# DEVELOPMENT OF A READY TO DRINK SORGHUM BEVERAGE THROUGH OPTIMIZATION OF MALTING AND FERMENTATION.

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**DEPARTMENT OF FOOD SCIENCE, NUTRITION AND TECHNOLOGY**
**FACULTY OF AGRICULTURE**
**UNIVERSITY OF NAIROBI**

**2023**

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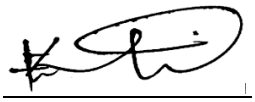
Signed  Date:11/10/2023

This thesis has been submitted with our approval as university supervisors.

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**DEDICATION**

This body of work is dedicated to my late brother Douglas Bwamu and my family for whom their support and love has been an immeasurable pillar of strength.

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**ABBREVIATIONS AND ACRONYMS**

| | |
|---|---|
| 3D | Three days of malting and fermentation at 20 $^0$C |
| 4D | Four days of malting and fermenting at 25 $^0$C |
| 5D | Five days of malting and fermenting |
| 5D1 | Five days of malting and fermenting at 30 $^0$C |
| 5D2 | Five days of malting and fermenting at 35 $^0$C |
| AOAC | Association of Official Analytical Chemists |
| ANOVA | Analysis of Variance |
| CFU | Colony Forming Units |
| DNA | Deoxyribonuclease |
| EABL | East Africa Breweries Limited |
| IMVIC | Indole, Methyl Red, Voges-Proskauer and Citrate test |
| KALRO | Kenya Agricultural and Livestock Research Organization |
| LAB | Lactic Acid Bacteria |
| MRVP | Methyl Red/ Voges-Proskauer media |
| MUFA | Monounsaturated fatty acids |
| NCD | Non-communicable disease(s) |
| PH | Potential Hydrogen |
| PUFA | Polyunsaturated fatty acids |
| RDA | Recommended Dietary Allowance(s) |
| SCOBY | Symbiotic Culture of Bacteria and Yeast |

**GENERAL ABSTRACT**

There has been a growing trend in production and marketing of a range of beverages with poor nutritional quality in Kenya resulting in public health concerns such as diabetes, overweight and obesity especially among children and adolescents. As such, development of a commercially viable and nutritious beverage from underutilized cereal such as sorghum has been limiting. This study was therefore designed to develop an alternative sorghum-based beverage with high nutritional and sensory quality compared to commercially readily available beverages in the Kenyan market. Four beverage formulations containing sweetened sorghum malt extracts were developed through malting (3, 4 and 5 days respectively) and fermentation using *kombucha* culture at different temperatures (between 20 ºC – 35 ºC) and analyzed for chemical composition and nutritional characteristics (protein, ethanol, sugars, calories, total phenolics, tannins, vitamin C, iron and zinc) using standard analytical procedures. For the nutritional components, the values ranged between 2.01- 3.63 % for protein, ethanol was absent, 1.42-2.47% for sugars, 13.19- 23kcal/ml for calories, 0.13 - 1.66 GAE/100ml for phenolics, 1.29 - 40.08mg/100ml for tannins, 4.35 - 6.13 mg/100ml for vitamin C, 1.25- 29.56g/l for iron and 0.7-2.11g/l for zinc. Iron was used in the process optimization to select the beverage with high nutritional and sensory quality. The best formulation based on the process optimization was obtained from an optimum of four days malting and fermentation temperature of 25 ºC (4D).

The developed beverages were tested for their antimicrobial properties using food borne pathogens *E. coli* and *S. aureus*. Halo diameters were measured with significant differences ($p<0.05$) in diameters being noted for *E. coli* between the different samples, with the beverage formulation 3D forming the smallest halo zone compared to the rest. The minimum inhibitory concentration for the formulated beverages to be effective antimicrobials was found to be 8%. Microbial enumeration results showed dominance of lactic acid bacteria and yeasts compared to acetic acid bacteria. The microbial counts overall increased with increasing malting days and temperatures of fermentation. Total viable counts were highest in 5D2 with the least being in 4D and 5D1.

Sensory analysis was conducted on the developed beverages and compared to a control commercial beverage under the brand name ‘Malto’ purchased from the local supermarket by a semi trained panel of 15 people composed of university staff and students to determine their ranking in terms of color, aroma, taste and overall

acceptability. The best beverage was 3D in terms of taste (4.9), color (4.40), aroma (4.90) and overall acceptability (4.87).

Shelf-life analysis was also conducted on this best beverage to find out its shelf life at 4 ºC and 30 ºC. The shelf life was calculated according to Q10 factor that was found to be 1.89 leading to shelf-life values of 103 days and 17 days at 4 ºC and 30 ºC respectively. The study concluded that a nutritious and shelf stable sorghum based *kombucha* beverage is a viable alternative to current product variants in the market. The study however shows a need for further research on the effects of the *kombucha* culture on the functional and nutritional properties on sorghum and ways to optimize the beverage. The study nevertheless indicates the potentiality of sorghum as a viable alternative in food product development.

# CHAPTER ONE: GENERAL INTRODUCTION

## 1.1 BACKGROUND INFORMATION

The level of global undernourishment has been on the rise peaking at 10.9% as reported by the World Health Organization (WHO) report (WHO, 2018). Worldwide, 53 countries are on course to prevent an increase in the prevalence of overweight among children <5 years of age, which currently affects 5.6% of children. Very few countries are on course to meet the targets for diet-related NCDs. No country is on course to halt the rise of obesity, with 15.1% of adult (aged 18 years or over) women & 11.1% of adult men living with obesity globally. Also, diabetes is estimated to affect 7.9% of adult women and 9.0% of adult men, with very few countries on course to prevent these numbers from increasing.

So as to achieve our goals on human development, we need to be diverse in our diets for purposes of nutrition balance. In addition to the diversity, of more importance is the availability of foods that are nutritious and wholesome so that people can grow and thrive to their maximum potential for positive contribution to the country's socio-economic development (KDHS, 2014). For many people in Africa and Asia, sorghum is a major source of minerals, calories and proteins. Due to its drought tolerance and the way it is adapted to dry ecosystems, both tropical and subtropical the world over, it is considered a very important subsistence crop (Taylor & Kruger, 2019). Its utilization commercially, especially in East Africa is poor despite its numerous benefits such the nutritional qualities it possesses and the research efforts made in the past in many parts of Africa (Ngugi *et al*., 2013).

In Kenya, sorghum is ranked third after maize and wheat in terms of cereal production. Traditionally all farming communities regard it as an important part of the diet and as a food secure crop so it is grown in all agro-ecological zones. Area under sorghum in recent years has increased from 12236 hectares (ha) in 2005 to 225782 ha in 2010 (FAO, 2018). The cereal has great potential to stimulate regional development and improve food security. For many low-income households in Kenya, it is a staple crop. It is the only cereal indigenous to Kenya and is cultivated in most parts of the country including areas considered poor in terms of agricultural potential. Majority of the sorghum produced is grown in Coast, Eastern and Western regions (Njagi *et al*., 2019). Apart from sea level, it can grow up to 2,500 meters altitude and requires about 250 mm and 10$^{o}$C rainfall

and temperature minimums respectively per year (Mrema et al., 2017). In terms of utilization, 1% of the production is consumed as feed, more than 50% as food, one fifth is processed and close to fifteen per cent lost through post-harvest losses and in the field (Njagi *et al*., 2019). Rural households in Kenya are the major consumers of sorghum and they usually mill it to make *'ugali*'. Some is sold in urban markets after processing into flour by commercial mills. In many cases, before being sold to consumers, it is used to enrich cassava flour (Chemonics, 2010).

Foods based on cereals are fermented for purposes of preservation. It is a simple technology; carried out at home and millions of people have been fed from it. Currently, cereals are being used to produce a variety of fermented foods at household and at the semi-industrial levels (Ukwuru and Ohaegbu, 2018). The major types of fermentation reported for cereals are lactic fermentation mediated by lactic acid bacteria or fermentation by a mixed population of bacteria which are acid-producing and yeasts. Bacteria take part in flavor development and lactic acidification, whereas yeasts affect development of aroma and bioavailability leading to a variety of foods. It is however worth noting that many cereals fermented by yeast produce alcoholic beverages (Galati et al., 2014). The different products are known by different names in various parts of the world.

In the fermentation process of cereals, the modification of the grains is through various steps that result in biochemical changes within the endosperm in preparation for action by microbes (Mizuta et al., 2020). Fermentable sugars are produced through synthesis and enzymatic activities. Antinutrients such as tannins, enzyme inhibitors and polyphenols which are present in cereals, are removed by fermentation and there is enhancement of physicochemical properties, which lead to nutrients bioavailability (Sharma et al., 2020). Hence, incorporation of human-derived probiotic strains into cereal substrates under conditions which are controlled would result into essential benefits in terms of health.

Similarly, fermentation also removes mycotoxins in cereals (Ukwuru and Ohaegbu, 2018). Other benefits of fermentation include easy assimilation of nutrients in addition to the increased food digestibility (Msheliza *et al*., 2018). Nutrients and enzymes are also retained. These are usually lost during processing (Mohammed *et al*., 2011). On another note, the nutraceutical beverages market is fast growing worldwide (Heckman *et al*., 2010) and more recently, there has been a surge of interest by consumers in functional foods. Therefore, the food industry must make it a priority

to develop such high quality and safe products. Lately, an essential strategy for improvement of human health through diet has emerged, which is the consumption of fermented foods containing live microorganisms (Marco et al., 2017). This is aimed at having improvement of the health of the host by influencing the gut composition in terms of the microbiome and also reducing the incidence of chronic diseases (Liptáková *et al*., 2017). This shows that market trends for the Kenyan beverage industry should feature a shift towards health-oriented wellness drinks so that consumers of beverages currently in the market get the key nutrients they require to meet RDAs and also improve their health. They should not only be enjoying sweet tastes and getting empty calories.

Despite the numerous nutritional benefits sorghums possesses and the research carried out in African countries, in East Africa, it has not been adopted by many, and production, utilization and commercialization is poor (Ngugi *et al*., 2013). This cereal has the potential to alleviate negative health effects of cardiovascular disease, obesity and other chronic diseases, among other health benefits. This beverage development is aimed at adding onto the list of existing limited value-added sorghum products and provision of essential nutrients and positive contribution to the health of beverage consumers.

## 1.2 RESEARCH PROBLEM

There are diverse brands of beverages currently in the Kenyan market, many of which contribute no or limited nutritional benefits with exception of carbohydrates and energy. They are just sweetened and flavored beverages packed with empty calories. Research shows sugar-sweetened beverage consumption is significantly related to the risk of obesity and related lifestyle diseases and this is seen to be true due to the increased prevalence of lifestyle related diseases among adolescents and young people, who are the major consumers of these beverages (Katzmarzyk et al., 2016).

This lag in commercialization of sorghum compared to that of other important cereals (maize, rice and wheat) is due to low and variable production levels, high costs of assembly, high costs of processing, and grain prices which are uncompetitive (Amelework *et al*., 2016). These accompanied with the low adoption and adaptation of current existing sorghum products has resulted in low production and uptake due to limited technologies which have yet to be fully

exploited for utilization of commercially viable sorghum-based foods and beverages. However, success of sorghum has been in production of alcoholic beverages, but the trend has not been fully explored in production of non-alcoholic nutritious beverages. This is against the backdrop of growing market for nutraceutical-based food and beverages (Heckman *et al*., 2010) and more recently, there has been a surge of interest by consumers in functional foods. In particular, is the growing use of utilization of *kombucha* culture in various beverage formulations for the nutritional and functional benefit especially to gut health microbiome (Dahiya and Nigam, 2023). Lately, an essential strategy for improvement of human health through diet has emerged, which is the consumption of fermented foods containing live microorganisms (Marco et al., 2017). This is aimed at having improvement of the health of the host by influencing the gut composition in terms of the microbiome and also reducing the incidence of chronic diseases (Liptáková *et al*., 2017).

However, use of sorghum in production of a *kombucha* based beverage is yet to be fully explored and commercially available in line with growing consumer interest. Therefore, the food industry must make it a priority to develop such high quality, safe and commercially viable products.

### 1.3 JUSTIFICATION

In addition to nutrients, sorghum has the potential to alleviate negative health effects of obesity, cardiovascular disease and other chronic diseases (Rhodes, 2014; Stefoska-Needham et al., 2015a). These benefits which are absent in majority of beverages present in the market will be obtained by consumption of the drink.

Development of the beverage will strengthen the existing sorghum value chain due to the minimal number of products from sorghum currently in the market. It will cater for the lag in commercialization of sorghum in terms of products from it compared to other cereals (Njagi *et al*., 2019).

There has been an increase in consumer awareness about the relationship between health and nutrition which has in turn created a supporting environment for the concept of functional food (foods or food ingredients which provide beneficial effects on health in addition to their nutritional value) (Dahiya and Nigam, 2023). Additionally, the functional food market is also expanding (Kechagia et al., 2013). This novelty will have a place in this market.

Essential micronutrients and large numbers of microorganisms beneficial to the gut can be obtained from consumption of fermented foods (Rezac et al., 2018). Fermentation has also been

in use for a long time for purposes of preservation and enhancement of texture, shelf life, flavor and a food's functional properties (Rezac et al., 2018).

## 1.4 OBJECTIVES

### 1.4.1 Main objective:

To develop a ready to drink sorghum beverage through optimization of malting and fermentation processes.

### 1.4.2 Specific objectives

i. To formulate a ready to drink sorghum beverage by optimization of malting and fermentation processes.
ii. To evaluate the nutritional and functional properties of the developed sorghum beverages.
iii. To determine the antimicrobial properties of the developed sorghum beverages.
iv. To evaluate the shelf life and acceptability of the sorghum beverage through sensory analysis

## 1.5 HYPOTHESES

i. A nutritious and sensory acceptable beverage can be developed by optimized malting and fermentation processes.
ii. The developed sorghum-based beverage has improved nutritional and functional properties than commercial-based beverage of similar type.
iii. The developed sorghum beverage has effective antimicrobial properties against foodborne pathogens.
iv. The developed sorghum beverage will exhibit a prolonged shelf life under ambient temperature.

# CHAPTER TWO: LITERATURE REVIEW

## 2.1 OVERVIEW OF SORGHUM VALUE CHAIN IN KENYA

### 2.1.1 Input Sellers

There has been local development of sorghum varieties which have improved traits by universities and research institutions. One major actor in this sector is the Kenya Agricultural and Livestock Research Organization (KALRO) which accounts for 46% of the improved varieties released into the market (Chimoita et al., 2017). Agro-dealers supply farmers with seeds, while seed producers and several society organizations coordinate to provide seed through Community Based Organizations (Muui *et al*., 2013). To overcome some of the challenges faced for example access to seed and fertilizer, contract farming has been brought into play especially for commercial purposes. Inputs are provided to farmers who are contracted by the output buyers who purchase the crop which has been harvested (Njagi *et al*., 2019).

### 2.1.2 Middlemen, small traders and wholesalers

These are found at the county level in the main areas where sorghum is produced and play a vital role by aggregating sorghum especially from farmers who are small holders and selling to wholesalers (Njagi *et al*., 2019). However, the wholesalers play an important aggregation of sorghum due to their ability to handle relatively large grain quantities that they then aggregate and sell to retailers, millers and processors (Chemonics, 2010).

### 2.1.3 Processors/millers

Milling and malting are the main types of processing that exist. Sorghum is milled for food products (Taylor and Kruger, 2019) with the by-products being used as feed for animals (Stefoska-Needham *et al*., 2015). The opportunity for sorghum processing in Kenya has been expanded by the recent entry of East Africa Breweries Limited who use the cereal for their alcoholic beverage production, and this has resulted in huge demand for sorghum (Njagi *et al*., 2019).

### 2.1.4 Sorghum output market and linkages

An opportunity to access the market has been created by the commercialization of sorghum farming, market presence with good prices for the crop to majority of the small scale farmers (Orr et al., 2016). The sorghum market has been expanded by use of sorghum in brewing with projected consumption set to increase with provision of incentives such as contracts, inputs provision, financial assistance which guarantees them a good sale price of their produce (Kilambya and witwer, 2013; Njagi *et al*., 2019). In addition, there is growing demand for sorghum-based syrups, animal feeds and bio-ethanol products, which has increased the cereal demand.

Despite the market potential for sorghum and its products, the market is disorganized and is characterized by inconsistent product quality, inadequate resources for marketing and strategies, inability to fully exploit the economies of scale and lack of competitiveness as exhibited by narrow product range (Dayakar Rao, 2018). The linkages in the market place are broken and majority of farmers, producers and consumers are not aware of progress made. For farmers, it has been illustrated that access to quality farm inputs and information has a positive return in high quality produce thus able to command a higher price for their harvest (Masese *et al.,* 2018).

## 2.2 CHALLENGES IN THE SORGHUM VALUE CHAIN

Production of sorghum is conducted majorly by subsistence farmers who produce just enough for their domestic use, seldom excess for sale purposes. Thus, production limitations vary from conventional to commercial scales (Omoro, 2013). The constraints faced by the sorghum farmers in Kenya vary in degree and combination from one area to another. Studies on sorghum have found that production constraints can be broadly classified into two; Biotic and Abiotic factors (Omoro, 2013). In the semi-arid areas of the world, drought is a hinderance to sorghum production (Njagi *et al.*, 2019); a problem bought about by varying climate, characteristics of soil, management, pests and in various cases political and socio-economic aspects. Low yields could be due to constraints in production such as use of seeds which are of low quality, lack of enough income for purchasing inputs, lack of market incentives, poor agronomic practices and pests and diseases (Muui *et al*., 2013). For the sorghum enterprise to thrive, these factors should be urgently addressed.

The lag in commercialization of sorghum compared to other cereals in Kenya is mainly due to grain prices that cannot compete with the other cereals, levels of production which are low and which also vary, high costs of assembly and high costs of processing. Constraints faced by business

enterprises dealing in sorghum and its products include fluctuating raw material (grain) quality, inadequate strategies and resources for marketing, lack of the ability to exploit economies of scale and due to high costs of production/processing, the products cannot compete in the market (Njagi *et al*., 2019).

