UNIVERSITY FOR DEVELOPMENT STUDIES

SHELF LIFE IMPROVEMENT OF SORGHUM BEER (PITO) THROUGH PASTEURIZATION AND THE ADDITION OF MORINGA OLEIFERA

BY

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DECLARATION

Student

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere:

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Supervisors'

I hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

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ABSTRACT

Pito is a traditional alcoholic beverage that is mostly brewed in the three northern regions of Ghana. Although widely consumed and used during many festivities, poor shelf-life limits its economic potential as an income generating venture for most women. The study was carried out to improve the shelf life through the addition of *Moringa oleifera* leaf extract and pasteurization. The study was in two phases, that is, the extract level testing phase and the storage phase. The former was carried out to ascertain suitable concentration of moringa extract that could be added to the pito, and the latter was to investigate storage effect of the treatments: untreated pito, pasteurized pito, moringa pito and pasteurized moringa pito, on microbial (fungi and coliform growth), physical (pH, sugar and alcohol levels), proximate (moisture, crude protein, crude fat, ash, carbohydrate, and energy levels) and consumer acceptability. The extract level testing phase revealed that pito treated with 25% moringa extract was suitable for the storage phase. The treated pito samples: pasteurized pito, moringa pito and pasteurized moringa pito showed significantly ($P < 0.05$), higher values in pH (3.54 - 3.17), sugar (7 - 5.2%) and alcohol content (3.93 - 2.10% alcohol by volume), and also low fungal (2.1 x 10^6-2.0x10^4 cfu/ml) and coliform (7.0x 10^5 - 8.0x 10^4 cfu/ml) growth than the untreated pito during storage. The pasteurized pito and pasteurized moringa pito had no significant change in fungal and coliform growth. The untreated pito had pH of 3.28 - 3.16; sugar 7 - 4.5%; alcohol content 3.93 - 1.76% alcohol by volume; fungi growth 3.7 x10^6- 4.6 x10^5 cfir/ml and coliform growth of 4.0x10^6 - 1.5x10^5 cfu/ml during storage. The pito samples with moringa extracts had significantly ($P < 0.05$) high crude protein of 0.029 g/100 g and crude fat of 0.2 g/100 g. There was high consumer

acceptability of pasteurized pito when stored for 4 weeks. However, the moringa treated pito was less acceptable to consumers during storage. The untreated pito was also acceptable up to a period of 1 week. Based on the findings of this research it can be concluded that pasteurization (75-80 °C) and/or the addition of Moringa oleifera leaf extract can help increase the shelf-life of pito to 4 weeks. However, pito with moringa extract was not acceptable to consumers. Additional research is suggested on other antimicrobial plants since it is proven that consumers did not like pito with the Moringa oleifera leaf extract.

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Indeed the LORD has sent rains at the proper time, from his rich treasury in the heavens, and has bless all the work I did which affirms that, his blessings makes a person rich without adding any sorrow to it. Thus, the LORD deserves all the glory, honour and adoration through his son Jesus Christ. I say THANK YOU LORD

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DEDICATION

This work is dedicated to Mr. And Mrs Ayirezang.

CHAPTER ONE

INTRODUCTION

1.1 Background

The brewing and drinking of traditional beverages are intrinsic part of the culture of African people. Among the problems of these traditional products, is the poor shelf life. The poor shelf-life of these traditional beverage products, usually processed in households by women, could be linked to perennial poverty prevailing in rural communities where the beverages are largely produced. One of such product that is worth considering is sorghum beer, pito, brewed and consumed by people of the West African sub region (Demuyakor & Ohta, 1993). The processing of pito is an income generating business which serves as a source of employment in areas where they are produced. However, pito production is limited by its short shelf-life, and the product needs to be consumed within a day (Demuyakor, 1994).

The spoilage of pito is mainly as a result of spoilage microorganisms due to poor food manufacturing practices. According to Doyle (2001), spoilage is due to undesirable changes in sensory characteristics in terms of texture, smell, taste, or appearance.

The spoilage of most beer is by a diverse array of bacteria and wild yeasts with lactic acid bacteria being the dominant spoilers, responsible for 60-90% of the spoilage that occurs in the breweries (Sakamoto & Konings, 2003). Microbial spoilage of alcoholic beverages is of critical importance and for this reason, different methods have been adapted to reduce spoilage. Among these methods employed are thermal treatment (pasteurization) and chemical treatments (preservatives).

The stability of some traditional sorghum beers is known to have improved through pasteurization, filtration and addition of artificial preservatives (Ellis, Oduro, & Terkuu, 2005; Onaghise & Izuagbe, 1989; Osseyi, Tagba, Karou, Ketevi, & Lamboni, 2011). Also, some of these methods can be sophisticated and expensive for the small scale operator to adopt. According to Onaghise & Izuagbe (1989) the shelf life of pito was improved to last for a period of 4 weeks when pasteurized at 75°C for 30 minutes with the addition of sorbic acid concentration of 5%. Osseyi, Tagba, Karou, Ketevi, & Lamboni (2011) were also, able to obtain stability in the tchoukoutou beer (sorghum beer) for at least 6 months through double fermentation and pasteurization (75-80°C for 15 min.). Ellis et al. (2005) also improved the shelf-life of filtered pito for eight weeks by pasteurization (60-70°C for 15 minutes) and the addition of sodium metabisulphite. The use of sodium metabisulphite in foods and beverages is known to have adverse effects such as respiratory tract irritation and anaphylactic symptoms which is life threatening (Pavord et al., 1991; Vally, Misso, & Madan, 2009).

Although synthetic antimicrobial and antioxidant agents are approved in many countries, its usage has created environmental and health concerns, which has called for natural, safe and effective preservatives by consumers and producers (Ortega-Ramirez et al., 2014; Regnier, Combrinck, & Du Plooy, 2012). Ortega-Ramirez et al. (2014) also proposed that medicinal plants, traditionally used to treat health disorders and prevent diseases can serve as a source of bioactive compounds for food additives. This is because these medicinal plants are rich in antimicrobial phytochemicals. *Moringa oleifera leaf,* in that regard is also found to possess antimicrobial properties (Eilert, Wolters, & Nahrstedt, 1981). Information on the use of *Moringa oleifera* in the brewing of sorghum beer has not received any attention. Therefore, using pasteurization and a natural preservative *(Moringa oleifera),* which is easily accessible in Ghana and in the

tropics (Fahey, 2005; Quarcoo, 2008) in pito comparatively can be less expensive. Also although other parts of the moringa plant, such as the seed, bark, and fruit have been reported to have antimicrobial properties, the leaf is easy to grow and also an ecological and economical vegetable in Ghana and other tropical countries (MAG, 2013).

In light of the highlighted above problem associated with the production of pito, novel ways are needed to produce pito of a better shelf-life. Therefore, the purpose of this study was to improve the shelf-life of pito by the addition of *Moringa oleifera* leaves in combination with pasteurization at 75-80°C for 15 minutes.

1.2 Objectives

1. To determine the level of moringa leaves incorporation that will be acceptable to consumers.

2. To investigate the shelf-life of pito by;

I. Determining the effect of pasteurization and/or the addition of moringa leaf extract on the microorganisms in pito.

II. Investigating the effect of the addition of moringa leaf extract and/or pasteurization on the physical properties of pito.

III. By determining the consumer acceptability of moringa incorporated pito in view of shelf life extension.

3. To investigate the effect of the addition of moringa leaf extract and/or pasteurization on proximate composition of pito.

CHAPTER TWO

LITERATURE REVIEW

2.1 African Traditional Sorghum Beer

The importance of beer in Africa is something that cannot be overlooked. Archaeological evidence indicates the production and consumption of beer in Egypt since 5500-3100 BC (Hornsey, 2003). The beers produced are mostly from the grains of maize, millet and sorghum (Steinkraus, 2004).

Sorghum beer is produced from the grains of sorghum *(Sorghum bicolor).* In Ghana sorghum is the third most produced cereal crop after maize and rice (MAFAP, 2013). It is produced in Guinea and Sudan savannah zones, where the Northern, Upper-East and Upper-West regions of Ghana can be located (MAFAP, 2013).

Sorghum beer can be in a clear or an opaque form. However, not all types of sorghum varieties are used for the production, since the type of grain used is dependent on the location in which it is produced (Demuyakor, 1994). The red type was reported by Lyumugabe, Gros, Nzungize, Bajyana, & Thonart, (2012) as the most preferred for beer production.

The brewing of sorghum beer generally involves malting, drying, milling, souring, boiling, mashing and alcoholic fermentation (Lyumugabe et al., 2012). The procedure, however, varies, due to the geographic localization (Haggblade & Holzapfel, 2004). African traditional beers are generally sour, less carbonation, and lack the addition of hops (Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003) and are often cloudy and yeasty (Oliver & Colicchio, 2011). The alcohol content of traditional brews ranges from 1-8% alcohol by volume, depending on the level of fermentation. However the

alcohol content of most sorghum beers is in the range of 3-4% alcohol by volume (Oliver & Colicchio, 2011).

The beer is also consumed during the process of fermentation (along with the fermenting microbes), with large amounts of fragments of insoluble materials which constitute starch and dextrin (Glennie & Wight, 1986). This contributes to the cloudy appearance associated with sorghum beer. Sorghum beer contains maltriose, the last sugar fermented by yeasts during the process of fermentation (Oliver & Colicchio, 2011). The drink is also rich in protein which contributes to foam stability, giving it a milk-like head (Lyumugabe, et al., 2012). Although mainly consumed as an alcoholic drink during social events, sorghum beer also serves as food and drink, which satisfies hunger and thirst respectively. The most prevailing problems associated with traditional sorghum beers are short shelf-life and poor quality. Lyumugabe, Kamaliza, Bajyana, & Thonart (2010) attributed this to their poor hygienic practices, low ethanol content, organoleptic difference and unsatisfactory preservation.

Traditionally produced African sorghum beers are not filtered which also contributes to the opaqueness of the beer (Glennie and Wight, 1986). It also does not undergo pasteurization process, which has the ability to halt the activities of the microbes; making possible continuous fermentation until the fermentable nutrients in the beer are exhausted.

In Ghana, the popularly known traditional sorghum beer is pito. Pito is produced by the people of West Africa, especially in countries like Ghana, Nigeria, Burkina Faso and Togo. In Ghana, this beverage is normally produced and sold by women (Demuyakor, 1994).

Ekundayo (1969), characterized pito as a product whose colour is golden yellow to dark brown, slightly sweet to very sour tastes, contains lactic acid, sugar, amino acids and alcohol percentage ranging from 2 to 3 %, with suspended material found on the surface (Demuyakor, 1994). In addition, it contains minerals such as calcium, magnesium and iron. These minerals are indispensable in regulating and building the living cells and also aiding in the fight of depression (Kolawole, Kayode, & Akinduyo, 2007). Pito has a probiotic effect due to fermentation. Such probiotic properties include hypolipidemic, hepatoproctective and antibacterial which is effective in treating gastroenteritis in man and animals due to the lactic acid bacteria (Aderiye, Laleye, & Odeyemi, 2007).

2.1.1 Pito production

Pito like other African indigenous fermented sorghum beer production is by spontaneous and uncontrolled mixed fermentation of microorganisms (Demuyakor & Ohta, 1991, 1993; van der Aa Kithle, Jesperen, Glover, Diawara, & Jakobsen, 2001; Vieira-Dalode' et al., 2007). Fermentation of pito is of two stages, and these are: acidic fermentation and alcoholic fermentation. In Ghana, fermentation of pito occurs in two stages: the first stage involves lactic acid bacteria (Demuyakor, 1994), with traces of acetic and formic acid being formed (Ekundayo, 1969) followed by the production of alcohol by yeasts.

There are different styles of pito brewing in Ghana (kasina, dargarti, nandom and kokomba pito). Their differences come from the wort extraction and fermentation processes (Duodu et al., 2012). A pito brewing process is shown in figure 1.

