucrose	Xylose	*Organism*
+	-	-	-	-	+	+	+	-	A/G	A/G	A/G	A/G	A/-	*L. planetarium*
+	-	+	-	-	+	+	+	-	A/G	A/G	A/-	A/G	A/G	*L.brevis*
+	+		+	+	+	-	+	-	A/-	A/-	A/-	A/-	A/-	*B.subtilis*
+	-	+	-	-	-	-	-	-	A/G	-/-	A/-	A/-	A/-	*Corynebacterium*
+	-	-	-	+	+	+	+	-	A/G	A/G	A/G	-/G	A/G	*L. fermentum*
+	+		+	-	+	+	-	+	A/-	A/-	A/-	A/G	A/-	*S. lactis*
+	-		+	+	+	-	-	+	A/-	A/-	A/-	A/-	A/-	*S.aureus*
+	+	+	-	-	+	+	-	-	A/G	-/-	A/G	A/-	A/-	*S.thermophillus*
+	-	-	-	-	+	+	+	-	A/G	A/-	A/G	A/G	A/G	*L.bulgaricus*
+	+	-	+	-	-	-	+	-	A/G	-/-	A/G	A/G	A/G	*L. mesenteroides*
+	-	-	+	+	-	+	-	-	A/G	-/-	-/-	-/-	A/-	*Micrococcus sp*
+	+	-	-	+	-	+	+	-	A/G	A/G	-/G	A/-	A/G	*Enterococcus*
+	+	+	+	-	+	+	+	-	A/G	-/-	A/G	-/-	-/-	*Bacillus cereus*
+	+	-	+	+	+	+	+	+	A/-G	A/G	A/G	A/G		*L.. licheniformis*
+	-	-	+	+	+	-	-	-	A/-	A/-	A/-	A/-	A/-	*B. megaterium*
-	+	-	+	+	-	-	+	-	A/-	A/G	A/-	A/-	A/-	*E. aerogens*
+	+	-	+	+	-	+	-	+	A/G	A/G	A/-	A/G	-/-	*Citrobacter*
-	+	-	+	+	+	-	+	-	A/G	-/-	A/-	A/G	A/G	*Enterobacter*
+										A/G				*S.epidermis*
+				+	-	-	-	-	A/G	A/G				*L. lactus*
+	+		+	+	+	-	+	-	A/-	A/-	A/-	A/-	A/-	*B. subtilis*

Key; A- acid production, G-gas production, + - positive, - - negative

The sweet substrate goes through spontaneous fermentation, which encourages the growth of yeasts and bacteria to change it into a range of metabolites, mostly ethanol, lactic acid and acetic acid (Ouoba *et al.,* 2012; Santiago-Urbina *et al.,* 2013).

Lactobacillus species produce lactic acid in the digestive tract, which is crucial for overall health. Some nutritional benefits of lactic acid bacteria include enhanced digestion of lactose, suppression of certain kinds of cancer and regulation of blood cholesterol levels (Chandrasekha *et al.,* 2012). Lactic acid bacteria that are extracted from fermented foods produced bacteriocins which are proteinaceous, antibacterial compounds (Holzapfel, 2002) that limits the emergence of pathogenic organisms such as *Bacillus cereus, Listeria monocytogenes, Clostridium dificile and Staphylococcus aureus* (Holzapfel, 2002). *Lactobacillus plantarum* and *Leuconostoc mesenteriodes* were the dominant lactic acid bacteria which give the gruel its sour taste and decreases pH during fermentation; they also prevent the development of undesirable microorganisms like *enterobacteria* through production of acid (Alcántara-Hernández *et al.,* 2010).

Lactobacillus plantarum in addition also have antimicrobial property, has hepatoprotective effect, anticholesterolaemic effect and immunostimulatory effect. *Lactobacillus acidophilus* is a probiotic bacterium mainly found in the small intestine of humans, where it secretes natural antibiotics called “lactocidin” and “acidophillin” Chandrasekha *et al.,* (2012). *Leuconostoc* increases the nutritive quality, produces characteristic flavor and also produces CO_2 that speedily lowers the pH and inhibits the production of unwanted microbes in foods (Ranjana *et al.,* 2020 Rhee *et al.*, 2011). *Bacillus* strains were also present in the fermenting substrates; they are usually harmless and has some antibiotic property, *Pediococcus* species help to maintain the digestive system's microbiota is in a healthy balance (Efremenkova *et al.,*2019; Sabina, 2014).

L.bulgaricus and S.thermophilus are yoghurt cultures and are known to destroy pathogens such as *Pseudomonas, E.coli, Staphylococcus and Shigella spp,* this is likely due to its ability to synthesize methanol acetone which is a powerful anti-pathogenic agent. *S.thermophilus* isolated from the ogi is also able to improve the nutritional value of foods by producing micro nutrients, (Efremenkova *et al.*, 2019 Sabina, 2014). Acetic acid bacteria of the genera *Gluconobacter* and *Acetobacter* were present in *ogi* inoculated with palm wine. The functions of acetic acid bacteria during palm wine

fermentation are related to acetic acid production and aroma (Santiago-Urbina and Ruíz-Terán, 2013). Addditionally, like lactic acid bacteria, acetic acid bacteria also contributes to the souring and inhibition of unfavourable organisms (Ouoba *et al.*, 2012). *Saccharomyces cerevisiae* is the major microbediscovered as being in charge of alcohol synthesis and odorant generation.

According to Arora *et al*. (2010), probiotic fermentation and germination are two potential mechanisms for boosting the nutritional value of food mixes made from coarse cereals.When it is realized that fermentation of indigenous foods with probiotic organisms is an approach that could lead to creation of products with better quality, therefore, the findings made in this study could be valuable in the development of novel non-dairy probiotic formulations.

4.14 Proximate Composition of sorghum *ogi* produced by induced fermentation methods

The proximate compositions of sorghum *ogi* processed by induced fermentation techniques are shown in Table 4.20. Malting resulted in a rise in protein, crude fibre and ash contents of the samples and also caused a breakdown of fat and carbohydrate. The protein content of the ogi samples was between 7.00 and 10.34%, with samples fermented with yoghurt having the highest value. Unmalted spontaneously fermented sorghum had the least value of 6.90 while malted spontaneously fermented yoghurt had 8.46. Malted samples had higher protein content than unmalted samples. Ijarotimi and Keshinro (2012) also noticed a substantial rise in protein content with sprouting. Induced fermentation brought about an increase in the protein content resulting from production of enzymic protein by the germinating seeds. The activity and growth of the lactic acid bacteria present during fermentation also caused a rise in the protein content of the ogi samples. This trend is consistent with the research of Afoakwa *et al*,(2004) who reported that Protein content and product functionality could both be enhanced by fermentation.. Increased protein in *ogi* produced by induced fermentation is an important observation that can be used to boost the nutritional intake of infants as well as adults and the convalescent. In this study, the problem of bulkiness was addressed by producing a less viscous *ogi* with more nutrients and

Table 4.20: Proximate Composition (%) of sorghum *ogi* produced by induced fermentation methods

	Protein	Fat	Fibre	Moisture	Ash	Carbohydrate
UR	7.41 ± 0.48^{b}	3.54 ± 0.26^{a}	2.88 ± 0.07^{cd}	9.47 ± 0.12^{e}	1.47 ± 0.09^{b}	74.75 ± 0.58^{e}
MR	8.42 ± 0.37^{a}	3.44 ± 0.45^{a}	3.09 ± 0.14^{a}	11.20 ± 0.05^{a}	1.73 ± 0.11^{a}	72.43 ± 0.19^{f}
USF	6.90 ± 0.06^{c}	2.61 ± 0.03^{b}	2.43 ± 0.01^{e}	10.07 ± 0.02^{d}	0.38 ± 0.02^{ef}	77.01 ± 0.08^{a}
SF	8.46 ± 0.06^{a}	2.56 ± 0.01^{b}	2.98 ± 0.01^{b}	10.36 ± 0.04^{b}	0.43 ± 0.02^{def}	75.81 ± 0.12^{cd}
UBFS	7.00 ± 0.03^{c}	2.51 ± 0.03^{b}	2.46 ± 0.01^{c}	10.05 ± 0.02^{d}	0.37 ± 0.01^{f}	77.07 ± 0.09^{a}
BFS	8.38 ± 0.01^{a}	2.48 ± 0.01^{b}	2.93 ± 0.03^{bc}	10.22 ± 0.03^{c}	0.42 ± 0.03^{def}	76.10 ± 0.01^{c}
UPWFS	7.11 ± 0.09^{bc}	2.62 ± 0.02^{b}	2.46 ± 0.02^{c}	10.11 ± 0.03^{d}	0.46 ± 0.01^{cde}	76.80 ± 0.08^{ab}
PWFS	8.71 ± 0.02^{a}	2.58 ± 0.01^{b}	2.83 ± 0.02^{d}	10.21 ± 0.07^{c}	0.53 ± 0.02^{c}	75.58 ± 0.01^{d}
UYFS	7.21 ± 0.01^{bc}	2.72 ± 0.01^{b}	2.45 ± 0.01^{e}	10.08 ± 0.01^{d}	0.41 ± 0.01^{def}	76.59 ± 0.03^{b}
YFS	10.34 ± 0.06^{a}	2.64 ± 0.01^{b}	2.93 ± 0.02^{bc}	10.25 ± 0.01^{c}	0.48 ± 0.01^{def}	75.61 ± 0.04^{d}

