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PROCESS DEVELOPMENT AND PRODUCT CHARACTERIZATION OF A FERMENTED RICE-BASED BABY FOOD

BY

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DECLARATION

I, Victoria Yemoteley Odoi, do hereby declare that, except for other people's works which have been cited and duly acknowledged, this work is my original research and this dissertation has not been presented for another degree elsewhere, either in part or as a whole.

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DEDICATION

I dedicate this work to my God Almighty, for His grace, favour, guidance and blessings throughout this work. I also dedicate this research work to my entire family, most especially to my mother, Mrs. Elizabeth Kate Nana Adjua Faidua Odoi, for all her love and sacrifices; I love you so much mum! Thanks for pushing me this far and believing in me.

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ABSTRACT

Oryza glaberrima is a local rice variety that is native to West Africa. Its tough texture and robustness produces milled rice with a large quantity of broken grains when dehusked (polished) accounting for the huge gap in quality. Consequently, imported rice is much more consumed in Ghana than locally cultivated and milled rice. The objective of this study was to add value to local rice by developing and characterizing a rice-based baby food from low grade locally milled and parboiled rice by using simple traditional food processing technologies of fermentation and size reduction. A 2χ 4 factorial design for a local rice cultivar (milled and parboiled) and fermentation time (0, 12, 24 and 48 hours) was used to develop drum dried, pre-cooked fermented rice product as baby food. The physico-chemical, functional and sensory characteristics of the drum dried fermented rice product were evaluated.

The drum dried products had moisture content of 5.88 -7.84 % and 5.12 - 11.2 % for milled and parboiled rice respectively. Fermentation time and milling method influenced the physical characteristics of the final products. Parboiled products were lighter in colour (ΔE= 4.27 - 2.69) and showed significantly lower pH values (4.13-3.38) with corresponding higher total acidity values (0.046-0.150)% lactic acid as fermentation time increased. Lower water absorption index (WAI) values and corresponding higher water solubility index (WSI) values were recorded for parboiled products as fermentation progressed due to the incidence of degraded starch molecules. The SDS-PAGE patterns of rice proteins showed three bands corresponding to proteins of molecular weights 14kDa, 30kDa and 45kDa respectively in both milled and parboiled rice samples in all three buffer systems. Protein solubility increased with fermentation time until twelve hours then it decreased through to 48 hours fermentation time. Parboiling and increasing

fermentation time also significantly favoured the formation of accessible protein thiols. Pasting profiles of products indicated that milled products had a higher tendency to disintegrate as well as undergo retrogradation and thus were more likely to give thinner gruels when hot water was used for re-constitution than parboiled rice. The 48-hour fermented products from both milled and parboiled rice exhibited the highest protein and starch digestibilities. Sensory profiles revealed altering intensities of sensory attributes of appearance, aroma, flavour, aftertaste and texture (mouth feel and touch sensation) using powdered and re-constituted forms of the eight fermented milled and parboiled rice-based products. Parboiled samples were described as more fermented, rich, sour with sour after-taste as fermentation time progressed by the quantitative descriptive analysis (QDA). Principal component analysis (PCA) scores were used to classify baby food products based on aroma and flavour from quantitative descriptive analysis (QDA) data as well as e-sensing measurements(electronic nose and tongue). These findings suggest that local rice would be very useful as a baby food product and demonstrates the function of quantitative descriptive analysis and e-sensing in evaluating and identifying product attributes that could be essential to consumers and food processors.

CHAPTER ONE

INTRODUCTION

1.1 Background and Study Rationale

Rice is one of the most universally consumed grains around the world (Adeniran *et al*., 2012; Boboye & Daramola, 2011; Amissah *et al.*, 2003). In Ghana, its consumption has steadily grown over the years (Tomlins *et al.*, 2005, Adu-Kwarteng *et al.*, 2003). CARD (2010) reported a rise in the annual per capita consumption of rice in Ghana from 12.4kg/person to 20kg/person from 1980 to 2005. This figure doubled to 30kg/person by 2009 with a projected estimate of 63kg/person by 2015 (USAID, 2009). However, more than half of all the rice consumed in Ghana is imported (Kranjac-Berisavljevic *et al*., 2003).

Rice is mainly composed of carbohydrates (about 75-80%), protein (about 7%) and some minerals and vitamins B1, B2 and B3 (Oko *et al*., 2012). Because the main carbohydrate portion of rice is starch (Deepa *et al*., 2010), it contributes to the body's energy needs (Ratnayake &Jackson, 2008) when consumed. The unique properties of rice starch have increased its application in many products especially gluten-free products. Rice starch has a high glycaemic index indicating high digestibility (Mir *et al.*, 2013); one reason why it is incorporated in most processed shelf stable infant cereal mixes and blends. A number of research studies on rice starch digestibility on infant nutrition have been reported (Dreher *et al*., 1984; Fernandez-Artigas *et al*., 2001). Rice proteins constitute the next most abundant nutrient in rice after starch. The proteins in rice are of a very good quality (Juliano, 1985). Rice protein has also been found to have a high

digestibility (Ramirez-Jimenez *et al.*, 2003). Like all cereals, rice is also rich in some minerals. These include the macrominerals; calcium, magnesium and phosphorus. Micronutrients such as iron, zinc, copper and manganese are present in trace amounts (Yousaf, 1992). Adu-Kwarteng *et al.* (2003) reported that local rice varieties have a higher nutritional content as compared to their foreign counterparts based on a study conducted involving ten local varieties in Ghana. However, local rice continues to suffer low patronage.

Globally, there are two commonly cultivated rice varieties: *Oryza sativa* and *Oryza glaberrima* (ODI, 2003). In West Africa, the *Oryza glaberrima*, also known as African rice (AfricaRice, 2010) is cultivated and constitutes most of the locally produced rice in Ghana. *Oryza sativa* is native to Asia and it is the main variety imported into the country. These two varieties are among the few domesticated types that are cultivated out of the existing twenty one different wild species of rice belonging to the genus *Oryza* (Juliano, 1985; Santos *et al.*, 2013).Severallocal rice types (*Oryza glaberrima*)are cultivated locally and they include *Vionor, Emokokoo, Togo marshall, Amavi, Abodwese, Emofitaa, Abibifoomo and Mui* (Adu-Kwarteng *et al*., 2003). However, the post-harvest handling of rice in Ghana is inefficient and leads to poor quality rice. Raw rice grains are first harvested as paddy (raw rice) and dried to moisture content below 14% (usually 12-14% moisture content) then de-husked to obtain brown rice, broken de-husked rice and husk (chaff). In Ghana, the paddy may be processed by parboiling before milling or by raw milling, with the former representing about 46% of locally produced rice (Tomlins *et al*., 2005). Parboiling is a process that involves soaking, steaming and drying of the paddy before milling (de-husking or polishing). The soaking time ranges from a few hours to about three days and in certain situations warm water is used for this process. The essence of the soaking process is to allow the paddy to

imbibe as much water as possible to eventually reduce the amount of brokenness during milling (Ayamdoo *et al*., 2013). Although parboiling has reported benefits such as increasing the nutrient density of rice, the procedure brings about a characteristic strong flavour, discolouration of grains, firmer grain texture and prolonged cooking time and these features are undesirable. On the other hand, raw milling of the husked rice, where the pericarp, tegmen and aleuron layer are removed produces the preferred white rice which may be whole or broken. Frequently, the percentage of broken rice in locally milled rice is unacceptably high, leading to low grade or poor quality product.

Rice is graded on the basis of the assessment of certain quality characteristics: physical attributes, physicochemical qualities and the organoleptic characteristics. Grading of rice ranges from Grade 1 (premium) to Grade 5 (least in quality); this may however differ slightly from country to country. For instance, according to Ghana Standards Authority, most of the locally produced rice fall under the category 4.7 while USAID (2009) reported that 83% of domestic rice production falls within grade 5. Milling of most of the local varieties produces high amounts of broken (about 13.60-32.70%) (Diako *et al*., 2010). These poor quality attributes of local rice consisting of mostly broken explains consumers' low patronage for this rice type.