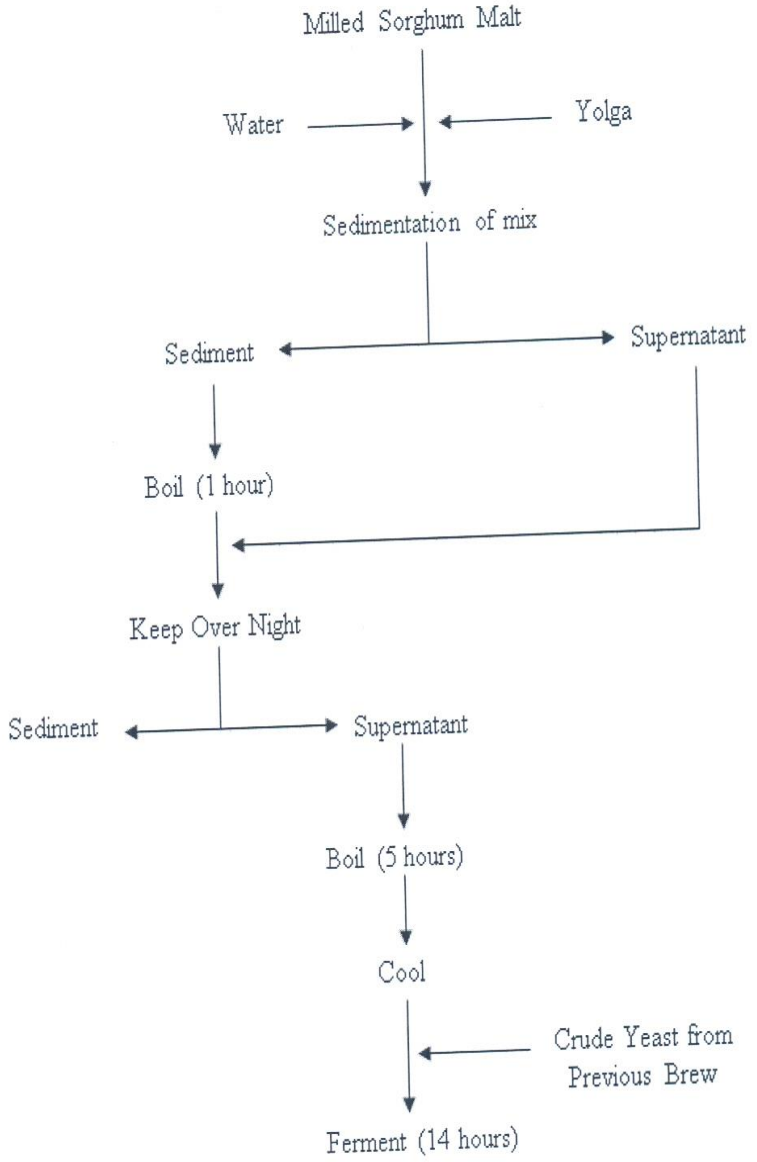

Figure 1: A flow chart of the brewing process of pito (Demuyakor, 1994)

There is no added value to the finished product of pito, which gives it the tendency to spoil rapidly. After production it is left in the brewing pots or poured into barrels and afterwards, fetched from the pots or plastic barrels and served into calabashes for customers (Demuyakor, 1994). Therefore, the need to upgrade the indigenous fermented foods and beverage is of outmost importance as discussed by Achi (2013). Bottling, pasteurization and the addition of natural preservatives, as done in most

western beers, will help develop this industry and create more avenues for income generation in rural communities

2.2 Microbial Spoilage of Sorghum Beer

Microorganisms can multiply anywhere except in the atmosphere (Ray & Bhunia. 2013). Their ability to survive is dependent on environmental and nutritional factors such as temperature, oxygen availability, pH, water availability, and energy sources (Nester, Anderson, Roberts, Pearsall, & Nester, 2001).

Beer in general is known to be microbiologically stable (Sakamoto & Konings, 2003; Vaughan, O'Sullivan, & Sinderen, 2005). The reason for this as reported by Sakamoto & Konings (2003) was that, beer is an unfavourable medium for many micro-organisms due to the presence of; ethanol concentration (0.5-10% w/w), hop bitter compounds (approx. 17-55 ppm of iso-a-acids), the high content of carbon dioxide (approx. 0.5% w/v), the low pH (3.8-4.7), the extremely reduced content of oxygen (<0.1 ppm) and the presence of only traces of nutritive substances such as glucose, maltose and maltotriose.

Hop bitter acids, added to confer bitter flavour of beer, also exert antibacterial effects, providing an additional layer of defence against bacteria, which may inadvertently gain entry into finished beer products (Suzuki, Asano, Iijima, Kuriyama, & Kitagawa, 2008). Indigenous beers spoil between 1-5 days (Haggblade & Holzapfel, 2004), with the sorghum beer (pito) taking a day (Demuyakor & Ohta, 1993). The reasons for the poor shelf-life of traditional beers are that, they are actively fermenting, with a wide range of organisms flourishing in the rich medium (Lyumugabe et al., 2010) additionally, it is not hoped (Blandino et al., 2003). According to Lyumugabe et al. (2012), during fermentation of sorghum beer, yeasts initially increase in number and then in the later stage of logarithmic growth, the production of ethanol starts and proceeds during the

stationary phase. Lyumugabe et al. (2012) also detected that during this time, very little or no increase in the number of contaminating organisms seems to occur. However, at the end of fermentation, the yeast die, or else they undergo autolysis and their cell constituents are released into the beer which may change the flavour of the beer. Although Western beers have higher stability than the traditional sorghum beers, there is still a niche of microorganisms that are capable of growth in the malt, wort and beer (Sakamoto & Konings, 2003; Vaughan et al., 2005).

The rich nutritional value of pito beverage makes them attractive to microorganisms, which however, have the potential of causing spoilage or being pathogenic (Bankole & Ogunmusere, 2006; Kolawole et al., 2007). Most of the traditional brewers in Ghana prefer previously used yeast in pito fermentation, which gives distinct taste profile to the beer. However, this practice may contribute largely to the poor quality of the pito beer, which might be characterized by the growth of toxic and carcinogenic mycotoxin-producing fungi (Pitt, Hocking, & Diane, 2009), off-flavours and development of turbidity due to the growth and metabolic activity of wild yeasts, certain lactic acid bacteria (LAB) and anaerobic Gram negative bacteria (Vaughan et al., 2005).

Bankole & Ogunmusere (2006) identified *Escherichia coli, Klebsiella* sp. *Proteus vulgaris*, *Salmonella* Typhimurium and *Shigella sonnei*, *Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer* and *Saccharomyces cerevisiae.* Kolawole et al. (2007) also isolated microorganisms like *Escherichia coli, Staphylococcus aureus, Bacillus subtillis, Streptococcus species, Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus, Saccharomyce cerevisiae, Mucor species,* and *Proteus* species, from the sorghum beer pito. Lyumugabe et al. (2010), reported on some microorganisms obtained from spoiled sorghum beers; *Acetobacter* sp, *Pediococcus* spp, *Lactobacillus*

spp, *Leuconostic* spp, *Zymomonas* spp, *Obesumbacterium* spp, *Candida* spp, *Hansennula* spp, *Saccharomyces* spp (wild strains).

For these microbes to cause a detectable change in the quality and sensory attributes of the beer, they must multiply to attain certain levels, referred to as the "spoilage detection level" which is dependent on the type of microbe causing the spoilage (Ray & Bhunia, 2013). Depending on the nature of spoilage and microbes involved, the spoilage detection level can range from 10^6- 10^8 cells/ml (Ray & Bhunia, 2013). During spoilage, there is often a succession of different populations that rise and fall as different nutrients become available or are exhausted. Some microbes are very competitive and are able to secrete compounds to inhibit competitors. Examples of such microbes are lactic acid bacteria and molds (Gram et al., 2002).

Certain practices such as mashing, low temperature during the fermentation and the distribution of the sorghum beer during the brewing of sorghum beer have also been observed to support the growth of contaminants, (Steinkraus, 2004; Lyumugabe et al., 2012). These contaminants may find their way into the pito beer, through the air and water and also by the activities of small animals, particularly insects (Doyle, 2001; Deak, 2007). This can be prevented if good manufacturing and hygienic practices are followed prior to and during preparation of pito beer. Lactic acid bacteria, *Pediococcus* and *Streptobacterium,* along with other undesirable acetic acid bacteria *(Acetobacter)* are responsible for over souring as well as the production of volatile off-flavours. The lactic acid bacteria *Leuconostoc* and *Betabacterium,* produce acetic acid or ethanol and carbon-dioxide in addition to lactic acid (Steinkraus, 2004).

Spoilage microorganisms and their microbial metabolites that cause repugnant taste and odours, though not desirable, are generally not harmful (Nester et al., 2001) if pathogens and toxins are not present (Doyle, 2001). Chemical reactions that cause offensive sensory changes in pito beer may be mediated by a variety of microbes. Ray and Bhunia (2013) mentioned that the only way for food to spoil by microorganisms is through microbial growth in mass or number and by enzymes excreted by the microorganisms. Spoilage by microbial growth is however faster than by the enzymes excreted. Some spoilage can be detected at either lower or higher microbial load. Food with higher initial loads of spoilage microbes with shorter generation time will spoil more rapidly than foods with lower initial loads of spoilage microbes with longer generation time (Ray & Bhunia, 2013). This means that indigenous fermented foods and beverages can take a long time to spoil if the microorganisms can be reduced and the generation time of pathogens is increased. This can be achieved when the foods and beverages are pasteurized or through the addition of antimicrobial compounds.

2.3 Enhancing Microbial Stability in Beer

It is impossible to prevent access of microorganisms into beer through the environment. However, it is possible to control their access into the medium (beer) to reduce initial load and minimize microbial spoilage and health hazards after processing (Ray & Bhunia, 2013). The microbial safety and stability, the sensory and nutritional quality, and the economic potential of many foods are maintained using a combination of preservative factors (Vriesekoop, Krahl, Hucker, & Menz, 2012). Some of the preservative methods used are discussed in the following section.

2.3.1 Biological preservation

Biological preservation uses competitive or antagonistic microorganisms or their antimicrobial metabolites to improve the safety of beverages (Schillinger, Geisen, &

Holzapfel, 1996). There are certain yeast strains that produce metabolites detrimental to other yeast strains (Golubev, 2006). Also, lactic acid bacteria (LAB) produce certain antimicrobial compounds, such as bacteriocin (Schillinger et al., 1996) and pediocin (Biswas, Ray, Johnson, & Ray, 1991) which causes unfavourable conditions for other microorganisms to survive (Stiles, 1996). During the acidification stage of pito brewing, LAB are the predominant microorganism (Demuyakor, 1994). Also, they can be found in the final product. This indicates that there may be the possibility of the killing of sensitive bacteria and fungi that may be present, consequently reducing to some extent the range of microorganisms in the traditional sorghum beer. A report by Fadahunsi, Ogunbanwo, & Fawole (2013), showed that there was a reduction of microbial growth in pito beer during storage and this was attributed to metabolites produced by competitive or antagonistic microbe. These microorganisms were likely to be lactic acid bacteria and some strains of yeast.

Biological preservation might be one of the reasons why some researchers have used selected microorganisms as starter cultures for the improvement of indigenous fermented beverages to improve its shelf-life and organoleptic properties (Gadaga, Mutukumira, Narvhus, & Feresu, 1999; Holzapfel, 2002; Leroy & De Vuyst, 2004; Zorba, Hancioglu, Genc, Karapinar, & Ova, 2003).

2.3.2 Antimicrobial plants

Beer is more prone to spoilage by pathogenic or spoilage organisms when antimicrobials are absent or present at a reduced level (Vriesekoop et al., 2012). Therefore, increasing the concentration of the antimicrobials will have adverse effect on these microorganisms. An increase in the concentration of antimicrobial compounds has been reported to be effective against microorganisms (Devendra, Srinivas, Prasad

Talluri, & Latha, 2011; Peixoto et al., 2011). The antimicrobial activity of plant oils and extracts has been recognized for many years (Hammer, Carson, & Riley, 1999). Some of these plants have extensively been exploited in medicine (Cowan, 1999; Mathur et al., 2010; Silva & Fernandes Junior, 2010), food (Bukar, Uba, & Oyeyi, 2010; Ortega-Ramirez et al., 2014; Regnier et al., 2012), and beverages (Carlsen et al., 2010; Quarcoo, 2008; Sakamoto & Konings, 2003; Van Wyk & Gericke, 2000). In the brewing of beer, the most used antimicrobial plant is the hop (Vaughan et al., 2005), but there are specialty beers brewed with certain spices (sassafras, cinnamon and ginger), that have antimicrobial compounds (Vriesekoop et al., 2012). The hop plant has been known for thousands of years, nevertheless its usage in beer is recent, compared to the existence of beer (Sakamoto & Konings, 2003). The hop plant, however, is not available in Ghana hence there is the need to investigate other plants for their antimicrobial properties.

Adenuga, Olaleye, & Adepoju (2010) substituted bitter leaf *(Vernonia amygdalina),* bitter kola *(Garcinia kola)* and `utasi' leaf *(Gongronema latifolium)* extract for hops in sorghum beer production. Neem *(Azadirabta indica)* extract was also used as hop substitute in African traditional brewed beer by Ajebesone & Aina, (2004). Although the sensory, physico-chemical and the proximate properties were analysed, the antimicrobial effect was not studied. Therefore, there is the need to further investigate the antimicrobial properties of these leaf extracts in sorghum beer, and the proportions which will be suitable.

Various publications have documented other plant extracts that have antimicrobial properties (Awuah, 1989; Boakye-Yiadom, Fiagbe, & Ayim, 1976; Ncube, Afolayan, & Okoh, 2008; Rabe & Van Staden, 1997; Rios & Recio, 2005). Among the plants

investigated is. *Moringa oleifera* (Bukar et al., 2010; Chen, 2009; Oluduro, 2012; Sayeed, Hossain, Chowdhury, & Hague, 2003). Although *Moringa oleifera* lacks the bitterness compound, and therefore may not be substituted for hop in traditionally made sorghum beers, it can be added to help reduce or prevent the growth of microorganisms present. There are some special beers in which aged hops are added, solely for the antimicrobial property (Vriesekoop et al., 2012).

2.3.21 Moringa oleifera as a food and beverage preservative

Moringa oleifera is also known as "Miracle Tree" (Kumari, Sharma, Srivastava, & Srivastava, 2006). It also has a lot of medicinal uses with high nutritional value. Different parts of this plant consist of important minerals, and are a good source of protein, vitamins, β - carotene, amino acids and various phenolics (Anwar, Latif, Ashraf, & Gilani, 2007). Mustapha & Babura (2009) reported that *Moringa oleifera* leaves also contained 10.1% carbohydrate, which was equivalent to 110 mg/ml concentration. Because of its impressive nutritional value, it has been used as a low-cost solution to undernourishment, as well as remedy for common ailments of the locals (Manaois, Morales, & Abilgos-Ramos, 2013). This makes it a natural, cost effective and non toxic antimicrobial agent (Chen, 2009). Moringa leaves have been reported to be a good source of natural antioxidants (Dillard & German, 2000). The mechanism of antioxidant is to inhibit oxidation in foods (Brewers, 2011).