Values represent mean of three values ± standard deviation
Means with the same superscript along the columns are not significantly different from each other.
UR- Unmalted raw sorghum *MR – Malted raw sorghum*
USF – Unmalted Sorghum Fermented Spontaneously *SF – Malted Sorghum Fermented spontaneously*
UBFS – Unmalted Sorghum Fermented with burukutu *BFS – Malted Sorghum Fermented with burukutu*
UPWFS – Unmalted Sorghum Fermented with palmwine *PWFS – Malted Sorghum fermented with palmwine*
UYFS – Unmalted Sorghum fermented with yoghurt *YFS – Malted Sorghum fermented with yoghurt*

probiotics with possible therapeutic properties. Fermentation with starter cultures has been shown to have good potentials for enhancing the methionine and lysine levels of *ogi* (Banwo *et al*., 2022).

The fat content of the sample was in the range of 2.48 and 2.82% and samples fermented with palmwine had the highest values of 2.82 for the unmalted sample followed by samples fermented with yoghurt culture which had 2.72 %. The fat content of all the samples did not differ significantly ($P<0.05$). The decrease in fat content of the fermented samples was caused by the breakdown of fatty acids and glycerol by lipolytic organisms present in the sample during fermentation, this in turn led to an enhanced flavour, taste, texture, smell and shelf life. This was also observed by Ojoko and Bello, (2014).

The ash content of the samples varied from 0.37 and 0.53 % with malted sample fermented with palmwine culture having the highest value. Dry-milling prior to fermentation results in a lower ash content and could be attributed to the substrate's large surface area, which leads to mineral leaching. Fermentation resulted in a rise in the ash content of the samples. Since the amount of ash in a food sample is a measure of the total amount of minerals present therein, an increase in its concentration during microbial fermentation may be caused by the fermenting organisms' inadequate mineral utilisation during metabolism, the fermented sorghum samples' ash content agrees with the findings of Pikuda and Ilelaboye, (2013).

The crude fibre content of the fermented sorghum was between 2.43 and 2.98 %. Malting resulted in an increase in the fibre content of all the samples. The value for malted sorghum fermented spontaneously was the highest followed by sample fermented with yoghurt inoculum. Fermentation of milled sorghum led to a decline in the crude fibre content of the fermented sorghum, the reduction could be brought about by the lactic acid bacteria's use of the fibre as a carbon source during fermentation, which breaks down the fibre enzymatically and turns the product into microbial biomass, lowering the food's fibre content. Ojoko and Bello, (2014) also observed a decrease in fibre with fermentation but an increase with malting after fermentation of millet-soybean blends.

The carbohydrate values of sorghum ogi were between 75.58 and 77.07%. Samples of sorghum dry-milled before fermentation had a higher carbohydrate values but other nutrients were lower because nutrients are leached in water due to the larger surface area represented by the powdered granules. Pikuda and Ilelaboye, (2013) also observed a higher carbohydrate content in steeped powdered grains than in steeped whole grains but a reduction in other nutrients in steeped powdered grains, this decrease was made up for in this work by the activities of the microorganisms in starter cultures used for fermentation.

Malting gave rise to a reduction in carbohydrate content of the sorghum, This decrease in carbohydrate content was probably brought on by the fermenting lactic acid bacteria using some sugars for development and other metabolic processes.The reduction in carbohydrate contents of fermented malted sorghum noticed in this study are consistent with the findings of Tamilselvan and Kushwaha, (2020) that carbohydrate contents reduction during fermentation is caused by the activities of fermenting microbes. The reduction in carbohydrate levels can also be related to bacteria that ferment carbohydrates into glucose. which utilizes the glucose as energy source. Anyiam *et al*.,(2022), also reported a decrease in the amount of carbohydrates present when cassava products were fermented.

4.15 Antinutrients (mg/100g) in sorghum *ogi* produced by induced fermentation methods

Results for phytate, tannin and oxalate contents of *ogi* prepared by induced fermentation were as presented in Table 4.21. Phytate content of the samples was between 0.08 mg/100g to 0.23mg/100g; tannin from 0.08 to 0.27mg/100g and oxalate from 0.99 to 1.36mg/100g. Ogi from milled grains generally contained lesser anti nutrients. Fermentation and malting generally gave rise to reduction in anti -nutrients with reduction of oxalate being more pronounced in samples whose fermentation processes were induced than spontaneously fermented samples, this observation is consistent with Sanni *et al., (*2013) who noticed that the mode of fermentation and species of organisms play a vital role in fermentation process.

Table 4.21: Antinutrients (mg/100g) in sorghum *ogi* produced by induced fermentation methods

	Phytate	Tannin	Oxalate
USF	0.23 ± 0.01^{c}	0.27 ± 0.01^{c}	1.36 ± 0.04^{c}
SF	0.17 ± 0.01^{e}	0.16 ± 0.00^{def}	1.01 ± 0.01^{fg}
UBFS	0.21 ± 0.01^{d}	0.23 ± 0.01^{cd}	1.34 ± 0.01^{c}
BFS	0.13 ± 0.00^{f}	0.16 ± 0.01^{def}	1.03 ± 0.02^{f}
UPWFS	0.18 ± 0.01^{e}	0.23 ± 0.01^{cde}	1.25 ± 0.02^{d}
PWFS	0.09 ± 0.01^{s}	0.11 ± 0.02^{f}	1.02 ± 0.01^{f}
UYFS	0.17 ± 0.01^{e}	0.20 ± 0.01^{cde}	1.22 ± 0.01^{e}
YFS	0.08 ± 0.01^{h}	0.08 ± 0.014^{ef}	0.99 ± 0.01^{s}

Values represent mean of three values ± standard deviation
Means with the same superscript along the columns are not significantly different from each other.
USF – Unmalted Sorghum Fermented Spontaneously
SF – Malted Sorghum Fermented spontaneously
UBFS – Unmalted Sorghum Fermented with burukutu
BFS – Malted Sorghum Fermented with burukutu
UPWFS – Unmalted Sorghum Fermented with palmwine
PWFS – Malted Sorghum fermented with palmwine
UYFS – Unmalted Sorghum fermented with yoghurt
YFS – Malted Sorghum fermented with yoghurt

Phytate combines with zinc, iron, calcium and magnesium to produce complexes that reduce their availability. Protein is bound and precipitated by tannins, which are naturally occurring plant polyphenols. This prevents protein from being absorbed and digested. In this study, fermentation reduced these anti nutrients and thereby increasing their digestion and absorption. Elifiato *et al.,* (2014) also observed a reduction in anti- nutrients with fermentation.

4.16 Loosed Bulk Density, Packed Bulk Density. Hausner ratio and Carr index.

Table 4.22 showed the properties of ogi powder in terms of loose bulk density, packed bulk density and the flow behaviour. The loose bulk density was in the range 0.44 and 0.62g/mL and packed bulk density from between 0.58 and 0.80g/mL. From the results obtained in this study, o*gi* samples processed by induced fermentation had a lower packed bulk density as compared to samples allowed to ferment spontaneously except the samples fermented with *burukutu* which had a value of 0.72 and 0.80g/m respectively for unmalted and malted samples, the values of bulk densities obtained in this work was similar to the one obtained by *Charles et al.,* (2016).

Bulk density is a measurement of a product's weight that indicates how much packaging material is needed, making it a crucial consideration in the preparation, handling, packaging, storage, and distribution of food. It is a very crucial index in specification of products obtained from drying or size reduction operations (Ojo and Enuijuighua, 2016). High bulk density implies greater compactness of the particles (Falade and Olugbuyi, 2010). In general, high bulk density is required for easier dispersibility and reduction in paste thickness. Malting increases packed bulk densities of samples and This is advantageous since it aids in reducing paste thickness, which is crucial for infant and convalescents nutrition.