## 2.3 SORGHUM NUTRITIONAL COMPOSITION

### 2.3.1 Carbohydrates

The carbohydrates in sorghum and millets have been reported to contain starch enveloped with soluble sugars, and dietary fiber (Serna-saldivar et al., 2019). Starch makes up the dominant fraction in sorghum followed by fiber and soluble sugars in its morphological composition (Bean et al., 2019). The composition of starch component is influenced by genetic strains of the sorghum and environmental growing conditions. (Hill *et al*., 2012). The starch component represents 51%-78% of the cereal's weight, of which is composed of amylose and amylopectin units (Serna-saldivar et al., 2019). Shegro et al. (2012) reports amylopectin and amylose contents being (81.0–96.5%) and (3.5–19.0%) respectively. Among cereals, its starch digestibility is the lowest because of tannins, starch granules and proteins being strongly bound between each other (Mkandawire et al., 2013). Arabinoxylans are the main insoluble fibers accounting for 75-90% of non –starch polysaccharides (6g-15g/100g). The other polysaccharides (non-starch) are soluble fibers which range from 10.0%–25.0% (Martino et al., 2012).

### 2.3.2 Proteins

The genetic variability and environmental conditions have varying effect on protein composition of sorghum. The morphological composition of sorghum proteins are comprised primarily of water-soluble abumins, salt soluble globumins, alcohol soluble prolamins and acid-alkali soluble glutelins (Awika, 2014). The sorghum prolamins are the predominant protein fraction comprising 70% of total whole grain composition (Awika, 2014), but they are noted by their low lysine content (Bean et al., 2019). The main prolamins of sorghum – kafirins- are α-kafirins, β-kafirins and ∞-kafirins with their quantities reported in ranges of 66-84%, 8-13% and 9-21% respectively (Mokrane et al., 2010). After cooking, the digestibility of its proteins compared to wheat and maize is lower (Mokrane et al., 2010). Lysine is the main limiting amino acid in sorghum and the proteins are rich in non-polar amino acids –leucine, proline, and alanine and glutamic acid. In humans, the

kafirins in sorghum do not trigger allergies or autoimmune reactions (Mesa-stonestreet *et al*., 2010). This renders it very safe for those suffering from celiac disease (Morais Cardoso et al., 2014).

### 2.3.3 Lipids

The lipid content is low, ranging between 1.24g to 3.07 g/100 g main composition being of fatty acids (unsaturated) (83–88%) (Martino et al., 2012). In most varieties, the monounsaturated fatty acids (MUFA) are lower in quantity than the polyunsaturated fatty acids (PUFA). Its main fatty acids are palmitic (12.4–16.0%), oleic (32.2–42.0%), linolenic and linoleic acids (45.6–51.1%) (Morais Cardoso et al., 2014).

### 2.3.4 Minerals and vitamins

Depending on where it is cultivated, the cereal's mineral content varies. The major minerals present in sorghum are iron, zinc, phosphorus and potassium. Ajiboye et al. (2014) reports some mineral and vitamin content as calcium (10 mg), iron (3.5 mg) and niacin (1.7 mg) respectively. The Minerals bioavailability is still quite unknown. Availability of iron in the grain ranges from 6.6% to 15.7% and zinc is between 9.7% and 17.1%. Sorghum is a good source of B vitamins (B1, B2 and B3) and fat soluble vitamins D, E, and K (Morais Cardoso et al., 2014).

### 2.3.5 Phenolic compounds

In some sorghum varieties, phenolic acid content varies from 135.5mg to 479.40 mg/g with major amounts of the ferulic (120.5mg to 173.5 mg/g) and protocatechuic (150.3mg to 178.2 mg/g) acids and minimal amounts of syringic (15.7mg to 17.5 mg/g), p-coumaric acid (41.9mg to 71.9 mg/g), vanillic (15.4mg to 23.4 mg/g), cinnamic (9.8 to 15.0 mg/g), caffeic (13.6mg to 20.8 mg/g), p-hydroxybenzoic (6.1mg to 16.4 mg/g) and gallic (14.8mg to 21.5 mg/g) acids (Afify *et al*., 2012).

In cereals, phenolic acids are in most cases lignin or arabinoxylans chain-bound. The colon microbiota ferments these phenolic acids. They do not undergo hydrolysis by digestive enzymes in the gut which cause their bioavailability to be decreased (Chiremba *et al*., 2009). Tannins (phenolic compounds) which are present in many plants defend them against predators and pathogens. The availability of starch, minerals and proteins of the sorghum are reduced by tannins. However, tannins, in terms of ability to scavenge radicals, are more effective than other simple phenolics despite their anti-nutritional effect (Kaufman *et al.*, 2013).

## 2.4 PRODUCTS FROM SORGHUM

### 2.4.1Bakery products

#### 2.4.1.1 Bread, Cakes and Biscuits

Bread from sorghum is made by blending with wheat flour up to 40-60%. Modified starches can also be used in the same, for example carboxymethyl. Sorghum flour of fine particle size is mixed with other bread making ingredients (salt, sugar, fat and bread improvers) and is made into dough. After addition of baker's yeast to the dough, fermentation is done for a longer period than that of wheat bread. The dough is then molded and baked (one hour). More yeast and gluten (external) are added for purposes of improvement of the leavening and softness (Ratnavathi and Patil, 2014).

The drawback of using sorghum flour is the reduction in specific volume of the bread and overall acceptability of the final bread with increased crumb hardness (Jafari *et al*., 2017). Sorghum cakes are prepared in a similar way as cakes from wheat. Sorghum flour of fine particle size is used. Comparing Sorghum flour to wheat flour in terms of cake preparation, it is superior. The fine grain flour is blended with eggs, sugar, fat and emulsifiers in the right quantities (Ratnavathi and Patil, 2014).

Fine sorghum flour is used in biscuits preparation from fine sorghum flour in combination with wheat flour upto 15%. Sorghum flour is mixed with wheat flour, sugar, vegetable fat and baking powder (15%) together with the essences which are required. Mixed dough is then molded and baked at the correct temperature. Higher substitution can be increased by improved techniques of milling to produce flour having particle size which is comparable to that of wheat flour (Ratnavathi and Patil, 2014).

### 2.4.2 Noodles and pasta

Sorghums of hard endosperm can be used to make high quality noodles while composite flours of wheat and sorghum could be used for pasta. For best pasta products, sorghums used should be of soft texture, endosperms which are yellow with white pericarps and having unpigmented testa. Incorporation of sorghum in the noodle making technology reduces the price of the product and also caters for health since sorghum products are rich in B vitamin and fibre (Ratnavathi and Patil, 2014).

### 2.4.3 Extruded products

Their commercial value is high and are very important nutritionally. Sorghum has excellent extrusion properties which are equal to those of maize and rice. Extruded products have the major advantage of larger output from the original flour despite high infrastructure and machinery costs. Waxy (high in amylopectin) lines are suitable for production of dry breakfast cereals, snacks and beverages (Ratnavathi, 2016).

### 2.4.4 Flakes and other related products

Ready-to-eat cereals are prepared using a number of techniques including flaking, shredding and puffing in wheat, corn and rice (Alavi *et al*., 2018). Sorghum flakes, though suitable processing, might be feasible to produce. Cereal flakes are among the cereals which are ready to eat and popular. Despite missing information on preparation of flakes from sorghum, there have been attempts to make sorghum semolina and other related products.

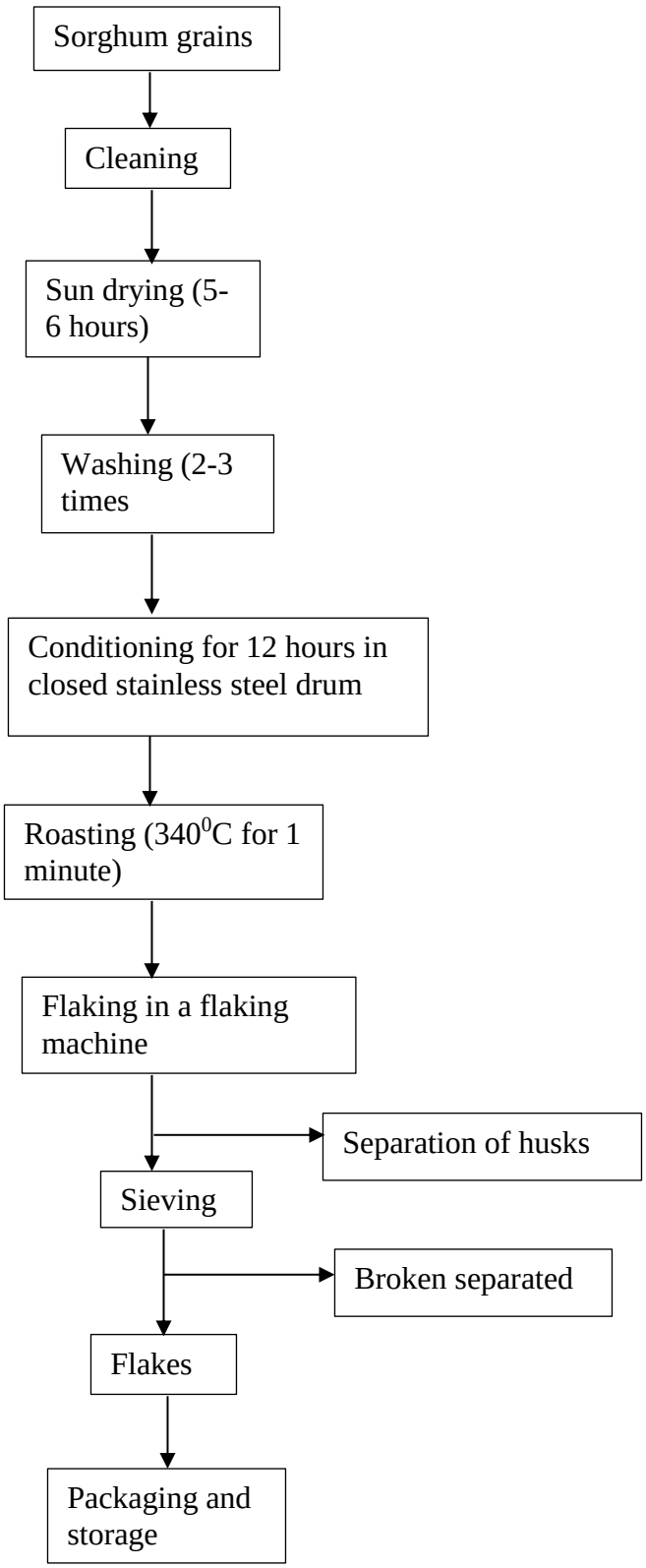


**Figure 2. 1: General procedure for sorghum flake preparation**

### 2.4.5 Sorghum Beverages

The main ingredient in most non-traditional food and beverage products and successful novelties is sorghum. Non-alcoholic malt beverages are very popular for example in Nigeria. They include "brewed" non-alcoholic malt drinks such as "Malta", Dolo in Burkina Faso and Mali, tchakpalo or tchoukoutou in Togo and Benin, burukutu or pito or kunu in Nigeria and Ghana, Niger and Côte d'Ivoire and Oshikundu, a beverage which is fermented but non-alcoholic and which is popular in certain regions of Namibia (Embashu et al., 2013).

The main ingredients of the beverages include water, sorghum flour and flour from pearl millet (mahangu). Pearl millet bran may be optionally added. However, the beverage has a short shelf life, which is below 6 hours (Ashekele *et al*., 2012). Production of oshikundu first involves stirring after adding boiled water to from pearl millet flour. The mixture cools at ambient temperatures, sorghum malt is added followed by stirring. Bran from pearl millet can optionally be added here at this stage. To the mixture, cold water is added to the volume desired and consistency. Back-slopping is done using previously fermented Oshikundu and the batch fermented for 4-6 hours in an area with shade (Cheikhyoussef and Kahaka, 2012).

In Sudan, there's a traditional non- alcoholic beverage prepared from thin flakes of malted and non malted sorghum flours to which spices have been added for purposes of taste known as *Hulu-mur* and *Abreh,* meaning *'sweet and sour'*.

In South Africa, there's a traditional lactic acid bacteria-fermented sorghum drink locally known as *Motoho and mageu* in Lesotho (Alavi *et al*., 2018). In Kenya there's locally produced sorghum beer which was released in 2003 under the brand name Senator and which was later changed to Senator keg in 2004. As the name suggests, it is a product targeted at the consumers who wanted to upgrade to bottled beers from the normal drinks brewed at home (illicit drinks). In Kenya, this beer is the second-most popular as per the analysis done by Euromonitor International (Orr and Mwema, 2016). Sorghum beer products are inferior compared to barley beers in terms of attractiveness due to a high starch gelatinization needed and the low enzyme activity of sorghum (Garzón *et al*,, 2016).

There are also coffee substitutes from sorghum for those who are sensitive to caffeine and also those who choose not to take caffeinated beverages. The substitutes are parts of roasted plants made into products which when mixed with hot water provide a brew which resembles coffee. There is also the malt extract from sorghum which is a sweet wort rich in sugar and which is also

a beverage by itself, but can be made into a flavored syrup through concentration or powder by evaporation of the extract into a product which is dark-colored (Elgorashi *et al*., 2016).

## 2.5 MALTED AND FERMENTED CEREAL PRODUCTS INCLUDING SOME FROM SORGHUM

Sorghum *tortillas* that are in the form of very fine circles are a traditional Mexican food, manufactured through the process of nixtamalization in which calcium oxide is used. *Roti / bhakri* is made with a sorghum flour (whole and fine) that is baked on rolling. *Injera* is a bread made from dough fermented for about 48 h and which is later baked for 2-3 minutes. *Kanji or ambali* is a porridge prepared from whole sorghum flour that is of low consistency and is consumed in the southern India, Central America and Africa. *Tô* is made from sorghum grain which has been decorticated that is made into flour and cooked for about an hour in water (1:4) with minimal amount of tamarind or lemon juices and allowed to cool for an hour.

*Annam* is made of sorghum grains which have been dehulled and placed in water (1:3) until soft and excess water drained off. *Dosa and Idli* are breakfast foods which are fermented. *Idli* is moulded and then cooked in steam, while *Dosa* is an oily pan cake which is thin in nature. *Ogi* is made from sorghum which has been dehulled or whole milled and is cooked with vegetables, water, meat and other ingredients producing a type of soup. *Kisra* is prepared by mixing water and sorghum flour (whole) in the ratios of 60%and 40% and fermenting for 12-24 h until the degree of sourness required is obtained (Hernandez *et al*., 2016).

**Table 2. 1: An overview of traditional fermented products and beverages**

| Fermented Food | Country(s) | Cereal(s) used/ raw material | Microorganism(s) |
|---|---|---|---|
| Indli | South India, Sri Lanka | Rice | *Leuconostoc mesenteroides*<br>*Enterococcus,*<br>*Torulopsis* |
| Dosa | India | Rice | *Leuconostoc mesenteroides*<br>*Streptococcus faecalis*<br>*Torulopsis candida* |
| Kishk | Egypt, Syria | Wheat, Milk | *Lactobacillus plantarum*<br>*Lb. casei*<br>*Lb. Brevis* |
| Tarhana | Turkey | Wheat, Yoghurt | *Lactobacillus bulgaricus*<br>*Streptococcus thermophilus*<br>*Saccharomyces cerevisiae* |
| Ogi | West Africa | Maize, millet, sorghum | *Lactobacillus plantarum*<br>Yeast<br>Molds |
| Pozol | Mexico | Maize | Molds<br>Yeast<br>Bacteria |
| Injera | Ethiopia | Sorghum, maize | *Candida guilliermondii* |
| Sake | Japan | Rice | *Saccharomyces sake* |
| Bouza | Egypt | Wheat | LAB |
| Boza | Albania, Turkey | Wheat, Millet | LAB<br>*Saccharomyces cerevisiae*<br>*Leuconostoc* |
| Mahewu | South Africa | Maize | *Lactococcus lactis* |
| Chicha | Peru | Maize | *Aspergillus*<br>*Penicillium*<br>Yeast<br>Bacteria |
| Uji | Kenya, Uganda | Maize, millet, sorghum | *Lactobacillus plantarum*<br>*Leuconostoc mesentroides* |

Source: (Liptáková *et al*., 2017)

## 2.6 MALTING AND FERMENTATION PROCESSES OF CEREALS

Malting is the germination of cereal grains in moist air under controlled conditions. Its primary objective is to mobilize the hydrolytic enzymes (endogenous) for them to cause a modification in the chemical composition of the grain (Udeh *et al*., 2018). This modification enables rapid enzymatic and physical solubilization during brewing producing a medium containing all nutrients required for rapid yeast fermentation producing carbon dioxide and ethanol (Debabandya et al., 2017). Alpha and beta amylases are the most important enzymes in malt hydrolysis, producing maltose, a fermentable sugar from starch in addition to other participatory enzymes in malting process including endopeptidases, and, lipases and phytases for mineral bioavailability (Taylor and Duodu, 2019).

### 2.6.1 Unit Operations and the science in malting

The malting process involve steeping, germination and drying as the main processing steps. During the steeping process, usually for one day, where the grains are soaked in water to initiate the germination process (Onyango et al., 2013). The process is terminated when the seedling radicle that is emerging becomes visible. Use of a sack cloth is used in traditional germination practice, while today, the process is done on concrete floor. The period of germination is approximately five days, depending on the ambient temperature with watering done periodically to maintain the dampness (Ojha et al., 2018). The length of the shoots and roots are determined when germination should be terminated, with that of sorghum being about 3-5 times the length of the seed. The seedlings from germination should have slight sweetness and be softened fully due to starch hydrolysis and endosperm modification by enzymes (Opeyemi *et al*., 2016).

Direct sunlight was used for drying purposes of the "green malt". The seedlings are spread out on sacking cloth and are periodically turned. If it is to be used immediately for brewing, partial drying is done or complete drying if to be stored. To know whether the shoots and roots were completely dry, rubbing of the seedlings between fingers would be done and if well dry, the shoots and roots would break off cleanly (Taylor and Kruger, 2019).

Water uptake (imbibition), which is the first step, leads to subsequent growth due to the rise of metabolism. It is a process with three stages: the water content increases rapidly once the seed is exposed to water because the dry seed had low water potential. Protein synthesis begins, respiration and energy metabolism reactivate and damage to DNA and membranes caused by

storage, rehydration and dehydration are repaired (Weitbrecht *et al*., 2011).