Moringa oleifera plant is rich in compounds containing the simple sugar, rhamnose, and a fairly unique group of compounds called glucosinolates and isothiocyanates (Bennett et al., 2003). The composition of *Moringa oleifera* extract is reported to have antimicrobial compounds such as 4-(4'-O-acetyl-a-L-rhamnopyranosyloxy) benzyl isothiocyanate (Eilert et al., 1981), which acts on several bacteria and fungi (Eilert et

al., 1981). There has also been the identification of a protein called the Flo protein responsible for the antibacterial property (Fisch, Suarez, & Mermoud, 2004). The mechanism of these proteins may be, disrupting cell membranes or synthesis of essential enzymes thereby inhibiting microbial growth (Fisch et al., 2004). While these compounds are relatively unique to the Moringa family, it is also rich in a number of vitamins and minerals as well as a more commonly recognized phytochemicals such as the carotenoids (including (3-carotene or pro-vitamin A) (Fahey, 2005). The antimicrobial property of *Moringa oleifera* is thus as a result of the phytochemicals present (Bukar et al., 2010; Eilert et al., 1981).

2.3.3 Pasteurization

For decades, thermal treatments have been used to prevent beer spoilage (Portno, 1968). Some methods employed are; Low Temperature Long Time, and High Temperature Short Time pasteurization methods (Early, 1998). Pasteurization is heating food or beverage at high temperatures to kill pathogenic microorganisms present and also destroy large proportion of spoilage organisms in order to extend the shelf life. Pasteurization has been used and currently being used to improve beer quality by extending its shelf life. According to Douglas (2014), during pasteurization the rate of destruction of the microorganisms is logarithmic, as is their growth rate. That is, the microorganisms subjected to heat are killed at a rate that is proportional to the number of organisms present. Meaning, the number of microorganisms in pasteurized beer depends, among other factors, on the number of microbial cells before treatment (Zufall & Wackerbauer, 2000). In reference to this, if the initial microorganisms in food or beverages are high, then there would be the likelihood of some amount of microorganisms remaining after pasteurization.

Pasteurization of beer results in the killing of a large proportion of yeast cells (Haggblade & Holzapfel, 2004). This is because *Saccharomyces* species are easier to inactivate than other spoilage microorganisms in beer (Zufall & Wackerbauer, 2000).

Indigenous beers take short time to spoil because there is no further boiling or heat treatment applied. In addition, conditions during mashing and especially alcoholic fermentation favour the growth of the contaminants (Haggblade & Holzapfel, 2004).

The enhancement of microbial stable sorghum beers has been achieved through pasteurization (Ellis et al., 2005; Haggblade & Holzapfel, 2004; Osseyi et al., 2011). Conversely, trying it the first time proved to be a failure (Novellie, 1968). This failure led to an increase in the beer viscosity, extra gelatinization of starch, elimination of amylolytic enzymes and the removal of the beer's characteristic effervescence by destroying the active yeast (Novellie, 1968).

According to Onaghise & Izuagbe (1989) the shelf life of pito was improved for a period of 4 weeks when pasteurized at 75°C for 30 minutes with the addition of sorbic acid at 5% concentration. It has been confirmed that sorbic acid cannot inhibit yeast growth (Stratford & Anslow, 1998); thus the inhibition of microbial growth in the pito, therefore, might be solely as a result of the heat treatment applied by Onaghise & Izuagbe (1989). Osseyi, Tagba, Karou, Ketevi, & Lamboni (2011) used double fermentation method and pasteurization in a water bath at 75-80°C for 15 minutes to extend the shelf life of tchoukoutou beer (sorghum beer) for at least 6 months. Microbiological analysis results showed that the bottled tchoukoutou did not contain any microbes after pasteurization proving that the thermal treatment was sufficient to destroy the fermenting microorganisms. On the other hand, the traditional beverage in

which the fermenting microbes were still alive was loaded with yeasts, molds, lactic and acetic acid bacteria.

Ellis et al, (2005) also reported that pito, which was filtered, can be stored for eight weeks through pasteurizing at 75°C for 15 minutes, and the use of sodium metabisulphite as preservatives. Nevertheless, the use of sodium metabisulphite as food preservative has health implications such as dermatitis, urticaria, flushing, hypotension, abdominal pain and diarrhoea, respiratory tract irritation and anaphylactic symptoms which are life threatening (Pavord et al., 1991; Vally et al., 2009). Therefore the use of a natural, safe and effective preservative from plants, such as moringa, could be considered as a better alternative.

Although literature has proven the effectiveness of pasteurization on sorghum beer, the process is not applied by the commercial brewers in pito production, and because of this, microorganisms resistant to low pH continue to proliferate (Steinkraus, 2004) and consequently leading to spoilage. There are limited publications on pasteurization of traditional sorghum beers, especially on pito. Therefore, there is the need to conduct more research on pasteurizing traditional sorghum beer. According to Zufall & Wackerbauer (2000), there is a complete killing of microorganisms in filtered beers when pasteurized than in cloudy beers. However, the improvement of filtered sorghum beers through pasteurization is already known, and there is the need, therefore, to investigate if the shelf life of pito in its cloudy state can be improved through pasteurization.

2.4 Effect of Storage on Acidity and pH of Indigenous Alcoholic Beverages

Sorghum beer is mostly contaminated by lactic acid and acetic acid bacteria (Steinkraus, 2004). The environmental condition during, and after the brewing process,

may determine their ability to multiply. Lactic acid bacteria along with other undesirable bacteria, acetic acid bacteria *(Acetobacter)* were reported to be responsible for over souring as well as the production of volatile off-flavours, fruity odours, and pellicles in indigenous sorghum beers, which is unacceptable to consumers (Lyumugabe et al., 2012; Steinkraus, 2004). Traces of acetic acid and formic acid were also found in pito by Ekundayo (1969). However, lactic acid bacteria are primarily responsible for the spoilage of sorghum beer (Lyumugabe et al., 2012).

The acid produced by these bacteria reduces the pH of the beverage. The pH range for traditional sorghum beer is 3-4 (Ellis et al., 2005; Kolawole et al., 2007). During the storage of pito and burukutu (beer made of sorghum) there was a significant decrease in pH (Fadahunsi et al., 2013), which might have resulted from an increase in organic acids present. Nwachukwu, Achi, & Ijeoma (2010), reported that an increase in lactic acid bacteria will result in a decreased pH. The low pH of beers enhances the entry of weak organic acid into cells, leading to intracellular acidification, the destruction of enzyme systems and reduction in nutrient uptake, and result in metabolic exhaustion of undesirable microorganisms (Ray & Bhunia, 2013; Vriesekoop et al., 2012).

It should be noted that microorganisms have a specific pH range in which they can survive in food or beverages. According to Deak (2007) yeast and molds are able to grow at lower pH than bacteria. The pH range for the growth of molds is 1.5-9.0, and for yeast it is 2.0-8.5 (Deak, 2007). Also gram-negative bacteria cannot survive at low pH compared to gram-positive bacteria (Ray & Bhunia, 2013). Example of gram positive bacteria is lactic acid bacteria which produce organic acids and are able to grow and survive at low pH (Nezhad, Stenzel, & Britz, 2010), although not as compared to fungi. Many researchers have reported on the dominance of fungi followed by lactic

acid bacteria in freshly brewed traditional sorghum beer (Demuyakor, 1994; Glover, 2007; Haggblade & Holzapfel, 2004; Lyumugabe et al., 2012; Steinkraus, 2004). However, during storage of traditional sorghum beer there may be the likelihood of types of microorganisms increasing and falling due to the accumulation of organic acids during storage. Charalampopoulos, Pandiella, & Webb (2002), reported the accumulation of lactic acid and acetic acid during fermentation of fermentable sugars.

The pH of sorghum beer can therefore be stabilized if there is microbial stability in the beer. Ellis et al., (2005) reported a reduction of pH of pasteurized pito from 3.4 — 3.3; however, when the pito was pasteurized and chemically preserved, the pH was the same at 3.4 after two months of storage. It should be noted that before pasteurization and the chemical preservation, there was a filtration of the pito. However, Ellis et al. (2005), could not specify the type of filtration which can be used in order to achieve similar results. Moreover, if sterile filtration was used, it can be expensive and laborious (Portno, 1968), when added to the pasteurization process for the poor commercial traditional pito brewer.

2.5 Effect of Storage on Alcoholic Content of Alcoholic Beverages

According to Tan (2005), acetic acid bacteria have the ability to convert ethyl alcohol, C_2H_5OH, into acetic acid, CH_3CO_2H. Efficient ethanol production can be affected by the pH of the medium. Lin et al., (2012) recorded an optimal pH range of 4.0-5.0 for ethanol production process. Sanni, Onilude, Fadahunsi, & Afolabi (1999) and Fadahunsi et al. (2013), also reported a reduction of alcohol production during the storage of pito. Alcohol decreased from 3% to 1% in about 72 hours (Sanni et al., 1999). This might have resulted from oxidation of alcohol by acetic acid bacteria (Christensen et al., 2006), and in effect indicating an increase in the acetic acid present.

The acceptable alcohol level in beers, in most breweries is 5% alcohol by volume (Ellis et al., 2005). This may be because high ethanol production in beer provides one of the antimicrobial mechanism against spoilage microorganisms (Vriesekoop et al., 2012). The alcohol percentage of most traditionally brewed sorghum beer is below 5% (Demuyakor, 1994; Ellis et al., 2005; Fadahunsi et al., 2013; Sanni et al., 1999). This may be one of the factors in the rapid spoilage of these beers. It has been reported that moderate consumption of alcoholic beverages boost a person's resistance to pathogenic infections (Vriesekoop et al., 2012).

Ellis et al., (2005) reported an increase in alcohol content when pito pasteurized (6070°C) and/or chemically preserved was stored for 8 weeks. This signifies that the stabilization methods used did not stop the activities of microorganisms in the pito but rather reduced their activity. Osseyi et al. (2011), however, reported on lack of microbial growth after sorghum beer, Tchoukoutou, was pasteurized (75-80°C), therefore stabilizing the pH and alcohol content. Higher pasteurization temperature at 75-80°C for 15 minutes seems to be more effective than pasteurizing at 60-70°C for 15 minutes. However, increased pasteurization temperature and/or time effect on the sensory attributes of pito has not extensively been investigated.

2.6 Sensory Analysis of Beer during Storage

Most often foods and beverages are subjected to changes during storage. While it is preferred that flavour improves during the beer maturation process, formation of undesirable flavours inevitably occurs during beer storage. Beer ageing problems occur during beer storage and can damage the beer style (Cao, Zhou, Guo, & Li, 2011). These problems are mostly related to off-flavours which distorts the quality of the beer (Harayama, Hayase, & Kato, 1991). Rodrigues et al. (2011), reported that the

deterioration of the organoleptic qualities of beer on aging is due to the formation of volatile carbonyl compounds. Vanderhaegen et al., (2003) also reported on changes in flavour during beer storage for six months. Vanderhaegen and co-workers monitored specific flavour compounds in beer which caused changes in the sensory evaluation results. These flavour changes results in the staleness of the beer (Strating & Eerde, 1973).

Sensory evaluation of pasteurized sorghum beer, by a panel of 80 who were familiar with the product, showed consumer acceptance when stored for 1, 3 and 6 months. Also pasteurization and the double fermentation applied to the beer showed no significance difference when compared to the traditionally brewed sorghum beer (Osseyi et al., 2011). The sensory analysis conducted by Ellis et al. (2005), on pasteurized and chemically preserved beer was also accepted by taste panel of 20 when stored for 2 months. This shows that the stabilization methods employed by the authors above, was efficient in preserving sorghum beer.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Experimental Design Overview

This project had two phases: Extract level testing phase and the storage phase. The purpose of the extract level testing phase was to ascertain the suitable proportion of moringa leaf extract that could be added to the pito for the shelf life study.

3.2 Collection and Preparation of Samples

3.2.1 Sample collection

Dagarti Pito samples were obtained from a commercial brewer into a keg. *Moringa oleifera* leaves were also collected from the Nyankpala community into a clean polyethylene bag. The leaves were washed thoroughly under tap running water, and then air dried at room temperature (30-31°C) and relative humidity of 20-30% (Danso, Drechsel, Obuobie, Forkuor, & Kranjac-Berisavljevic, 2014) for 72 hours. The dried leaves were milled into fine powder using Philips blender HR2000/16 for 3 minutes and the moringa powder was collected into a clean airtight bowl, and then stored in a refrigerator at 5°C.

3.2.2 Extraction from moringa leaves

Dried moringa powder (50 g) was added to 500 ml distilled water in 1 litre conical flask, stoppered and kept for 1 week in a refrigerator (5°C) with periodical shaking for uniform mixture. The extract was filtered using a clean, sterilized muslin cloth, and then boiled for 30 minutes with continuous stirring.

3.3 Extract Level Testing Phase

This involved the addition of moringa extract to pito at 20, 25 and 30% replacement levels to obtain a final volume of 2000m1. Thus for 20% (1600 ml of pito added to 400 ml moringa extract), 25% (1500 ml of pito added to 500 ml moringa extract) and 30% (1400 ml of pito added to 600 ml moringa extract). The final mixtures were investigated for consumer acceptance and the antimicrobial effects of the added moringa on growth of fungi and coliforms in pito.

3.3.1 Moringa extract level consumer preference test

Sensory analysis using the paired preference test method as described by (Stone and Sidel, 2004) was adapted for 50 taste panellists known to be familiar with the pito product. Coded samples (Appendix I) of the products were given to the assessors to indicate their choice of preference. Assessors were informed about the inclusion of moringa in the pito samples.

The treated samples were paired equally and positioned in a way to obtain equal chance of selection by a panellist. The order of serving is therefore presented as follows:

- 20% versus 25%;
- 25% versus 30%; and
- 30% versus 20%.