4.17 Pasting Properties

The pasting properties of *ogi* samples examined in this study are shown Table 4.23. The stiffness of the starch granules determines the paste characteristics, which in turn impacts the granules' capacity to swell and the quantity of amylase leaching into the solution. Fermentation and malting significantly ($P < 0.05$) affected the pasting properties of *ogi*.

Table 4.22: Loosed Bulk Density, Packed Bulk Density. Hausner ratio and Carr index of sorghum *ogi* produced by induced fermentation

	LBD	PBD	HR	CI
UR	0.53 ± 0.00^{b}	0.74 ± 0.00^{cd}	1.41 ± 0.00^{a}	28.95 ± 0.00^{a}
MR	0.54 ± 0.00^{bc}	0.76 ± 0.01^{bc}	1.40 ± 0.05^{ab}	28.36 ± 0.00^{ab}
USF	0.50 ± 0.02de	0.69 ± 0.01^{ef}	1.39 ± 0.07^{ab}	28.05 ± 3.04^{ab}
SF	0.59 ± 0.02^{a}	0.77 ± 0.03^{b}	1.31 ± 0.09^{bc}	23.43 ± 5.20^{bc}
UBFS	0.51 ± 0.00^{cd}	0.72 ± 0.03^{de}	1.40 ± 0.05^{ab}	28.51 ± 2.62^{ab}
BFS	0.62 ± 0.03^{a}	0.80 ± 0.00^{a}	1.33 ± 0.05^{abc}	24.55 ± 2.84^{abc}
UPWFS	0.53 ± 0.02^{bc}	0.71 ± 0.01^{e}	1.36 ± 0.06^{abc}	28.26 ± 3.26^{abc}
PWFS	0.49 ± 0.01^{e}	0.58 ± 0.01^{f}	1.39 ± 0.03^{ab}	28.04 ± 1.84^{ab}
UYFS	0.44 ± 0.00^{f}	0.59 ± 0.01^{g}	1.29 ± 0.01^{c}	21.68 ± 1.00^{c}
YFS	0.53 ± 0.00^{bc}	0.70 ± 0.01^{ef}	1.34 ± 0.02^{abc}	25.11 ± 1.16^{abc}

Values represent mean of three values ± standard deviation

Means with the same superscript along the columns are not significantly different from each other.

UR- Unmalted raw sorghum

MR – Malted raw sorghum

USF – Unmalted Sorghum Fermented Spontaneously

SF– Malted Sorghum Fermented spontaneously

UBFS – Unmalted Sorghum Fermented with burukutu

BFS – Malted Sorghum Fermented with burukutu

UPWFS – Unmalted Sorghum Fermented with palmwine

PWFS – Malted Sorghum fermented with palmwine

UYFS – Unmalted Sorghum fermented with yoghurt

YFS – Malted Sorghum fermented with yoghurt

LBD- Loosed bulk density

PBD- Packed bulk density

HR Hausner ratio

CI- Carr index

Table 4.23: Pasting properties of sorghum ogi prepared by induced fermentation

Sample	Peak viscosity(RVU)	Holding strength (RVU)	Breakdown viscosity (RVU)	Final viscosity (RVU)	Set back viscosity (RVU)	Peak time (min)	Pasting temperature (^{0}C)
UR	56.06 ± 6.6^{f}	55.50 ± 2.02^{f}	1.08 ± 0.83^{c}	123.83 ± 0.75^{b}	68.92 ± 1.00^{a}	5.18 ± 0.27^{d}	84.00 ± 0.75^{e}
MR	3.47 ± 0.05^{h}	1.33 ± 0.08^{h}	2.14 ± 0.13^{c}	2.42 ± 0.00^{f}	1.08 ± 0.08^{h}	3.64 ± 0.04^{e}	Error
USF	126.83 ± 2.59^{a}	93.61 ± 0.52^{a}	8.13 ± 1.05^{b}	133.30 ± 0.32^{a}	46.99 ± 0.13^{cd}	6.25 ± 0.11^{b}	84.47 ± 0.71^{de}
SF	103.71 ± 0.35^{b}	82.46 ± 0.47^{b}	7.98 ± 1.95^{b}	106.22 ± 0.81^{d}	50.09 ± 0.07^{bc}	5.77 ± 0.15^{c}	85.57 ± 0.45^{d}
UBFS	91.20 ± 0.73^{c}	82.63 ± 3.54^{b}	8.66 ± 2.83^{b}	133.03 ± 2.91^{a}	50.30 ± 0.64^{bc}	5.96 ± 0.10^{c}	87.22 ± 0.07^{c}
BFS	84.24 ± 1.08^{de}	68.44 ± 1.24^{b}	8.08 ± 1.08^{a}	124.16 ± 0.50^{b}	54.72 ± 0.53^{b}	5.79 ± 0.05^{c}	87.85 ± 0.09^{bc}
UPWFS	80.28 ± 10.22^{de}	72.14 ± 5.22^{d}	8.14 ± 8.54^{a}	116.19 ± 10.93^{c}	44.06 ± 6.18^{de}	6.69 ± 0.30^{a}	88.73 ± 1.97^{b}
PWFS	86.53 ± 3.04^{cd}	77.35 ± 1.16^{c}	8.58 ± 3.00^{b}	129.44 ± 5.54^{ab}	51.50 ±5.50^{bc}	5.92 ± 0.02^{c}	88.98 ± 0.30^{b}
UYFS	78.36 ± 1.25^{e}	72.78 ±0.67^{d}	5.58 ± 0.58^{bc}	113.50 ± 1.50^{c}	40.67 ±0.83^{e}	5.93 ± 0.07^{c}	88.23 ± 0.42^{bc}
YFS	67.47 ± 0.05^{g}	32.19 ± 0.63^{a}	5.11 ± 0.54^{bc}	60.78 ± 0.63^{e}	28.42 ± 0.83^{f}	6.03 ± 0.03^{bc}	93.77 ± 0.12^{a}

Values represent mean of three values ± standard deviation

Means with the same superscript along the columns are not significantly different from each other.

UR- Unmalted raw sorghum *MR – Malted raw sorghum*

USF – Unmalted Sorghum Fermented Spontaneously *SF– Malted Sorghum Fermented spontaneously*

UBFS – Unmalted Sorghum Fermented with burukutu *BFS – Malted Sorghum Fermented with burukutu*

UPWFS – Unmalted Sorghum Fermented with palmwine *PWFS – Malted Sorghum fermented with palmwine*

UYFS – Unmalted Sorghum fermented with yoghurt *YFS – Malted Sorghum fermented with yoghurt*

Peak viscosities of samples of sorghum *ogi* ranged from 67.47 RVU to 126.83 RVU with unmalted, spontaneously fermented sample showing the highest peak viscosity while malted sorghum fermented with yoghurt had the lowest value. Peak viscosity measures the strength of the pastes as a result of gelatinization during food processing and a high peak viscosity reflects a higher thickening ability of the starch, from the results obtained in this work, malted samples had a lower peak viscosity than unmalted ones, this is because malting leads to production of weaker pastes and less viscous liquids.

The holding strength of samples of sorghum *ogi* examined in this work ranged from 32. 19 to 93. 61 RVU. The sample that was dry milled prior to fermentation and allowed to ferment spontaneously had the highest holding strength as the holding strength of other samples was reduced with malting. The holding strength of sorghum *ogi* samples were reduced with induced fermentation compared to when the samples were allowed to ferment spontaneously with the malted sample fermented with yoghurt culture having the lowest.

Break down viscosities of samples of sorghum fermented with yoghurt culture were significantly lower ($P< 0.05$) than others with a value of 5.11and 5.58 RVU for malted and unmalted samples respectively, suggesting possible formation of complexes with starch component of the sorghum samples and the culture (*Lactobacillus thermophillus and Lactobacillus bulgaricus*). (Osungbaro *et al.,* (2010), attributed the interaction of lipids with starch molecules for the breakdown viscosity reduction, which promotes resistance to hydrothermal partitioning during gelatinization.The holding strength and breakdown viscosities are indicators of the ability of the samples to remain intact when exposed to constant high temperature for a long time and the ability to resist decomposition during cooking

Final viscosities of the sorghum *ogi* samples ranged between 60.78RVU and 133.30RVU malting generally reduced the final viscosities of the sorghum *ogi* samples and samples fermented with yoghurt culture had significantly ($P< 0.05$) lower final viscosity when compared with others. Final viscosity is an indicator of stability of cooked sample.

Set back viscosity is an indication of retrogradation of cooked sample (Ojo and Enurghia, 2016) and it ranged from 28.42RVU to 54.72 RVU for the sorghum *ogi* samples examined in this study. From the results obtained, malting generally increased the set back viscosity of sorghum *ogi* samples which implied that the malted samples had a tendency to retrograde faster than the unmalted samples.