In order to add value and increase the overall acceptability and utilization of the poor quality local rice, basic food processing operations such as fermentation and size reduction could be applied. Size reduction transforms the physical properties and opens up further options for the food application of rice. For instance, broken rice is converted into flour which can be used to produce

baby foods or extruded into snacks and breakfast cereals or even used as a coating. Fermentation is also a popular traditional processing technique applied to several food crops, particularly cereals in Ghana. It is used in the development of a popular traditional complementary food, 'koko', which is a thin cereal porridge commonly prepared from maize, sorghum or millet. Also, there have been several publications on other fermented cereal-based foods in Ghana (Mensah *et al*., 1991; Annan *et al*., 2003; Blandino *et al.*, 2003; Asante *et al*., 2013). In their study, Annan *et al.* (2003) pointed out that spontaneous fermentation of corn dough used in the production of Ga kenkey produced acids including 75 other compounds that contributed to the product's aroma profile. Strong evidence exists that the presence of these acids in fermented foods disallows the growth of food pathogens and prolongs shelf life (Steinkraus, 1996; Oyewole, 1997; Deshpande,2000; Beaumont, 2002; Annan *et al.*, 2003; Kalui *et al.*, 2009).Fermented rice, though not very common in Africa has reported frequent use in many forms in Asia; 'dosa' (rice pan cake), 'sake' (rice wine), 'idli' (steamed rice balls) and 'khanom jeen' (fermented rice noodles) (Soni *et al.*,1985; Wantanabe *et al*., 1998; Nagaraju & Manohar, 2000; Keatkrai & Jirapakkul, 2010).

Apart from the variation in dishes, fermentation has also been found to increase the nutrient quality, for instance, the availability of lysine in rice (Hamad & Fields, 1979) and modify the flavor profile of the product. In Africa, a study by Kabeir *et al.* (2004) improved the nutritional value of 'medida', a thin porridge made from fermented rice used as complementary food in Sudan while in Nigeria, Boboye & Daramola (2011) regulated the genes of the microorganism *Micrococcus luteus* for the synthesis of glucose and protein during the fermentation of 'Igbimo',

an *Oryza sativa* variety of Nigerian rice. However, in Ghana, use of naturally fermented rice for complementary or other foods has not yet been established.

The otherwise small market for low grade rice can be transformed by using a simple fermentation process to produce a rice-based food product that requires size reduction such as complementary or baby food. Complementary foods are important to the nutrition and healthy growth of babies in the age group between 6 months and 2 years. They offer the major nourishment to infants alongside breast feeding from six months to two years (Oyarekua *et al.*, 2011). This is one of the most vulnerable periods in the child's life because at this stage the infant needs adequate nutrients for good health and development. Consequently malnutrition is prevalent among infants in most third world countries (UNICEF, 1990). In these countries most of the traditional complementary foods are usually low in essential micronutirents, protein of high biological value and particularly energy because they are often consumed in much diluted quantities (Kapil, 2000), leading to growth deficits (WHO/UNICEF/ICCIDD, 2001). Additionally, a number of studies on the usage of α-amylase-treated complementary foods to increase nutrient uptake by infants have been recounted (Moursi*et al.*, 2003; Chakravarthi & Kapoor, 2003; Den Besten *et al.*, 1998). Owino *et al.* (2007) confirmed that the incorporation of α-amylase in a cereal-legume complementary food blend improved its acceptability when a thinner porridge consistency was obtained from the blend. This demonstrates that α-amylase positively affect the final product acceptability when added before or during fermentation process (Mok, 1992).

There is a pressing need to improve the value of local rice varieties in Ghana. Thus, besides reduction of postharvest losses, many food processing methods can be used to solve the quality problems associated with locally produced rice which are usually branded low grade. The use of simple technologies such as size reduction and fermentation in the presence of alpha amylase to produce an easily digestible baby food might tremendously improve the market value of locally milled, low grade rice.

1.2. Main Objective of Study

The main objective of this study was to develop and characterize a fermented rice-based baby food using low grade locally milled rice.

1.2.1 Specific Objectives

The specific objectives of this study are to:

1. develop a process for the production of a pre-cooked, ready-to-eat fermented rice-based baby food.
2. determine the physicochemical changes of the fermented rice-based dough and final product.
3. determine the functional properties of the final product.
4. determine the starch digestibility and protein digestibility of the final product.
5. evaluate the sensory attributes of the product.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Ready-to-eat baby foods have recently become very popular in the last two decades because mothers have become increasingly busy. The driving force of the continuous growing purchase of these accessible baby foods is convenience. 'Cerelac', 'Beechnut' and 'Purity' are amongst the commonly recognized baby food brands in the country. Most of these baby food brands are cereal-based, may or may not contain milk and are reconstituted before feeding to baby. In Ghana, cereals such as maize, sorghum and millet are locally fermented and used in the preparation of porridges to feed weaning infants at home. These porridges have been reported as thin, dilute and lack all the nutrients crucial to the proper development of growing babies (Lartey *et al.*, 1999). Over the years, a number of researchers have studied possible ways of improving the nutrient density of these cereal-only baby food blends (Afoakwah *et al.*, 2010; Saalia *et al.*, 2012). Some findings have suggested a combination of cereals with other protein-rich sources such as dry fish powder and legumes including cow pea, soy bean, groundnut and melon seeds (Egoulety, 2002; Amankwah *et al.*, 2009). Lartey *et al.* (1999) reported a study that was conducted in Ghana by UNICEF and Ministry of Health, Nutrition Department to develop 'Weanimix', a baby food blend made up of maize, cowpea or soy bean and groundnut. Much of these published works have centered on the use of fermented maize as the cereal base-meal in baby food production. However, the use of tubers including sweet potato and yam as carbohydrate and other nutritional sources for infant foods in Ghana and other African countries have also been reported (Nnam, 1999; Amagloh *et al.*, 2012).

Rice flour has been enlisted as part of the list of ingredients in many ready-to-eat baby food products attributable to the desirable properties of this cereal grain. There is however paucity of data on the source of these rice flours suggesting that few of these rice types belong to local rice varieties. Elsewhere in Sudan, Kabeir *et al.* (2004) suggested a formulation that improved the nutritional value of 'medida', a fermented brown rice flour porridge also used as food for weaning infants. Though the cereal-base used in this case was fermented rice, it was also not a local variety. The use of fermented rice is not popular in Ghana and utilizing local rice as a main cereal-base meal in the production of a ready-to-eat infant food is yet to be studied.

2.2 Categories of Baby Foods in Ghana

Baby foods are semi-solid foods given to infants from six months until they attain age two. These foods may be home-made or available commercially in ready-to-eat or ready-to-use formulae. Although these foods may contain a mishmash of various food types sometimes including pureed meat, fish, fruits and vegetables, cereals normally form the base meal though they may at certain times serve as added ingredients (Lartey *et al.*, 1999; Odumodu, 2007). Ready-to-eat baby foods could be classified based on their base-ingredients and these include milk-based infant foods, infant cereals with or without milk, baby biscuits, fruit-based baby food, dairy-based desserts, and mixtures of pureed fruits, vegetables, fish and meat (USFDA, 1994). These baby foods may be liquid or solid in the form of dry powders that could be reconstituted with water or milk to make the food soft or molten in a way that the infant can easily swallow or chew. Ready-to-eat infant cereals that are common on the Ghanaian local market have not been reported to be sourced from the local variety rice market even when rice is enlisted as part of the incorporated ingredients.

2.2.1 Cereal-based baby foods

Cereals are the first source of food locally used for complementary feeding in developing countries. Maize, millet and sorghum are cereals from which 'koko'; a common home-made porridge is prepared for feeding infants in Ghana. Many studies have demonstrated improved nutritional properties of cereal-based infant foods to which non-cereals such as legumes and sweet potato have been added (Lartey *et al.*, 1999; Afoakwah *et al.*, 2010; Saalia *et al.*, 2012). Amankwah *et al*. (2009) investigated maize-soybean blends and the effect of fermentation and malting on their viscosities. Muhimbula *et al.* (2010) conducted a study which involved the formulation of complementary food from a combination of cereals (maize, sorghum and finger millet) and legumes (cow pea and green peas) in Tanzania and evaluated their sensory attributes. Saalia *et al.* (2012) developed and evaluated low viscosity porridge ('koko') prepared by co-fermenting maize flour with millet malt to be used for complementary feeding. Amagloh *et al.* (2012) studied the carbohydrate composition, viscosity, solubility, and sensory acceptance of maize-based and sweet potato-based complementary foods and found that the formulation of the latter showed significant nutritional benefits. Results from these studies have shown that there is a strong inclination to the frequent use of fermented maize in complementary feeding which is perhaps driven by the availability, rheological properties and favourable sensory attributes of this cereal which have been handed down through generations over the years in the sub-region.