Water was used as a neutralizer before and in-between tasting.

3.3.2 Effect of *Moringa oleifera* extracts on microbial growth in pito

The coliform and fungi growth in pito were determined and the treatments used were; untreated pito (pito without the moringa extract), pito with 20% moringa extract, pito with 25% moringa extract and pito with 30% moringa extract.

The storage effect of the moringa extract: The effect of storage on fresh moringa extract in pito and moringa extract stored for 7 days in pito were considered. Using the pour plate method, each treatment was serially diluted to 10^5 and plated on PDA and MacConkey agar in triplicates.

3.4 Storage Phase

Based on the two studies: paired preference test and the microbial enumeration presented above (with detailed results in chapter 4) the 25% was selected for the storage trial.

Pito was subjected to four treatments, which are: Untreated pito, pasteurized pito, moringa pito and pasteurized moringa pito. Purchased pito, 32 litres was used for the experiment. A total of 9 litres of product was used for each treatment.

Addition of **Moringa leaf extract (ME):** Methods of moringa extraction as well as pito preparation was the same for the extract level testing phase.

3.4.1 Untreated pito

Nine (9) litres of pito were dispensed from kegs into 300 ml glass bottles and crowned immediately using a manual hand crowner, then packaged into boxes and labelled ***untreated pito.***

3.4.2 Moringa pito

A 6.75 - litre portion of pito was added to 2.25 litres of moringa leaf extract. The mixture was uniformly mixed and distributed into thirty 300 ml glass bottles and crowned. Bottled products were packed into a box and coded ***moringa pito.***

3.4.3 Pasteurized pito

Nine (9) litres of pito was dispensed from a keg into thirty 300 ml litres glass bottles. They were immediately crowned. The bottles with the pito were then pasteurized at 75 ± 5°C for 15 minutes using the water bath. The pasteurized samples were left to cool, packaged in boxes and labelled ***pasteurized pito.***

3.4.4 Pasteurized moringa pito

A 6.75 - litre portion of pito was added to 2.25 litres moringa leaf extract, mixed and filled into thirty 300 ml glass bottles and crowned. They were pasteurized using a water bath at 75 ± 5°C for 15 minutes and left to cool. They were packed into a box and labelled ***pasteurized moringa pito.***

3.5 Media Preparation for Microbial Analysis

3.5.1Potato dextrose agar (PDA) for yeast growth

About 39 g of PDA, obtained from Sigma-Aldrich, was suspended in 1 litre of distilled water, using recommended protocol from manufacturer. It was boiled for 1 minute, stirred constantly with a magnetic stirrer to dissolve. The media was autoclaved at 121°C for 15 minutes.

3.5.2 MacConkey media for coliform growth

In accordance with manufacturer's protocol 50 g of the medium, from Sigma-Aldrich, was suspended in I litre of distilled water, heated until completely dissolved and after which autoclaved at 121°C for 15 minutes.

3.5.3 Phosphate buffered saline (PBS) in 1 L of H_20

PBS was prepared according to (Dulbecco & Vogt, 1954) with modification. About 8 g of sodium chloride, 0.2 g of potassium chloride, 0.24 g of potassium dihydrogen, and

0.75 of sodium phosphate were dissolved in one litre of distilled water and autoclaved at 121°C for 15 minutes.

3.5.4 Growth and enumeration of microorganisms of pito samples

The pour plate method as described by Hoben & Somasegaran (1982) was used. Serial dilutions of the pito samples (untreated pito, pasteurized pito, moringa pito and pasteurized moringa pito) were made. Ten (10) millitres of each pito sample was added to 90 ml of the diluent (PBS). Each sample was further diluted; one (1) milliliter of each sample was aseptically transferred to 9 ml of the diluent in a separate test tube and mixed vigorously. The process was continued until the 5^{th} diluents. Appropriate serial dilutions of 0.2 ml were aseptically plated. The PDA and the MacConkey agar were poured into the plates then left to solidify. Plates containing MacConkey agar were incubated at 37°C and colonies counted using the colony counter after 24 hours of incubation. The plates containing PDA were incubated at 30°C and colonies counted after 72 hours of incubation. Coliform and fungal counts were done on MacConkey and Potato Dextrose agar respectively.

3.6 Analysis of Physico-chemical Properties

Four replicates of each of the pito samples (untreated pito, pasteurized pito, moringa pito and pasteurized moringa pito) were sampled and examined for pH, sugar and alcohol levels.

3.6.1 pH determination of pito samples

The pH of the pito samples were determined using Basic 20 pH meter. The pH meter was calibrated using the alkaline and acidic buffer.

3.6.2 Sugar determination

The hand held refractometer was used to determine the sugar levels of each pito sample.

3.6.3 Alcohol determination

The alcohol content was carried out using the Specific Gravity method described by Mathapati, Ghasghase, & Kulkarni, (2010) as follows. A hundred milliliters measuring cylinder were filled with pito beer, however, making sure it doesn't spill over. The hydrometer was dropped in slowly and then allowed to float in the beer. The specific gravity was read by noting the level at which the surface of the fluid contacts the glass when the hydrometer is floating in the pito. The specific gravity of the wort, that is when yeast is not added, was recorded; this first reading is the original specific gravity (OG). When yeast was added to the pito beer and it was fermented (before bottling), the specific gravity was also recorded, which is read as final specific gravity (FG).

Mathematically;

$$\%abw = (OG - FG) \times 105\ \%$$

$$\%abv = abw \times 1.25$$

Where abw = alcohol by weight

Abv = alcohol by volume.

3.7 Proximate Analysis

The moisture, ash, crude protein, and crude fat content were determined using the standard method of AOAC (2005) and the energy was calculated using the FAO (2003) method. The analyses were conducted in triplicate and all reagents were of analytical grade.

3.7.1 Moisture determination of pito samples

Empty moisture dishes were weighed using an electronic balance. About 2 ml of each sample were poured into the moisture dishes and also weighed. They were then placed in a hot air oven at a temperature of 105°C. After 90 minutes the dishes were removed

- after all the moisture had evaporated, then they were weighed. The percentage of moisture content was then measured using the mathematical formulae below:

***% moisture* = (*c* - *a* ÷ *b* - a) x 100** Where;

a = empty moisture dish

b = weight of dish + sample before evaporation

c = weight of dish + sample after evaporation

3.7.2 Ash determination of pito samples

Ash is the inorganic residue that remains after combustion. It provides a measure of the total amount of minerals within a food.

Using a modified AOAC method, the ash content of pito and modified pito samples were determined.

The weights of empty crucibles were measured using an electronic balance. Each sample was poured into it respective crucible and the weight measured. The crucibles containing the samples were put in furnace at a temperature of 600°C. After 75 minutes they were removed from the furnace and the crucible containing the ashes of the samples where weighed. Then the percentage of ash in each crucible was measured.

Mathematical formulae

% ash = (c - a ± b - a) x 100

Where;

a = empty crucible

b = weight of crucible + sample before combustion

c = weight of crucible + sample after combustion

3.7.3 Crude fat

Procedure

Pito samples were poured into moisture dishes and placed in a hot air oven at 105°C for 75 minutes for evaporation to occur. Empty thimble was weighed (a) and 5 ml of the dried samples were measured and weighed (b). After that cotton wool was used to cover the mouth of the thumble, to prevent spillage, and then also weighed (c). Samples were put in the fat extraction chamber which contained ethanol, serving as a solvent. It was then left to boil for 30 minutes for the extraction of oil. The thumbles were placed in the dessicator to dry then weighed (d). The percentage of fat was then determined by;

Crude fat % = (C - d ÷ a - b) x 100

Where;

a = weight of empty thumble

b = weight of Sample and thumble

c = weight of sample, cotton wool, and thumble.

d = dried sample in the thumble with the cotton

3.7.4 Crude protein

Nitrogen is an important component of protein, and each protein has a unique nitrogen content. It is one of the five major elements found in organic matter such as proteins. This fact was recognized by a Denish chemist, John Kjeldehl. Using his method commonly known as the micro Kjeldahl, protein content in the pito samples was determined.

This process consists of three steps;

- Digestion
- Distillation
- titration

Digestion of pito samples

About 8 ml of the samples were weighed into a digestive flask. 15 ml of concentrated sulpheric acid was added. A catalyst, composing of 50% potassium sulphate and 50% copper sulphate, of about 5 g, was added. The mixture was then heated on a digestion burner until a greenish blue color was obtained; this indicates complete digestion of sample. Mixture was removed from the digestion burner then allowed to cool.

Distillation

The purpose of distillation is to separate the ammonia, which is the nitrogen, from the digestion mixture. This was done by; raising the pH of the mixture by adding concentrated sodium hydroxide (100% NaOH). This had the effect of changing the

ammonium (NI-I4^{+}) ions, which were dissolved in the liquid into ammonia gas. The process is as follows

Concentrated NaOH was added to the mixture until there was a change in color. The temperature of the mixture was raised to a boiling point, this was to separate the nitrogen from the mixture. Volatile gas, ammonia, from the mixture was trapped in a special solution (indicator) of about 4m1 and 0.1N of H_2SO_4. The trapping flask was removed and the condenser was rinsed with distilled water to make sure all the ammonia had been dissolved.

Titration

The ammonia in the trapped flask was back titrated using a standard base solution of NaOH. The amount of ammonia distilled off from the digestive solution was then calculated, hence the amount of nitrogen.

Calculation of protein in the sample

$$\% \text{ Nitrogen} = \frac{\text{volume of } H_2SO_4 \text{ (collection) - volume of NaOH (titration x 0.1N x I4 x100)}}{\text{Sample weight (mg)}}$$

To convert nitrogen to protein

% Protein = % of nitrogen x 6.25

Carbohydrate

It was determined by subtracting the crude protein, crude fat, moistureand ash contents from100.

Mathematically;

***Carbohydrate = 100 — (crude** protein + crude fat + moisture + ask)*

3.7.5 Energy determination

Energy was determined using the FAO (2003) method.

Total energy ***(kcal/g) = ((protein x 4) + (carbohydrate x 4) + (fat x 9) + (alcohol x 7)***

3.8 Consumer Sensory Evaluation of Pito during Storage

The four different pito samples: untreated, pasteurized, moringa and pasteurized moringa pito, were assessed by 150 sensory panelists in order to reduce the effect of any limitations in the test environment or inexperience of the consumer (Stone & Sidel, 2004). The panelists consisted of students and lecturers of UDS Nyankpala Campus, as well as community members from Nyankpala, selected based on their familiarity with pito. The samples were stored for two months at ambient temperature. Consumer preference of the samples listed above were compared at Zero (0), Seven (7) Fourteen (14), Twenty eight (28) and Fifty six (56) days of storage.

Sensory ballot sheet (Appendix II**)** was provided for the subjects and the sensory scale adopted was the 5-point hedonic scale, and the just-about - right scale (Stone and Sidel, 2004).

Samples were coded and presented to the assessors to indicate their preferences on appearance, colour, aroma, taste, sourness and acceptability. Samples were served in clean, transparent plastic containers with tight lids.

3.10 Statistical Analysis

Data obtained from the paired preference test were analysed using the Microsoft Excel Programme. All data, with the exception of the paired preference test, were subjected to analysis of variance (ANOVA) for variation of means of treatments with their

respective period of storage, using the Genstat Discovery edition 4. Multiple mean comparison was also carried out with the Minitab. Statistical significance was set at $P < 0.05$.

CHAPTER FOUR

RESULTS

4.1 Extract Level Testing Phase of Shelf Life Improvement of Pito

4.1.1 Consumer preference of the proportions of moringa extract

Data on consumer preference from the extract level testing phase are presented in Table 1. Variations in the preference of treatments were observed in each set of the trials. The higher preference in the first, second and third trials were 70%, 66% and 62% respectively. However, with the exception of the third trial, significant differences were observed in the first and second sets. Out of the three trials, the 25% moringa treated pito was mostly preferred.

Table 1: Percentages of moringa extract and preference rating

Serving order	% of extract	Count
First set	20	15
	25	35
Second set	25	33
	30	17
Third set	30	19
	20	31

N (number of panellists) is 50. Statistically significant ($p < 0.05$), if count is ≥ 33 (Stone & Sidel, 2004).

4.1.2 Effect of moringa extracts on microbial growth in pito

Microbial enumeration of pito with various proportions of moringa extract (Table 2) indicated a general decline in counts for both coliform and fungi growth.

The concentration of *Moringa oleifera* extract showed no significance difference ($p>0.05$) for coliform growth. There was, however, a general reduction with increasing incorporation of moringa extract with a fold decrease of 1.3-, 1.7-, and 3.1-, respectively. Also, the treated pito had a lower count of coliform than the untreated pito

The same trend observed for coliform growth was also obtained for the fungal counts. However, fungi growth was observed to be significantly different ($P < 0.05$).

Table 2 Microbial population with moringa extract

	% of extract in pito				
Parameter	Untreated pito	20	25	30	Fpr < 0.05
Coliforms ($\times 10^6$) cfu/ml	4.8 ± 1.2^{a}	3.5 ± 2.7^{ab}	3.1 ± 2.2^{ab}	1.7 ± 1.0^{bc}	0.093
Fungi ($\times 10^6$) cfu/ml	4.9 ± 0.5^{a}	2.9 ± 1.7^{ab}	1.9 ± 1.6^{bc}	0.6 ± 0.1^{c}	0.001

Values are means and standard deviations of coliform and fungi forming units; values within row that do not share the same letter are significantly different ($P < 0.05$).