Stagnation of swelling and water uptake cause germination process to enter the second phase. Here, respiration and metabolism are increased further and in embryonic tissues, the reserve mobilization begins. Elongation of the embryonic axis starts and tissues surrounding the protruding radicle are weakened. This leads to the rupture of these tissues. After radicle protrusion, mobilization of reserves also occurs, causing accumulation of osmotically active compounds of low-molecular weight from storage reserve hydrolysis, causing water uptake to increase further (Weitbrecht *et al*., 2011). Malting gets rid of antinutrients in legumes, roots, tubers and cereals (Opeyemi *et al*., 2016).

### 2.6.2 Fermentation process of cereals

The process of fermentation of cereals depends on microorganisms which are either inherently natural or may be incorporated as a starter culture. The main aim of cereal fermentation is preservation, which relies on acidification from production of acids such as lactic, propionic and acetic acid, alcohol production, enhancement of safety of end products through pathogen growth inhibition, nutritional improvement and enhancement of bioavailability of certain components such as phenolic compounds (Ojha et al., 2018).

The fermentation of cereals is affected by certain variable characteristics such as cereal type which determines the quantity of nutrients, fermentable substrates, growth factors, minerals, temperature and duration of the fermentation process (Liptáková *et al*., 2017).

#### 2.6.2.1 Alcoholic Fermentation

Studies of African traditional beers have brought out revelations such as *Saccharomyces cerevisiae* being the yeast species which is predominant and responsible for the fermentation which produces alcohol (Lyumugabe et al., 2012). Traditional brewing of opaque beer involves back sloping of wort or simply relying on the flora of yeast inherent to the clay pot that has been repeatedly used for fermentation. Today, brewers use active sun-dried yeast sediment from previous successful fermentation. Millet and sorghum traditional beers are unpasteurized and consumed while still undergoing fermentation. Fermentation is still continuing in most opaque beers. On the contrary, for cloudy beers, fermentation by yeast is complete but fermentation by LAB is still proceeding (Taylor and Kruger, 2019).

#### 2.6.2.2 Lactic Acid Fermentation

Organisms responsible for fermentation of cereals are lactic acid bacteria. These microorganisms have gained popularity due to their role which is beneficial in preservation, enhancement of the nutritional value of food, detoxification and production of aroma and flavor (Lu et al., 2018). The genera which are highest in number are *Lactococcus, Leuconostoc, Lactobacillus* and *Pediococcus*. Mold species which may also be involved include penicillium, *Aspergillus, Fusarium* and *Cladosporium* (Lu et al., 2018).

The inoculation of the culture (inherent or externally added) and metabolic activities which ensue result in acidity increase of the medium leading to elimination of microorganisms which are not the lactic acid type (Petrova, 2020). A synergy between yeasts and the surviving LAB is formed. Due to competitive activities between the microbes, the fermentation becomes spontaneous. Some strains which have rapid growth adapt best and hence dominate others at certain stages of fermentation (Achi and Asamudo, 2015).

#### 2.6.2.3 Acetic acid fermentation

Bacteria responsible for this type of fermentation are commonly found on fruits, flowers and other plants as these environments are aerobic and rich in sugar alcohols/ ethanol and carbohydrates (Ashaolu and Reale, 2020). This enables these bacteria, through a particular respiratory chain, to convert these substrates through incomplete oxidation into organic acids for energy production (Gomes et al., 2018). Acidification occurs, preventing competitors from growing while the cells involved possess mechanisms of tolerating acidity, utilize the organic acids that have accumulated to further aid their growth (Gomes et al., 2018). Acetic acid bacteria capable of production of cellulose are retained on the culture surface after forming biofilms. This is favorable for their survival since they are strictly aerobic (De Roos and De Vuyst, 2018).

These bacteria are gram negative, cylindrical or ellipsoidal in shape, and under the microscope appear in pairs, singly, aggregated and in chain form. They are aerobic, with oxygen being the final electron acceptor, however from time to time, other compounds act on behalf of oxygen, enabling their survival in anaerobic conditions, for example during wine fermentation (Gomes et al., 2018). These bacteria are found on sugar/alcohol containing substrates such as wine, beer, cider, fruit juice and vinegar (Mas et al., 2014). There is incomplete oxidation of sugars and

alcohols resulting into organic acids such as gluconic and acetic acid accumulating (Mas et al., 2014).

Due to how they behave while growing in culture media, these bacteria responsible for acetic fermentation are considered “fastidious”. They have low cultivability is and many strains of acetic acid bacteria lose some features after growth in culture media, for example the ability for production of substantial quantities of acetic acid (Mas et al., 2014).

Some additional benefits of sorghum and other cereal fermentations are: The digestibility of sorghum protein may be boosted by germination and fermentation despite the decrease in digestibility of its protein after wet heat cooking (Afify et al., 2012b). The application of probiotic strains in cereal fermentations and cooking processes which are specific can cause an increase in the free phenolic acids content, thereby causing improvement in their bioavailability (N’Dri et al., 2013).

Fermentation decreases the antinutritional components of sorghum with time. It also makes digestion and nutrient assimilation easier. For those components destroyed by processing (vitamins, enzymes and other nutrients) they are retained. The amino acid content is decreased by fermentation alone (Mohammed *et al*., 2011).

## 2.7 EFFECTS OF VARIOUS TREATMENTS ON PHYSICOCHEMICAL PROPERTIES OF SORGHUM

### 2.7.1 Milling

Milling of grains is preceded decortication and dehulling which involves the removal of the bran layers (germ and pericarp) (Duodu, 2015). The grain endosperm is composed of a harder outer part and a softer inner part which is floury. The vitreous endosperm cannot be reduced to a particle size which is fine. Hence, starch damage can result from milling the hard endosperm to flour size and this can severely affect the flour quality in terms of bread making (Schober *et al*., 2007). Removal of bran majorly affects composition of the flour with increase in protein due to pericarp removal, while germ removal lowers the lysin content by approximately 20% (Duodu, 2015). Lipid content is also reduced by removal of the germ-the tocopherols inclusive. B vitamins and minerals are also reduced due to them being in high concentration in the aleurone layer and the germ (Awika, 2005). Bioavailability of minerals on the other hand may be improved as the level of antinutrients such

as phytic acid which binds iron, calcium and zinc is reduced because it is also found in the germ. The potential of the flour in terms of antioxidants also decreases on bran removal due to the removal of phenolics which are concentrated in the pericarp and testa (Adeyeye et al., 2019).

### 2.7.2 Kilning/Roasting

In the kilning process, melanoidins, which are colored are formed from combination of breakdown products of germination, that is the carbohydrates and proteins (Cardoso et al., 2014). Alpha and beta amylases are decreased slightly with beta amylase being inhibited to a greater extent than its counterpart, at curing temperatures above 100°C, proteolytic enzymes are destroyed (Pradhanang, 2013). Catalase activity strongly diminishes during kilning, and the temperatures of curing completely inactivate it. Polyphenols activity is not affected and lipase is slightly inactivated (Pradhanang, 2013).

## 2.8 BENEFITS OF CONSUMPTION OF FERMENTED FOODS AND BEVERAGES AND PROBIOTICS

Gut microbiota can be controlled through consumption of adequate doses of probiotics which can confer health benefits to the consumer (Marsh et al., 2014a). From epidemiological studies it has been shown that there is a reduction in risk of contracting diseases and ultimately an improvement in health associated with consumption of fermented foods. For example, there has been an association between yogurt consumption and reduction in gaining weight (Fung et al., 2015). Yoghurt has also been shown to improve lactose tolerance due to the microbes releasing *B*-lactosidase (Savaiano, 2014) adults in Korea, there was a correlation between reduced atopic dermatitis and asthma incidence on consumption of vegetables which were fermented and Kimchi (Kim et al., 2016). There was also an association between reduction in the risks of high blood pressure and diabetes type 2 among adults in Japan and consumption of foods from fermented soybean which was rich in biologically active peptides and plant estrogens (Nozue et al., 2017).

Health benefits may be imparted by some fermented foods even in the absence of probiotics. Ethanol that is produced early during fermentation of red wine causes enhancement in the extraction of polyphenols from the skins. Vitamins and other biologically active molecules originally not present in a food may be produced from metabolism by the microbes. Deficiency of folic acid as a global health problem has been noted by Saubade *et al.* (2017) and suggestions have been made those fermented foods could deliver folic acid to the populations that are at risk. Some

LAB produce small amounts of folate and the quantities may not be enough to hit the threshold (Saubade et al., 2017).

The probiotic bacteria can affect health positively because they out-compete the pathogens for resources, use carbohydrates which are available to produce fatty acids which are short chain, have ability for production of anti-microbial agents, contribute to homeostasis of the immune system and are also responsible for vitamin production (Derrien and Vlieg, 2015).

### 2.8.1 Probiotics

They are live microorganisms which when taken in amounts which are adequate, improve the health of the host (Kechagia et al., 2013). Bacteria of different genera and strains (*Bifidobacterium, Lactobacillus, Lactococcus, Pediococcus, Leuconostoc, S. salivarius* subsp. *thermophilus, E. faecalis, E. faecium, B. subtilis, E.coli , B. coagulans, B.cereus, B. licheniformis and B. polyfermenticus* ) and yeast (*Saccharomyces boulardi)* are used as probiotics especially in dairy products (yoghurt, milk and probiotic cheeses), soy milk drink, juices and fermented cereal products (Dey, 2018; Setta *et al*., 2020).

The choice of microbe to use is determined by factors such as: their safety (have to be safe), non-pathogenicity, and non-toxicity, survival through the gut and adherence to the intestinal mucosa and production of organic acids, lactic and acetic acids (Chavan et al., 2018). The nutritional benefits of probiotics known include anti-mutagenicity, anti-carcinogenic, anti-allerginic properties, serum control and blood pressure control (Acin-Albiac et al., 2021; Xu et al., 2020).

*S. boulardii* administration as a biotherapeutic yeast which is non-pathogenic plays an essential role in the prevention or treatment of diarrhea associated with antibiotics caused by *C. difficile* among others (Liptáková *et al*., 2017). Other benefits are: improvement of the health of the gastrointestinal tract, lowering of the cardiovascular disease risk and type two diabetes (Rezac et al., 2018). The components of the immune system are stimulated, the immune response of the gut is rejuvenated and also homeostasis in the intestines is maintained (Savard et al., 2011); diarrhea is prevented and also treated; faecal properties are improved and also the microbiota, treatment of irritable bowel syndrome , constipation and inflammatory bowel disease; improvement of *H. pylori* eradication treatment system (Wilhelm *et al*., 2011); supporting the response of the immune system of HIV-infected children and adults (Hemsworth *et al*., 2012), and due to low molecular

weight molecules being produced by probiotic bacteria, there are immune-stimulatory properties obtained (Kmoníčková et al., 2012).

### 2.8.2 *Kombucha* (Fermented tea)

It is a beverage which has been fermented and contains a specialized culture added to tea which has been sweetened. The culture is a combination between bacteria and yeast which are in a symbiotic relationship (SCOBY). Acetic acid producing bacteria of the genera *Gluconacetobacter, Acetobacter*, *Gluconobacter*, and LAB are the bacteria present in the beverage. The Saccharomyces species of yeasts are the dominant type of yeasts present although other genera may also be within (Coton et al., 2017).

**Table 2. 2: The microbial population in Kombucha**

| Bacteria | Yeast |
|---|---|
| *Acetobacter xylinum* | *Bret anomycessp* |
| *Acetobacter aceti* | *Bret anomycesbruxellensis* |
| *Acetobacter ketogenum* | *Bret anomycesintermedius* |
| *Acetobacter pasteurianus* | *Candida* |
| *Bacterium gluconicum* | *Candida guilliermondii* |
| *Bacterium katogenum* | *Candida famata* |
| *Bacterium xylinum* | *Candida stellata* |
| *Bacterium xylinoides* | *Mycoderma* |
| *Gluconobacter oxydans* | *Mycotorula* |
| | *Pichia* |
| | *Pichia fermentans* |
| | *Pichia membranaefaciens* |
| | *Saccharomyces cerevisiae subsp. aceti* |
| | *Saccharomyces cerevisiae subsp. cerevisiae* |
| | *Saccharomycodes ludwigii* |
| | *Schizosaccharomyces pombe* |
| | *Torulaspora delbrueckii* |
| | *Torulopsis famata* |
| | *Zygosaccharomyces bailii* |

Source: (Kumar and Joshi, 2016)

Kombucha, Japanese tea fungus, Tea Kvas, Manchurian or Indonesian tea fungus are the names commonly used for the osmophilic yeast and bacteria which are in a symbiotic relationship in form of a membrane which is thick and jelly like and which is cultured in sweetened tea. It is a slightly sweet beverage, flavored by acetic acid and is also called tea cider. Different flavor compounds which include alcohols, ketones, aldehydes, amino acids and esters in the fermented broth have been identified.

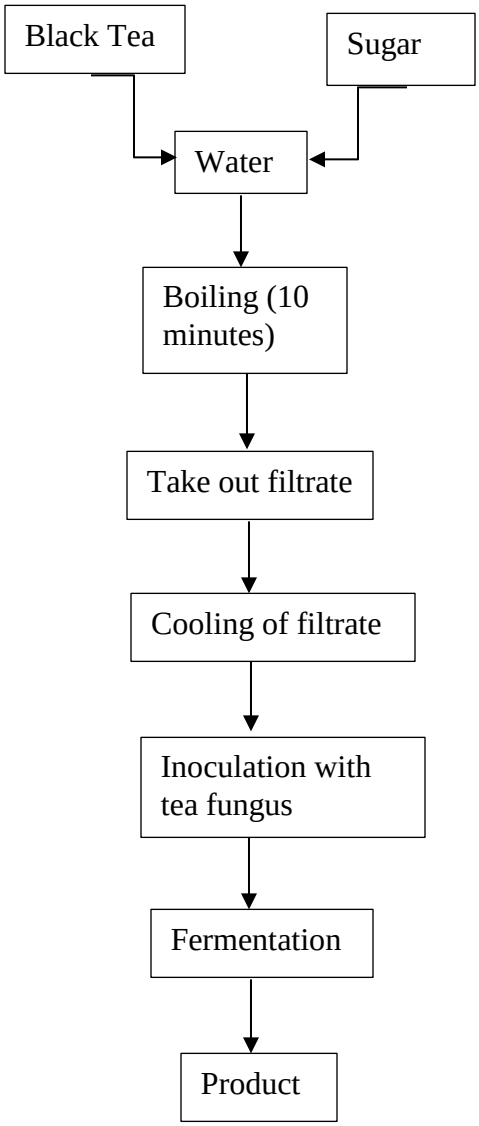


**Figure 2. 2: General process for kombucha preparation**

Traditionally, the sweetened black tea has been used for kombucha preparation and the beverage is produced through aerobic fermentation for a period of 6 to 10 days, at temperatures of between 20ºC and 30ºC(Amarasinghe *et al*., 2018). The first step of the fermentation process results in production of ethanol produced through action of yeast fermentation, which is further oxidized to acetic acid which causes a reduction in the overall pH of the medium. Besides black tea, orthodox and herbal teas have been used in production of apple tea win through inoculated and natural fermentation (Kapp and Sumner, 2019). The metabolism of kombucha produces small amounts of ethanol, fructose, glucose, $CO_2$, Folic acid and vitamins C, $B_1$, $B_2$, $B_3$, $B_6$, $B_{12}$, and the organic acid acetic acid as product (Villarreal-Soto et al., 2018). The alcohol content in Kombucha is usually less than 0.5% thus classified as a non-alcoholic beverage. If the Kombucha stays for long, becoming more acidic, the content may rise to 1.0% or 1.5%, depending on whether high quantities of sugar and yeast have been used and also more brewing time which is anaerobic. Most of the ingredients present in tea like caffeine and tea catechins are also present. The beverage is considered a prophylactic agent and of benefit to human health (Kumar and Joshi, 2016).

## 2.9 SUGAR SWEETENED BEVERAGES

The intake of sugar sweetened beverages has been shown to cause weight gain, diabetes, dental carries and metabolic syndrome (Singh et al., 2015). Since 1950, soft drinks consumption has increased by a factor of 5. Some analysis suggest that the consumption of beverages sweetened with sugar exposes one to being susceptible to metabolic syndrome, diabetes and cardiovascular disease. In certain trials, features of fatty liver and the metabolic syndrome were induced from consumption of two sixteen-ounce beverages sweetened with sugar daily for 6 months. Some controlled trials in children and adults that lasted between six months to two years showed a reduction in weight gain from lowering of soft drink intake (Bray and Popkin, 2014).

## 2.10 GAPS IN KNOWLEDGE

The utilization and commercialization of sorghum is poor despite its numerous benefits nutritionally and in terms of health. Beverage products which are purely from sorghum or have it incorporated and which exploit malting and fermentation processes are also limited in the market.

The use of *Kombucha* culture as an innovative method to improve the sensory and nutritional value of beverages especially the sorghum type of beverages has not been exploited to the maximum. This product development will help in filling the commercial gap in sorghum value added products, exploitation of fermentation and malting techniques in production of novelties and use of the culture described.

# [1] CHAPTER THREE: OPTIMIZATION OF MALTING AND FERMENTATION PROCESS OF A READY TO DRINK SORGHUM BEVERAGE AND EFFECTS ON NUTRITIONAL QUALITY.


## ABSTRACT

Sorghum is a nutritious and underutilized cereal whose potential in development of nutritious ready-to-drink beverages remain unexplored. This study aimed at optimizing malting and fermentation conditions to obtain a nutritionally superior new sorghum-based beverage product. Four beverage formulations containing sweetened sorghum malt extracts were developed through fermentation using *kombucha* culture at different temperatures (between 20ºC - 35ºC). The formulations were also malted for three, four and five days and analyzed for nutritional characteristics (protein, calories, total phenolics, tannins, vitamin c, iron and zinc) using standard analytical procedures.