Another important cereal that has reported use in infant feeding is rice (Amankwah *et al.*, 2009). Typically, rice flour is produced from rice grains that are broken or very small in size. In recent times, the use of fermented rice for varied products has been investigated (Nicolau *et al.*, 2011).

In the southern part of China, Lu *et al.* (2003) examined the influence of fermentation of rice used to produce noodles and concluded that favorable sensory properties were attained. In most Asian countries, complementary foods are usually rice-based for instance in the Philippines (Perlas & Gibson, 2012). In their study, Perlas & Gibson (2012) used soaking to enhance the bioavailability of iron and zinc in rice-based complementary foods used in the Philippines.

The benefit of using fermented rice is that it retains its nutrients even when used to develop products that go through heat processing such as ready-to-eat food products (Tongnual & Fields, 1979). It is possible that rice is usually incorporated in several complementary cereal mixes for this reason and also because of its hypoallergicity, bland taste and digestibility (Lu *et al.*, 2003).

2.3 Nature and Composition of Rice as a Feeding Grain

2.3.1 Structure of the Rice Grain

The rice grain belongs to the grass family. It is usually harvested as paddy rice. The paddy is either parboiled before milling or the raw paddy is milled directly after harvesting. According to Li (2004) the structure of the rice grain is made up of fundamentally, the husk and the caryopsis (brown rice). The caryopsis is considered as the 'fruit' part of the rice grain and constitutes the bran which consists of the pericarp, aleurone layer and tegumen; the nutrient-rich embryo and the starchy endosperm. The bran constitutes the outer layer of the grain and is rich in fibre. The lemmas and palea are clutching outer coverings that enclose and protect the fruity caryopsis during grain development (Krishnan & Dayanandan, 2003). According to the same authors, the

rachilla, sterile lemma and radicle are related structures that form part of the rice spikelet (Juliano &Goddard, 1986).Figure 1 below shows the structure of the rice grain.

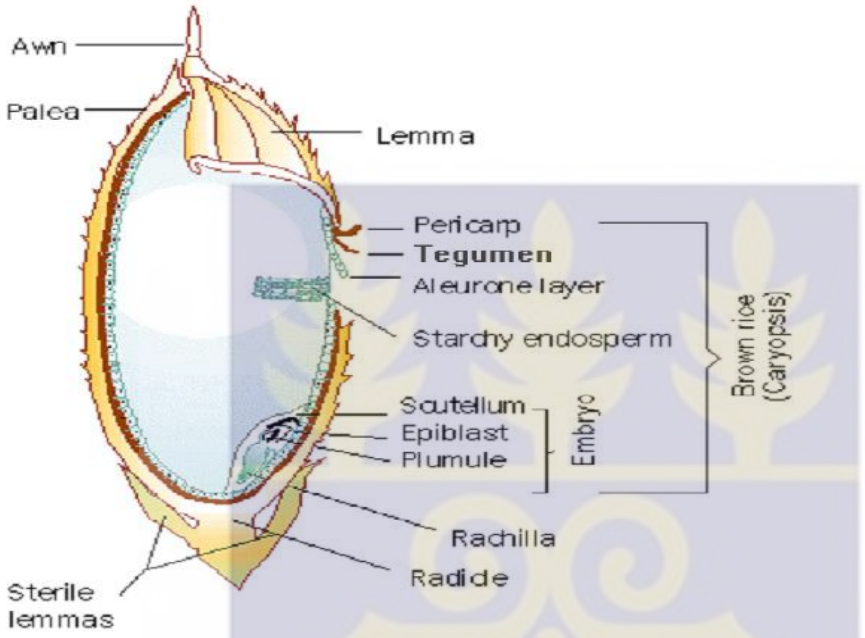

Figure 1 The Structure of the Rice Grain

Source: www.riceweb.org/planthtm. Accessed 27th January, 2015.

2.3.2 Rice Composition

Rice is mostly composed of starch (about 80%), proteins (7%) and little fat (about 1.5 -1.7%) (Juliano & Goddard, 1986). The protein quality of rice is good because it contains all the essential amino acids (Kabeir *et al.*, 2003). It is also rich in minerals such as phosphorus (P), potassium (K), calcium (Ca), sodium (Na), iron (Fe) and zinc (Zn). Rice is also a good source of the B vitamins; thiamin, niacin, riboflavin, cobalamin (Boboye & Daramola, 2011). Most of the nutrients in rice are concentrated in the bran.

2.4 Rice Varieties, Their Production and Applications in Food

Oryza sativa and *Oryza glaberrima* are the two main cultivated species of rice worldwide (ODI, 2003). The *Oryza glaberrima* is native to West Africa (Djedatin *et al.*, 2011) and constitutes the local rice variety. The local rice grain is short, oblong and thick in size. The grains are usually difficult to mill (de-husk); the total head rice obtained after milling or de-husking is low. *Oryza glaberrima* is hardy; it is resistant to drought, pests and diseases (Linares, 2002) and this explains the prehistoric evidence of its original cultivation in African countries such as Ghana, Zimbabwe, Senegal, Mali, Guinea, Liberia, Ivory Coast, Sierra Leone and Nigeria (Djedatin *et al.*, 2011). On the contrary, it has a low yield and longer growth period. Since *Oryza sativa* has some desirable characteristics such as long, thick grain size and higher yield, hybrid varieties have been developed over the years from these two varieties. NERICA 1 and NERICA 2 are among the hybrid varieties that have been developed by AfricaRice and which have successfully been cultivated in Ghana and other African countries (Sie *et al.*, 2012). About seventy local rice varieties have been enlisted in a report by Crop Research Institute (2007) with about 29 varieties cultivated in upland regions and the remaining 41 grown lowland in Ghana. Classification of local varieties has been poor because of inadequate funds and research leading to a high probability of same varieties being called differently by farmers who also cultivate these rice grains in different localities (CRI, 2007).

In Ghana, rice is grown locally by farmers largely in the Northern region as well as the Upper-East and Volta regions. Even though a significant increased yield potential has been observed over the years, a report by Ministry of Health (2009-2011) indicated that the local average yield has been 2.5 tons/hectare/year. This was confirmed by a survey conducted by some rice

institutions: Crops Research Institute (CRI), Savannah Agricultural Research Institute (SARI), and International Food Policy Research Institute (IFPRI), where the achievable yield potential was purged at 6 to 8 tons/hectare after a national average yield of 2.2tons/hectare per season was recorded. Diako *et al.* (2010) established that consumers' preference for imported varieties was largely related to taste and aroma of the cooked rice in urban areas; thus rice growers have increased production of locally improved aromatic varieties such as Togo Marshall, Aromatic Short and Jasmine 85 to compete with those imported (Osei-Asare, 2010). However, the market value of local rice is still low; mostly utilized by rural dwellers and a few health-conscious people in urban areas.

Rice is usually consumed mostly as cooked (boiled) rice. According to Gayin *et al.* (2009), rice is used in varied forms in Ghana; in pureed form for feeding infants or as breakfast meal, in the form of balls served with soup and sometimes as milled into flour. Amankwah *et al.* (2009) in his study on complementary blends for weaning infants in Ghana reported the addition of rice flour as one of the starch-based ingredients. Rice has also been applied to complementary foods in the form of malt (Odumodu, 2007). The rice used in most of these studies is of the variety *Oryza sativa.* Ghana produces a higher quantity of local rice as compared to other West African countries (Assuming-Brempong, 1998). However, poor postharvest handling, lack of storage facilities, insect infestations, high percentage of brokens, high amount of foreign materials (stones, weeds, dead body parts of insects) and poor packaging are among the reasons why local rice varieties are usually branded low grade and undesirable to the Ghanaian consumer. Processing of cereals has been described as a suitable technique used to transform cereals to

enable their applications in more acceptable forms(Fernandez-Artigas *et al.*, 2001). Thus, local rice (*Oryza glaberrima*) which is nutrient dense (Adu-Kwarteng *et al.*, 2003) could easily be manipulated to enhance micronutrients bioavailability, flavour and digestibility and used as complementary food for infants.