4.1.3 Effect of freshly prepared *Moringa oleifera* extract and 7 days stored *Moringa oleifera* extract on microbial growth in pito

There was a significant difference ($P < 0.05$) in the coliform growth with respect to the days of storage of moringa extract in pito (Figure 2). Freshly prepared moringa extracts (20%, 25% and 30%) in pito recorded the highest coliform counts whereas moringa extracts (20%, 25% and 30%) stored for 7 days in pito had the lowest number of colonies. A similar trend was obtained for fungi growth (Figure 3).

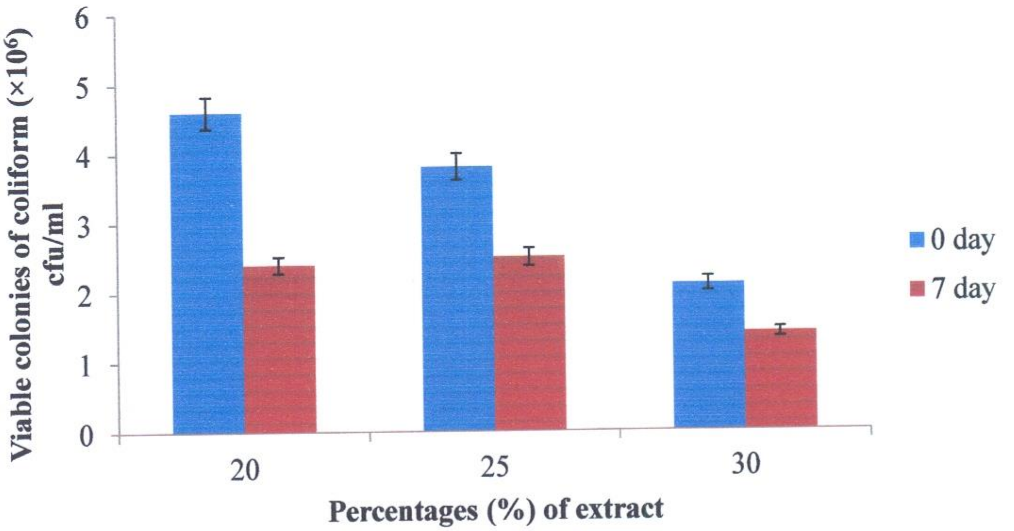

Figure 2: Effect of freshly prepared moringa extract and 7 days stored moringa extract on coliform counts in pito

Bar values are means and standard deviations of colony forming units per millilitre. Bar with different letters are significantly different (P < 0.05)

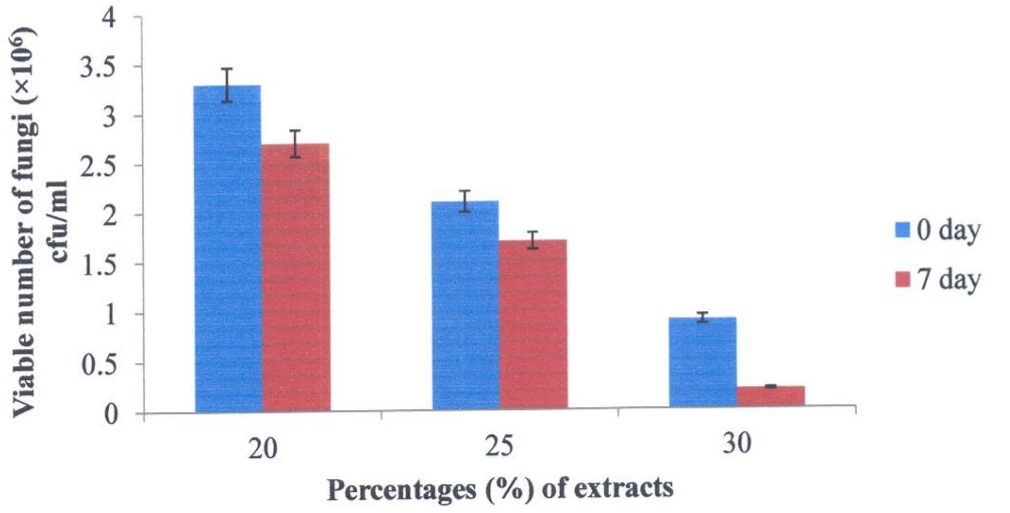

Figure 3 Effect of freshly prepared moringa extract and 7 days stored moringa extract on fungi growth in pito

Bar values are means and standard deviations of fungi forming units per millilitre. Bars with different letters are significantly different (P < 0.05)

4.2 Storage Phase of Shelf life Improvement of Pito

From the extract level testing phase, pito with 25% *Moringa oleifera* leaf extract, and extract stored for 7 days, were the suitable treatment option for the storage phase.

4.2.1 Effect of different treatments on coliform growth in pito

There was a general decline in coliform counts during storage (Figure 4). Coliform growth was significantly different during storage, deduced by F probability value of $P<0.001$ with respect to the treatment. Varied levels of coliforms were, however, observed with respect to treatments as well as the duration of storage. Pasteurized pito, moringa pito and pasteurized moringa pito had similar ($P > 0.05$) coliform count. The treated pito (pasteurized, moringa and pasteurized moringa pito) showed a relative reduction of coliform growth as compared to the untreated pito at 0 day of storage. The reduction of the untreated pito was from $4.0x\ 10^6$ to $2.0x\ 10^5$ cfu/ml; and for pasteurize pito, moringa pito, pasteurized moringa pito, the reductions from the initial count were $3.0x\ 10^5$ to $2.0\ x\ 10^5$ cfu/ml, $7.0x\ 10^5$ to $1.0x\ 10^5$ cfu/ml; $1.0x10^5$ to $8.0x10^4$ cfu/ml respectively. The reduction of coliform counts in pasteurized moringa pito was 2 times that of the pasteurized pito and 4 times that of the moringa pito. The untreated pito recorded the highest coliform counts compared to the treated pito samples.

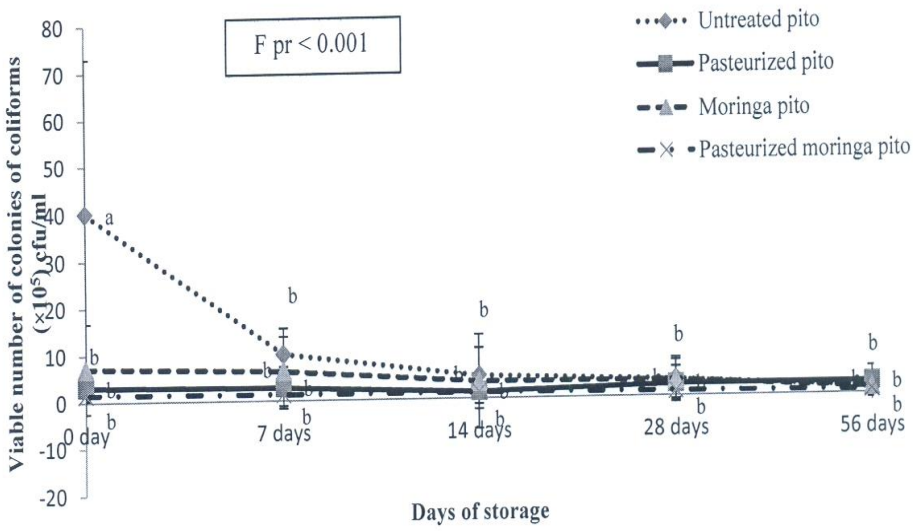

Figure 4: Viable number of coliforms when untreated and treated pito samples were stored for 2 months.

Values are means and standard deviations of colony forming unit per millilitre; values that do not share the same letter are significantly different (P < 0.05). All treatments were stored at an ambient temperature.

4.2.2 Effect of different treatments on fungal growth in pito

As in the coliform growth there was also a general decline of fungi growth during storage (Figure 5). The effect of the treatment investigated: untreated pito, pasteurized pito, moringa pito, and pasteurized moringa pito, showed an overall significance difference for fungi growth with F probability value of $P < 0.001$. Fungal count for pasteurized pito and pasteurized moringa pito were similar ($P > 0.05$). Fungal count for untreated pito was decreased from 3.6×10^6 to 5.0×10^5 cfu/ml; and for pasteurized pito, moringa pito, pasteurized moringa pito the reduction from the initial count were 2.0×10^5 to 9.0×10^4 cfu/ml, 2.1×10^6 to 1.0×10^5 cfu/ml, and; 7.0×10^4 to 2.0×10^4 cfu/ml repectively. Pasteurized moringa pito had the lowest fungi growth followed by pasteurized pito, moringa pito and the untreated pito for all the days during the storage period. The reduction of growth in the pasteurized moringa pito was 3.5 times that of

the pasteurized pito, and 20 times that of the moringa pito. The untreated pito obtained the highest fungi growth than the treated pito samples during the storage period.

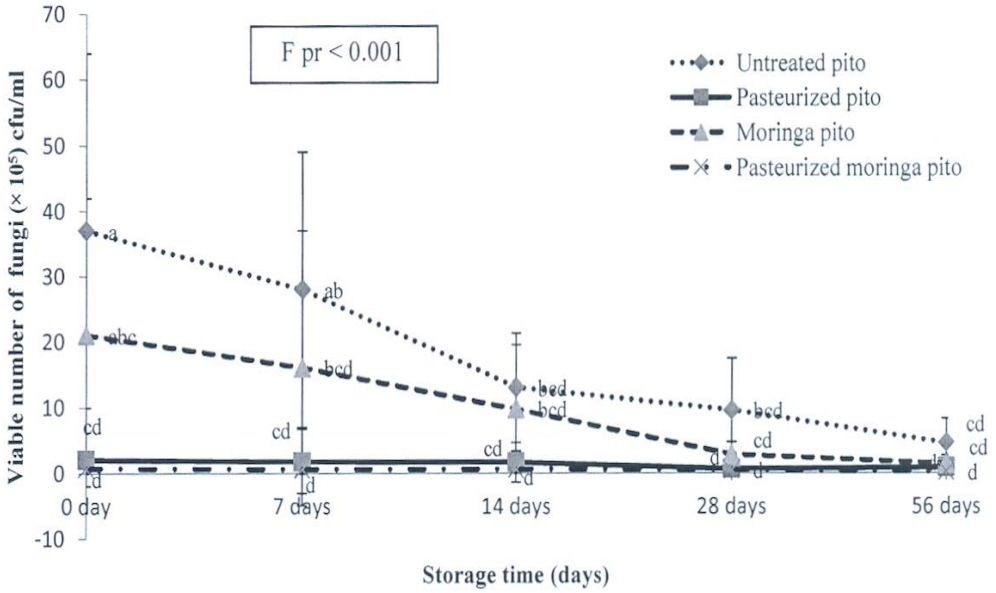

Figure 5: Viable number of fungi for untreated and treated pito samples stored for 2 months.

Values are means and standard deviations of fungi forming units per millilitre; values that do not share the same letter are significantly different (P< 0.05). All treatments were stored at an ambient temperature.

4.3 Physico-chemical Composition of Untreated and Treated Pito Samples during Storage

4.3.1 pH levels in pito products

In general, pH of pito decreased during storage irrespective of treatment (Figure 6). The pasteurized pito, and pasteurized moringa pito, showed significantly ($P < 0.05$) higher pH values than the untreated pito from the 0 day to the 28th days of storage and with moringa pito from 7th days to the 14th days of storage.

The level of pH reduction in the untreated pito, pasteurized pito and moringa pito was 0.2, 0.04 and 0.1 times that of pasteurized moringa pito respectively.

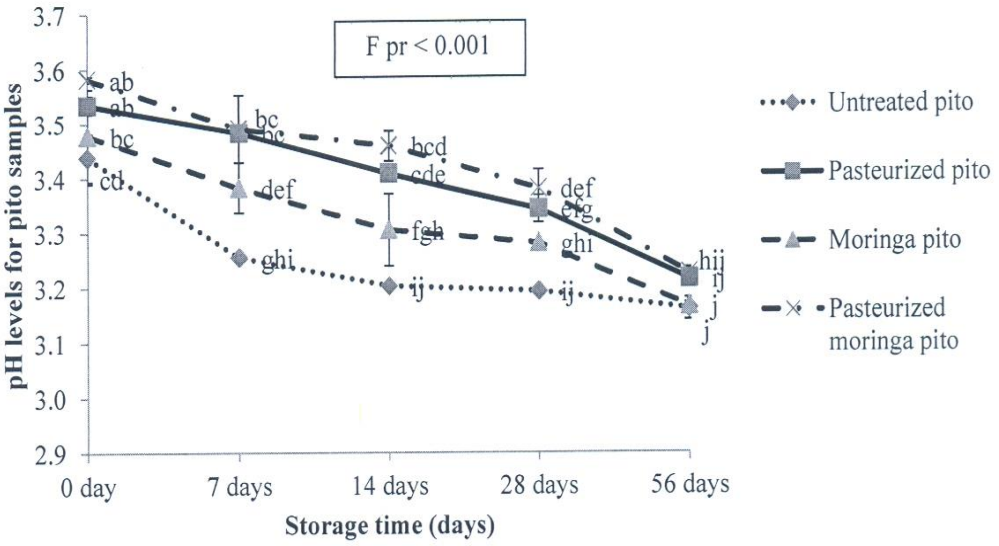

Figure 6: Levels of pH during 56 days of storage of untreated and treated pito samples.

Values are the means ± standard deviation of quadruple determinations. Values that do not share the same letter are significantly different (P< 0.05). All treatments were stored at an ambient temperature.

4.3.2 Sugar level in pito products during storage

Expectantly, there was a general decline in sugar content (Figure 7). Pasteurized pito, and pasteurized moringa pito were significantly ($P < 0.05$) higher in the sugar content than in the untreated pito from the 7th day to the 28th day of storage, and for the moringa pito from the 7th day to the 56th day of storage. The sugar content in all the treatments reduced with increasing duration of storage. Untreated pito, pasteurized pito, and moringa pito recorded a percentage difference of 19%, 8%, and 11% in that order with respect to pasteurized moringa pito during storage.