The protein content was not significantly different ($p>0.05$) and ranged between 2.01 g/100ml and 3.63 g/100ml. Total sugars, Calories, vitamin C contents were significantly different ($p<0.05$) and ranged between 1.42% to 2.47%; 13.19 kcal/ml to 23.00 kcal/ml; and 4.25 mg/100ml to 6.13 mg/ml respectively. The mineral contents of the beverages were significantly different ($p<0.05$), with iron content being used in the process optimization to selected best beverage with high nutritional and sensory quality. The mean contents of iron in the beverages ranged from 1.25 mg/kg (formulation 5D1) to 29.56mg/kg (formulation 4D). The best formulation was obtained from an optimum of four days malting and fermentation temperature of 25ºC (4D). The functional properties of the beverages indicated the phenolic content ($p<0.05$) ranged between 0.1328 to 1.6601mg GAE/100ml, tannic content ranged between 1.29 mg/100g and 40.08 mg/100g respectively.

The findings indicate that nutritious beverages can be developed from sorghum by employing different malting days and fermentation conditions to come up with products having varying levels of iron, vitamin C and phenolics depending on your process or consumer needs.


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## 3.1 INTRODUCTION

Sorghum is a major source of minerals, calories and proteins for many people in Africa and Asia (Hadebe *et al*., 2017). It has drought tolerance and is adapted to both tropical and subtropical ecosystems hence is a very important subsistence crop (Amelework et al., 2016). In terms of cereal production in Kenya, it is ranked third after maize and wheat and is a staple crop for many low-income households (Kilambya and Witwer, 2013). It is the only indigenous cereal to Kenya, being cultivated even in areas considered poor in terms of potential for agriculture. This cereal has great potential to stimulate regional development and improve food security. The composition and contents of starch in the grain are influenced by the grain's growth conditions (Serna-saldivar et al., 2019). It usually ranges between 32.1-72.5 g/100 g with amylopectin being (81.0–96.5%) and amylose (3.5–19.0%) (Shegro et al., 2012). Due to starch granules, proteins and tannins being bound together strongly, it has the lowest starch digestibility among cereals (Mkandawire et al., 2013). Prolamins and non-prolamins are the main proteins present in the sorghum grain. Of the total protein composition, prolamins range between 77–82% (7-15 g/100 g) with glutelins, globulins and albumins occupying the other minor proportion (Mokrane et al., 2010).

The cereal's mineral content varies depending on area of cultivation with the minerals being phosphorus, potassium, iron and zinc. Their bioavailability is still unknown and availability of iron and zinc in the grain ranges from 6.6%-15.7% and 9.7%-17.1% respectively (Morais Cardoso et al., 2014). The phenolic acid content in some sorghum varieties varies from 135.5mg-479.40 mg/g with ferulic and protocatechuic acids as the major contributors with 120.5mg to 173.5 mg/g and 150.3mg to 178.2 mg/g respectively (Afify., *et al.*, 2012). Tannins, which are a group of phenolic compounds and are found in many plants, play part in defense against pathogens and predators. They reduce the availability of minerals, starch and proteins. Despite their anti-nutritional effect, they are better radical scavengers compared to other simple phenolics (Kaufman *et al.*, 2013). Sorghum has a lot of health benefits some of which include alleviation of the negative effects brought about by cardiovascular disease, many chronic diseases and obesity (Salazar-López et al., 2018).

The lag in commercialization of sorghum compared to other cereals in Kenya is mainly due to grain prices that cannot compete with the other cereals, levels of production which are low and which also vary, high costs of assembly and high costs of processing (Njagi *et al*., 2019).

Production of sorghum is also conducted majorly by subsistence farmers who produce just enough for their domestic use, seldom excess for sale purposes. Thus, production limitations vary from conventional to commercial scales (Omoro, 2013). Malting involves germination, under controlled conditions but in most air of cereal grains. Its main objective is modification of the chemical composition of the grain through mobilization of the endogenous enzymes. As a result, physical and rapid solubilization are enabled during brewing resulting in a nutritionally rich medium for yeast fermentation which produces carbon dioxide and ethanol (Taylor and Kruger, 2019). In hydrolysis of malt, the most important enzymes are alpha and beta amylases that cause production of fermentable maltose from starch (Taylor and Kruger, 2019).

Fermentation of cereals is usually aimed at preservation, which comes from acids production. The acids include lactic, acetic and propionic or alcohol production which is often combined with a reduction in water activity; safety enhancement of the final products by inhibition of pathogenic microorganisms; enhancement of sensory properties (color, aroma, texture and taste); nutritional value improvement by removal of anti-nutrients such as tannins, phytic acid, enzyme inhibitors and polyphenols and bioavailability enhancement of some components and carbohydrates, and indigestible poly and oligosaccharides reduction (Liptáková *et al*., 2017). Epidemiological studies have shown that consumption of fermented foods leads to an improvement in health and a decline in the risk of disease contraction. Probiotics consumption in adequate doses can confer health benefits to the consumer (Rezac et al., 2018). The probiotics use carbohydrates that are available to produce short chain fatty acids, out-compete pathogens for resources, produce antimicrobial agents, they balance the immune system and also produce vitamins (Derrien and Vlieg, 2015).

In terms of sorghum beverages, in Kenya there's locally produced sorghum beer targeted at consumers who wanted to upgrade to bottled beers from illicit drinks (Orr *et al*., 2014), coffee substitutes from sorghum for those sensitive to caffeinated beverages (Omer and Abou-zaid, 2022). There is also the malt extract from sorghum which is a sweet wort rich in sugar and which is also a beverage by itself, but can be made into a flavored syrup through concentration or powder by evaporation of the extract into a product which is dark-colored (Elgorashi *et al*., 2016). This beverage development will contribute to the sorghum value chain by adding onto the list of existing sorghum products.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Study design

A completely randomized block design was used with malting and fermentation chosen as the blocks in the experimental design.

### 3.2.2 Sample collection and preparation

Sorghum *(Sorghum bicolor L. Moench)* was purchased from Busia, finger millet (*Eleusine coracana*) from the local market in Kangemi, *kombucha* culture bought from Kombucha Kenya Company (a mushroom-like consortium of yeasts and acetic acid bacteria which are in a symbiotic relationship suspended in previously fermented broth), previously fermented *kombucha* broth and white sugar purchased from a local supermarket.

## 3.3 METHODOLOGY

### 3.3.1 Product development

1/2 a kilo of Sorghum grains were malted for 3, 4 and 5 days after which malt extraction was conducted resulting into malt extracts which were further subjected to 7 days fermentation at temperatures between 20 $^0$C - 35 $^0$C by the SCOBY (Table 3.1). Resulting beverages were analyzed for their nutritional components and the nutrient which had a significant number of reported deficiencies nutritionally and also had significant differences between the formulations was chosen as the determinant factor during the process optimization. The product flow for the beverages is as outlined in Figure 3.1.

**Table 3. 1: Experimental design used for this study**

| Formulations | Malting temperatures and days | Fermentation temperatures and days | Culture % |
|---|---|---|---|
| 1 | 20°C for 3 days | 20°C, 7 days | 2.4 |
| 2 | 20°C for 4 days | 25°C, 7 days | 2.4 |
| 3 | 20°C for 5 days | 30°C, 7 days | 2.4 |
| 4 | 20°C for 5 days | 35°C, 7days | 2.4 |

*Note: (Equal sorghum and finger millet ratios were used in all formulations and also equal culture inoculation rates)

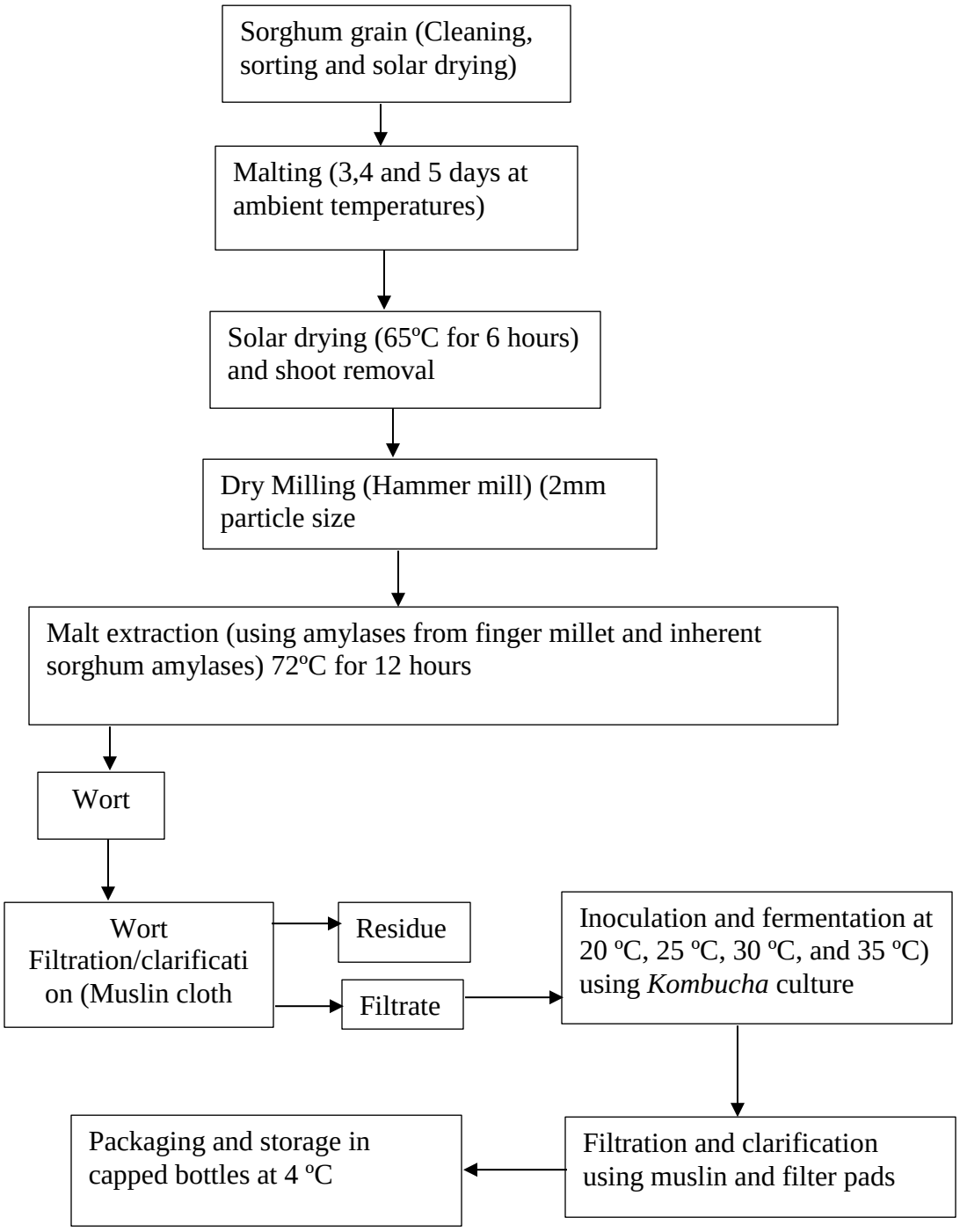


**Figure 3. 1: Process Flow Diagram for Production of A Ready to Drink Sorghum Beverage**

### 3.3.2 Malting

The malting method according to Aluge *et al*. 2016 was used, with variations being applied in the germination days per formulation. Foreign matter was sorted out and sorghum grain soaked with

potable water for 12 hours in the ratio 1:2(500g Sorghum;1000ml water). The sorghum grains were rid of water by draining then sprouted by spreading on wet blankets on malting trays on wooden benches and finally covered for the sprouting process. Daily sprinkling was done onto the grains and 50ml absolute ethanol (78% alcohol) sprayed onto the grains to prevent growth of mold. Oven drying (memmert oven supplied by GmbH and Co. Stavendamm22 model Schutzart DIN 40050-IP20) for 6 hours at 65 $^{0}$C was done to the germinated grains. After drying, meshed trays were used to remove the shoots by rubbing the sprouts off them (Hassani *et al*., 2014).

#### 3.3.2.1 Malt extraction/brewing

Slight modifications were applied to the hot water extraction method by Peter *et al*. 2014. 2 litres of portable water at ambient temperature were mixed with ½ a kilo of milled sorghum malt (supplemented with 40% malt(200g) from finger millet). Finger millet malt was essential in boosting the amylase enzyme, that is inadequate in sorghum malt during the fermentation process. The mash was left to sediment for 1 hour. 40% (1litre) of the supernatant extract composed of enzymes was decanted and kept aside using calibrated jars. 30 minutes boiling in stainless still cooking vessels of the remaining mash which was thick was conducted and the enzyme extract added after cooling.

The cooked mash was then incubated at 72 °C ± 1 °C (memmert oven supplied by GmbH and Co. Stavendamm22 Schutzart DIN 40050-IP20) overnight for saccharification then cooled to room temperature and an adjustment made to the weight to reach 3 kg with distilled water. Filtration was done to the resulting wort using a muslin cloth then finally through filter pads.

### 3.3.3 Inoculation and Fermentation

The methods according to Jayabalan *et al.* 2014 and Kumar and Joshi, 2016 with slight modifications were used. Into a wide-mouthed clean vessel which had been sterilized with boiling water, 1litre of the cool sorghum malt sweetened with 100g sucrose at ambient temperature was poured, 100ml of previously fermented *kombucha* to prevent the growth of undesirable microbes by lowering the pH and a mat of the culture (24g) placed on the surface of the infusion and the jar was hygienically covered using clean muslin cloths and properly fastened using rubber bands. The preparations were placed at different incubation temperatures (between 20 $^{O}$C and 30 $^{O}$C) for 7 days.

## 3.4 ANALYTICAL METHODS

### 3.4.1 Nutritional analysis

#### 3.4.1.1 Protein

AOAC 2012 method 991.20 was used for determination of crude protein. Into a Kjedahl flask, 0.5g of the accurately weighed samples were placed while folded in a nitrogen free filter paper. Sulphuric acid and a catalyst tablet were added to digest the sample in a fume chamber. Phenolphthalein indicator was used to indicate the end point before connection of the flask to a distillation unit. For back titration, 40% NaOH solution was used against 0.1N NaOH solution. The standard conversion factor used was 6.25.

#### 3.4.1.2 Vitamin C

AOAC 967.21 (2006) was used whereby 5ml of the test solution was titrated against prepared standard solution of ascorbic acid until the end point which was a faint pink color.

#### 3.4.1.3 Iron and zinc

The method AOAC 999.11 according to 2006 AOAC was used. The beverage samples were subjected to ashing in a muffle furnace overnight and the resulting ashes collected, acidified with nitric acid to remove acid soluble minerals and heated on a hot plate. The resulting clear solutions were diluted up to 100mls in volumetric flasks with distilled water then subjected to Buck Scientific Atomic Absorption Spectrophotometer (Model 210VGP) to obtain the mineral content readings directly using the different cathode tubes made from the elements of interest (Fe and Zn).

### 3.4.2 Total sugars

Done according to Islam *et al.*, 2013 with slight adjustments. 4 ml of anthrone reagent was added into an aliquot of pipetted 1ml beverage extract in test tubes. This was cooled after boiling for ten minutes. Preparation of a reagent blank was done and it was treated the same way. The resulting solutions' absorbances were measured at 630nm in a Perkin Elmer UV-VIS spectrophotometer model 166351. A glucose standard curve was also prepared and used in calculating the concentrations from absorbances obtained.

Total sugar content per 100ml sample was calculated using the formula:

$$\% \text{ Total sugar} = \frac{\text{Quantity of sugar obtained X 100}}{\text{Sample weight}}$$

### 3.4.3 Alcohol content determination

The procedure according to Park *et al.* (2004) was used. Distillation was performed to a final volume of 50 ml after filtration of 100 ml samples through a strainer. Distilled water was used to readjust the distillate to 100 ml. An alcohol hydrometer was used to determine the strength at room temperature.

### 3.4.4 Calorific value

This was done according to Mohammed. (2011). Calculations of calorific value were done using the Atwater factors: 4Kcal/g for carbohydrates, 4Kcal/g for protein, 9Kcal/g for fat and 7kcal/g for alcohol.

### 3.4.5 Total Phenolics

Done according to Singleton *et al*. (1999). The Folin-Ciocalteu method was used with some modifications. 50 ul of the diluted sample was mixed with Folin-Ciocalteu reagent (100ul) and deionized water was used as the diluent and control. Final dilution was done to a total volume of 1,150 ul with deionized water and mixed thoroughly. 10 minutes incubation at room temperature was done then 500 ul of 20% $Na_2CO_3$ solution added with mixing immediately and this was further incubated for 2 h at room temperature. Absorbance was recorded at 765 nm with all samples being measured in duplicate. Gallic acid (1 mg/ml) was used as the standard and the quantification of total phenolic compounds was done in milligrams per 100 ml gallic acid equivalents (mg GAE/100 ml).

### 3.4.6 Tannins determination

The method according to (Adeyeye et al., 2019) was used with modifications. 1ml sample was weighed and soaked with a solvent mixture 100ml with acetic acid and acetone in the ratio 1:4 respectively for 5 hours so as to extract the tannins. The samples were filtered and absorbance of the filtrate determined using a Perkin Elmer UV-VIS spectrophotometer model 166351 according to AOAC. A calibration curve for the standard (tannic acid) was prepared.

## 3.5 STATISTICAL ANALYSIS

The analysis was done in triplicates and data analyzed using one-way ANOVA on Genstat statistical software version 15.1. Means obtained were compared using Least Significant Difference at 5% and comparisons performed by Tukey's multiple range test.

## 3.6 RESULTS

### 3.6.1 Nutritional quality of developed sorghum beverage

The nutritional quality of raw ingredients and of the developed beverage are outlined in Table 3.2 and Table 3.3 respectively. Most nutritional components in the raw materials were significantly increased ($p<0.05$) while the non-nutritional components and sugars were reduced after processing. There was no significant difference ($p>0.05$) between the protein contents of the developed beverages. Protein content of raw unprocessed sorghum was recorded at 8.44% DM (Table 3.2), while the developed beverage was between 2.01 and 3.63 g/100ml (Table 3.3). Total sugars varied significantly ($p<0.05$) between the different formulations, with ranges between 1.42 and 2.47%, with formulation recording the highest sugar content. This could probably be due to the limited number of malting days such that sugars had not been used up a lot by the germinating seedling compared to the other formulations which have lesser sugars as the days of malting increase, the least being beverage formulation 4 (Table 3.3).