Pasting temperature of sorghum *ogi* samples varied between 84.47^{o}C to 93.77^{o}C. The results of this study suggested that malting increased the pasting temperature of samples. Pasting temperature is a measurement of the minimum temperature needed for cooking. (Ladunni *et al.,* 2016). Of all the samples examined in this study, samples fermented with yoghurt starter culture had higher pasting temperature. It serves as a marker for the temperature at which the viscosity of flour begins to increase while cooking.and gives a clue on the energy cost required for cooking. Malted sorghum fermented with yoghurt starter culture had relatively higher pasting temp which is responsible for its lower peak viscosity. The pasting temperature of malted raw sorghum was undetectable in this study and implies it is not able to form a paste within the scope of the experiment. Peak time of malted sample fermented spontaneously was the lowest with 5.77 minutes while the unmalted sample fermented with palm wine had the highest value of 6.69 minutes.

4.18 Colour Determination

The colour parameters of *ogi* powder evaluated in relation to the CIF tristimulus colour parameters (L*, a,* and b*) were presented in Table 4.24. The L* values of the samples ranged between 51.27 to 65.66 where the malted unfermented (raw) sample had the highest value and the unmalted spontaneously fermented sample had the lowest value of 65.66 and51.27 respectively. Malting increased the lightness index of all the samples of sorghum ogi, as a result of tannin reduction with malting, tannin imparts reddish to brownish colour on sorghum. Malting is one of the ways of reducing tannin content of ogi and hence increase its lightness. The introduction of cultures for controlled fermentation also increased the lightness of ogi and this had a desirable influence on consumer acceptability of the product. The highest l* values associated with unfermented samples indicated that fermentation and drying had tendency of reducing lightness of ogi as observed also by Costa *et al.,* 2011.

The a* value measures the degree of redness and greenness and the values for ogi ranged from +13.27 to +10.08 These values indicated that the samples tended towards red than green. For samples allowed to ferment spontaneously, the unmalted grains had a red value of 12.28 while the malted grains had a value of 10.12, this reduction in redness with malting was also observed in samples fermented with palm wine and *burukutu*, suggesting that malting reduces the redness value of *ogi*.

The b* value is an indication of the degree of yellowness or blueness in a material Colour of foods affects consumer acceptability and purchasing decision directly (Costa *et al.,2011*) as consumers may have a bad perception of quality if the colour differs from what is expected.

Table 4.24: Colour parameters of sorghum *ogi* produced by induced fermentation

SAMPLE	l*	a*	b*
UR	62.51±0.08^{abc}	10.08±0.02^{gh}	13.52±0.01^{d}
MR	65.66±0.12^{a}	10.53±0.06^{g}	11.35±0.02^{e}
USF	51.27±0.01^{d}	12.28±0.03^{d}	16.01±0.01^{c}
SF	57.68±1.12^{c}	10.12±0.01^{h}	11.35±0.02^{e}
UBFS	62.55±0.02^{abc}	13.27±0.03^{a}	18.79±0.01^{a}
BFS	58.73±0.13^{bc}	10.95±0.01^{e}	13.66±0.03^{d}
UPWFS	59.63±0.05^{bc}	12.72±0.02^{b}	18.21±0.55^{b}
PWFS	60.27±0.10^{bc}	10.75±0.02^{f}	13.50±0.01^{d}
UYFS	60.23±0.04^{bc}	12.40±0.02^{c}	17.95±0.02^{b}
YFS	63.17±5.64^{ab}	12.62±0.21^{b}	17.90±0.02^{b}

Values represent mean of three values ± standard deviation

Means with the same superscript along the columns are not significantly different from each other.

UR- Unmalted raw sorghum

MR – Malted raw sorghum

USF – Unmalted Sorghum Fermented Spontaneously

SF – Malted Sorghum Fermented spontaneously

UBFS – Unmalted Sorghum Fermented with burukutu

BFS – Malted Sorghum Fermented with burukutu

UPWFS – Unmalted Sorghum Fermented with palmwine

PWFS - Malted Sorghum fermented with palmwine

UY FS– Unmalted Sorghum fermented with yoghurt

YFS – Malted Sorghum fermented with yoghurt

4.25: Invitro Protein digestibility of sorghum ogi samples produced by induced fermentation

Sample	IVPD (%)
UR	46.65 ± 1.23^{e}
MR	66.68 ± 0.35^{d}
USF	84.30 ± 0.63^{c}
SF	86.25 ± 0.15^{abc}
UBFS	85.79 ± 1.17^{bc}
BFS	86.41 ± 0.27^{ab}
UPWFS	87.45 ± 0.82^{ab}
PWFS	88.05 ± 2.03^{a}
UYFS	86.05 ± 2.06^{abc}
YFS	88.12 ± 0.11^{a}

Values represent mean of three values ± standard deviation

Means with the same superscript along the columns are not significantly different from each other.

UR- Unmalted raw sorghum

MR – Malted raw sorghum

USF – Unmalted Sorghum Fermented Spontaneously

SF– Malted Sorghum Fermented spontaneously

UBFS – Unmalted Sorghum Fermented with burukutu

BFS – Malted Sorghum Fermented with burukutu

UPWFS – Unmalted Sorghum Fermented with palmwine

PWFS- Malted Sorghum fermented with palmwine

UYFS – Unmalted Sorghum fermented with yoghurt

YFS – Malted Sorghum fermented with yoghurt

4.19 Sensory attributes of sorghum ogi produced by induced fermentation

Table 4.26 showed that the average scores for sensory qualities of *ogi* gruel produced from sorghum fermented with palmwine and processed by dry milling before fermentation was the most preferred by the panelists while ogi that was fermented spontaneously was the least. There were no significant differences ($p < 0.05$) between the scores in terms of overall acceptability of the samples. For dry milled samples, the inclusion of starter culture helped to boost the sensory scores of all the attributes, this implies that induced fermentation improved the acceptability of ogi besides improving the nutritional value.

The scores for colour ranged from 4.62 to 7.13. Samples whose fermentation were induced had higher scores than samples that was fermented naturally, malting also improved the colour of the ogi samples, this is evident with higher scores obtained for malted samples than unmalted samples. Malted sample fermented with palm wine had the highest value of 7.13 in terms of colour.

The sensory score for taste of the samples ranged from 4.02 to 7.02, malted sample fermented with palm wine had the highest value as a result of lactic acid and acetic acid bacteria responsible for the taste of the *ogi*. Malting generally improved the taste of the *ogi.*

The scores for mouth feel was relatively low compared with other attributes, this is because the samples were not sieved but the values obtained but were still within acceptable range. The scores for the attributes except aroma and mouth feel was higher in samples that were first dry milled before fermentation. Otitodun (2009) also observed that ogi gruels prepared by dry milling is acceptable to the consumers.

For all the attributes, dry milled samples were preferred to ogi processed by the conventional wet milling method, this is because wet milled ogi has a stronger taste and aroma which is not always acceptable to the consumers, and wet milling also increased the tartness of ogi.

Table 4.26: Sensory attributes of sorghum ogi processed by induced fermentation

	Colour	Aroma	Taste	Mouthfeel	Overall acceptability
UR	4.62^{c}	4.23^{c}	4.02^{c}	5.12^{a}	4.27^{c}
MR	5.34^{b}	5.46^{b}	5.97^{b}	5.23^{a}	5.46^{b}
USF	5.71^{b}	5.81^{b}	6.34^{a}	5.13^{a}	6.22^{a}
MSF	5.92^{b}	5.93^{b}	6.46^{a}	5.24^{a}	6.43^{a}
UBFS	6.14^{ab}	6.14^{ab}	6.42^{a}	5.31^{a}	6.68^{a}
BFS	6.22^{ab}	6.22^{ab}	6.56^{a}	5.40^{a}	6.82^{a}
UPWFS	6.92^{a}	6.43^{a}	6.91^{a}	5.42^{a}	7.21^{a}
PWFS	7.13^{a}	6.62^{a}	7.02^{a}	5.51^{a}	7.28^{a}
UYFS	6.65^{ab}	6.98^{a}	6.61^{a}	5.43^{a}	6.81^{a}
YFS	6.74^{a}	6.74^{a}	6.70^{a}	5.46^{a}	6.78^{a}

Values represent mean of three values ± standard deviation

Means with the same superscript along the columns are not significantly different from each other.