2.5 Nutritional Properties of Rice and Its Functionality in Food

2.5.1 Rice Starch

The starch in rice represents the bulk of the nutrients present in the grain. Its granules are small in size (between 2-7µm) shaped like a polyhedron (Juliano, 1985; Lii & Chang, 1991; Vandeputte & Delcour, 2004). Like all starch types, it is made up of amylose and amylopectin. Amylose is made up of linear α-(1-4) linked glucose units (Vandaputte & Delcour, 2004) while amylopectin is a branched polymer with α-(1-4) and α-(1-6) glycosidic linkages (Buleon *et al.*, 1998). Rice may be classified as waxy or non-waxy depending on the ratio of the two starch polymers; amylose and amylopectin. When the amylose content of rice is higher in comparison to its amylopectin rate, it is said to be waxy (0-2% amylose) and non-waxy in the contrary (Juliano *et al.*, 1981). The amylose forms the amorphous portions of starch while amylopectin acts like a crystalline backbone. The ratio of these polymers contributes to starch functionality in food.

When starch is heated in the presence of excess water, the granules swell, increase in size leading to the formation of a viscous solution with the simultaneous leaching of amylose; this process is termed gelatinization (Copeland *et al.*, 2009). A prolonged heating time completely breaks down the configuration of the granule, improves solubility and an eventual reduction in viscosity is

observed. In a recent study comparing pasting properties of local rice with imported rice, Diako *et al.* (2010) proposed that flour from local rice could be utilized as food thickeners. He also observed higher breakdown viscosity values for local rice and recommended their use in marshy or pasty foods. Retrogradation generally occurs after starch cools and forms a crystalline network from the aggregation of one of glucose polymers due to hydrogen bonding (Hoover, 2001). Retrograded starch is attributed to amylose or amylopectin; the latter retrogrades at a faster rate forming double helical linkages involving up to 70 glucose units (Jane & Robyt, 1984). According to Sievert & Wusch (1993), the linear structure of amylose allows a high tendency of molecules to realign themselves and recrystallize. Retrogradation due to amylose (Champagne *et al.*, 1999; Tang & Copeland, 2007) and amylopectin (Lai *et al.*, 2000; Han & Hamaker, 2001) has been well investigated.

The pasting profile of rice could be measured with a Viscoamylograph or a Rapid Visco-analyzer. According to Copeland *et al.* (2009), high peak viscosity values show the susceptibility of starch to increased water absorption. Rice which is high in amylose content will also record high values for setback. Tran *et al.* (2004) declared a high breakdown value in relation to a better tasting cooked rice product. The properties of rice starch for most of the imported cultivars of rice have been well studied (Hsu *et al.*, 2000; Sodhi & Singh, 2003; Noosuk *et al.*, 2005; Singh *et al.*, 2007). In the African sub-region, Falade *et al.* (2014) made a report in literature on the functional and physiochemical properties of improved varieties of African rice. Lawal *et al.* (2011) also isolated rice starches from improved cultivars of rice and characterized them according to their rheological and functional qualities. The cooking behavior of rice starch is useful for rice-starch

based applications. Figure 2 gives an illustration of a simple to a simple viscosity-temperature profile obtained from using Rapid Visco-Analyzer (RVA).

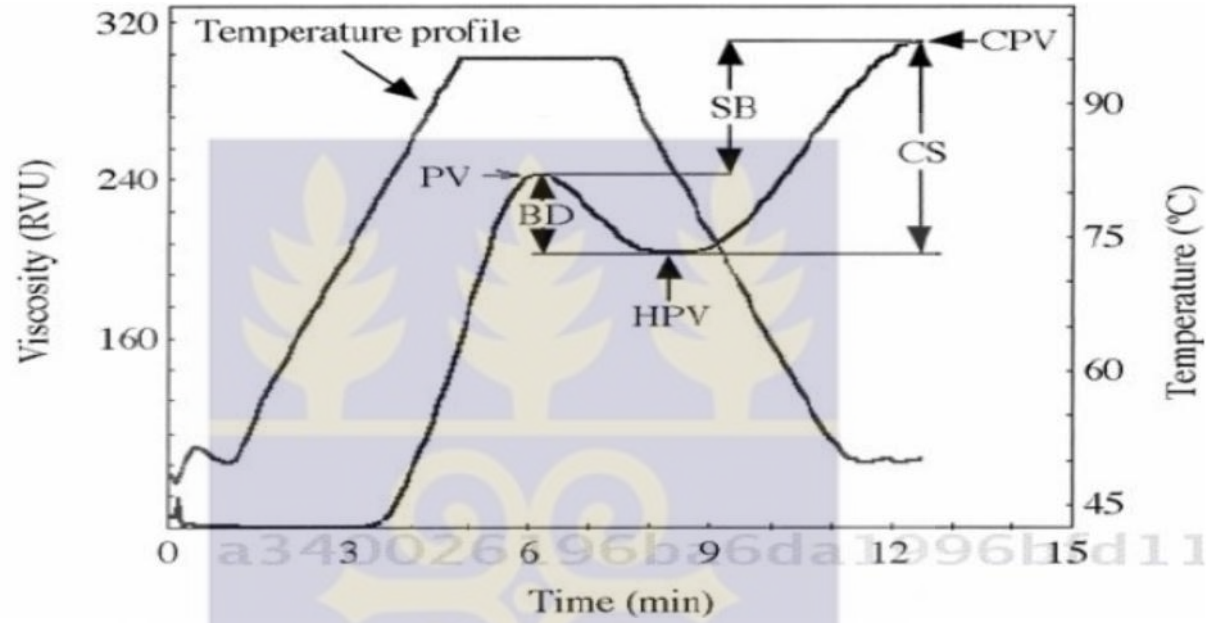

Figure 2a A simple viscosity-temperature profile obtained from using Rapid Visco-Analyzer. PV: peak viscosity; BD: breakdown viscosity; HPV: hot paste viscosity; SB: set back viscosity; CPV: cold paste viscosity; CS: consistency (Ibanez et al., 2007)

2.5.2 Proteins in rice

Proteins present in rice are a small percentage (Singh *et al.*, 2000); nonetheless they contribute to the overall nutritional, cooking and eating quality of rice. Like most cereals, rice proteins include globulin, albumin, prolamin and mostly glutelin (about 80%) (Juliano, 1985; Martin & Fitzgerald, 2002). Comparatively local varieties have higher protein levels per a study conducted by Adu-Kwarteng *et al.* (2003) involving ten local rice varieties in Ghana. Another study carried out in Nigeria by Osaretin & Abosede (2008) revealed a relatively high protein content (>7 %) in a local

variety native to that country known as 'Ofada'. These rice proteins could individually affect rheological and physicochemical properties of rice. Baxter *et al.* (2004) showed that the presence of prolamin influenced water absorption of rice. It has also been documented that the major protein in rice, glutelin, and another starch synthase protein (60kDa) have an influence on the adhesiveness and overall texture of rice starch-based products (Chrastil, 1992; Hamaker & Griffin, 1993). The total protein content values and the amount of extracted water-soluble proteins in local rice varieties significantly correlated positively in a report by Adu-Kwarteng *et al.* (2003).

The variations in rheological behavior of rice attributable to the amount of protein present have been documented. It was reported that when protein content was low in rice flour it results in an increase in the peak viscosity (Tan & Corke, 2002). Rice with low protein content is suitable for cooked rice dishes which require rice grains to be sticky (Okadome, 1999; Lyon *et al.*, 2000).

The protein quality of cereals, in general, is considered poor because of the unavailability of lysine. Many cereal blends are therefore supplemented with legumes and other protein rich foods such as milk and fish powder (Amankwah *et al.*, 2009). Fermentation has also been found to be capable of correcting this deficit in rice (Mugula *et al.*, 2003). Kabeir *et al.* (2004) proposed the addition of skim milk and malting to improve the energy and protein content of 'medida' (fermented rice porridge). However, Ramìrez-Jimẽnez *et al.* (2004) in his study disputed this fact when he found out that the inclusion of milk in infant cereals caused the absorption of water of the product which upon storage led to the loss of lysine. Though previous studies have also shown the loss of lysine in powdered milk stored at about thirty degrees Celsius (El & Kanvas,

1997), the key culprit has been an increase in the water activity of the cereal food with or without milk products (Ramìrez-Jiménez *et al.*, 2004).