From 0 day to 14th days of storage there was no significant ($P > 0.05$) reduction of sugar of the pasteurized pito and pasteurized moringa pito.

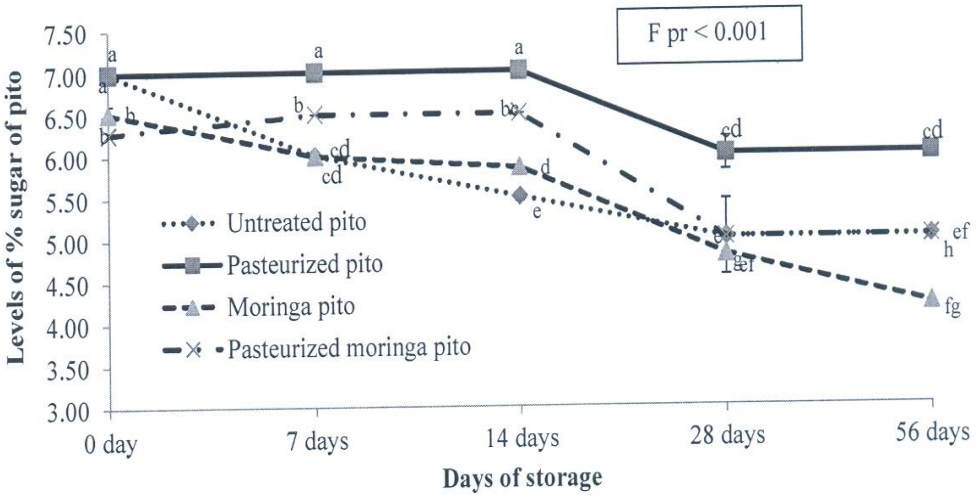

Figure 7: Percentage of sugar during 56 days of storage of untreated l and treated pito samples.

Values are the means ± standard deviation of quadruple determinations. Values that do not share the same letter are significantly different (P< 0.05). All treatments were stored at an ambient temperature.

4.3.3 Alcohol levels in pito products during storage

The alcohol content decreased during storage (Figure 8). There was an overall significant difference in the alcohol value in the samples investigated. The treated pito: pasteurized pito, moringa pito, and pasteurized moringa pito, significantly ($P < 0.05$) recorded higher alcohol content than the untreated pito from the 7th day to the 56th day of storage except on day 28 when there was no significance difference between the moringa pito and the untreated pito. The percentage difference with respect to pasteurized moringa pito of the untreated pito during the 56 days of storage was 33%; and pasteurized pito, moringa pito, recorded 20%, and 24% respectively.

Statistically, no significant ($P > 0.05$) reduction in the alcohol levels was observed when the treated pito samples: pasteurized pito, moringa pito and pasteurized moringa pito, were stored for a week.

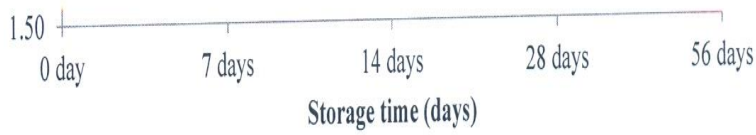

Figure 8: Percentage of alcohol by volume (% ABV) during 56 days of storage of conventional and treated pito.

Values are the means ± standard deviation of quadruple determinations. Values that do not share the same letter are significantly different ($P < 0.05$). All treatments were stored at an ambient temperature.

4.4 Proximate Composition of Untreated and Treated pito

Proximate composition and calculated energy content of the pito samples (Table 3) shared similar ($P > 0.05$) moisture, ash, CHO and energy. Generally, the moringa treated pito samples had crude fat and crude protein more than those without moringa

Table 3: Proximate composition of untreated and treated pito before storage

	Pito samples				
Parameter(g/100g)	Untreated pito	Pasteurized pito	Moringa pito	Pasteurized moringa pito	Fpr < 0.05
Moisture	98.53±0.38[a]	98.47±0.26[a]	98.08±0.21[a]	97.43±1.10[a]	0.181
Ash	0.13±0.10[a]	0.146±0.12[a]	0.242±0.05[a]	0.198±0.04[a]	0.419
Crude fat	0.03±0.02[a]	0.04±0.02[a]	0.1±0.08[b]	0.2±0.09[b]	0.009
Crude protein	0.0016±0.0003[a]	0.0014±0.0006[a]	0.0292±0.0124[b]	0.0272±0.0097[b]	0.003
CHO	1.31±0.51[a]	1.35±0.17[a]	1.57±0.14[a]	2.15±1.17[a]	0.416
Energy(kcal/100g)	27.53±1.84[a]	27.74±0.73[a]	27.42±2.80[a]	28.83±3.98[a]	0.8

Values are means and standard deviations of triplicate determinations; values within row that do not share the same letter are significantly different ($P < 0.05$).

4.5 Consumer Preference of Pito Samples before and during Storage

The findings of this study (Table 4) clearly showed significant difference ($P < 0.05$) among the consumer preference attributes: appearance, colour, aroma, taste, and overall degree of liking.

Pito samples without moringa extract were generally preferred by the assessors than those with moringa extract. The untreated pito was found objectionable by assessors after a week of storage. Pasteurized pito was liked throughout the storage period of 4 weeks.

Table 4: Presentation of the acceptability of treatments during storage

		Pito Sample				
Parameter	Storage(days)	Untreated pito	Pasteurized pito	Moringa pito	Pasteurized moringa pito	Fpr<0.05
Appearance	0	4.53±0.63^{a}	4.47±0.70^{a}	3.55±1.27^{cdefg}	3.53±1.36^{defgh}	0.001
	7	4.23±0.74^{ab}	4.39±0.66^{ab}	3.49±1.11^{efghi}	3.67±1.14^{cdef}	
	14	4.00±1.11^{bc}	4.25±0.58^{ab}	3.11±1.28^{ghi}	3.07±1.22^{hi}	
	28	3.95±1.25^{bcde}	3.99±1.26^{bcd}	3.35±1.20^{fghi}	3.04±1.49^{i}	
	56	3.43±1.49^{fghi}	3.57±1.28^{cdefg}	2.37±1.13^{j}	1.89±1.11^{k}	
Colour	0	4.53±0.60^{a}	4.38±0.74^{a}	3.73±1.22^{c}	3.59±1.40^{c}	0.001
	7	4.33±0.64^{a}	4.44±0.67^{a}	3.46±1.19^{cd}	3.58±1.09^{c}	
	14	4.23±0.97^{a}	4.21±0.59^{ab}	3.09±1.23^{de}	2.95±1.18^{e}	
	28	3.55±1.21^{c}	3.77±1.24^{bc}	3.07±1.12^{de}	2.85±1.31^{e}	
	56	3.49±1.44^{cd}	3.55±1.19^{c}	2.37±1.13^{f}	2.06±1.12^{f}	
Aroma	0	4.40±0.71^{a}	4.29±0.71^{a}	2.74±1.19^{ef}	2.75±1.19^{ef}	0.001
	7	4.06±0.85^{ab}	4.29±0.73^{a}	2.53±1.25^{fg}	2.61±1.25^{f}	
	14	3.46±1.14^{cd}	4.31±0.81^{a}	2.45±0.97^{fg}	2.45±1.11^{fg}	
	28	3.42±1.57^{cd}	3.64±1.22^{bc}	2.52±1.36^{fg}	2.74±1.30^{ef}	
	56	2.67±1.56^{f}	3.15±1.25^{de}	2.09±0.98^{gh}	1.98±0.97^{h}	

Table 4 *continued*:

Parameter	Storage(days)	Pito Sample: Untreated pito	Pasteurized pito	Moringa pito	Pasteurized moringa pito	Fpr<0.05
Taste	0	4.32±0.77^{a}	4.32±0.80^{a}	2.63±1.23^{de}	2.52±1.26^{e}	0.001
	7	3.85±0.99^{ab}	4.19±0.90^{a}	2.39±1.23^{ef}	2.63±1.29^{de}	
	14	3.08±1.32^{cd}	4.12±0.92^{a}	2.29±1.06^{ef}	2.47±1.13^{e}	
	28	3.17±1.41^{c}	3.49±1.44bc	2.37±1.29^{ef}	2.44±1.42^{e}	
	56	2.50±1.48^{e}	3.04±1.37^{cd}	1.91±0.93^{f}	1.92±1.02^{f}	
Overall	0	4.41±0.70^{a}	4.27±0.75^{ab}	2.86±1.19^{efg}	2.83±1.27^{efg}	0.001
Degree	7	3.87±0.99^{bc}	4.29±0.69^{ab}	2.60±1.22^{g}	2.78±1.24^{fg}	
of	14	3.26±1.23^{de}	4.23±0.73^{ab}	2.69±1.08^{fg}	2.65±1.11^{fg}	
Liking	28	3.30±1.38^{de}	3.61±1.36^{cd}	2.55±1.37^{g}	2.65±1.31^{fg}	
	56	2.51±1.51^{g}	3.08±1.36^{ef}	1.87±0.92^{h}	1.91±1.09^{h}	

Values are means and standard deviations of triplicate determinations; values within interactions of individual sensory attributes that do not share the same letter are significantly different (P< 0.05). A 5-hedonic scale was used (1= dislike extremely/least acceptable; 3= neutral; 5= like extremely/ highly acceptable) for the sensory attributes.

4.5.1 Consumer preference for the degree of sourness of pito samples

The degree of sourness of pito samples (**Figure 9**) showed that untreated pito samples were very sour after 0 day of storage; followed by moringa pito, then the pasteurized pito samples (pasteurized pito and pasteurized moringa pito) which became very sour after 14 days and 28 days of storage respectively.

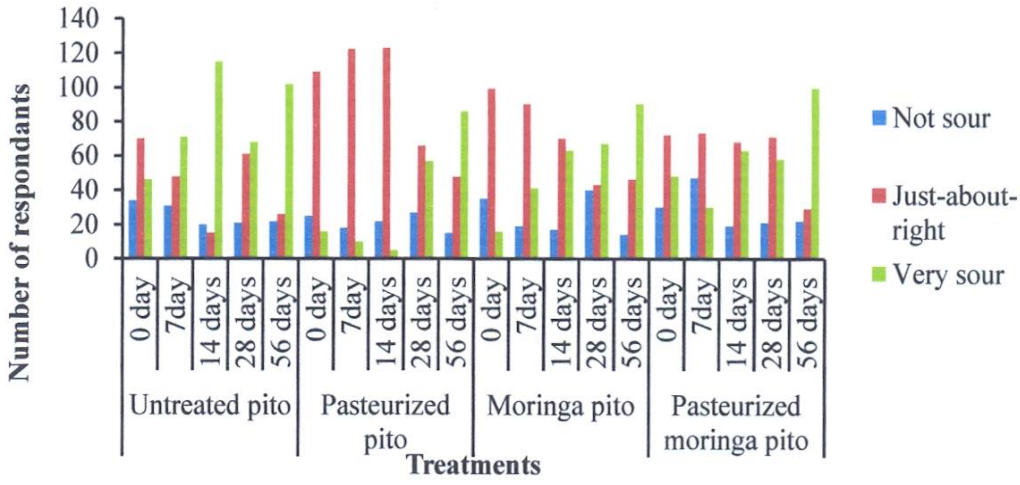

Figure 9: The degree of sourness of pito samples

The scale used was the three-category-just about-right scale.

4.5.2 Market acceptability of pito samples

The preference of pito samples in the Ghanaian market **(Figure 10)** showed that respondents favoured pito samples without moringa extract in the Ghanaian market than those with the moringa extract.

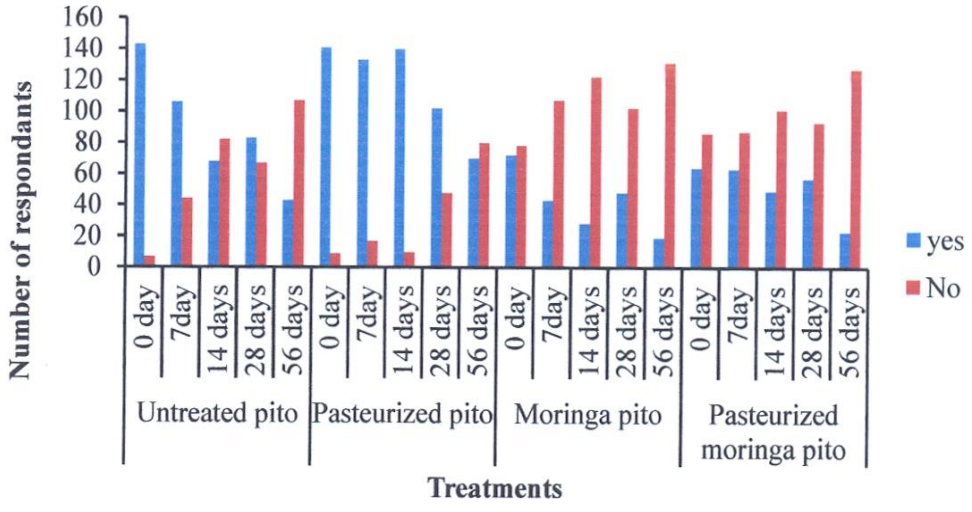

Figure 10: Preference of pito samples in the Ghanaian market

CHAPTER FIVE

DISCUSSION

5.1 Outcome of the Extract Level Testing Phase

The consumer preference and the microbial analysis of pito treated with different proportions of moringa leaf extract indicated that pito treated with the 25% moringa and the extract stored for 7 days before being added to the pito was suitable.