There were significant differences ($p<0.05$) in mineral content of the beverage formulations with iron ranging between 1.32 -29.56 g/l and zinc ranging between 0.7 – 2.11 g/l respectively. The mineral contents increased with days of malting and fermentation temperatures up to the fourth day then decreased sharply. For both minerals, formulation 2 had the highest concentrations, with 3 and 4 having the least concentrations that were not of significant difference between the two. From the above statistics, it is evident that formulation 2 (Four days malting and 1week fermentation at $25^0$C) is superior in terms of the content of iron, which increases with days of malting and temperature of fermentation up to the $4^{th}$ day of malting and $25^0$C fermentation then subsequently decreases with increase in both factors. Zinc had no significant difference between the different formulations (Table 3.2). In terms of vitamin C content, formulation 3 (five days malting and 1 week fermentation at $35^0$C) was the most superior while 2 and 4 had the least quantities (Table 3.2).

**Table 3. 2: Average means obtained for the different nutrients on analysis of the raw sorghum (60%) and finger millet (40%) composite flour before development of the beverage.**

| Raw materials | Protein % | Total Sugars (%) | Calories kCal/100g) | Iron (mg/100g) | Zinc (mg/100g) | Tannin (mg/100g) | Total Phenolics (GAE/100g) |
|---|---|---|---|---|---|---|---|
| Sorghum | 8.44±0.34 | 74.55±0.44 | 331.95±0.80 | 60.66±0.75 | 20.88±0.94 | 105.66±0.58 | 0.83±0.134 |

**Table 3. 3: Means obtained for the different nutrients present in the beverages on blending the raw finger millet (40%) and raw sorghum (60%)**

| Beverage formulation | Protein (g/100ml) | Total Sugars (%) | Alcohol (%) | Calories (Kcal/ml) | Vitamin C (mg/100ml) | Iron (g/l) | Zinc (g/l) |
|---|---|---|---|---|---|---|---|
| 1 | $3.28^{a}$±0.06 | $2.47^{c}$±0.001 | ND | $23.00^{b}$±0.21 | $5.04^{b}$±0.14 | $13.31^{b}$±1.37 | $1.51^{a}$±0.08 |
| 2 | $3.63^{a}$±0.56 | $1.99^{b}$±0.001 | ND | $22.48^{b}$±2.17 | $4.35^{a}$±0.28 | $29.56^{c}$±13.83 | $2.11^{a}$±0.84 |
| 3 | $3.63^{a}$±0.56 | $2.02^{b}$±0.001 | ND | $22.59^{b}$±2.20 | $6.13^{c}$±0.28 | $1.25^{a}$±0.29 | $1.21^{a}$±0.59 |
| 4 | $2.01^{a}$±0.62 | $1.42^{a}$±0.001 | ND | $13.19^{a}$±1.65 | $4.65^{a}$±0.14 | $1.32^{a}$±0.26 | $0.70^{a}$±0.36 |

Mean values with common superscript letters in a column indicate no significant difference among samples (P>0.05) from Tukey's mean test. ND – not detected

Beverage 1:3 days malting and 7 days fermentation at $20^{0}$C

Beverage 2: 4 days malting and 7 days fermentation at $25^{0}$C

Beverage 3:5 days malting and 7 days fermentation at $30^{0}$C

Beverage 4:5 days malting and 7 days fermentation at $35^{0}$C

### 3.6.2 Non-nutritional composition of developed sorghum beverage

There was a significant difference ($p<0.05$) in the tannin content between the different sorghum beverage formulations with formulation 1 having the highest tannin contents (40g/100g) and total phenolic content (1.66mg/100g) as outlined in Table 3.4 respectively. They were highest in formulation 3D and lowest in 4D and 5D2. The malting days for the cereals to have less tannins were 4 days as from the values obtained (Table 3.4).

For phenolics, despite significant differences between the formulations, the trend was not well defined since 4D and 5D1 had the least phenolics content and 5D2 the highest. Formulation 4 (5 days malting and 1-week fermentation at $35^{0}$C) is characterized by the highest total phenolics

content (Table 3.4).

**Table 3. 4: Mean total phenolics and tannin contents of the different formulations**

| Beverage Formulation* | Tannins (mg/100g) | Total Phenolics (GAE/100 ml) |
|---|---|---|
| 1 | $40.08^{c}$±0.26 | $0.35^{b}$±0.01 |
| 2 | $1.29^{a}$±0.04 | $0.13^{a}$±0.05 |
| 3 | $2.17^{b}$±0.07 | $0.17^{a}$±0.02 |
| 4 | $1.31^{a}$±0.07 | $1.66^{c}$±0.06 |

Mean values with common superscript letters in the same column indicate no significant differences among the formulations (P>0.05) from Tukey's mean test.

*Beverage 1 – 3 days malting and 7 days fermentation at $20^{0}$C

Beverage 2 – 4 days malting and 7 days fermentation at $25^{0}$C

Beverage 3 -5 days malting and 7 days fermentation at $30^{0}$C

Beverage 4-5 days malting and 7 days fermentation at $35^{0}$C

### 3.6.3 Malting effects on nutritional quality of developed sorghum beverages

Malting or fermentation as treatments yield nutritionally superior products. The contributions of malting to the different nutrients can be seen especially on tannins and iron content on which there are significant differences between the samples. The fermentation temperatures used also cause significant differences ($p<0.05$) in the content of sugar, calories, vitamin c, iron, phenolics and tannins as outlined in Table 3.4. The results outlined in Table 3.5 indicate that there was no interaction between fermentation temperatures and the days of malting owing to the experimental design used.

**Table 3.5: Effect of fermentation temperatures on the nutritional quality**

| Temperature (°C) | Vitamin C (mg/100ml) | Phenolics (GAE/100ml) | Tannins (mg/100ml) | Calories (kcal) | Iron (g/l) | Protein (g/100ml) | Zinc (g/l) | Sugars (%) |
|---|---|---|---|---|---|---|---|---|
| 30 | $6.13^{b}$ | $0.17^{a}$ | $2.17^{b}$ | $22.59^{b}$ | $1.25^{a}$ | $3.63^{a}$ | $1.12^{a}$ | $2.02^{b}$ |
| 35 | $4.65^{a}$ | $1.66^{c}$ | $1.31^{a}$ | $13.19^{a}$ | $1.32^{a}$ | $2.01^{a}$ | $0.70^{a}$ | $1.42^{a}$ |
| 20 | $5.04^{a}$ | $0.35^{b}$ | $40.08^{c}$ | $23.001^{b}$ | $13.31^{a}_{b}$ | $3.28^{a}$ | $1.51^{a}$ | $2.47^{c}$ |
| 25 | $4.35^{a}$ | $0.13^{a}$ | $1.29^{a}$ | $22.49^{b}$ | $29.56^{b}$ | $3.63^{a}$ | $2.11^{a}$ | $1.99^{b}$ |
| P | 0.001 | <0.0001 | <0.0001 | 0.013 | 0.041 | 0.085 | 0.204 | 0 |
| Significant | Yes | Yes | Yes | Yes | Yes | No | No | Yes |

Mean values with different superscript in a column are significant at $p<0.05$. Means separated and compared with Tukey's test at $p<0.05$

### 3.6.4 Effects of fermentation on nutritional quality of developed sorghum beverages

Table 3.6 outlines the effect of fermentation on the nutritional quality of the developed sorghum-based beverages. The results indicate that fermentation temperatures were found to have a significant effect ($p<0.05$) on the levels of vitamin c, phenolics, tannins, calories, iron and sugars but had no effect ($p>0.05$) on protein and zinc contents.

The number of malting days used were also found to have a significant effect ($p<0.05$) on the levels of tannins and iron content. There was no significant effect ($p>0.05$) on the levels of vitamin C, phenolics, calories, protein, zinc and sugars (Table 3.6).

**Table 3 6: Effect of malting days on the nutritional quality of the beverage formulations**

| Malting days | Vitamin C (mg/100ml) | Phenolics (GAE/100ml) | Tannins (mg/100ml) | Calories (kcal) | Iron (g/l) | Protein (g/100ml) | Zinc (g/l) | Sugars (%) |
|---|---|---|---|---|---|---|---|---|
| 5 | 5.39[a] | 0.92[a] | 1.74[a] | 17.89[a] | 1.28[a] | 2.82[a] | 0.91[a] | 1.72[a] |
| 4 | 4.35[a] | 0.13[a] | 1.29[a] | 22.49[a] | 29.56[b] | 3.63[a] | 2.11[a] | 1.99[a] |
| 3 | 5.04[a] | 0.35[a] | 40.08[b] | 23.01[a] | 13.31[a] | 3.28[a] | 1.51[a] | 2.47[a] |
| P | 0.309 | 0.407 | <0.0001 | 0.381 | 0.009 | 0.566 | 0.105 | 0.061 |
| Significant | No | No | Yes | No | Yes | No | No | No |

Mean values with different superscript in a column are significant at $p<0.05$. Means separated and compared with Tukey's test at $p<0.05$

## 3.7 DISCUSSION

Compared to most of the beverages currently in the market, the protein content of the developed beverages is higher and can contribute towards meeting the daily protein requirements. Protein content forms an important basis for the quality of a beverage. The interactions among proteins, amino acids and phenols greatly influence the stability and organoleptic characteristics of the beverage. The amino acids also form a great component of the beverage's aromatic compounds. There was increase in protein content with increase in fermentation period which can be attributed to an increase in microbial mass (Correia et al., 2010). This could be supported by the favorable pH for the growth of lactic acid bacteria with the progress of the fermentation which in turn could cause extensive hydrolysis of the protein molecules to amino acid and other simple peptides (Das et al., 2015). It is also worth noting that increased fermentation time yielded lower protein content. This is because the fermenting microorganisms also uses amino acid which could lower the protein content and quality of some fermented food (Pranoto *et al*., 2013).

Sugars form an important part for calories provision in an individual. Beverage F1 had the highest sugar content. The initial days of malting facilitated the enzymatic breakdown of carbohydrates into simple sugars through activation of endogenous enzymes such as α-amylase thereby improving digestibility as a result of degradation of starch to provide energy for the seed development (Nkhata et al., 2018). The rest of the beverage formulations had decreased sugars, an occurrence that can be linked to the malting periods. In earlier stage of germination, large portions of soluble sugars are expected to be used up during respiration and not enough α-amylase has been synthesized or activated to hydrolyze starch, leading to less increase in sugars (Okolo et al., 2020). However, after 36–48 hr of germination, the dormancy is lost as the amylolytic enzymes synthesized in the aleurone layer migrate into the endosperm and initiate the hydrolysis of starch granules. Glucose and fructose levels are generally low in the raw cereals at this moment, however, on germination, the two soluble sugars increase significantly such that their levels supersede that of sucrose activation of invertase which hydrolyzes sucrose into glucose and fructose during germination (Oseguera-Toledo et al., 2020). This explains the gradual increase then decrease to a further increase in sugar content in the formulations depending on malting periods. Calories were not significantly different between formulations 1, 2 and 3 while 4 had the least calories due to also having the least sugars.

Cereals have most of the nutritional elements bound. Malting ensures the bound mineral components are released. This explains the increase in mineral content (iron and zinc) up to day 4. The increase could be due to leaching of the antinutritional factors that bind the minerals. It has been hypothesized that the remarkable increase in phytase activity during germination helps reduce phytic acids, which bind minerals subsequently leading to increased mineral availability (Nkhata et al., 2018). After day 4, the bound elements have been released hence accounting for the sharp decrease. The initial increase in tannin content could be attributed to hydrolysis of condensed tannins such as proanthocyanidin. While the eventual decrease may be due to their binding with cotyledon endosperm that are usually undetected by routine method due to their insolubility in solvent or may be due to microbial phenyl oxidase action as explained by (Osman, 2011).

Phenolic content increased with increasing days of fermentation, a factor which may be attributed to an increase in the level of free soluble phenolics, due to hydrolysis of the glycosidic bonds of bound phenolics by hydrolytic enzymes secreted by microorganisms in the culture (Elkhalifa and Bernhardt, 2018). Phenolic compounds provide the antioxidant compounds in a beverage. These

phenolic compounds have several functional properties in the beverage and influence its colloidal stability, flavor and color (Adebo and Medina-Meza, 2020). Phenolic compounds are also important antioxidants, and owing to this antioxidant capacity and low alcoholic content, consumption of beer helps to improve the plasma antioxidant activity and reduce the risk of cardiovascular diseases (Das et al., 2015). For phenolics, they affect the taste of products especially when they are very high in concentration. They were highest in this formulation due to increased number of malting days as suggested by (Carciochi et al., 2016) and also fermentation temperature (Aguilar et al., 2019).

For vitamin C, consumption of 300ml of either of the four beverages is enough to meet the daily requirements of the nutrient. Osman, (2011) confirmed that malting and fermentation increase the quantity of vitamin C. Vitamin C can be synthesized during malting by the hydrolysis of starch using amylases and diastases that avail glucose for this process. This enhanced content of glucose is the one that acts as a precursor to formation of vitamin c. This study confirmed that C-6 of glucose could be oxidized to form the carboxyl carbon of the ascorbic acid concluded that the same could happen in plants during fermentation or malting.

For process optimization, iron content was chosen as the standard due to the fact that previous studies have shown that phenolics can contribute to bitter taste especially when in exceeding amounts, for example in olive oil as suggested by Shahidi and Ambigaipalan (2015) despite them having health benefits against cancer and cardiovascular diseases. Based on the recommended dietary allowances issued by WHO (8mg/day), this beverage will easily meet the requirements of all individuals. Formulation 4D is well balanced in terms of phenolics which are lower in amounts compared to the rest hence the issue of bitterness may not be present and also in terms of the content of vitamin C- it contains an amount which is almost similar to the other formulations when the means are compared.

## 3.8 CONCLUSION

It is quite interesting to note that varying the number of malting days and use of different fermentation temperatures can lead to considerable differences nutritionally in the final cereal products despite similarities in certain nutrient compositions as in the above scenario. The best formulation for the beverage development for good nutritional output is four days malting at $25^0$C

fermentation temperature for one week. Sensory analysis output can also be used for optimization purposes then the products during commercialization can be improved nutritionally via fortification as is done by most food industries.

## CHAPTER FOUR: FUNCTIONAL AND MICROBIAL PROPERTIES OF A SORGHUM BEVERAGE DEVELOPED THROUGH MALTING AND FERMENTATION


### ABSTRACT

Sorghum grain has been showed to contain bioactive compounds that have been observed to exhibit health promoting functionalities such as antidiabetic activity, antioxidant and antimicrobial activity among other properties. These therapeutic health benefits of sorghum can be added onto the list of existing sorghum products and also for purposes of providing consumers of soft drinks and energy drinks with other nutritional and health benefits apart from sweet tastes only. This study thus evaluated the effect of fermentation with *Kombucha* culture on the functional and microbial properties of a sorghum-based beverage. Malting was done for 3 to 5 days and fermentation of the malt extract done at temperatures between 20 $^0$C to 35 $^0$C using *Kombucha* culture after which phenolics, tannins and microbial properties were analyzed. Total phenolics differed significantly ($p<0.05$) between the formulations (0.1328 GAE/100ml - 1.6601GAE/100ml), tannins also had a significant difference between the formulations (1.29 - 40.08 g tannic acid equivalents per 100ml). For the antibacterial tests which proved the beverages to be effective against food borne pathogens *E. coli* and *S. aureus* halo diameters were measured with significant differences ($p<0.05$) in diameters being noted for *E. coli* between the different samples, beverage 3D forming the smallest halo zone least compared to the rest. The minimum inhibitory concentration for the beverages to be effective antimicrobials was found to be 8%. Microbial enumeration results showed dominance of lactic acid bacteria and yeasts compared to acetic acid bacteria. The microbial counts overall increased with increasing malting days and temperatures of fermentation. Total viable counts were highest in 5D2 with the least being in 4D and 5D1. Enumeration of coliforms showed that the beverages were contaminated but which could be avoided by using distilled water and ensuring proper hygiene during processing. The study results indicate that optimum processing conditions are essential in ensuring production of wholesome and nutritious beverages from sorghum. In addition, the study also shows the enhanced functional and microbial properties, that need further studies to understand how processing conditions can be optimized further to extract full helath benefits from sorghum-based beverages.

## 4.1 INTRODUCTION

Sorghum possesses numerous benefits nutritionally and a lot of research has been done on it in Africa but in East Africa, its production, adoption, utilization and commercialization has been poor (Ngugi *et al*., 2013). Sorghum has been heralded for its nutritional and therapeutic effects in alleviating lifestyle diseases such as cancers, cardiovascular diseases and obesity (Duodu and Awika, 2019; Salazar-López et al., 2018). The composition and contents of starch in the grain are influenced by the grain's growth conditions (Hill *et al*., 2012). It usually ranges between 32.1-72.5 g/100 g with amylopectin being (81.0–96.5%) and amylose (3.5–19.0%) (Gerrano et al., 2012). Due to starch granules, proteins and tannins being bound together strongly, it has the lowest starch digestibility among cereal (Mkandawire *et al*., 2013). Non-starch polysaccharides in the grain are of two kinds-soluble and insoluble fibers with arabinoxylans being the main insoluble fibers. They account for 75-90% of non –starch polysaccharides (6-15g/100g). The soluble fibers in the grain range from 10-25% (Martino et al., 2012).

Prolamins and non-prolamins are the main proteins present in the sorghum grain. Of the total protein composition, prolamins range between 77–82% (7-15 g/100 g) with glutelins, globulins and albumins occupying the other minor proportion (Girard and Awika, 2018). Kafirins are the main prolamins of sorghum and are of three types: a, b and g-kafirins with their quantities are 66-84%, 8-13% and 9-21% respectively (Mokrane et al., 2010). The cereal's mineral content varies depending on area of cultivation with the minerals being phosphorus, potassium, iron and zinc. Their bioavailability is still unknown and availability of iron and zinc in the grain ranges from 6.6%-15.7% and 9.7%-17.1% respectively. Sorghum is also a good source for fat soluble vitamins (D, E, and K) and B vitamins (B1, B2 and B3) (de Morais Cardoso et al., 2017).