2.5.2 Other nutrients in rice

The most common minerals in rice that have been investigated are iron and zinc. This is because their bioavailability has been found to positively correlate with phytate concentration (Graham *et al.*, 1999). The presence of thiamine, riboflavin and niacin in rice has been scrutinized. A study carried out on thirty foreign rice varieties developed at International Rice Research Institute (IRRI) and five pigmented native Philippine rice varieties indicated that pigmented varieties from Philippine recorded higher values for riboflavin but thiamine levels were similar in all the rice varieties studied (Villareal&Juliano,1989). Local rice has been found to record relatively high values for macronutrients. Calcium has been found to be present in some local varieties with significantly higher concentrations ranging from 20.27 to 26.70 mg/100g (Adu-Kwarteng *et al.*, 2003). Also, potassium levels in these same local rice varieties studied were relatively high in the range 80.89–103.88 mg/100 g except for the variety 'Emo kooko' which recorded a value of 20.63mg/100g potassium. Phosphorus values were above 100.32mg/100 g (Adu-Kwarteng *et al.*, 2003).

2.5.3 Starch Digestibility of Rice

Rice starch could be classified according to its resistance to amylolytic enzymes; rapidly/readily digestible starch, slowly digestible starch and resistant starch (Newton *et al.*, 2011). Resistant starch is the type of starch that is not broken down by amylolytic enzymes. According to Sasaki *et*

al. (2009), rice starches containing a higher amylose ratio are less likely to be hydrolyzed by enzymes; thus more resistant starch will be formed. The rapidly or readily digestible starch is hydrolyzed in twenty minutes; slowly digestible starch is hydrolyzed between 20 and 120 minutes and resistant starch is not hydrolyzed within 120 minutes. The glycemic index (GI) is a key factor that determines the starch digestibility of food. According to Roberts & Lui (2009), the glycemic index is the overall glycemic response following the ingestion of about 50g of carbohydrate when compared to the value of glucose or white bread. From research, the lower the amylose content, the higher the glycemic response value (Hu *et al.*, 2004).

Starch digestibility is crucial for infants. A study carried out by Verity & Edwards (1994) found starch in the faeces of children three years and younger. This could be attributed to the presence of surpluses of non-digestible carbohydrates (Brown, 1997) in certain cereal based-complementary foods which cause malabsorption and sometimes diarrhoea in infants (Rowland, 1986). Even though there is documented evidence of benefits relating to the intake of non-digestible carbohydrate in adults in managing certain diseases (Zhang & Hamaker, 2009); in the early stages of life, very high or very low levels of non-digestible carbohydrate may influence the overall well-being of infants over a long period of time (Agett *et al.*,2003). Rice that is sticky will have a high glycemic index; local rice with such property when used to produce rice-based complementary foods would therefore promote digestibility in infants.

2.5.4 Protein Digestibility of Rice

The quality of protein in cereal depends on its digestibility (Hahn *et al*., 1982) because it is a reflection of the amino acid content. When proteins are heated, they denature and are more susceptible to enzymatic breakdown. Hence food substances that have undergone a high temperature cooking treatment are more likely to have highly digestible proteins. In vitro digestibility of proteins could be measured using two key enzymes: pepsin which is involved in gastric digestion and pancreatin which is involved in duodenal digestion (Petitot *et al.*, 2009). A study by Petitot*et al.* (2009) found that increased amounts of wheat proteins in pasta were digested when pepsin digestion preceded pancreatic digestion *in vitro*. It has also been reported that increased protein digestibility have resulted in corresponding increased starch digestibility of some rice products (Mujoo & Ali, 1998).

2.6 Technologies Used in Processing of Rice Paddy

Rice is harvested as paddy and needs to be processed to obtain the edible grains. Paddy rice is usually harvested at a moisture content between 12-14%. Raw milling is the processing technology adopted to remove the husk from the rice grain. Locally, especially in the northern regions, rice is parboiled before milling because the parboiling treatment decreases the amount brokenness of the rice after milling (Ayamdoo *et al*., 2013). His study elaborated the parboiling methods and advocated for the adoption of the improved parboiling method as the most feasible and suitable method in the country.

Parboiling of rice is a common technique widely practiced in Ghana and other parts of the world. Ayamdoo *et al.* (2013) explained that parboiling of rice involves three main processes: soaking, steaming and drying and suggested the improved method of parboiling as ideal and cost effective (Figure 2b). The soaking time ranges from a few hours to about half a day in order to soften the paddy, that is, separate the husk from the caryopsis. This is followed by steaming of the paddy to about 100℃ until gelatinization. The steamed paddy is then allowed to cool and sun dried before milling. The nutritional benefits of parboiling are that nutrients from the bran are able to penetrate the endosperm (Kyritsi *et al.*, 2011) and hence, even after milling there is a reduction in the overall essential nutrients lost. Parboiled rice has a harder texture because it results in the formation of retrograded starch.

Milling or de-husking of rice involves abrasion to de-hull the paddy rice and to remove the bran from de-hulled rice to obtain the white polished rice grains (Li, 2004). After milling, the broken and whole grain kernels are collectively termed as the total head rice produced. Milling of the rice gives the rice a better storing capability. Rice with a good milling degree is rice which has as little remaining bran on the rice grains as possible after milling (IRRI, 2009).

The marketability of rice has a lot to do with the way the paddy was processed. After milling, the total head rice should fall between 75% and 100%, otherwise, the value of the milled rice grains depreciates to about half the original price (Trop Rice International Rice Research Institute, 2004). Correa *et al.* (2007) has argued that attention should be drawn to the operational machinery used during processing of rice paddy. He continued to explain that unitary operations affect the

processing of rice paddy rather than the variety. Either way, a large percentage of brokens are still produced from local rice processing in Ghana.