5.1.1 Consumer acceptance of moringa pito

Assessors preferred the pito treated with 25% moringa extract than the samples with the 20% and 30% of the extract, because it appealed to their senses in terms of its appearance, colour, taste and aroma. The 20% extract added to the pito might have been very low and assessors could not sense the extract effect in the product, and also the 30% extract in the pito might have overshadowed the sensory attributes of the pito.

5.1.2 Effect of moringa leaf extract on microbial growth in pito

The addition of moringa extract to pito was capable of reducing coliform and fungi growth in the pito samples. Pito with 25% and 30% had higher inhibitory effect than pito with 20% moringa extract. This result agrees with the findings of Peixoto et al., (2011) that increased concentration of moringa leaf extract was efficient against microorganisms.

It was expected that increasing the concentration of moringa extract would produce different results due to their level of potency. Interestingly, that did not happen with coliform and fungi growth when pito was treated with 25% and 30 % extract. This may be because the amount of antimicrobial composition in both treatments was similar or

the antimicrobial compounds in the 30% extract were not enough to cause a significant change in fungi or coliform growth.

5.1.3 Effect of *Moringa oleifera* on microbial growth during storage

Although the addition of fresh extract to pito reduced fungal and coliform growth, moringa extract kept for 7 days added to the pito was more efficient. This then confirms why some authors store moringa extract for sometime before using it against microbial activity (Bukar et al., 2010; Rahman et al., 2009). The reason for this observance is, however, not very clear yet.

5.2 Effect of Different Treatments on Coliform Counts in Pito

The insignificant ($p > 0.05$) reduction of coliform growth in the treated pito samples: moringa pito, pasteurized pito and pasteurized moringa pito reveals that moringa and pasteurization can enhance microbial stability in pito during 56 days of storage.

The moringa treatment and pasteurization extended the shelf life owing this to the antimicrobial property (Eilert et al., 1981; Fisch et al., 2004) and possible destruction of coliform bacteria (Devendra et al., 2011; Rahman et al., 2009) respectively.

The high coliform growth ($p < 0.05$) of the untreated pito at the initial stage which decreased during storage also may be linked to the development of unfavourable condition in the pito. Indigenous sorghum beers have been reported to have an accumulation of lactic acid and acetic acids during storage, which are detrimental to some sensitive bacteria (Ekundayo, 1969; Fadahunsi et al., 2013). Moreover, some coliforms cannot survive at low pH values (Ray & Bhunia, 2013), therefore explaining the drastic reduction.

The reduction might also have resulted from inaccessibility of nutrients in the pito sample (Fadahunsi et al., 2013), which also explains the insignificant ($p > 0.05$) coliform growth after 7 days of storage.

The presence of coliform in the pito samples may have resulted from unhygienic practices during and after the brewing process of the pito, causing the relatively high increase of coliform growth at the initial stage of storage in the untreated pito. The high coliform count in the fresh pito might have led to the presence of coliform in the pito samples after pasteurization and/or the addition of moringa extract (Douglas, 2014).

5.3 Effect of Different Treatments on Fungi Growth in Pito

The significant reduction of the number of fungi growth in the untreated pito during storage might have resulted from the exhaustion of nutrients in the products, thus reducing the overall food availability for the microorganisms as reported by other researchers (Fadahunsi et al., 2013).

Also the growth of fungi might have been impeded by unfavourable conditions, such as decrease in pH which might have resulted from the production of lactic acid and acetic acid by lactic acid bacteria and/ or acetic acid bacteria (Fadahunsi et al., 2013; Nwachukwu et al., 2010; Steinkraus, 2004).

The insignificant ($p > 0.05$) fungi counts among the treated pito samples (moringa pito, pasteurized pito and pasteurized moringa pito) as compared with the untreated pito during storage indicates a possible microbial stability of the pito samples. The addition of moringa extract and/or the heat treatment might have been the major contributing factor, influencing the overall reduction of fungal growth in moringa pito, pasteurized pito and pasteurized moringa pito.

The moringa, known to contain antifungal compounds (Eilert et al., 1981), might have aided in the reduction of fungal growth. This confirms the findings made by some researchers, on the inhibitory effect of *Moringa oleifera* leaf extract on some selected fungal strains (Bukar et al., 2010; Devendra et al., 2011).

Pasteurizing the pito samples might have also caused the inactivation of fungi cells in the product and consequently, explaining the low fungi growth in the pasteurized products. This corroborates with previous reports that pasteurization is capable of inactivating microbial activity in traditionally brewed sorghum beers (Ellis et al., 2005; Osseyi et al., 2011).

It was expected that after pasteurization and the addition of moringa leaf extract, there should be no visible growth of fungi in the pito. Interestingly the results showed otherwise. This was in contrast to reports made by Osseyi et al. (2011), who recorded insignificant fungi growth in sorghum beer after pasteurization. The high load of fungi ($3.7x10^6$cfu/m1) found in the fresh, untreated pito might have been a contributing factor to this observation. Douglas (2014) reported that, microorganisms subjected to heat are killed at a rate which is proportional to the number of organisms present. That is, the number of microorganisms in pasteurized beer depends, among other factors, on the number of microbial cells before treatment (Zufall & Wackerbauer, 2000). The same could be said about other preservative mechanisms such as chemical and natural preservatives. It can therefore be inferred from the experiment that, reduction of the fungi growth in treated pito products is substantially relative to the microbial load in the untreated pito.

5.4 Physico-chemical Composition of Conventional and Treated Pito

5.4.1 pH levels in pito products

The pH values confirmed previous findings in pito that ranges from 3-4 by other researchers (Ellis et al., 2005; Kolawole et al., 2007). The outcome of the results revealed changes in the pH of the treated pito during storage. This finding was in contrast to reports from other researchers, whereby the pH of pasteurized sorghum beer was stabilized for 8 weeks (Ellis et al., 2005) and 6 months (Osseyi et al., 2011) during storage. The reason might be that there were still microorganisms in the pito samples after pasteurization and/or the addition of moringa extract, which was not the case for Ellis et al. (2005), and Osseyi et al. (2011) who reported no microorganisms in sorghum beer.

The lowest pH values recorded in the untreated pito during storage is an indication of microbial activity which substantiates reports made by Fadahunsi et al. (2013). This may be due to the production of organic acids by some of the microorganisms that might be present (Balstasar et al., 2010). It has been reported that the souring in sorghum beer is due to the presence of lactic acid bacteria or acetic acid bacteria (Demuyakor, 1994; Ekundayo, 1969; Lyumugabe et al., 2012; Steinkraus, 2004).

The treated pito samples (pasteurized pito, pasteurized moringa pito and moringa pito) comparatively had higher pH values than the untreated pito, making them less acidic. This signifies low microbial activity in the treated pito samples. This is an indication therefore, that, pasteurization and/or the addition of *moringa oleifera* leaf extract is capable of improving the pH in pito.

5.4.2 Sugar level in pito products

The reduction of sugar in the untreated pito observed could be due to the active utilization of the sugar as a carbon source for energy by the microorganisms present. This observation confirms earlier findings of Demuyakor & Ohta, (1993), that the sugar content in pito reduced during storage.

The sugar content in moringa pito and pasteurized moringa pito was low at the initial stage of storage; this might be mainly due to the 25% reduction in the volume of pito to allow for the addition of the moringa extract. In as much as *Moringa oleifera* leaves is found to contain carbohydrate (Mustapha & Babura, 2009), conversion rate into fermentable sugars may be low, and in addition *Saccharomyces cerevisiae* conversion ability of starch (the largest proportion of carbohydrate in sorghum) is noted to be inefficient (Lyumugabe et al., 2010) translating into the low percentages of sugar in the pito at the initial stage of storage when treated with moringa extract (moringa and pasteurized moringa pito).

5.4.3 Alcohol levels in pito products

It was expected that, the amount of the sugar utilized should be equivalent to alcohol produced. Interestingly, that was not so because the stability of the levels of alcohol was observed for a week in pasteurized pito, pasteurized moringa pito and moringa pito. After that there was a decrease in the alcohol content. These irregularities indicate that the efficient conversion of sugar to alcohol involves other factors (Demuyakor & Ohta, 1993; Lin et al., 2012).

The significant reduction of alcohol in the untreated pito indicates deterioration in the sample. After 2 weeks of storage, the alcohol level in the untreated pito stabilized,

which might have resulted from inadequate carbon source for the microorganisms present. The treated pito significantly had higher alcohol content than the untreated pito which shows low microbial activity with regard to the treated pito samples. This reveals that the moringa and/or pasteurization were capable of improving the alcohol content in the pito during storage.

5.5 Proximate Composition of Conventional and Treated Pito

According to Oluwalana, Ade-Omowaye, and Adedeji (2013), the high moisture content is a characteristic of a good beverage. However, high moisture content encourage the growth of microbes during storage. The high moisture content recorded in the pito samples tends to confirm Oluwalana et al. (2013) assertion that sorghum beer satisfies thirst.

The relatively high levels of crude fat and crude protein in the pito treated with moringa extract (moringa pito and pasteurized moringa pito) may be due to the addition of the moringa in the pito samples. This confirms results by Quarcoo (2008) who reported some amount of fat and protein content in moringa beverage.

5.6 Consumer Sensory Acceptance of Untreated and Treated Pito before and after Storage

The appearance and colour of food are known to complement each other (Kemp, Hollowood, & Hort, 2011) which confirms the observation made between the colour and appearance of the treatments investigated: untreated pito, moringa pito, pasteurized moringa pito and pasteurized pito.

The colour of the moringa pito and pasteurized moringa pito, were not appealing to the panelists relative to the products with no moringa extract because the consumers might have been influenced by the unfamiliarity with the colour of the moringa treated pito samples.

The moringa pito and pasteurized moringa pito were found to be less accepted as compared to the pito samples without the moringa extract, and this might also be due to the unfamiliarity with the aroma. Furthermore, the panelists complained of the aroma being similar to that of herbal medicine, thereby influencing their general unacceptability of the treated products with the moringa extract.

The aroma of the untreated pito was only liked when stored for 7 days, which shows deterioration in the untreated pito decreasing the pito quality. Rodrigues et al. (2011) reported, volatile off-flavours in stored beer might have distorted the aroma of the beer causing deterioration. And these off-flavours might have resulted due to an increase in lactic acid and acetic acid bacteria (Demuyakor, 1994; Ekundayo, 1969; Lyumugabe et al., 2012; Steinkraus, 2004), thereby increasing the acidity of the pito during storage.

The taste of the untreated pito was acceptable when stored for 7 days, which is contrary to what was reported by Demuyakor (1994) that pito will go bad within a day of storage. This may be attributed to proper packaging in a tightly sealed clean container. However, it was observed that immediately after packaging there were breakages in some of the glass bottles in the fresh untreated pito. This was caused by internal pressure from the buildup of carbon dioxide by the actively fermenting microorganisms in the untreated pito.

The taste of the pasteurized pito was acceptable for 28 days, which showed an improvement over the unpasteurized pito. From the results it was observed that, the degree of liking of the aroma was proportional to that of the taste. The aroma might have therefore influence the taste of the products.

The intensity of the sourness is a reflection of the product acidity. The right degree of sourness is considered as part of the general characteristics of a good pito (Demuyakor, 1994) and if it becomes very sour it is an indication of deterioration. Also, the untreated pito was very sour after 7 days of storage indicating the impact of the decrease of pH level during storage. The panelists reported that the sourness was just about right for pasteurized pito, and pasteurized moringa pito, until after 28 days of storage, and the moringa pito after 14 days of storage.

5.6.1 Overall degree of liking (ODL) of products and market acceptability

The overall degree of liking and potential of products being accepted on the Ghanaian market mirrored the choices made by panelists in each of the sensory attributes discussed above. On the whole, pasteurized pito was mostly liked up to 28 days of storage. The untreated pito was further not preferred after 7 days of storage. The reason may be attributed to off-flavours which alter the quality of the beer causing it to deteriorate (Harayama et al., 1991; Rodrigues et al., 2011). Also the pito treated with moringa extract which was less liked throughout the storage period shows that consumers are not familiar with the product, and this confirms report by Barcellos et al. (2009), that, some consumers find it very difficult to change.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The 25% moringa extract stored for 7 days added to pito was suitable for the storage phase as it reduced microbial growth and consumer acceptance.

The addition of moringa extract and/or pasteurization reduced the fungi and coliform growth in the treated pito samples than the untreated pito.

The physic-chemical composition, that is, pH, sugar and alcohol levels were significantly influenced by pasteurization and the addition of *Moringa oleifera* leaf extract:

The untreated pito had lower pH than the treated pito samples during storage, comparatively causing the untreated pito to be very sour than the treated pito samples. Also, the pasteurized pito, moringa pito and pasteurized moringa pito had higher sugar and alcohol content than the untreated pito during storage.

The proximate composition in the pito was also significantly influenced by the addition of moringa extract with regards to the crude protein and crude fat content. Pito with moringa extract had relatively high crude protein and crude fat.

Although pito with the moringa did improve the shelf life, organoleptically, it was not liked by the assessors. The sensory results showed that pasteurized pito was more preferred by the consumers. Pasteurized pito was liked for up to 4 weeks of storage.

Based on the findings of this study, it could be concluded that, the shelf-life of pito can be improved through pasteurization and/or the addition of *Moringa oleifera* leaf extract

for 28 days. However, pito samples that contained the moringa extract was less favoured by consumers.

6.2 Recommendation

Based on the findings of the research, other antimicrobial plants should be used, since it was identified that, consumers do not like pito with the *Moringa oleifera* leaf extract.