UR- Unmalted raw sorghum

MR – Malted raw sorghum

USF – Unmalted Sorghum Fermented Spontaneously

SF – Malted Sorghum Fermented spontaneously

UBFS – Unmalted Sorghum Fermented with burukutu

BFS – Malted Sorghum Fermented with burukutu

UPWFS – Unmalted Sorghum Fermented with palmwine

PWFS – Malted Sorghum fermented with palmwine

UYFS – Unmalted Sorghum fermented with yoghurt

YFS – Malted Sorghum fermented with yoghurt

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

Ogi was processed using conventional wet milling method from unmalted and malted sorghum with varied fermentation periods of between 24 and 72 hours at a temperature of 48^{o}C with and without sieving as preliminary experiments to determine the combined effect of malting, sieving and fermentation periods on some quality attributes of the processed ogi. Ogi was also processed based on results of the preliminary experiments by dry milling prior to fermentation and introduction of inocula from palm wine, burukutu and yoghurt to determine the effect of using milled sorghum and induced fermentation on the physical, chemical, functional, pasting and sensory properties as well as in-vitro protein digestibility of the samples.

Fermentation for 48 hours gave the optimum result at the preliminary stage and was used as basis for further experiments. Ogi produced from unmalted-whole sorghum that was allowed to ferment spontaneously for 72 hours and not sieved had the lowest overall acceptability while the malted ogi fermented for 48 hours spontaneously had the highest score, the protein content of sieved samples was lower than that of the unsieved samples. Malting and not sieving retained the protein and mineral contents of the samples and reduced the antinutrients present in the samples. Protein digestibility of the samples were increased by malting and sieving and the colour, sensory and pasting properties of the samples were also affected. Induced fermentation improved the physical, functional, pasting and microbial properties of sorghum ogi.

5.2 Conclusion

In this research, physical, chemical and microbial modification of the grains and fermentation processes produced ogi with improved nutritional quality.The consequence of malting, sieving, processing methods and induced fermentation on the physical,

chemical, sensory, pasting, digestibility and microbial properties of *ogi* were determined. From the results, it could be concluded that:

i. At the preliminary stage of the experiment, the protein content of unsieved sorghum ogi that was fermented for 48 hours was the highest with malting. This might be due to release of nitrogen that has been stored which helps the grains to sprout.The protein content of the sieved samples were lesser when compared with unsieved samples, this is because much of the protein is concentrated in the bran.

ii. Fermentation was also found to be optimum at 48 hours at 48^{o}C secondary fermentation for all the parameters evaluated.

iii. Malting (germination for 72 hours, drying for 48^{o}C for 24 hours) and fermentation (48 hours) lowered the fat content and this could be caused by metabollic reactions taking place during the processes.

iv. Sieving of the samples significantly reduced the fibre content of the samples.

v. The mineral content of unsieved samples was higher for all the minerals evaluated, the same trend was also observed in malted samples. For samples produced by dry-milling method with induced organisms, because of the synthesis of enzymic proteins, the activities of lactic acid bacteria increased the protein content of the food. Increasing protein content with induced fermentation is beneficial for improving the dietary intake of infants, adults, and convalescents, who are the primary consumers of ogi.

vi. Malting and fermentation significantly reduced the antinutrients (tannin, phytate and oxalate) present in raw sorghum. The occurence of some grain modifications led to decrease in antinutrient levels during malting. The antinutrients were reduced by sieving because they are more concentrated in the pericarp which is being sieved out. Ogi produced from powdered grains contained lesser antinutrients than the ones produced by the wet milling method. There was pronounced phytate reduction in ogi with induced fermentation.

vii. Protein digestibility was higher in malted and sieved samples. This is due to the absence of outer layers of the grains which usually interfere with digestibility.

Malting had a positive effect on malted samples and this will in the long run negate the effect of cooking on digestibility. Samples that were dry milled prior to fermentation had higher protein digestibility. Induced fermentation also had a positive effect on digestibility. Malted sample whose fermentation was induced by yoghurt culture had the highest value for digestibility.

viii. Malted samples were lighter in colour as shown by the higher l^* and b^* values and lower a^* values. Malting increased the lightness index of all the samples due to tannin reduction with malting. The controlled fermentation also increased the lightness of the ogi samples, which had a positive impact on the samples' consumer acceptability.

ix. Fermentation and malting significantly influenced the pasting properties. Malted samples had lower viscosities.This will in turn give rise to a lower thickening power and a less dense paste. Malting also reduced the final viscosity values which indicate stability of cooked samples. Malted samples had higher set back viscosities; this implies that they have the tendency to retrograde faster.

x. The predominant probiotics present in *ogi* produced by induced fermentation were *Lactobacillus, Leuconostoc, Bacillus, Streptococcus* and *Saccharomyces* species. Fermentation was faster with inoculated substrate with formation of bubbles after 12 hours of fermentation. Fermentation was also very fast in malted samples and this could be attributed to the fact that the grains have been converted to fermentable sugars. The prevalent lactic acid bacteria found in *ogi* fermented with palm wine were *Lactobacillus plantarium* and *Leuconostoc mesenteroides. Lactobacillus, Staphylococcus, Streptococcus, Bacillus and Saccharomyces* were isolated from the substrates fermented by *burukutu* inoculum. *S.thermophilus* isolated from the ogi can also boost the nutritional value of foods by producing micro nutrients.

The results from this research work may be useful in the development of novel non-dairy probiotic formulations.

5.3 Recommendations

The following recommendations were drawn from the study:

i. The utilisation of starter cultures during the fermentation of sorghum ogi should be encouraged asthis will enhance its processing. Mass production of sorghum should be encouraged since it can adapt to drought and other adverse physical conditions during planting and growing on the field.
ii. It is also recommended that the practice of sieving of ogi after milling should be discouraged as nutrients are lost in the spent grains after sieving.
iii. The optimum secondary fermentation time recommended for ogi is 48 hours to conserve the flavor and the nutrients.
iv. A more convenient, faster and better method of ogi processing that involves dry-milling which helped in improving the nutritional and keeping quality of ogi should be encouraged.

5.4 Contributions to Knowledge

This study has made the following contributions to knowledge:

i. The study identified the effect of malting and sieving on the nutritional, functional and sensory properties of sorghum ogi, it has been established that malting sorghum without sieving it will give a better quality *ogi*.
ii. This study has also established that 48 hours is the optimum period of secondary fermentation for sorghum *ogi.*
iii. Organisms involved in the induced fermentation of *ogi* with *burukutu,* palmwine and yoghurt inoculums have been identified.
iv. This research has identified a faster and better way of processing sorghum *ogi* which involves dry milling and induced fermentation; this will give *ogi* improved digestibility, better nutritional, functional and microbial properties.