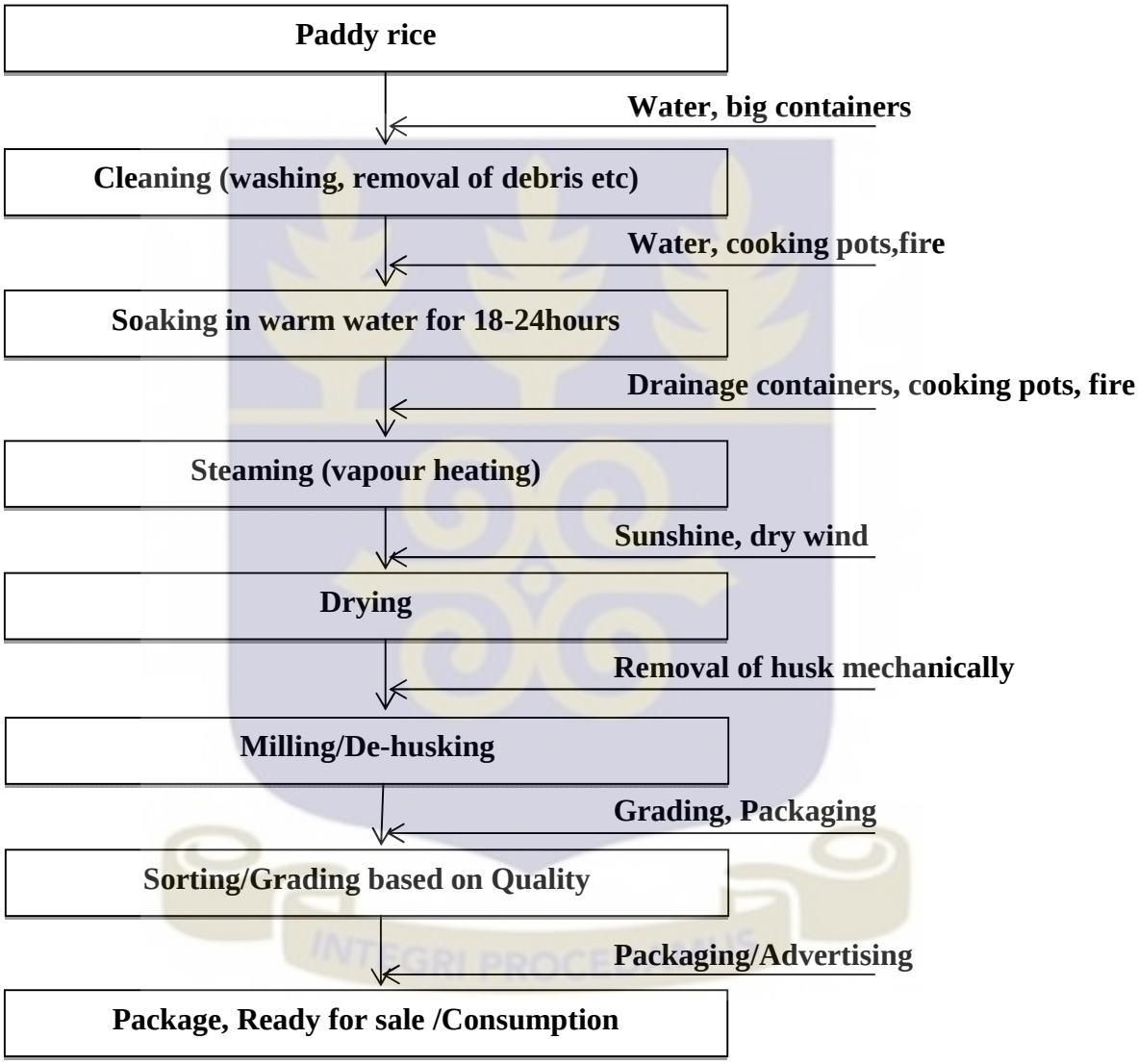

Figure 2b Improved method of parboiling paddy rice in Ghana (Ayamdoo *et al.*, 2013)

2.7 Industrial Technologies used in the Food Industry to Produce Ready-to-Eat Cereal Based Products

The current techniques used in the processing of ready-to-eat food products have emerged to suit modern day busy lifestyles. Extrusion cooking, flaking, milling, toasting and drum drying are all processing technologies that transform the appearance, texture and sensory qualities of cereal-based food products. Traditionally, cereals have been processed using (a combination of) simple technologies such as soaking, sprouting, milling, boiling, roasting or toasting and fermentation (Sefah-Dede*et al.*, 1991; Nout*et al.*, 1993) to produce a variety of ready-to-eat infant food blends, breakfast cereals and snacks (Afoakwah *et al.*, 2004; Amankwah *et al.*, 2009; Saalia *et al.*, 2012). Cereals are considered a dominant food group from which infant food production thrives. A combination of traditional and industrial processes could further improve the application of cereals.

2.7.1 Traditional Processing of Cereal Food Products

2.7.1.1 Fermentation

Fermentation of cereals is a common practice that has been in existence since the primeval era. In Ghana, fermented cereals are the main source of the first class of 'solid foods' that is given to infants between the ages six to twenty-four months; a period in the life of the infant when feeding with only breast milk is insufficient. Fermentation is one of the oldest food technologies which make use of the biochemical activities of microorganisms converting the organic substrate into organic acids or alcohols and carbon dioxide (William & Dennis, 2011). Several studies on fermented weaning blends have focused on maize; where the authors have suggested fortification

with locally available legumes, tubers and other raw materials (Afoakwah *et al.*, 2004, Amagloh *et al.*, 2011). Nonetheless, a few studies carried out in Sudan (Kabeir *et al.*, 2004) and Nigeria (Boboye & Terwase, 2004) have centered on the use of fermented rice-based products.

Natural fermentation of rice has been proven to involve mainly lactic acid bacteria, yeast and moulds (Toyada *et al.*, 1979; Uchimura *et al.*, 1991; Sanni *et al.*, 2002). These microorganisms improve nutritional properties of rice; for instance, protein quality of rice was enhanced by availability of essential amino acids such as lysine through proteolysis and the presence of debris of microbial cellular mass (Adams, 1990; Mugula *et al.*, 2003). In a study carried out by Yousif & ElTinay (2000), natural fermentation of maize dough improved its protein digestibility as well as soluble protein fractions; albumin and globulin. Similarly, starch digestibility was also improved by fermentation through the action of amylase enzyme where starch was broken down to glucose and other sugars (Karovičová, 2007) and was easily assimilated into the blood stream.

The physicochemical properties and the nutritional content of rice-based products are also influenced by fermentation. According to Lu *et al.* (2003), fermentation improved the whiteness of rice flour and decreased its pasting properties such as the gelatinization temperature and the peak viscosity. The rheological properties of noodles prepared from the fermented rice were also improved. There has been a report on the mineral content of complementary cereal-based food in Africa which was enhanced through fermentation (Nicolau *et al.*, 2011) and these findings encourage a similar application to the local rice processing industry in this country.

2.7.1.2 Soaking and Malting of Cereals

Soaking of cereals has been found to improve further processing of cereal into other products. It is a simple treatment that has been used over the years by mothers to prepare baby foods at home. When cereals are soaked in water, the outer covering absorbs moisture, softens it and leaches out antinutritional factors. Perlas & Gibson (2002) studied the effect of soaking on the phytic acid concentration of rice-mug bean-sesame flour and reported an increase in the bioavailability of iron and zinc in the rice-based complementary food. Soaking followed by decanting reduced the quantity of phytate in maize flour by about 57% but this also led to a significant loss of micronutrients (Hotz *et al.*, 2001).

Soaking has been reported to also give some nutritional benefits. When cereals are soaked for a longer period of time before fermentation or malting, it improves mineral bioavailability (Duhan *et al.*, 2002). Brown rice was soaked for more than twenty hours before germinating and the concentration of γ-aminobutyric acid (GABA) was retained even after sterilization (Komatsuzaki *et al.*, 2007). Chiang & Yeh (2002) established that soaking of rice grains led to a loosening fine structure and reduced starch damage when that treatment preceded wet milling of rice into flour.

Malting of rice has also been reported as very useful in the production of crude α-amylases to effect starch hydrolysis (Ayernor & Hammond, 2001) to produce locally fermented cereal-based products. Recent studies have confirmed successful varied use of malted or germinated cereals in addition to cereal blends (Kabeir *et al.*, 2004; Saalia *et al.*, 2012). Shelf stable malts are also commercially available for industrial purposes.

2.7.2 Industrial Processing of Cereal Food Products

2.7.2.1 Drum Drying

One of the processing technologies applied in the food industry to produce ready-to-eat breakfast cereals and baby food is drum drying (Valous *et al.*, 2002). Drum drying is a thermal process which involves the transformation of the main composition of the food product; in this case, the starch structure, and other functional properties. According to Falagas (1985), the drying process could be employed to concentrated food products such as fermented rice dough. Two types of drum-dryers are used in industry; the single and the double drum-dryer with the difference suggested by their names. Daud & Austrong (1987) have reported in a study that involved the use of a single drum-dryer to dry rice flour slurry. The double drum-dryer has been also used to dry fruit purees (Kitson & MacGregor *et al.*, 1982) and sweet corn pulp (Rosenthal & Sgarbieri, 1992). Valous *et al.* (2002) elaborated the drum-drying technology as one that uses high temperature from the surface of steam-heated drums to alter the wet food product by completely gelatinizing and dehydrating the food product to produce drum-dried flakes that are easy to reconstitute with water. In Ghana, the use of drum drying technology to process cereals for babies is used in some industries.

2.7.2.1 Extrusion cooking

In extrusion cooking, raw materials are converted into products through a cooking process at high temperatures for a relatively short time (Zhang *et al.*, 2014). Extrusion allows for a combination of raw materials and modification of their molecular structures to five a final product with transformed rheological properties. The twin screw and the single screw extruders are the two

types available. Extrusion has been widely used for production of breakfast cereals, baby foods and snacks (Meuser & van Lengerich, 1992).

Rice flour as an ingredient in extrusion has been widely investigated (Ilo *et al.,* 1999; Mouquet *et al.*, 2003). Ding *et al.* (2005) studied the effects of feed rate, feed moisture, screw speed, and temperature on the physicochemical and textural properties of an extruded rice snack product with a co-rotating twin-screw extruder. Using extrusion cooking, defatted rice bran was added to develop a ready-to-eat breakfast cereal (Charunuch *et al.,* 2014). Extrusion cooking has also been continuously proven to be a rewarding processing technology in the food industry.