The phenolic acid content in some sorghum varieties varies from 135.5mg-479.40 mg/g with ferulic and protocatechuic acids as the major contributors with 120.5mg to 173.5 mg/g and 150.3mg to 178.2 mg/g respectively. Other acids which are in minimal amounts are p-coumaric acid (41.9mg-71.9 mg/g), syringic (15.7mg-17.5 mg/g), vanillic (15.4mg to 23.4 mg/g), caffeic (13.6mg to 20.8 mg/g), cinnamic (9.8 to 15.0 mg/g), gallic (14.8mg to 21.5 mg/g) and p-hydroxybenzoic (6.1mg to 16.4 mg/g)acids (Afify *et al.*, 2012).

Tannins, which are a group of phenolic compounds and are found in many plants, play part in defense against pathogens and predators. They reduce the availability of minerals, starch and

proteins. Despite their anti-nutritional effect, they are better radical scavengers compared to other simple phenolics (Kaufman *et al.*, 2013).

Through metabolism, microbes can produce biologically active molecules and vitamins originally absent in a food. Probiotic microorganisms can positively contribute to health due to their ability to out-compete pathogenic microbes for available resources, production of anti-microbial agents, utilization of available carbohydrates resulting in production of short chain fatty acids, vitamin production and contribution to the immune system's homeostasis immune system (Derrien and Vlieg, 2015).

Thus, this study was designed to evaluate the effect of malting process and period on the development of functional properties and microbial properties in the developed sorghum-based beverage.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Study design

A completely randomized block design was used with malting and fermentation chosen as the blocks in the experimental design.

### 4.2.2 Sample collection and preparation

Sorghum *(Sorghum bicolor L. Moench)* purchased from Busia, finger millet (*Eleusine coracana*) from the local market in Kangemi, *kombucha* culture bought from Kombucha Kenya Company (a mushroom-like consortium of yeasts and acetic acid bacteria which are in a symbiotic relationship suspended in previously fermented broth), previously fermented *kombucha* broth and white sugar purchased from a local supermarket.

**Table 4 1: Experimental design used for the experiment**

| Formulations | Malting temperatures and days | Fermentation temperatures and days | Culture % |
|---|---|---|---|
| 1 | 20°C for 3 days | 20°C, 7 days | 2.4 |
| 2 | 20°C for 4 days | 25°C, 7 days | 2.4 |
| 3 | 20°C for 5 days | 30°C, 7 days | 2.4 |
| 4 | 20°C for 5 days | 35°C, 7days | 2.4 |

Note: (Equal sorghum and finger millet ratios were used in all formulations and also equal culture inoculation rates)

### 4.2.3 Product development

Sorghum grains(500g) were malted for 3, 4 and 5 days after which malt extraction was conducted resulting into malt extracts which were further subjected to 7 days fermentation at temperatures between 20°C-35°C by the SCOBY. Resulting beverages were subjected to tannins, phenolics and also microbiological analyses.

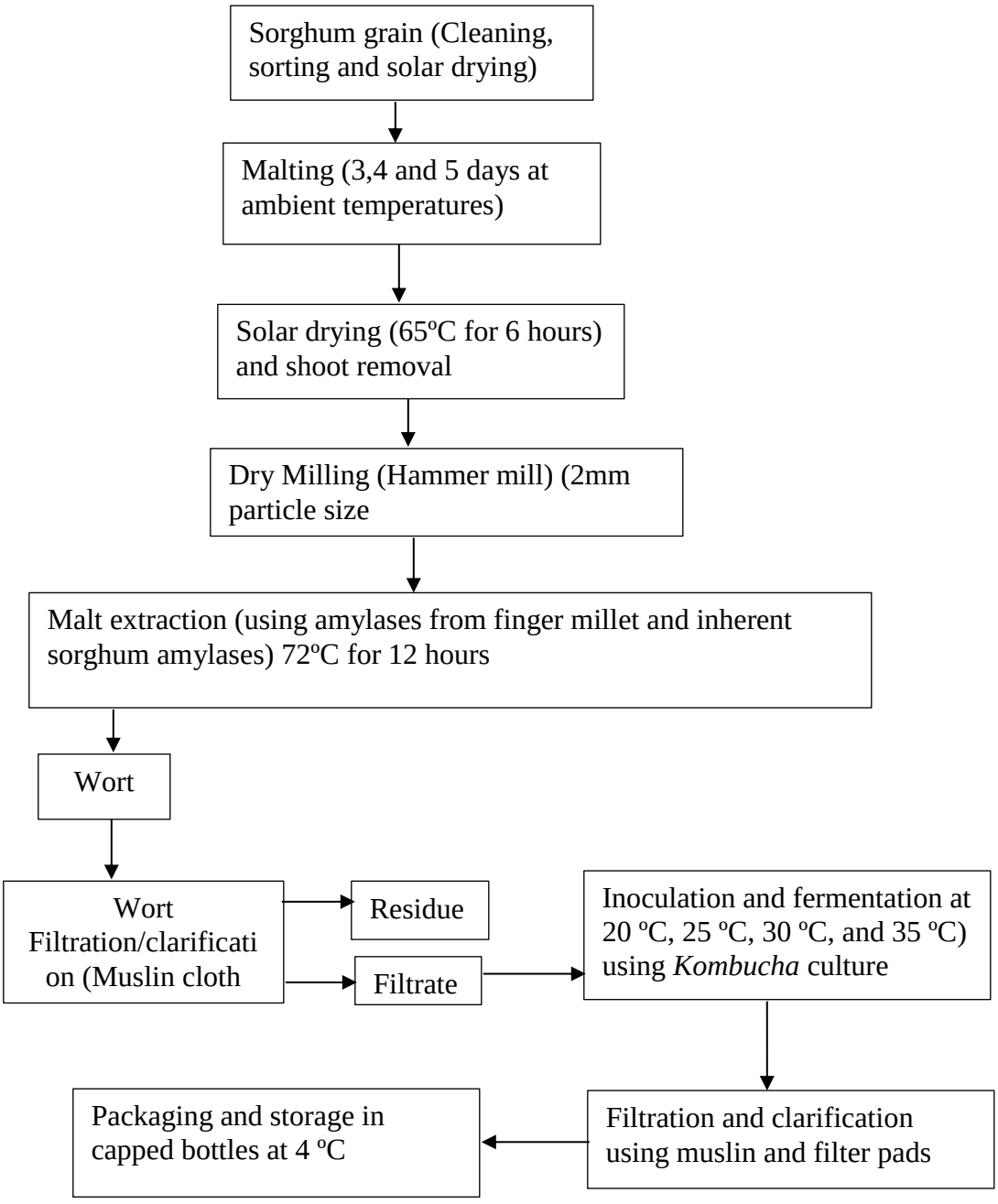


**Figure 4. 1: Process Flow Diagram for Production of The Ready to Drink Sorghum Beverage**

#### 4.2.3.1 Malting

The malting method according to Aluge *et al*. 2016 was used, with variations being applied in the germination days per formulation. Foreign matter was sorted out and grain soaking done in buckets with potable water for 12 hours in the ratio 1:2(500g:1litre). The grains were rid of water by draining then sprouted by spreading on wet blankets on malting trays on wooden benches and finally covered for the sprouting process. Daily sprinkling was done onto the grains and absolute ethanol (50ml) sprayed onto the grains to prevent growth of mold. Oven drying (memmert oven supplied by GmbH and Co. Stavendamm22 model Schutzart DIN 40050-IP20) for 6 hours at 65 $^{0}$ C was done to the germinated grains. After drying, meshed trays were used to remove the shoots by rubbing the sprouts on them (Hassani *et al*., 2014).

##### 4.2.3.1.1 Malt extraction/brewing

Slight modifications were applied to the hot water extraction method by Peter *et al*. 2014. 2 litres of portable water at ambient temperature were mixed with ½ a kilo of milled sorghum malt (supplemented with 40%(200g) malt from finger millet). The mash was left to sediment for 1 hour. 40% (1litre) of the supernatant extract composed of enzymes was decanted and kept aside using calibrated jars. 30 minutes boiling in stainless still cooking vessels of the remaining mash which was thick was conducted and the enzyme extract added after cooling.

The cooked mash was then incubated at 72 °C ± 1 °C (memmert oven supplied by GmbH and Co. Stavendamm22 Schutzart DIN 40050-IP20) overnight for saccharification then cooled to room temperature and an adjustment made to the weight to reach 3 kg with distilled water. Filtration was done to the resulting wort using a muslin cloth then finally through filter pads.

#### 4.2.3.2 Inoculation and Fermentation

The methods according to Jayabalan *et al.* (2014) and Kumar and Joshi, (2016) with slight modifications were used. Into a wide-mouthed clean vessel which had been sterilized with boiling water, 1litre of the cool sorghum malt sweetened with 100g sucrose at ambient temperature was poured, 100ml of previously fermented *kombucha* to prevent the growth of undesirable microbes by lowering the pH and a mat of the culture (24g) placed on the surface of the infusion and the jar was hygienically covered using clean muslin cloths and properly fastened using rubber bands. The preparations were placed at different incubation temperatures (between 20 and 30 $^{O}$C) for 7 days.

## 4.3 ANALYTICAL METHODS

### 4.3.1 Antioxidant activity

#### 4.3.1.1 Total phenolics

The method according to Singleton *et al*. (1999) with modification. It involved the use of Folin-Ciocalteu method with some adjustments. 50 ul of the diluted sample was mixed with Folin-Ciocalteu reagent (100ul) and deionized water was used as the diluent and control. Final dilution was done to a total volume of 1,150 ul with deionized water and mixed thoroughly. 10 minutes incubation at room temperature was done then 500 ul of 20% $Na_2CO_3$ solution added with mixing immediately and this was further incubated for 2 h at room temperature. Absorbance was recorded at 765 nm on a Perkin Elmer UV-VIS spectrophotometer model 166351 with all samples being measured in duplicate. Gallic acid (1 mg/ml) was used as the standard and the quantification of total phenolic compounds was done in milligrams per 100 ml gallic acid equivalents (mg GAE/100 ml).

#### 4.3.1.2 Tannins determination

The method according to Adeyeye *et al.* (2019) was used with modifications. 1ml sample was weighed and soaked with a solvent mixture 100ml with acetic acid and acetone in the ratio 1:4 respectively for 5 hours so as to extract the tannins. The samples were filtered and absorbance of the filtrate determined using a Perkin Elmer UV-VIS spectrophotometer model 166351 according to AOAC. A calibration curve for the standard (tannic acid) was prepared.

### 4.3.2 Microbial properties

#### 4.3.2.1 Antibacterial test

This was done according to Velićanski *et al.* (2014) with modifications. The antimicrobial activity of the developed beverages was tested. Test microorganisms were *Staphylococcus aureus* and *Escherichia coli*. The method known as agar well diffusion was used with slight modifications. To acquire the microbial cultures, isolation and characterization was done.

#### 4.3.2.1.1 *Escherichia coli*

For *E. coli,* effluent water from University of Nairobi Upper Kabete Pilot plant was used as the source. Gram staining was done to see whether the cells were present. The cells were gram negative (pink) and resembled short rods. Hichrome media was prepared and sterilized in an autoclave then used for the growth of the microbes at $37^0$C for 24hrs. To determine whether the culture was pure, IMVic test was done. IMVic media was prepared and it consisted of peptone water and 200mls distilled water and MRVP media and 200ml distilled water, which were boiled together. To prepare 200mls MRVP, the following were used: Peptone (7g/l), glucose(5g/l) and potassium dihyrogen (5g/l). 10mls of each media were poured into 6 test tubes, autoclaved and a loopful of the cells inoculated, turning the media to pink (Positive result). A colony counter was used to estimate the cell number per ml in CFU/ml of the stock solution. Homogenization of 9ml of melted Mueller Hinton agar with 1 mL microbial suspension was done and poured into petri dishes. 9 mm diameter wells were made in the plates containing agar using a sterile metal tube. 100 µL samples were transferred into the wells containing the inoculations. Incubation of the plates at 37 °C for 24 h was done and the halo zone diameters measured (mm).

#### 4.3.2.1.2 *Staphylococcus aureus*

For *Staphylococcus aureus,* media preparation and sterilization was done and a swab done around the nose area. Streaking was done on Baird Parker media for cultivation of the microbes at $37^0$C for 48 hours. They were subjected to gram staining to establish the positive colonies, which were subjected to a catalase confirmatory test.

### 4.3.2.2 Minimum inhibitory concentrations

After determination of the diameters, minimum inhibitory concentrations of the beverages which were effective against the microbes were determined. After media preparation and sterilization, 20mls media and 10%, 20%, 30%, 40% samples were homogenized together then 0.1 mls of respective microbes suspended in 10ml sterile distilled water were inoculated into the media containing the sample and incubated overnight at $35^0$C to see whether growth will occur. Sterile distilled water was used as the control. Afterwards, narrowing down was done to find the best concentration of sample that is effective against the microbes using the same procedure as above. Note: All analyses were carried out on the liquid components and not on the cellulose layer.

#### 4.3.3.3 Acetic acid and lactic acid bacteria enumeration

Done according to Ghariani *et al.* (2017) with moderate adjustments where acetic acid bacteria (AAB) were isolated by the spread plate method on Glucose yeast agar (0.8% yeast extract, 2% D-glucose, 0.5% ethanol, 0.3% $CaCO_3$, 0.5% peptone and 1.5% agar). This was supplemented with bromocresol blue to differentiate between glucobacter (turns to green) and acetobacter (retains the blue color). The plates were incubated aerobically at 30°C for 24 hours and the colony forming units recorded in logarithmic colony forming units/ml of sample (Log CFU/ml). The dilutions used were $10^{-1}$ to $10^{-3}$.

de Man, Rogosa and Sharp agar (MRS) supplemented with cycloheximide was used for the isolation and enumeration of Lactic acid bacteria (LAB). Cycloheximide prevents yeast and mold growth. Duplicates of 10 ml of the samples were suspended in 90 mL of sterile 2% (w/v) NaCl solution, homogenized aseptically and the results expressed as Log CFU/ml of sample.

#### 4.3.3.4 Total viable count test

Done as per the AACC method 42-11-01 with slight modifications. 25 ml beverage samples were put into 225 ml of 0.85% Nacl diluent and serial dilutions performed from $10^{-4}$ to $10^{-6}$. 1 ml of the serial dilutions were poured into the petri dishes and prepared Plate Count Agar poured on the plates which were then incubated at $37^0$C for 24 hours and enumerated using the colony counter technique. Results were expressed in Log CFU/ml.

#### 4.3.3.5 Yeasts enumeration

AACC 42-50-02 method was used. 25ml of the beverage samples were put into 225 ml 0.85% Nacl solution and serial dilutions performed from $10^{-3}$ to $10^{-5}$. The spread plate technique was used after preparation of Potato Dextrose Agar (containing 1% tartaric acid which acts as an antimicrobial agent for other organisms apart from yeasts and mould). The inoculated plates were incubated at $30^0$C for 48 hours then observed under a microscope. Results were expressed as Log Cfu/ml.

#### 4.3.3.6 Total coliforms

The method done as per the ISO method ISO 4831:2006 was used. The total coliforms were enumerated by the pour plate technique on Violet Red Bile Agar. After solidification, the inoculated plates were incubated aerobically at $37^0$C for 24 hours after which enumeration was done. Results were expressed as log Cfu/ml.

## 4.4 STATISTICAL ANALYSIS

Data was processed using one-way ANOVA on GENSTAT statistical software version 15.1. The method of least significant difference (LSD) (at 5% significance level) under Tukey test was used to compare means.

## 4.5 RESULTS

### 4.5.1 Tannin and Total Phenolic contents of the developed beverages

The tannin and phenolic content of the developed beverages are as shown in Table 4.2. The tannin content was significant ($p<0.05$) among the beverages with amount decreasing with number of malting days. Tannin content ranged between 1.31 mg/100 ml to 40.08 mg/100 ml, with highest tannin content recorded in formulation 3D, and lowest in 4D and 5D2.

The phenolic content of the developed sorghum beverages was significantly different ($p<0.05$) and ranged between 0.13 mgGAE/100 ml to 1.66 mg GAE/100 ml as outlined in Table 4.2. The formulations 4D and 5D1 had the least phenolics content and 5D2 the highest (Table 4.2).

**Table 4. 2: Tannin and Total phenolic contents of the developed beverages**

| Beverage | Tannins (mg/100 ml tannic acid equivalents) | Total Phenolics (mg GAE/100ml) |
|---|---|---|
| 3D | $40.08^{c}\pm0.26$ | $0.35^{b}\pm0.01$ |
| 4D | $1.29^{a}\pm0.04$ | $0.13^{a}\pm0.01$ |
| 5D1 | $2.17^{b}\pm0.07$ | $0.17^{a}\pm0.02$ |
| 5D2 | $1.31^{a}\pm0.06$ | $1.66^{c}\pm0.06$ |

Mean values with common superscript letters in a column indicate no significant difference among samples ($P>0.05$)

### 4.5.2 Antimicrobial activities of the developed sorghum beverages

The antimicrobial properties of developed sorghum beverages are as outlined in Table 4.3. For the antimicrobial properties, the diameters of the halos were measured and the beverages were found to exert some effect against the growth of the microbes. There was significant difference ($p<0.05$) in the diameters formed when the beverages were tested against *E. coli* with 4D, 5D1 and 5D2 having the largest diameters and 3D the least. There was no significant difference ($p>0.05$) between the beverage's effects on *S. aureus.* All had an equal effect (Table 4.3).

**Table 4. 3: Halo zone diameters showing the antimicrobial activities of the beverages against *E. coli* and *S. aureus***

| Formulated Beverage | *Escherichia coli* diameters (mm) | *Staphylococcus aureus* diameters (mm) |
|---|---|---|
| 3D | $13^{a}\pm0.01$ | $14.50^{a}\pm0.71$ |
| 4D | $16.5^{b}\pm0.71$ | $15.00^{a}\pm2.83$ |
| 5D1 | $18.5^{b}\pm0.71$ | $17.50^{a}\pm0.71$ |
| 5D2 | $17.5^{b}\pm0.71$ | $15.00^{a}\pm1.41$ |

Mean values with common superscript letters in a column indicate no significant difference among samples (P>0.05)

The minimum inhibitory concentration for the beverages was found to be below 10%. After doing trials (2%, 5% and 8%) it was discovered that the minimum concentration that causes no growth for both microbes is between 5% and 8%.