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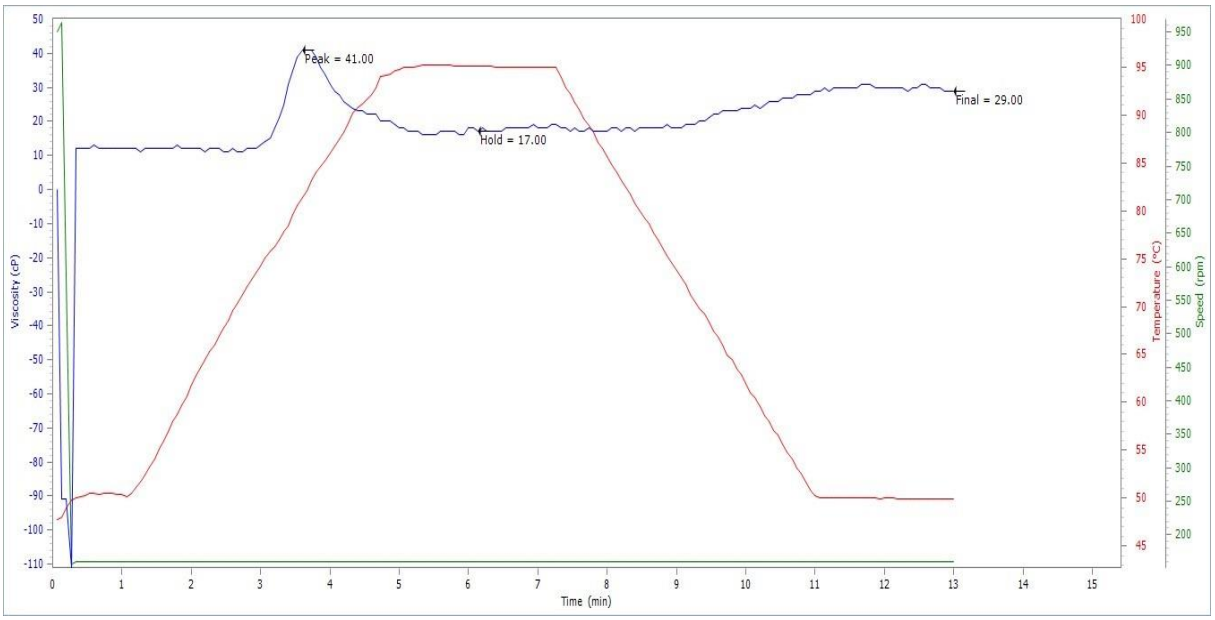
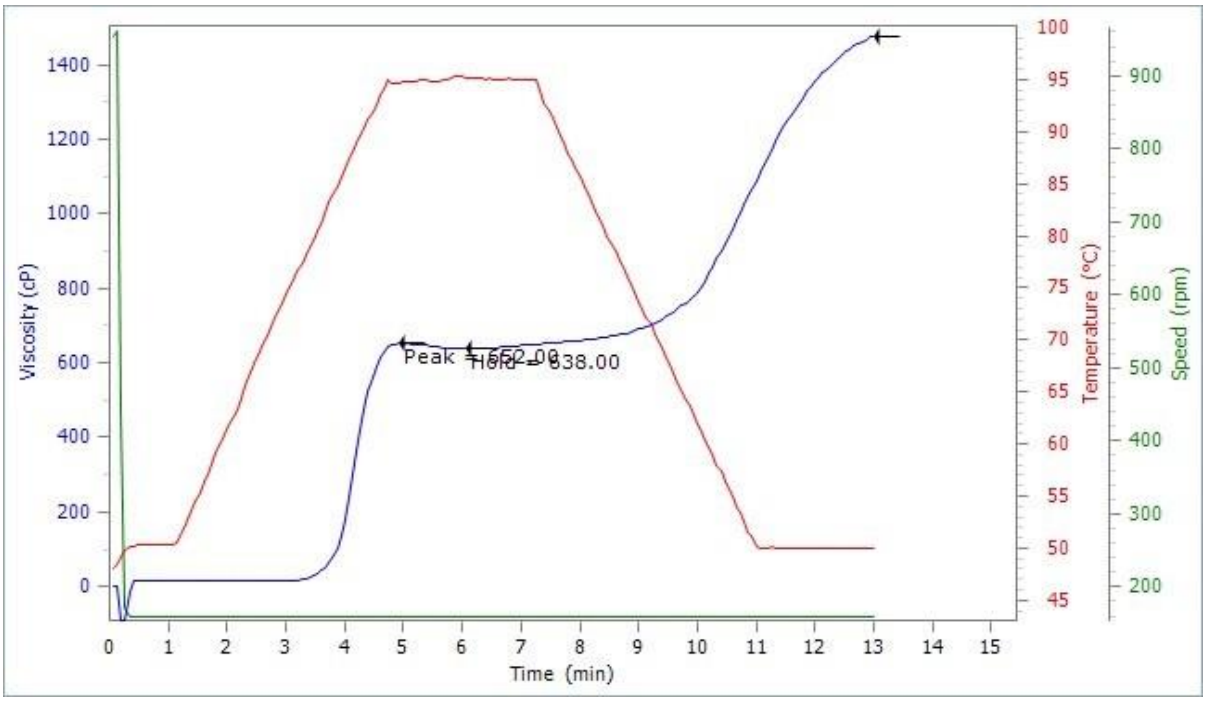
APPENDIX 1