2.8 Product Development Strategies

It is not unheard of that most of the new food products end their life span in research and development. This occurs because 33% to 60% of new products are not able to produce the intended profit margin to sustain the continuous marketing of the products (Schilling & Hill, 1988). In developing a new food product, companies normally used a stepwise approach where after the 'new idea' is identified, a concept is developed, the product is designed followed by the process before the product is finally commercialized. This process was replaced with the partial parallel sequence where the process is designed before the product or alongside the product formulation to enable manufactures input certain necessary requirements after the product leaves the 'laboratory table' onto pilot scale; since a product's manufacturing needs may differ at this point (De Meyer & Hooland, 1990).

The market for baby food is segmented; purchasing is done according to the availability and pricing. Thus there is a need to section out product development strategies in order to accomplish economic gain on local rice-based products. Strategies adopted by industries in the baby food products sector such as Nestle could be benchmarked to help acquire practical approaches in this respect.

2.9 Sensory Evaluation of Developed Products

Because the survival of a developed product on the market depends on the consumer, it is very necessary to ascertain the product's attributes from the consumer's point of view. Sensory evaluation is a scientific technique used to identify different sensory attributes of products which help to categorize them and asses product acceptability.

An appropriate sensory evaluation method that is advantageous in research in providing a comprehensive description of sensory attributes of products is the Quantitative Descriptive Analysis (QDA) (Stone *et al.*, 1974; Stone & Sidel, 2004). The QDA involves the use of a trained panel who are guided by a panel leader through several sensory training sessions where all noticeable attributes of products are listed and defined by the panelists without the interference of the panel leader. The list of descriptors (lexicons) generated in a reproducible manner are then used by the panelist to evaluate the product with the help of an unstructured line scale which is used to measure the intensity of each attribute enlisted (Sveinsdóttir *et al.*, 2010) and the intensity values of the different attributes measured are statistically analyzed with analysis of variance (ANOVA) and Principal Components Analysis (PCA). The QDA could be described as subjective

because it depends on the sensitivity of trained panelists and efficiency of training (Dijksterhuis & Byrne, 2005).

An objective procedure, that is, instrumental electronic sensing method could be used to determine the descriptive sensory profile (particularly aroma and flavour) of products specifically; the Electronic nose and Electronic tongue (Figures 3 and 5). These two instruments operate using electronic sensors. When using the electronic nose (Figure 3) for sensory analysis, the sample is prepared as would be consumed and placed in covered glass vials with a head space. Sampling is then carried out by taking an aliquot of the sample headspace, with a syringe, and injecting it into the detector (Figure 4). Samples with similar odours generally give rise to similar sensor response patterns while samples with different odours show differences in their patterns. The sensor array of the electronic nose PEN2 (Figure 3) is composed of 10 Metal Oxide Semiconductor (MOS) type chemical sensors: W1C (aromatic) W5S (broad range) W3C (aromatic) W6S (hydrogen) W5C (arom-aliph) W1S (broad-methane) W1W (sulphur-organic) W2S (broad-alcohol) W2W (sulph-chlor) W3S (methane-aliph). The sensor response is expressed as resistivity (Ω).

The interaction of volatiles with the array of gas sensors incites a series of signals through interactions between volatiles from the sample or analyte with the array of sensors which are then interpreted by specialized computer software (Figure 4) such as Minitab v. 18 . The human olfactory system has been found to be more sensitive (can detect volatiles at lower parts per trillion levels with a response time) to a wider range of odours than the electronic nose (Barnett, 1999; Haugen, 2001). Nevertheless, the use of electronic nose in sensory evaluation in recent times has been found to be very useful in the food industry (Peris & Escuder-Gilabert,

2009).Electronic tongues are also instruments that mimic taste perception by the human body. It utilizes electrodes connected to the detector which are immersed into the test sample or analyte (Figure 5).An electronic tongue is made up of two sensor arrays that are specific for liquid (Figure 5) and are able to evaluate tastes: sourness, saltiness, bitterness umami and astringency. The array of liquid sensors triggered are then interpreted by a specialized computer software such as MINITAB v. 18 (Escuder-Gilabert&Peris, 2010).The detecting part of the system consists of 7 sensors whose surface is attached with artificial lipid membranes having different response properties to chemical substances on the basis of their taste as reported in Table 1.

It was confirmed in a report by Winquist *et al.* (1998) that the combined use of electronic nose and electronic tongue for descriptive food sensory analysis provides a more extensive description of products analyzed. Overall, descriptive sensory analysis provide a widespread depiction of many food products and can serve as a foundation for differentiating sensory qualities that significantly reflect consumer acceptability of food products (Stone & Sidel 1993).

Figure 3 A portable Electronic Nose (PEN 2)

Figure 4 Operational procedure of the PEN 2 electronic nose showing interactions between array of sensors and volatiles from test sample

Figure 5A Simple Electronic Tongue (Taste-Sensing System SA 402B)

Table1 List and characteristics of electronic tongue detecting sensors.

Attribute	Name of detecting electrodes	Characteristics (Taste information)
Blend Membrane	AAE	Umami taste and umami richness
	CT0	Saltiness
	CA0	Sourness
Positively charged Membrane	C00	Bitterness and acidic bitterness
	AE1	Astringency
Negatively charged Membrane	AC0	Bitterness
	AN0	Bitterness

CHAPTER THREE

MATERIALS AND METHODS

3.1 Rice Samples

About 75 kg of milled rice and 100kg of paddy rice were procured from a local rice farmer near Sogakope in the Volta Region of Ghana. The local rice variety used was *Togo Marshall* belonging to the locally cultivated rice type *Oryza glaberrima*. The milled rice was sorted to remove stones as well as foreign materials and stored in clean airtight large transparent polyethylene bags at room temperature (about 28˚C)on a wooden pallet and the paddy was parboiled as described below.

3.2 Parboiling Procedure

This procedure was carried out as described by Ayamdoo *et al.* (2013). The parboiling process involved three main operations: soaking, steaming and drying. The rice paddy was first washed several times until the waste water was clear in colour. The immature seeds that floated during the washing process were removed using a sieve. The paddy was then soaked in water for about 8 hours and left overnight for 12 hours under standard conditions. Afterwards much of the soak water was drained off and just enough was left at the base of the pot. The pot was covered tightly with a lid to prevent the escape of steam during heating. It was then heated for about two hours and when about a quarter of the rice kernels at the topmost part of the pot began to split, it indicated the completion of the steaming process and so the heat source was removed. The steamed paddy was then thinly spread on polythene sheets to dry in a shaded open environment. The drying process lasted about 3 days (8 hours daily) avoiding direct sunlight. The dried

parboiled paddy was milled and packaged in clean air tight transparent polyethylene and stored at room temperature on a wooden pallet.

3.3 Dough Preparation and Fermentation

Twenty kilograms (20kg) of raw milled rice and same amount of parboiled rice were soaked in excess of de-ionized water (1 part of rice: 2 parts of water) at room temperature (about 28˚C) for 12 hours. Then the steep water was drained off and the rice grains washed twice with de-ionized water and drained. The wet rice grains were then milled into rice grist using a disc attriution mill.

The mean moisture content attained for the rice grist was about 36% and 45% for milled and parboiled rice respectively. Enough de-ionized water was added to prepare rice dough of 45% and 55% for the raw milled and parboiled rice respectively. About 5g of a starch hydrolyzing enzyme (alpha-amylase) was then mixed with the rice dough. The dough was then allowed to undergo spontaneous fermentation for 0, 12, 24 and 48 hours at room temperature (28˚C). At the end of each fermentation time, rice dough samples of both milled and parboiled rice were packaged in clean transparent stomaca bags and stored in a freezer at -20 ˚C. Figure 6 is a flow chart showing the production procedure of the drum-dried fermented rice-based ready-to-eat products.