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APPENDICES

APPENDIX I

Sensory codes for Paired Preference Test

Panellist	Code for 20% Moringa Extract	Code for 25% Moringa Extract	Code for 30% Moringa Extract
001	457	561	628
002	503	284	884
003	506	204	392
004	474	190	213
005	513	228	218
006	503	106	923
007	455	289	876
008	512	279	208
009	465	168	180
010	457	280	688
011	482	651	820
012	523	417	915
013	461	400	508
014	475	765	800
015	502	537	700
016	454	698	890
017	477	310	205
018	528	639	533
019	486	176	302
020	467	398	485
021	463	534	758
022	481	616	483
023	483	847	919
024	526	745	220
025	470	278	316
026	527	306	854
027	490	257	234
028	521	637	686
029	517	603	867
030	515	135	816
031	530	515	755
032	509	935	812
033	499	634	852
034	478	455	510
035	506	979	665
036	524	140	554
037	498	207	607
038	461	754	984
039	470	551	346
040	523	415	354

041	458	720	561
042	505	406	750
043	501	647	222
044	513	669	966
045	468	683	596
046	493	188	531
047	523	842	510
048	498	997	880
049	451	699	248
050	505	281	305

APPENDIX II

Sensory Ballot Sheet for Sensory Evaluation of Stored Pito

Panellist	Untreated Pito	Pasteurized Pito	Moringa Pito	Pasteurized Moringa Pito
001	222	305	525	723
002	171	292	497	711
003	163	323	486	708
004	161	327	517	702
005	169	350	458	710
006	210	290	463	738
007	161	307	467	734
008	248	319	501	717
009	208	315	473	653
010	216	275	488	708
011	246	289	489	717
012	164	347	485	696
013	198	269	509	699
014	238	310	529	685
015	239	283	459	718
016	204	250	463	687
017	198	348	488	740
018	166	294	507	722
019	242	254	491	670
020	200	350	480	679
021	194	343	516	660
022	159	339	489	726
023	214	291	508	738
024	244	279	516	691
025	153	261	528	704
026	207	325	511	685
027	161	292	519	704
028	229	317	454	700
029	192	265	517	734
030	168	329	506	739
031	227	324	510	738
032	217	335	499	747
033	196	296	484	663
034	219	281	509	676
035	221	282	491	670
036	182	276	492	691
037	167	257	461	681
038	228	263	501	664
039	177	326	451	697
040	241	350	462	723
041	206	317	466	681

042	244	300	507	663
043	182	266	502	657
044	193	347	526	693
045	234	328	473	748
046	174	272	516	732
047	196	283	475	667
048	153	281	453	694
049	177	343	518	734
050	232	292	476	654
051	154	779	947	966
052	185	770	931	962
053	189	773	851	998
054	150	845	900	963
055	220	823	887	982
056	206	759	921	957
057	207	846	850	963
058	192	765	900	994
059	205	781	894	999
060	174	846	929	976
061	144	753	923	901
062	176	767	945	956
063	188	802	930	957
064	148	776	916	950
065	159	783	903	985
066	204	813	861	971
067	131	764	896	979
068	170	825	872	987
069	130	827	852	954
070	181	816	887	977
071	156	829	905	996
072	138	834	860	965
073	220	786	923	986
074	155	750	948	965
075	154	847	949	990
076	140	832	914	978
077	207	831	915	992
078	213	822	872	983
079	200	833	887	979
080	223	837	946	954
081	134	839	908	997
082	182	751	928	993
083	147	757	854	902
084	227	818	888	995
085	194	848	891	972
086	202	770	871	968
087	228	821	931	973
088	184	769	921	994
089	210	798	856	952

090	161	816	948	671
091	163	762	912	979
092	141	772	934	967
093	184	841	931	964
094	185	849	927	975
095	132	780	867	981
096	196	848	948	998
097	142	796	851	967
098	228	787	940	962
099	173	772	893	973
100	210	764	878	957
101	245	438	567	671
102	270	526	533	708
103	251	518	538	630
104	410	525	561	667
105	353	463	558	713
106	388	470	580	693
107	246	477	581	722
108	297	505	566	636
109	278	457	553	707
110	330	452	530	663
111	350	489	562	728
112	403	431	607	722
113	231	475	623	689
114	375	494	574	637
115	267	459	550	648
116	284	479	628	697
117	351	522	539	653
118	379	470	573	712
119	318	433	553	719
120	309	435	590	686
121	360	439	609	655
122	261	443	537	651
123	297	489	557	709
124	408	501	548	632
125	260	499	538	645
126	250	528	534	673
127	281	476	592	690
128	263	488	530	662
129	281	460	586	699
130	363	512	583	630
131	343	481	565	669
132	353	439	602	675
133	286	481	574	725
134	407	492	629	682
135	260	522	569	686
136	389	438	601	720
137	391	462	614	639

138	320	516	546	643
139	271	493	604	666
140	251	482	547	709
141	240	515	560	630
142	370	507	540	730
143	366	495	560	667
144	336	437	598	645
145	427	431	546	725
146	300	487	614	658
147	395	530	582	665
148	344	488	584	652
149	340	439	618	639
150	338	515	627	697

APPENDIX III

Structured Questionnaire for Sensory

CONSENT FORM

THIS CONSENT FORM WILL BE HELD FOR 12 MONTHS FROM THE **DATE OF SIGNING**

Overview:

We have provided you with 4 pito samples, some of which are pasteurized and contain moringa leaf extract. We would like to test acceptability of all the samples presented.

DECLARATION

Please read the following and append your signature:

I have read and understood the information above and have had details of the study explained to me. My questions have been answered to my satisfaction, and I understand that

- I may ask further questions at any time
- I agree to voluntarily participate in this study based on the information provided
- I understand I have the right to withdraw from the study at any time and decline to answer any particular questions
- I understand that the products were prepared under hygienic conditions.

Participant's signature: _____

full name: _____

Age: _____

Date:5th July, 2014

CONSUMER ACCEPTABILITY TEST OF MODIFIED PITO

Panelist Code: _____ Sample

#: _____

Please evaluate the sample in front of you for appearance and other attributes by

checking (Ai) in the relevant box

1. Indicate your overall opinion on the APPEARANCE of the sample by checking only one box

❑	❑	❑	❑	☐
Dislike Extremely	dislike moderately	Neither like nor dislike	like moderately	like extremely

2. Indicate your overall opinion on the COLOUR of the sample by checking only one box

☐	☐	☐	☐	☐
Dislike Extremely	dislike moderately	Neither like nor dislike	like moderately	like extremely

3. Indicate your overall opinion on the **AROMA** of the sample by checking only one box

☐	☐	☐	☐	☐
Dislike Extremely	dislike moderately	Neither like nor dislike	like moderately	like extremely

4. Indicate your overall opinion on the **TASTE** of the sample by checking only one box

❑	❑	❑	❑	❑
Dislike Extremely	dislike moderately	Neither like nor dislike	like moderately	like extremely

5. Indicate your **OVERALL DEGREE OF LIKING** of the sample by checking only one box

Dislike Extremely	**dislike moderately**	**Neither like nor dislike**	**like moderately**	**like extremely**

6. Indicate your overall opinion on the sample's SOURNESS by checking only one box

☐ **Not sour** ☐ **just-about-right** ☐ too sour

7. Would you like to see this product on the Ghanaian market

☐ **Yes** ☐ No

APPENDIX IV

DESCRIPTIVE STATISTICS AND MICROBIAL ENUMERATION

Descriptive statistics of microbial growth of untreated and treated pito samples

Parameter		Pito Samples			
		Untreated pito	Pasteurized pito	Moringa pito	Pasteurized moringa pito
Fungi	Standard error	$3.1x10^5$	$0.5x10^5$	$2.4x10^5$	$0.08x10^5$
	minimum	0	0	0	0
	maximum	84×10^5	$14x10^5$	$60x10^5$	$2.0x10^5$
	count	40	40	40	40
	confidence level	$6.3x10^5$	$0.9x10^5$	$4.9x10^5$	$0.2x10^5$
	log reduction		1.11	0.26	1.56
Coliform	Standard error	$3.2x10^5$	$0.6x10^5$	$1.2x10^5$	$0.3x10^5$
	minimum	0	0	0	0
	maximum	$85x10^5$	$11X10^5$	$30x10^5$	lox 10^5
	count	40	40	40	40
	confidence level	$6.5x10^5$	$1.1x10^5$	$2.4x10^5$	$0.6x10^5$
	log reduction		0.68	0.44	1.01

Descriptive statistics of microbial growth during 2 months storage.

Parameter		Pito Samples during 56 days of Storage				
		Od	7d	14d	28d	56d
Fungi	Standard error	$3.9x10^5$	$3.2x10^5$	$1.4x10^5$	$1.0x10^5$	$0.5x10^5$
	Minimum	0	0	0	0	0
	Maximum	$84x10^5$	$56x\ 10^5$	$30x\ 10^5$	$20X10^5$	$9.9X10^5$
	count	32	32	32	32	32
	confidence level	$8.0x10^5$	$6.6x10^5$	$3.0x10^5$	$1.9x10^5$	$0.9x10^5$
	Log reduction		0.11	0.38	0.65	0.93
Coliforms	Standard error	$4.0X\ 10^5$	$1.1x10^5$	$1.1x10^5$	$0.7x10^5$	$0.4x10^5$
	Minimum	0	0	0	0	0
	Maximum	$85x10^5$	$23x10^5$	$29x\ 10^5$	14×10^5	$10x10^5$
	count	32	32	32	32	32
	confidence level	$8.2x\ 10^5$	$2.2x10^5$	$2.2x10^5$	$1.3x10^5$	$0.8x10^5$
	Log reduction		0.41	0.66	0.70	0.92

Microbial enumeration of untreated and treated pito samples

	Means of Pito Samples ($x10^5$ cfu/ml)				
Parameter	Untreated pito	Pasteurized pito	Moringa pito	Pasteurized moringa pito	Fpr < 0.05
Fungi	**18 ± 20**[a]	1.4±2.9[c]	10±15[b]	0.5±0.5[c]	0.001
Coliform	**12 ± 20**[a]	2.5±3.5[b]	4.3±7.4[b]	1.2±1.9[b]	0.001

Values are means and standard deviations of colony forming units; values within row that do not share the same letter are significantly different (p<0.05).

Microbial enumeration of untreated and treated pito samples during 2 months storage

	Means of Pito Samples during 56 days of Storage ($x10^5$ cfu/m1)					
Parameter	Od	7d	14d	28d	56d	Fpr < 0.05
Fungi	15 ± 22[a]	1 2±1 8[ab]	6.3±8.2[bc]	3.4±5.4[C]	1.8±2.6[c]	0.001
Coliforms	13 ± 23[a]	5.1±6.2[b]	2.8±6.2[b]	2.6±3.7[b]	1.6±2.2[b]	0.00₁

Values are means and standard deviations of colony forming units; values within row that do not share the same letter are significantly different (p<0.05)

APPENDIX V

Physico-chemical Composition of Untreated and Treated Pito

Table showing the levels of alcohol, pH and sugar of conventional and treated pito

	Means of Pito Samples				
Parameter	**Untreated pito**	**Pasteurized pito**	**Moringa pito**	**Pasteurized moringa pito**	**Fpr 5 0.05**
Alcohol	2.49±0.79[d]	3.35±0.62[a]	2.71±0.60[c]	2.94±0.30[b]	0.001
pH	3.21±0.05[a]	3.33±0.12[b]	3.27±0.19[c]	3.37±0.13[4]	0.001
Sugar	5.70±0.77[c]	6.60±0.50[a]	5.48±0.87[4]	5.86±0.74[b]	0.001

Values are means and standard deviations of four replications; values within row that do not share the same letter are significantly different.

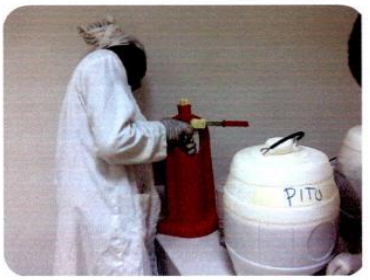
Table showing the levels of alcohol, pH and sugar of conventional and treated pito during storage

	Means of Pito Samples during 56 days of Storage					
Parameter	**0 day**	**7 days**	**14 days**	**28 days**	**56 days**	**Fpr < 0.05**
Alcohol	3.65±0.33[a]	3.28±0.48[b]	2.78±0.63[c]	2.33±0.31[d]	2.3 3±0.3 1 [d]	0.001
pH	3.45±0.13[a]	3.35±0.07[b]	3.27±0.05[c]	3.22±0.04[4]	3.19±0.03[e]	0.001
Sugar	6.70±0.38[a]	6.38±0.43[b]	6.21±0.61[c]	5.20±0.48[4]	5.05±0.66[e]	0.001

Values are means and standard deviations of four replications; values within row that do not share the same letter are significantly different.

APPENDIX VI
Pictures of the Experimental Set Up

Fresh and Moringa Pito



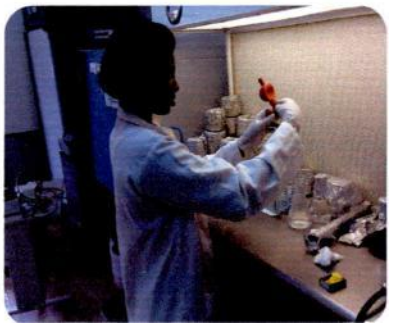
Crowning of Pito

Water bath with bottled pito

Wa

Bottled pito

Micro Kjeldahl Set up



Serial Dilution

APPENDIX VII

Pictures of Pito Brewing Processes

Malted sorghum

Milled malted sorghum

Mashed milled sorghum

"Yolga" in water

Boiling filtrate

Wort pitched with yeast

Fermented pito ready for drinking