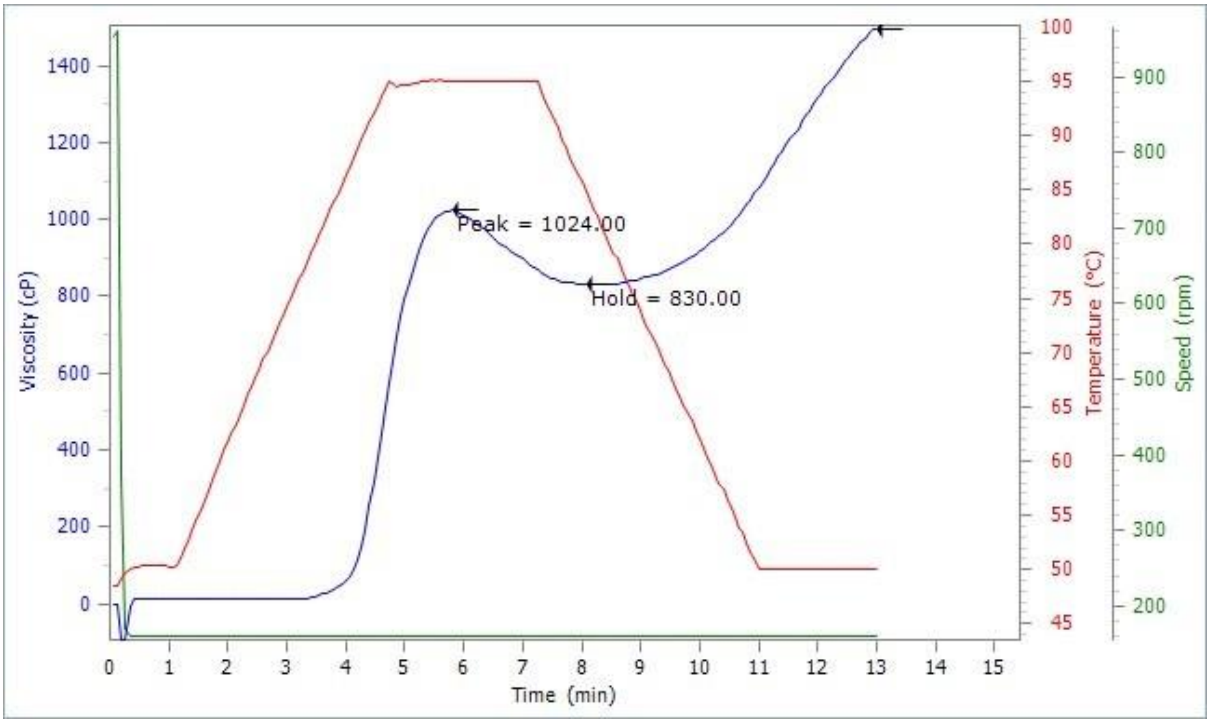
Unmalted natural

Appendix 2



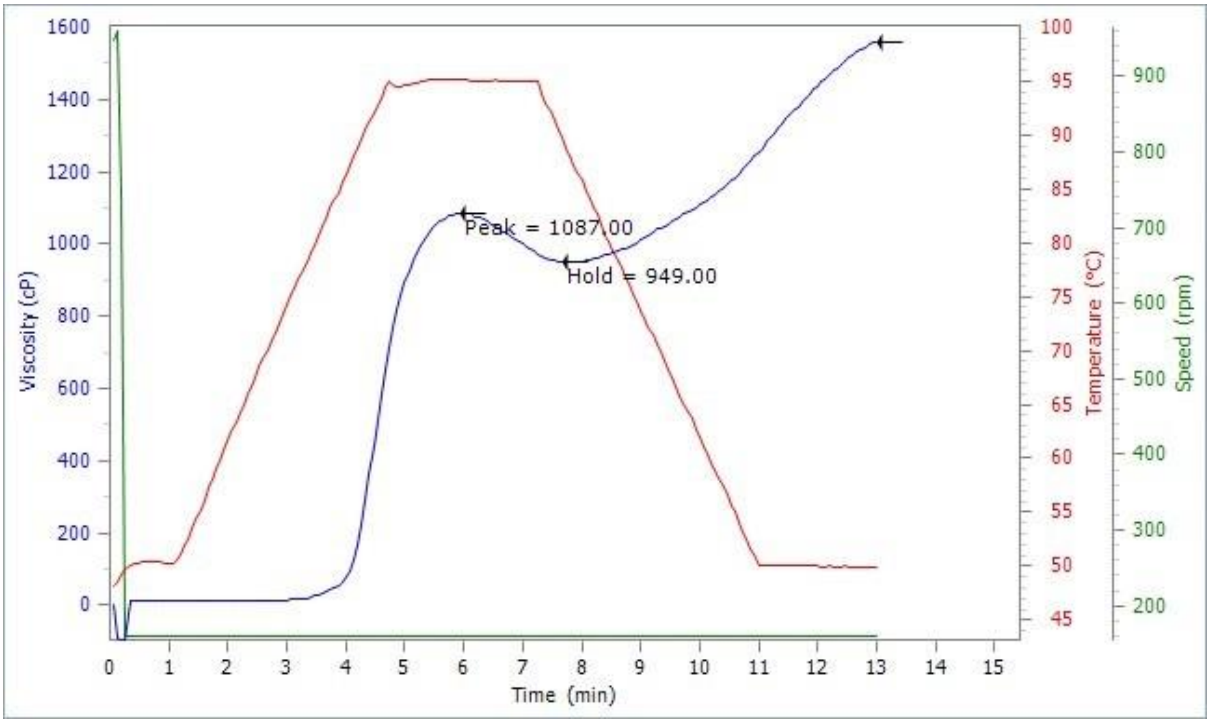
Malted natural

Appendix 3



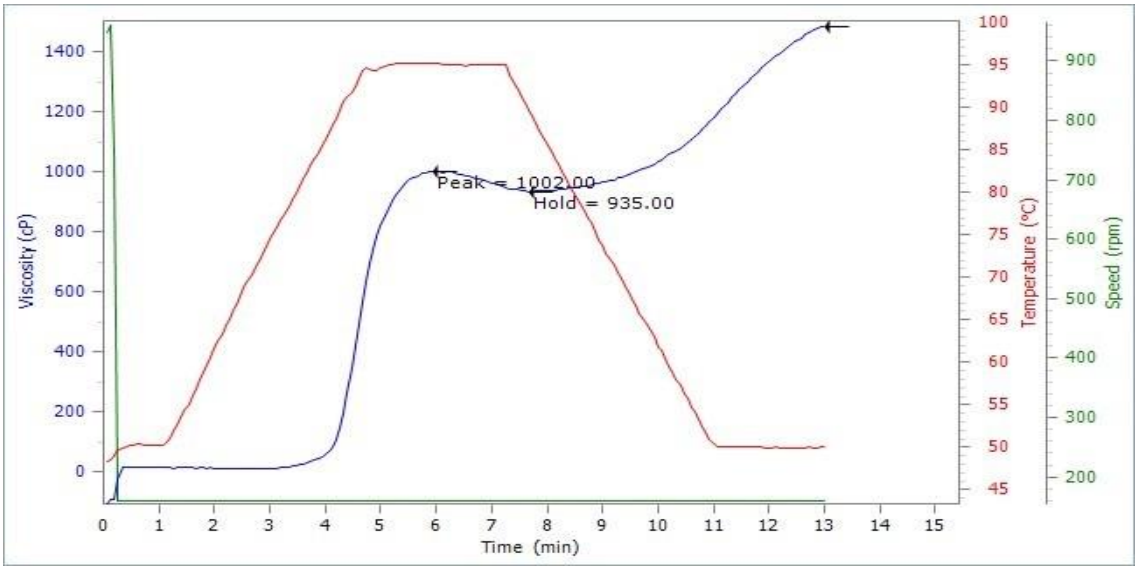
Unmalted sorghum fermented with burukutu