Acetic acid bacteria counts were lowest in formulation 3D (2.145 log cfu/ml) and highest in formulation 5D2 (4.855 log cfu/ml). The least counts of lactic acid bacteria were obtained in formulation 3D (4.455 log cfu/ml) and the highest in 5D2 (6.125 log cfu/ml). LAB counts were higher than Acetic acid bacteria counts (AAB) counts by 2 log cycles. Yeast counts were also higher than AAB counts with the highest counts being in 5D2 (4.795 log cfu/ml) and the least in 3D (3.815 log cfu/ml). The trend shown by yeast counts was similar to the one followed by AAB and LAB, counts increasing with increasing malting days and temperatures of fermentation. Highest TVC was found in beverage 5D2 with the least being in 4D and 5D1.

**Table 4. 4: Microbial counts of the different beverage formulations after enumeration**

| Fermented beverage* | Acetic acid bacteria (log cfu/ml) | Lactic acid bacteria (log cfu/ml) | Total viable counts (log cfu/ml) | Yeast (log cfu/ml) | Total coliforms (log cfu/ml) |
|---|---|---|---|---|---|
| 3D | $2.15\pm0.05^{a}$ | $4.46\pm0.04^{b}$ | $5.43\pm0.04^{b}$ | $3.82\pm0.05^{a}$ | $2.98\pm0.01^{a}$ |
| 4D | $2.46\pm0.04^{b}$ | $4.77\pm0.01^{c}$ | $4.59\pm0.16^{a}$ | $4.28\pm0.03^{b}$ | $3.88\pm0.02^{b}$ |
| 5D1 | $2.50\pm0.03^{b}$ | $4.63\pm0.03^{b}$ | $4.84\pm0.08^{a}$ | $4.53\pm0.02^{c}$ | $4.62\pm0.02^{c}$ |
| 5D2 | $4.86\pm0.01^{c}$ | $6.13\pm0.01^{d}$ | $7.18\pm0.01^{c}$ | $4.80\pm0.02^{d}$ | $5.08\pm0.01^{d}$ |

*Values with different superscripts along a row are significantly different at p<0.05. Means separated and compared with Tukey's test at p<0.05

## 4.5 DISCUSSION

### 4.5.1 Functional Properties

The increase in tannin during fermentation was attributed to hydrolysis of condensed tannins such as proanthocyanidin to phenols (Embashu and Nantanga, 2019). Tannins bind minerals and reduce their bioavailability depending on the duration of fermentation (Tamilselvan and Kushwaha, 2020). Prolonged fermentation decreased the tannin due to microbial phenyl oxidase action (Mokhtar et al., 2021). Nonuniform changes in the phenol concentration during fermentation obtained in our study can be explained by biotransformation of phenolic compounds, such as catechins (as well as other complex poly-phenols) by the enzymes extracted from symbiotic culture of bacteria and yeast (Velićanski et al., 2014). Additionally, catechins are released from acid-sensitive cells during kombucha fermentation which could be the reason for the increase in polypheolic content during fermentation. On the other hand, catechins may polymerize to molecules of higher molecular mass, and thus lower the content of polyphenols (Disharoon et al., 2021). Phenolics affect the taste of products especially when they are very high in concentration.

### 4.5.2 Microbial properties

Research on kombucha cultures has demonstrated its antimicrobial efficacy against pathogenic microorganisms of both Gram-positive and Gram-negative origin (Morales, 2020). Its antimicrobial activity is largely attributable to the presence of organic acids, particularly acetic acid, large proteins, and catechins (Hou et al., 2021). Acetic acid and catechins are known to inhibit a number of Gram-positive and Gram-negative microorganisms (Jayabalan et al., 2014). This indicates that consumption of the beverages can help protect against food poisoning especially in events where food is prepared by different people and served in masses for example weddings and funerals.

For the minimum inhibitory concentration, at 8% there was no growth of both microbes while at 5% it was minimal. It is safe to conclude that 8% is the minimum inhibitory concentration for the beverages to prevent growth and proliferation of these microorganisms which are responsible for majority of food poisonings (Hou et al., 2021). This proper antimicrobial effect is due to the presence of acetic acid in this symbiotic culture of bacteria and yeast used in the production of kombucha, and also low levels of pH can be beneficial (Diguță et al., 2020). Also, the proteins produced during the fermentation process and the catechins can be considered as critical factors

for the improvement of antibacterial properties. It is accepted that both catechins and acetic acid found in Kombucha cultures have bactericidal properties and also it was reported that Kombucha has antibiotic substances that lead to an increase in the antimicrobial performance (Dutta and Paul, 2019). The acetic acid in Kombucha not only improves the antimicrobial properties but also can enhance antifungal properties as is improved (Villarreal-Soto et al., 2018). Recently, due to the excellent antimicrobial activity of Kombucha, it can be extensively utilized as an agent to reduce the pathogens associated with human illnesses (Diguță et al., 2020). The scientists reported that the preparation process and fer-mentation time and also the origins could affect the anti-microbial and antifungal activity of Kombucha (Mousavi et al., 2020).

The count of acetic acid bacteria increases with malting days and fermentation temperatures. The intensity of microbial activity of the beverage is directly related to the concentration of the acetic acid. Acetic acid, as well as other organic acids, can influence the antimicrobial activity by two primary mechanisms: by cytoplasmic acidification and by accumulation of the dissociated acid anion to toxic levels (Velićanski et al., 2014). The difference in counts between acetic acid bacteria and yeasts and LAB shows that LAB were the predominant fermenters.

Temperature affects fermentation in many ways. At low temperatures yeasts tend to be less sensitive to the toxic effects of high alcohol concentration (Mizuta et al., 2020). The growth rate of yeast cells is strongly influenced by fermentation temperature. This is particularly evident during the exponential phase. At warmer temperatures (> 20°C), yeast cells experience a rapid decline in viability at the end of fermentation (Vohra et al., 2019). At cooler temperatures, cell growth is retarded, but viability is enhanced. Cool temperatures prolong the lag phase of fermentation and slow the rate of fermentation. Excessively high temperatures may disrupt enzyme and membrane functions, resulting in stuck fermentation (Mizuta et al., 2020). Although quick onset and completion of fermentation have advantages, the preferred temperature for vinification is often less than the optimum for ethanol production or yeast growth. The fermentation was to a large extent carried out by LAB and yeasts with AAB contributing a small proportion compared to the two.

The basic biochemical changes initiated by yeast and LAB in the fermentation are acidification of the culture with lactic and acetic acids produced by the LAB and the yeast (Sharma et al., 2020). Typical flavor and aroma development can be traced to biochemical activities of both lactobacilli and yeasts. LAB fermentations have other distinct advantages in that the foods become resistant

to microbial spoilage and toxin development (Sharma et al., 2020). Acid fermentations also modify the flavor of the original ingredients and often improve nutritive value. The total coliform counts were also high in the beverages suggesting poor hygiene/ contamination during the processing period of the beverage. The high counts can be avoided by proper preparation of the raw materials, proper hand washing, use of distilled / disinfected water as opposed to using tap water and working in a sterile environment during processing of the beverages (Sharma et al., 2020).

## 4.6 CONCLUSION

The developed beverages are rich in nutritional and functional value as attested by the results obtained above. The beverages contain sufficient amounts of tannins and phenolic compounds which are known for their antioxidant properties. In addition, they possess antimicrobial activities against common food poisoning microbes *Escherichia.coli* and *Staphylococcus aureus,* which gives them an edge over other currently existing beverages in the market. With slight improvement, for example fortification, they can be introduced and adopted as a healthier alternative to the beverages that currently exist in the market which are only composed of empty calories since the world is migrating to nutraceuticals.

# CHAPTER FIVE: EVALUATION OF THE STORABILITY AND CONSUMER ACCEPTABILITY OF A READY TO DRINK SORGHUM BEVERAGE.


## ABSTRACT

Shelf-life stability is normally crucial in new product development as it helps in assessing how long it takes (weeks, months or years) before significant biochemical and microbial changes happen in a product causing deterioration and consumer dislike of the product. The determination of storability of a product also helps evaluate the acceptability and preference by the target consumer. This study investigated the sensory acceptability and shelf-life stability of the developed sorghum-based beverage under normal storage conditions. The sensory analysis was conducted on the developed beverages and compared to a control commercial beverage. The beverage which had a high overall acceptability sensory score was subjected to accelerated shelf-life testing using Q10 factor. This most preferred and acceptable beverage was 3D with scores of 4.9 for taste, 4.4 for color, 4.9 for aroma, and 4.87 for overall acceptability. Storability and shelf-life testing were also done on this best beverage at storage temperatures of 14°C and 40ºC for prediction of the product's shelf life at refrigeration (4ºC) and room temperature (30ºC) respectively. The rate of reaction (Q10 factor) was used for prediction with titratable acidity being the chosen factor. The shelf life was calculated according to Q10 factor that was found to be 1.89 leading to shelf-life values of 103 days and 17 days at $4^0$C and $30^0$C respectively. The study showed that a desirable malted sorghum beverage with optimum retention of organoleptic qualities is shelf stable for 17 days and 103 days at storage temperatures of $30^0$C and $4^0$C respectively. Thus, a sorghum-based beverage has the shelf-life stability necessary for commercialization.

## 5.1 INTRODUCTION

Sorghum grain has been showed to be a power food packed with numerous nutritional and health benefits with extensive research having been carried out on the crop (Adetayo *et al.*, 2013). However, the adoption of sorghum and commercialization of sorghum value added products has been low despite sorghum possessing these numerous benefits in East Africa (Amelework *et al*., 2016). The production, utilization and commercialization levels of sorghum grains are poor with only 1% is consumed as feed, one fifth processed, more than 50% as food and its postharvest and field losses are about 15% (Njagi *et al*., 2019). Processing of raw materials adds economic value to them, converting perishables into storable and allowing them to be marketable products (Curi et al., 2017). In Kenya, rural households are top on the consumers list of sorghum, usually milling the grains to make *Ugali*. Apart from making *Ugali*, it is usually used to enrich cassava flour before sale (Chemonics, 2010). Apart from its mineral composition, sorghum has a lot of health benefits including alleviation to diseases such as cardiovascular diseases, obesity and other chronic diseases (Awika and Rooney, 2004).

The beverage sector is among the biggest players in the food industry for thirst quenching, refreshment, energy and for other purposes (Kaur et al., 2019; Nazir et al., 2019). The market of nutraceutical beverages is fast growing the world over and consumers' interests are shifting to functional foods (Kaur et al., 2019). Such shifts in trends should push the beverage industry to lean on developing products that fit this so that soft drink/ beverage consumers while refreshing themselves, get other benefits apart from only sweet tastes and empty calories. The carbonated drinks currently available such as fruit-flavored drinks, synthetic drinks and sweetened aerated drinks are poor nutritionally being only composed of water, synthetic colors and flavors (Nazir et al., 2019).

The application of *Kombucha* culture to improve the nutritional and sensory value of different beverages especially the sorghum-based beverages has not been exploited. Tea cider, also known as *Kombucha* is a type of tea which is fermented and often consumed for medicinal purposes (Dutta and Paul, 2019). A tea fungus composed of yeasts and acetic acid bacteria is used for the fermentation, producing a refreshing beverage that tastes like sparkling apple cider (Kechagia et al., 2013). From previous studies, the product that gives the best sensory characteristics is the one obtained after 7 days fermentation. Longer fermentation produces an over sour and unpleasant

liquor due to increased acidity (Kumar and Joshi, 2016).

In this study, sorghum malts were prepared then fermented at different temperatures using the culture then the beverages given to a sensory panel for evaluation of some sensory characteristics which included taste, color, aroma, flavor and overall acceptability.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Sample collection and preparation

Sorghum *(Sorghum bicolor L. Moench)* purchased from Busia, finger millet (*Eleusine coracana*) from the local market in Kangemi, *kombucha* culture bought from Kombucha Kenya Company (a mushroom-like consortium of yeasts and acetic acid bacteria which are in a symbiotic relationship suspended in previously fermented broth), previously fermented *kombucha* broth and white sugar purchased from a local supermarket.

## 5.3 METHODOLOGY

### 5.3.1 Product development

Sorghum grains(500g) were malted for 3, 4 and 5 days after which malt extraction was conducted resulting into malt extracts which were further subjected to 7 days fermentation at temperatures between 20 $^{0}$C-35 $^{0}$C by the SCOBY. Resulting beverages were analyzed for their nutritional components and the nutrient which had a significant number of reported deficiencies nutritionally and also had significant differences between the formulations was chosen as the determinant factor during the process optimization.

**Table 5. 1: Experimental design used for the product formulation**

| **Formulations** | **Malting temperatures and days** | **Fermentation temperatures and days** | **Culture %** |
|---|---|---|---|
| 1 | $20^0$C for 3 days | 20°C, 7 days | 2.4 |
| 2 | $20^0$C for 4 days | 25°C, 7 days | 2.4 |
| 3 | $20^0$C for 5 days | 30°C, 7 days | 2.4 |
| 4 | $20^0$C for 5 days | $35^0$C, 7days | 2.4 |

Note: (Equal sorghum and finger millet ratios were used in all formulations and also equal culture inoculation rates)

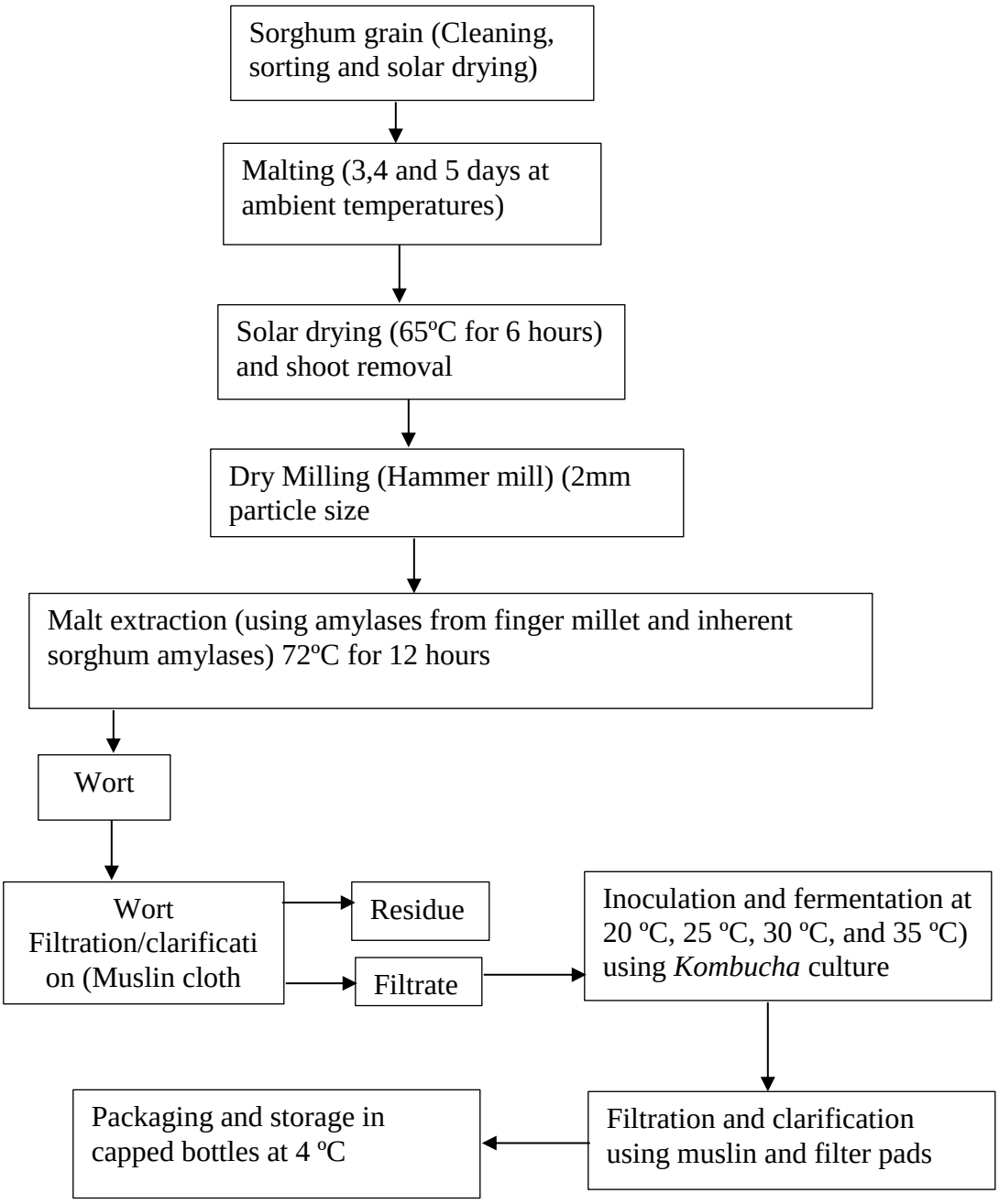


**Figure 5 1: Process Flow Diagram for Production of The Ready to Drink Sorghum Beverage**

### 5.3.2 Malting

The method according to Aluge *et al*. 2016 was used, with variations being applied in the germination days per formulation. Foreign matter was sorted out and grain soaking done in buckets with potable water for 12 hours in the ratio 1:2(500g:1litre). The grains were rid of water by draining then sprouted by spreading on wet blankets on malting trays on wooden benches and finally covered for the sprouting process. Daily sprinkling was done onto the grains and absolute ethanol(50ml) sprayed onto the grains to prevent growth of mold. Oven drying (memmert oven supplied by GmbH and Co. Stavendamm 22 model Schutzart DIN 40050-IP20) for 6 hours at 65 $^{0}$C was done to the germinated grains. After drying, meshed trays were used to remove the shoots by rubbing the sprouts on them (Hassani *et al*., 2014).

#### 5.3.2.1 Malt extraction/brewing

Slight modifications were applied to the hot water extraction method by Peter et al. 2014. 2 litres of portable water at ambient temperature was mixed with ½ a kilo of milled sorghum malt (supplemented with 40%(200g) malt from finger millet). The mash was left to sediment for 1 hour. 40% (1litre) of the supernatant extract composed of enzymes was decanted and kept aside using calibrated jars. 30 minutes boiling in stainless still cooking vessels of the remaining mash which was thick was conducted and the enzyme extract added after cooling.