Appendix 4



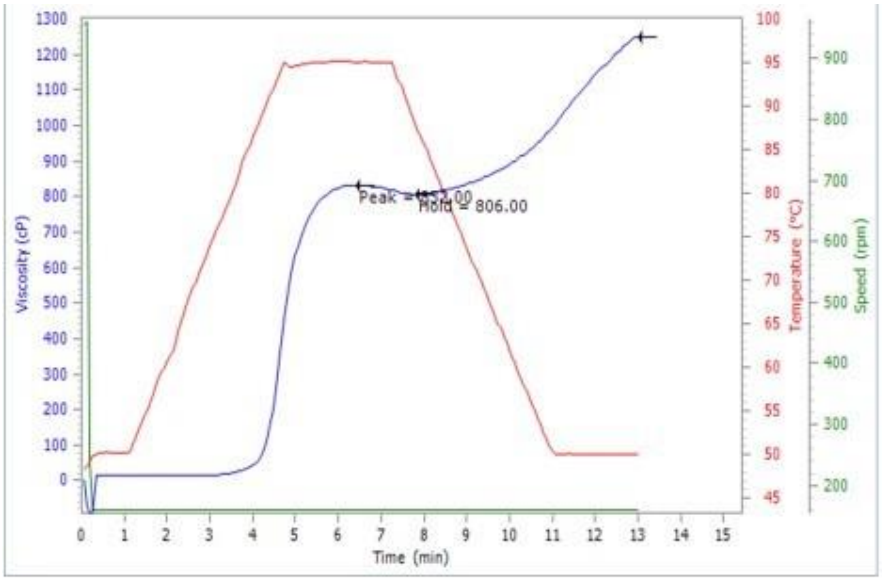
Malted sorghum fermented with burukutu

Appendix 5



Unmalted sorghum fermented with palm wine

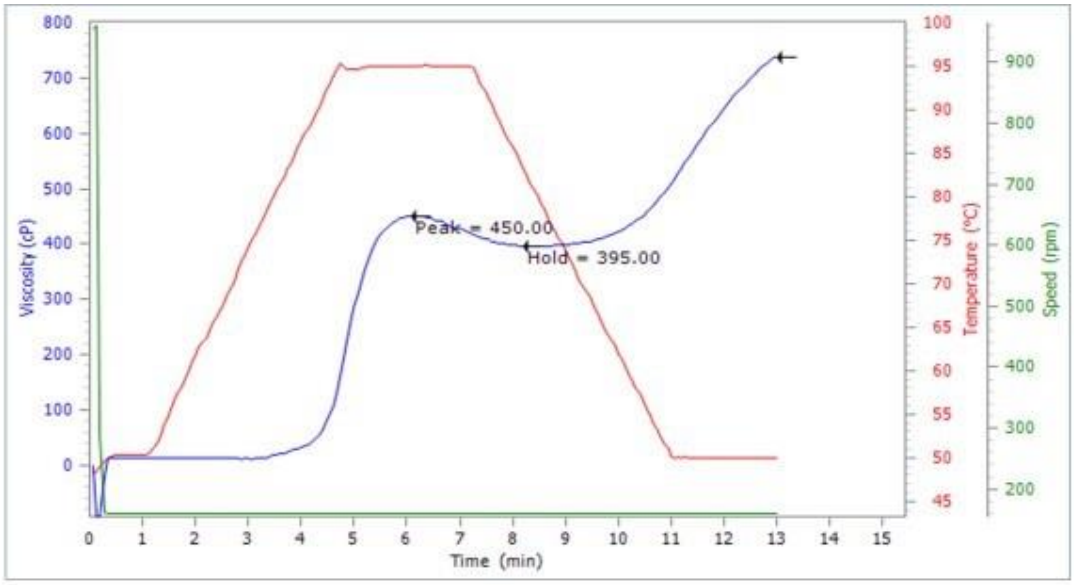
Appendix 6



MPc

Malted sorghum fermented with palm wine

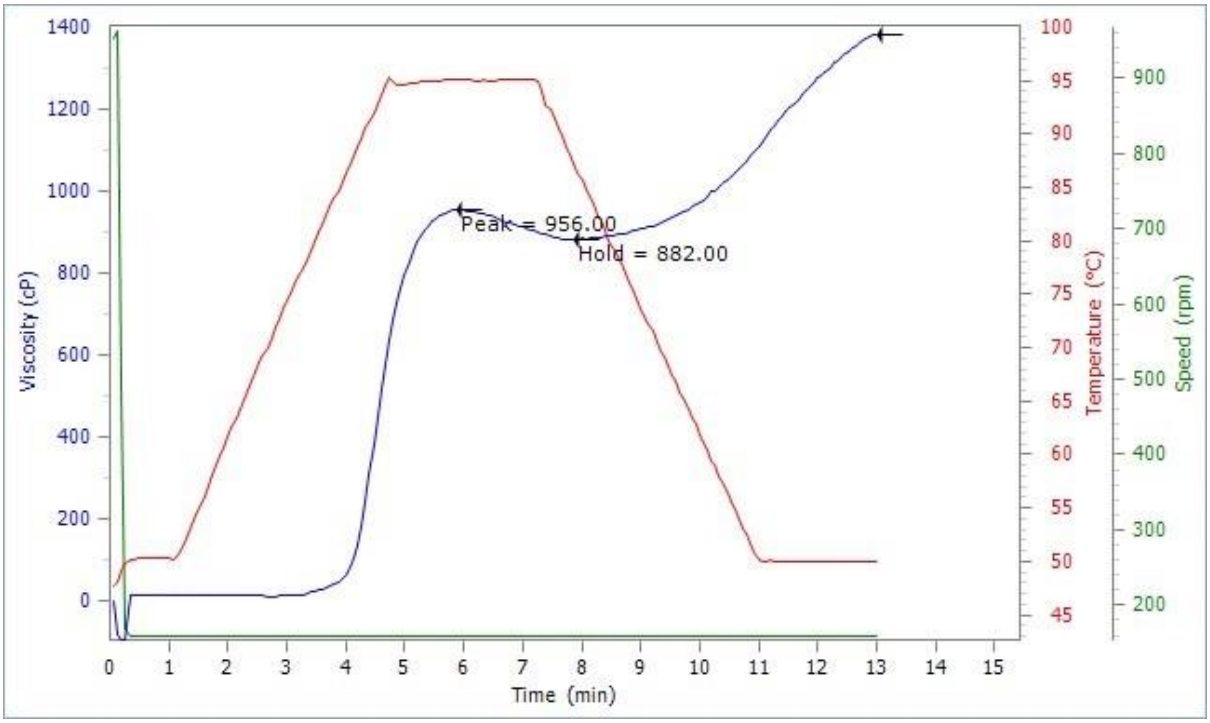
Appendix 7



UCb

Unmalted sorghum fermented with yoghurt

Appendix 8



Malted sorghum fermented with yoghurt

Appendix 9

Atomic Absorption Spectrophotometer

Appendix 10

High Performance Liquid Chromatography

Appendix 11

Rapid Visco Analyzer

Appendix 12

Sorghum grains during malting

Appendix 13

Sorghum *ogi* produced using innoculum from *burukutu*

Appendix 14

Sorghum *ogi* produced using innoculum from palmwine

Appendix 15

Sorghum *ogi* produced using innoculum from *yoghurt*

Appendix 16

Nucleotide sequences of bacteria isolated from burukutu

GGTTAAGTTAGAAAGGGCGCACGGTGGATGCCTTGGCACTAGGAGCCGATGAAGG
ACGGGCGAACACCGATATGCTTCGGGGAGCTGTAAGCAAGCTTTGATCCGGAGATT
TCCGAATGGGGAAACCCACCACTCGTAATGGAGTGGTATCCATATCTGAATTCATAG
GATATGAGAAGGCAGACCCGGGGAACTGAAACATCTAAGTACCCGGAGGAAGAGA
AAGCAAATGCGATTCCCTGAGTAGCGGCGAGCGAAACGGGATTAGCCCAAACCAAG
AGGCTTGCCTCTTGGGGTTGTAGGACACTCTGTACGGAGTTACAAAAGAACGAGGT
AGATGAAGAGGTCTGGAAAGGCCCGCCATAGGAGGTAACAGCCCTGTAGTCAAAAC
TTCGTTCTCTCCTGAGTGGATCCTGAGTACGGCGGAACACGTGAAATTCCGTCGGAA
TCCGGGAGGACCATCTCCCAAGGCTAAATACTCCCTAGTGACCGATAGTGAACCAG
TACCGTGAGGGAAAGGTGAAAAGCACCCCGGAAGGGGAGTGAAAGAGATCCTGAA
ACCGTGTGCCTACAAGTAGTCAGAGCCCGTTAACGGGTGATGGCGTGCCTTTTGTAGA
Bacillus subtilis strain
DKU_NT_02, CP022890.1

GCACAAGCGGTGGAGCCATGGTGGGTTTAATTCGAAGCAACGCGAAGAAC

CCTTACCAGGTCTTGACATA CTCGTACTAT TCCTAGAAGA ATAGGAGTTC

CTTCGGGACA CGGGAAAACA GGTGGTTGCA TGGTTGTCGT CAGCTCGTGT

CGTGGAGAAT GGTTTGGGTTTAAGTCCCGCA CGAGCGCACC CCCTATGTAG

TGCAATCAAT AGTGGCACTC TACGGAGACT GCGTGATAAC CGAGAAAACG

TTGAGATGAC GTCGATCATC ATGCCCTAAG ACTGGCTACA CTGCTACATG

GATGGTACAC GGATTTCCGC GGAAACAGTG ATG

Lactococcus lactus

AAAAAACTAGGTGTTGGTGAAGATATACCAAGTGATTATCCATTTTATAATGCTCAA
ATTTCAAACAAAAATTTAGATAATGAAATATTATTAGCTGATTCAGGTTACGGACAA
GGTGAAATACTGATTAACCCAGTACAGATCCTTTCAATCTATAGCGCATTAGAAAAT
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GAAAAATATTATTTCCAAAGAAAATATCAATCTATTAACTGATGGTATGCAACAAGT
CGTAAATAAAACACATAAAGAAGATATTTATAGATCTTATGCAAACTTAATTGGCAA
ATCCGGTACTGCAGAACTCAAAATGAAACAAGGAGAAACTGGCAGACAAATTGGGT
GGTTTATATCATATGATAAAGATAATCCAAACATGATGATGGCTATTAATGTTAAAG
ATGTACAAGATAAAGGAATG

|*Staphylococcus epidermidis* GU451307.1

TTCCGCCTGG GGAGTACGAC CGCAAGGTTG AAACTCAAGG AATTTFACGG

GGACCCGCAC AAGCGGTGGA GCATGTGGTT TAATTTCGAA GCAACGCGAG

AACCTTACCA GGTCTTGACA TCCTTTGAAG CTTTTAGAGA TAGAGTGTTC
TCTTCGGAGA CAAAGTGACA GTTGTTGCAT GTTCGTCGTC AGCTCGTGTC

GTGAGATGTT GGGTTAGTCC CGCAACGGAG GCGCAACCC

Leuconostoc mesenteriodes subspp mesenteriodes

TCCTACGGGA GGCAGCAGTG GGGAATATTG GACAATGGGG ICCAGCAATG

CCGCGTGTGT GAAGAAGGTT TTCGGATTGT AAAGCACTIT C GATGATGACG

GTACCCGTAG AAGAAGCCCC GGCTAACTTC GTGCCAGCAG ACGAAGGGGG

CTAGCGTTGC TCGGAATGAC TGGGCGTAAA GGGCGTGTAG CAGTCAGARG

TGAAATCCCC GGGCTTAACC TGGGAGCTGC ATTTGATACG GTGTGAGAG

AGGGTTGTGG AATTCCCAGT GTAGAGGTGA AATTCGTAGA ACACCGGTG

GCGAAGGCGG CAACCTGGCT CATAACTGAC GCTGAGGCGC GAGCAAACA

GGATTAGATA CCCTGGTAGT CCACGCTGTA AACGATGTGT GGTAAC'ITA

GTTATTCAGT GTCGCAGTTA

Acetobacter orientalis

CGGGAGGCAGCAGTAGGGMTCFTCCACM TGGGCGCAAG CCTGATGGAG

CAACACCGCG TGAGTGAAGA AGGGTTCGG CTCGTAAGC TCTGTTGTTA

AAGAAGAACA CGTATGAGAG TAACTGTTC ATACGTIGAC GGATTTACC

AGAAAGTCAC GGCTAACTAC GTGCCAGCAG CCGCGGTAAT ACGTAGGMC

AAGCGTTAT GAAAGCATGG GTAGCGCAGGAG CCCTGGTAGT CCATGCCGTA

AACGATGAGT GCTAGTGTTG GAGGGTTCC CGCCCHCAG TGCCGGAGCT

AACGCATTAA GCACTCCGCC TGGGGAGTAC GACCGAGGTTGAAAGTG

Lactobacillus fermentum

AACAGCTAGA ATAGGAAATG ATTTTAGTTT GACGGTACCA TACCAGAAAG
GGACGGCTAA ATACGTGCCA GCAGCCGCGG TAATACGTAT GTCCCG AGCG
TTATCCGGATT TATTGGGCGT AAAGCGAGC GCAGACGGTT TATTAAGTCT
GATGTGAAAG CCCGGAGCYC ACTCCGGAA TGGCATTGGA AACTGGTTAA
CTTGAGTGCA GTAGAGGTAA GTGGAACTCC ATGTG'Y AGCG GTGGAATGCG TAG
ATATATG GAAGAACACC AGTGGCGAAG
L.brevis

GGAGGCAGCAGTAGGGMTCFTCCACM TGGGCGCAAG CCTGATGGAG
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S. lactis

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GCCCATACTC CTACGGGAGG CAGCAGTAGG
Weissella cibaria

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Saccharomyces cerevisiae KU845301.2

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Micrococcus luteus strain trpE16 CP007437.1

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Lactobacillus Plantarium

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S. epidermis

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P. aerogenosa

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1 *Lactobacillus delbrueckii* subsp. *bulgaricus* CR954253.

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Streptococcus thermophilus strain EU01CP047191.1

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Lactobacillus Plantarium

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Lactobacillus licheniformis

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Lactobacillus fermentum

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Lactobacillus rhaminosus

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Bacillus subtilis BSn5 CP00468.1

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Micrococcus luteus strain trpE16 CP007437.1

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Leuconostoc mesenteriodes

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Lactobacillus fermentum

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C. tropicalis

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Micrococcus sp strain trpE16 CP007437.1

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Lactobacillus Plantarium

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Bacillus anthracis strain HYU01, CP008846.1

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A. orientalis

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Bacillus subtilis. ToIr10 KC806236.1

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|*Staphylococcus aureus* HF569113.1

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Saccharomyces cerevisiae KU845301.2