The cooked mash was then incubated at 72°C ± 1 °C (memmert oven supplied by GmbH and Co. Stavendamm22 Schutzart DIN 40050-IP20) overnight for saccharification then cooled to room temperature and an adjustment made to the weight to reach 3 kg with distilled water. Filtration was done to the resulting wort using a muslin cloth then finally through filter pads.

### 5.3.3 Inoculation and Fermentation

The methods according to Jayabalan *et al.* (2014) and Kumar and Joshi, (2016) with slight modifications were used. Into a wide-mouthed clean vessel which had been sterilized with boiling water, 1litre of the cool sorghum malt sweetened with 100g sucrose at ambient temperature was poured, 100ml of previously fermented *kombucha* to prevent the growth of undesirable microbes by lowering the pH and a mat of the culture (24 g) placed on the surface of the infusion and the jar was hygienically covered using clean muslin cloths and properly fastened using rubber bands. The preparations were placed at different incubation temperatures (between 20 and 30 $^{O}$C) for 7 days.

## 5.4 ANALYTICAL METHODS

### 5.4.1 pH and titratable acidity

Was done according to the procedure of Velićanski *et al.* (2014) with slight modification. pH and titratable acidity were measured on a daily basis during fermentation (24hrs) using an electronic pH meter model ST2100 from the day before fermentation up to the final day. Titration was carried out using a 20-mL sample against 0.1 moles/litre NaOH. Values of titratable acidity were expressed as grams acetic acid per litre of sample (Weight of acetic acid = 0.1M NaOH X volume of NaOH (in liters) X Molecular weight of acetic acid).

### 5.4.2 Sensory analysis

Done according to Curi *et al.*, (2017) with some modifications. 30 semi-trained panelists composed of university staff and students between the age of 18-45 evaluated the samples in duplicate, their scores being based on a 7 point hedonic scale (1–dislike very much, 7-like very much). The panelists were made familiar with the scale used and the characteristics to be evaluated which were color, taste, aroma and overall acceptability. The evaluation was done in one session by each of the panelists. Each taster was provided with 50 ml of the samples in transparent plastic cups coded with three digits already arranged in the booths that were separate from each other under white light and in a properly ventilated room at $25^0$C. A commercial malt drink (Malto) purchased from a local supermarket was used as the control sample. The panelists were shown how to use the scale and instructed to drink water between samples.

### 5.4.3 Shelf-life determination

Shelf life was estimated for the product that had the best scores in terms of color, aroma, taste and highest overall acceptability sensory score according to Hemanth *et al.* (2020) whereby the samples were stored at 14$^o$C and at $40^0$C for prediction of the product's shelf life at refrigeration (4$^o$C) and room temperature (30$^o$C) respectively. The rate of reaction (Q10 factor) was used for prediction with titratable acidity being the chosen factor. Q10= (R2/R1) (10/T2-T1). R1 and R2 are the rates of reaction at temperatures T1 and T2 respectively.

## 5.5 STATISTICAL ANALYSIS

Data was processed using one-way ANOVA on GENSTAT statistical software version 15.1. The method of least significant difference (LSD) (at 5% significance level) was used to compare means of the different scores and acidity values under Tukey test.

## 5.6 RESULTS

### 5.6.1 Shelf life and storability of the developed sorghum beverage

Figure 5.2 outlines the mean pH rate of changes of the developed sorghum beverages. In all beverages, the pH decreased steadily from day 1 of fermentation to the last day of fermentation (day 7), with significant differences in pH between the first and last days in each beverage formulation. Beverage 4D and 5D2 recorded the least final pH (2.91±0.0849 and 3.03±0.0424) while sample 3D (3.35±0.0424) and 5D1(3.25±0.212) had the highest. Before the beginning of fermentation, the malt obtained after three days had the highest pH while the malts obtained after 5 days had the lowest pH which was significantly different from the rest. On the fifth day of the fermentation, the samples differed significantly in terms of their pH values with 5D1 (3.55±0.127) as the highest and 5D2 (3.05±0.0212) the lowest. This is shown in Figure 5.2 below.

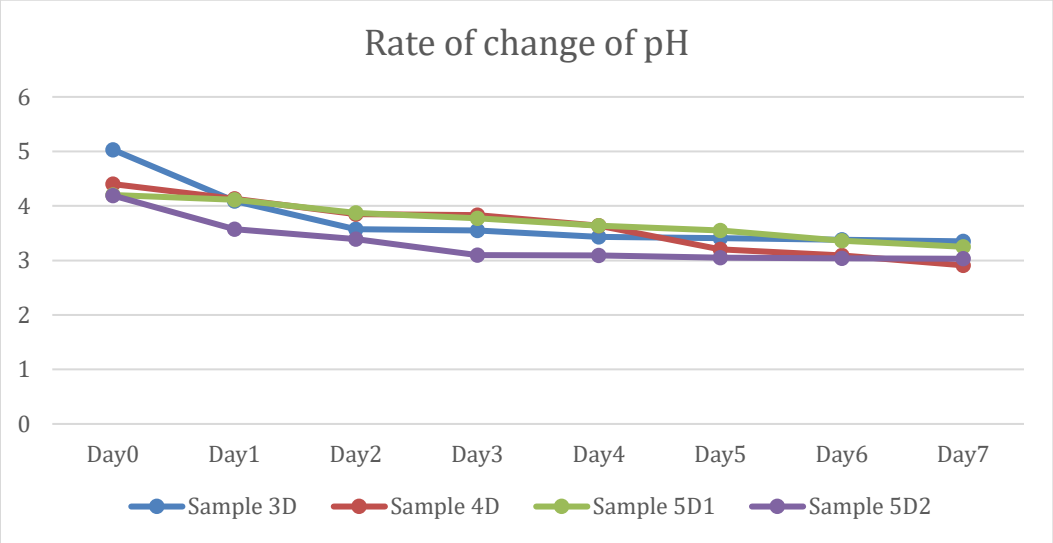


**Figure 5. 2: Rate of change of pH during fermentation of the beverages**

Figure 5.3 outlines the effect of fermentation on the trend in titratable acidities of the sorghum-based beverages. The general trend for titratable acidity was an increment during fermentation from the first to the last day for all beverage formulations. The highest final acidity value was obtained on beverage 5D1 (23.01±0.21) and the least acidity was obtained on beverage 3D (11.13±0.04) (Figure 5.3).

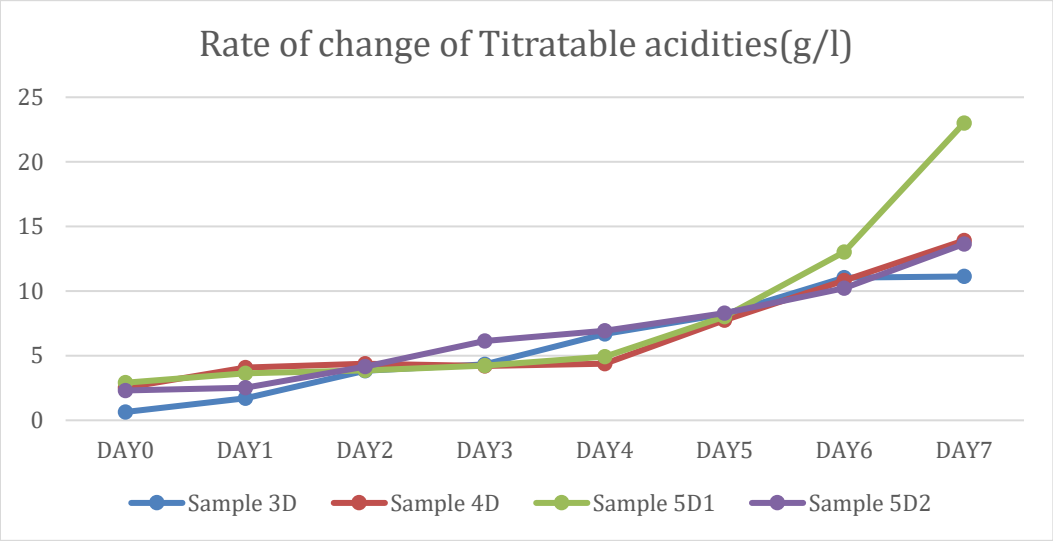


**Figure 5. 3: Rate of change of titratable acidities during fermentation of the beverages**

Table 5.2 presents the sensory scores of the developed sorghum-based beverage and the control sample. The results indiczted that the control sample had superior sensory scores for all attributes compared to the developed beverages. A comparison of the scores obtained for the different attributes between the different developed formulations revealed a superiority of beverage 3D in terms of taste and color. From the table, beverage 5D1 and 5D2 had the least taste scores, 5D2 the least score in terms of color. In terms of aroma, 3D and 4D had the highest scores, with no significant difference between them, 5D1 and 5D2 having the least with no significant difference between them. In terms of overall acceptability, 3D and 4D had the highest scores with no significant difference between them while 5D1 and 5D2 had the least scores also with no significant difference between their scores (Table 5.2).

**Table 5. 2: Multiple comparisons of mean sensory analysis scores for the developed beverages**

| Beverage Formulation(s)* | Taste | Color | Aroma | Overall Acceptability |
|---|---|---|---|---|
| 3D | $4.90\pm1.21^{c}$ | $4.40\pm1.38^{b}$ | $4.90\pm1.16^{b}$ | $4.87\pm1.17^{b}$ |
| 4D | $3.97\pm1.16^{b}$ | $3.53\pm1.31^{ab}$ | $4.37\pm1.19^{b}$ | $4.43\pm1.36^{b}$ |
| 5D1 | $2.60\pm1.30^{a}$ | $4.07\pm1.39^{ab}$ | $3.40\pm1.28^{a}$ | $3.47\pm1.38^{a}$ |
| 5D2 | $2.13\pm1.28^{a}$ | $3.40\pm1.13^{a}$ | $3.00\pm1.58^{a}$ | $2.77\pm1.48^{a}$ |
| C | $6.37\pm0.85^{d}$ | $6.33\pm1.29^{c}$ | $6.47\pm0.90^{c}$ | $6.30\pm0.99^{c}$ |

*Means within the same column with different superscript letters are significantly different ($p<0.05$).

Figure 5.4 shows the rate of change of acetic acid at varying temperatures of sample beverage 3D. Beverage 3D was picked as the best beverage due to possession of high scores in the two mentioned attributes and for also having the highest overall acceptability score. During accelerated shelf-life study of the beverage with highest sensory scores, % acetic acid at $40^{0}$C from the first to the last day of the study was higher than at $14^{0}$C (Figure 5.4).

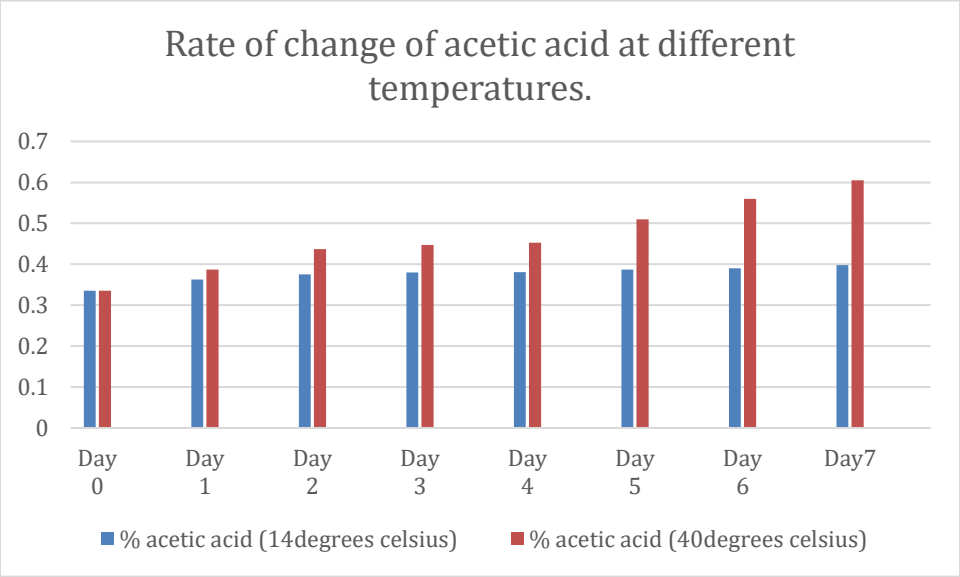


**Figure 5. 4: Rate of change of acetic acid at different temperatures during accelerated shelf-life testing of the best beverage**

## 5.8 DISCUSSION

After the third day of fermentation, the concentration of acetic and citric acid starts to increase accounting for the decrease in pH. Yeasts and bacteria are involved in such metabolic activities that utilize substrates by different and in complementary ways. Yeasts hydrolyze sucrose into glucose and fructose by invertase and produced ethanol via glycolysis, with a preference for fructose as a substrate (De Vuyst and Leroy, 2020). Acetic acid bacteria make use of glucose to produce gluconic acid and ethanol to produce acetic acid (Gomes et al., 2018). The pH value of the beverage decreases due to the production of organic acids during fermentation (Jayabalan et al., 2014). Despite the constant rise of organic acids during the fermentation process, independently of applied different temperatures, no significant changes of pH values were observed. It may be attributed to some buffering effects of the fermentation broth, during fermentation, carbon dioxide is released, and the obtained water solution of $CO_2$ dissociates and produces the amphiprotic hydrocarbonate anion (Velićanski et al., 2014). This anion easily reacts with hydrogen ions from the organic acids present in the fermentation broth, preventing further changes in the pH, thus contributing to the buffer character of the system (Velićanski et al., 2014). However, low pH can contribute to a decrease of general sensory quality of the beverage to an unacceptable level (Kapp and Sumner, 2019; Neffe-Skocińska et al., 2017). The low pH was due to increase in the quantity of acetic acid produced by the bacteria as the duration of fermentation increased, similar to findings by Jayabalan *et al.* (2014).

The first titratable acidity content would likely only be from the mother liquid, which would have previously produced acids, mainly organic acids (Khosravi et al., 2019). However, as the fermentation progressed, the current microorganisms would have produced new acids, partially dependent on the growing conditions and whether they were favorable. The lactic acid bacteria are responsible for the production of acids, meaning that the samples with a higher titratable acid had better growing conditions for bacteria to grow and produce acids (Alderson et al., 2021).

As the sugar concentration is related to sugars and organic acids levels, consumers have a preference for sweeter juices. However, higher acidity may be desirable by the fact that, to some extent, this parameter can contribute to enhance the flavor of the fruit (Curi et al., 2017). As fermentation progresses, the taste of kombucha beverage turns from a delightful fruity, sour and frothy flavor to a light vinegarlike flavor, thereby increasing the consumer acceptability of the flavor and other sensory aspects of the drink (Marsh *et al.*, 2014).

The rate of reaction rates increases rapidly with an increase in the temperature of storage. The calculated Q10 factor obtained was 1.89 from titratable acidity values. The predicted shelf life using Q10 was 103 days at 4ºC and 17 days at 30ºC. At the end of the seven day test period, the sample stored at 14ºC had no strong off-odor and had no floats as opposed to the sample stored at 40ºC which had a strong off-odor and also had floats due to more growth of the culture.

## 5.9 CONCLUSION

From the sensory results obtained, malting for three days followed by seven days fermentation at $20^0$C produces a beverage that is most desirable by consumers in terms of taste, aroma, color and overall acceptability. Acidity and final pH obtained after fermentation also play a part in determining the sensory acceptability (they have a direct impact on flavor) of the beverage produced with less acidic and higher pH beverages being preferred. Shelf life at $4^0$C and $30^0$C temperatures was also determined to be 103 and 17 days respectively.

# CHAPTER SIX: GENERAL CONCLUSIONS AND RECOMMENDATIONS

## 6.1 GENERAL CONCLUSIONS

The study noted that varying the number of malting days and use of different fermentation temperatures can lead to considerable differences nutritionally in the final cereal products despite similarities in certain nutrient compositions as in the above scenario. The best formulation for the beverage development for good nutritional output is four days with malting at 25 $^0$C fermentation temperature for one week. Sensory analysis output can also be used for optimization purposes then the products during commercialization can be improved nutritionally via fortification as is done by most food industries.

The developed beverages are rich in nutritional and functional value as attested by the results obtained above. The beverages contain sufficient amounts of sugars, protein, vitamins, minerals, tannins and phenolic compounds which are known for their antioxidant properties. In addition, they possess antimicrobial activities against common food poisoning microbes *E. coli* and *S. aureus,* which gives them an edge over other currently existing beverages in the market. With slight improvement, for example fortification, they can be introduced and adopted as a healthier alternative to the beverages that currently exist in the market which are only composed of empty calories since the world is migrating to nutraceuticals.

From the sensory results obtained, malting for three days followed by seven days fermentation at 20$^0$C produces a beverage that is most desirable by consumers in terms of taste, aroma, color and overall acceptability. Acidity and final pH obtained after fermentation also play a part in determining the sensory acceptability (they have a direct impact on flavor) of the beverage produced with less acidic and higher pH beverages being preferred. Shelf life at 4$^0$C and 30$^0$C temperatures was also determined to be 103 and 17 days respectively.

## 6.2 GENERAL RECOMMENDATIONS

With value addition, sorghum can be made to impact greatly on the lives of the farmers who are cultivating it. Thorough trainings should be conducted for purposes of awareness creation. Commercialization of the products should also be done on a wider scale since the general public have very little or no information on sorghum and the products that can be obtained from it. Further research using more advanced techniques such as High-performance liquid chromatography (HPLC) can be done on the processed beverages sugars composition to identify and quantify the individual sugars that result from enzyme hydrolysis of the malt and their presence after fermentation. The trends in the succession of the microbes present in kombucha culture during fermentation of the malt can also be studied.

Further improvements can be made to the best beverage obtained for example varying the type of sweetener apart from table sugar to for example honey and non-nutritive sweeteners, use of different flavoring agents to make the flavor richer, variation of colors of the beverage by adding food grade coloring compounds such as caramel among others so as to increase the aesthetic appeal of the beverage for purposes of commercialization.

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