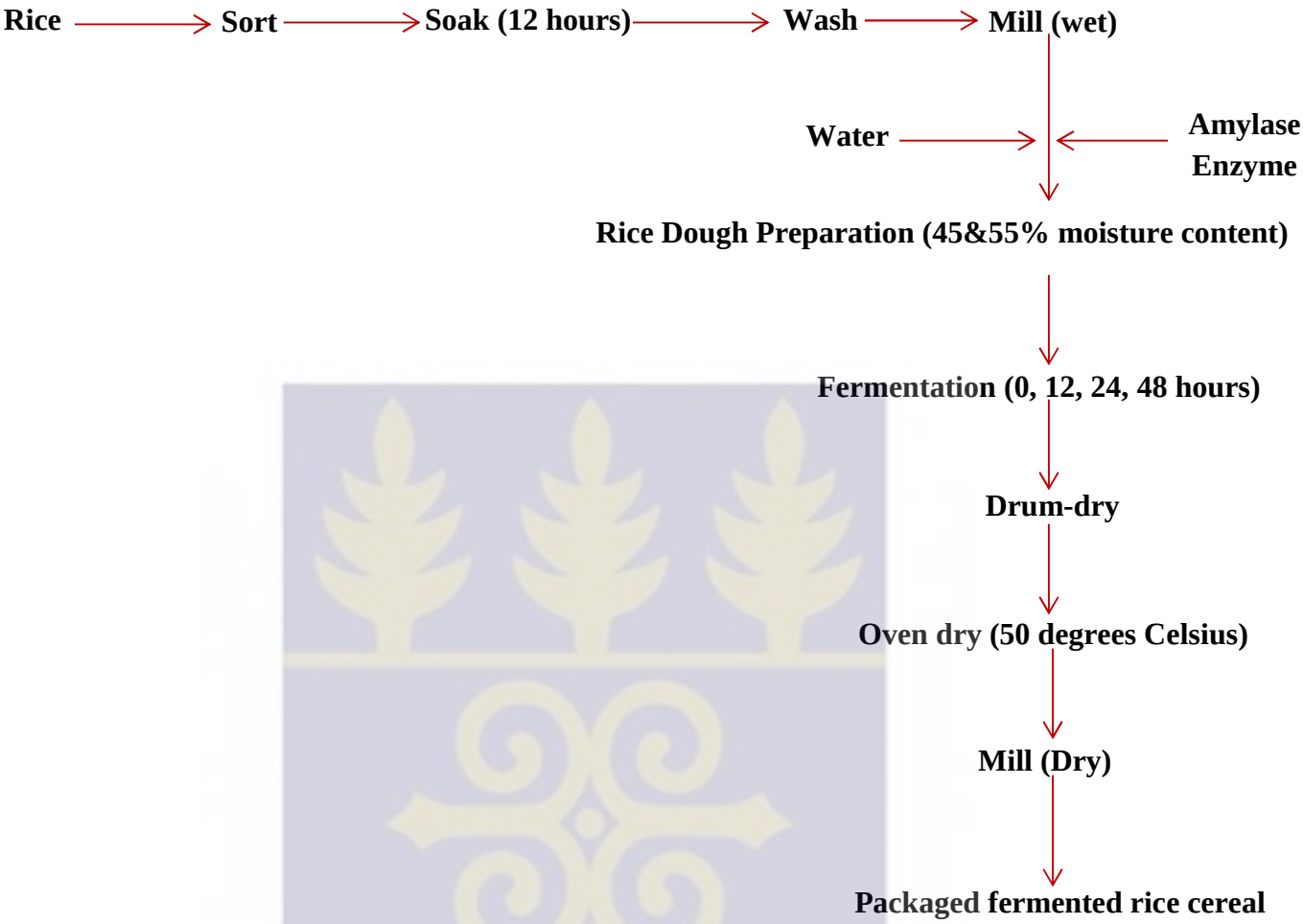

Figure 6 A flow chart showing the preparation of fermented rice-based baby food

3.4 Drum-Drying Process

The double drum dryer was used in this experiment as described by Valous *et al*. (2002). The counter rotating drums were heated with steam at 45psi and rotated at 5rpm. The dough samples were fed unto the rotating drums to form a thin paste on them. The dried flakes obtained as the drums turned were collected and further dried in an air oven preset at 50 $^{\circ}$C for about an hour and half to ensure a well dried product. The oven-dried drum-dried flakes were then milled into a dry

powdered final product using a hammer mill (Size 8 laboratory mill, Christy and Norris Ltd, Chelmsford, England) equipped with sieve size 4mm. The final product were then packaged into air-tight transparent plastic bags and sealed.

3.5 Analytical Procedures

All chemicals used were of analytical grade and obtained from Sigma-Aldrich, St Louis, USA.

3.6 Proximate analysis

3.6.1 Moisture content

Moisture content was determined on the raw rice samples, soaked rice samples, milled dough grist and drum dried products for both milled and parboiled rice. Moisture analysis was performed according to the AOAC Official Standard Method (AOAC, 2000). Measurements were done in duplicates.

3.6.2 Water activity

Water activity values were determined using a Rotronic Hygrometer (Model DT) at 25 ˚C according to Reh *et al.* (2004). Instrument was calibrated before use with reference standards. Measurements were carried out in duplicates.

3.6.3 Protein Solubility and Gel Electrophoresis Pattern

With the characterization of the proteins present in the baby food samples, the amount of protein dissolved was determined in native and denaturing conditions using the Bradford method (Bradford, 1976)while the gel electrophoretic pattern of proteins was determined using SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis).

3.6.3.1 Protein Solubility

This experiment involved three extraction buffers: **Buffer A (Saline Buffer)**; NaCl (100mM) + $NaH_2PO_4.2H_2O$(50mM), **Buffer B (Saline-Urea Buffer)**; NaCl (100mM) + $NaH_2PO_4.2H_2O$(50mM) + Urea (6M) and **Buffer C (Saline-Urea-DTT Buffer)**; NaCl (100mM) + $NaH_2PO_4.2H_2O$(50mM) + Urea (6M) + dithrethiol (10mM).The proteins were extracted by vortexing a solution of about 4ml of each buffer added to each of three samples weighed in duplicates at about 0.120g. The resulting sample solutions were then stirred on a shaker for an hour, then centrifuged at 3000**g* for 30 minutes at room temperature. The supernatant was then collected for both Bradford and SGS-PAGE analysis. The Bradford procedure was used to determine protein concentrations by diluting the extract or supernatant with Buffer A (Iametti *et al.*, 2006) using spectrophotometric procedure with wavelength (λ) set at 595nm (Bradford *et al.*, 1976). Soluble protein content of the supernatant was calculated as mg soluble protein /g baby food sample.

3.6.3.2 Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis Pattern (SDS-PAGE) of extracted proteins

Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis Pattern (SDS-PAGE) was carried out according to Iametti *et al.* (2006). The supernatant collected during the previous step was diluted with 1/1 (v/v) concentrated SDS-PAGE denaturing buffer (0.125 M Tris-HCl, pH 6.8; 50% w/v glycerol, 1.7% w/v SDS; 0.01% w/v Bromophenol Blue) containing 1% β-mercaptoethanol (v/v). The protein denaturing process took place after heating to 100 °C for five minutes. The electrophoresis was performed on a stationary porosity gel (12% monomer, XT Mes 20x, BioRad, Richmond,VA, USA), first at constant of 10 mV per gel for thirty minutes followed by a final increase to 16mV per gel for about two hours. For clearer viewing and identification of bands, all gels were stained overnight with Coomassie Brilliant Blue R250 (Simply Blue Safestain, Invitrogen, Carlsbad, CA, USA) with molecular weight markers ranging from 96 to 14 kDa (BioRad and Novex, Richmond, VA). The gel was then destained by washing several times with a destaining solution) until bands were clear on viewing. The gels were scanned using ScanMaker 4 (Microtek, Carson, CA, USA) at a resolution of 1000 dots *per* inch.

3.7 *In vitro* Protein Digestibility

Protein digestibility was carried out by a two-step proteolysis using pepsin followed by pancreatin as described by Petitot *et al.* (2009) with slight modifications. In vitro protein digestibility tests were carried out by using pepsin and pancreatin. For the *in vitro* pepsin digestion, 1.0g of finely ground sample was weighed into polypropylene test tube. About 10 ml of HCl 0.05 M was then added. Proteins were hydrolyzed by gastric pepsin (porcine stomach mucosa, EC 232-629-3, ref

P7012, Sigma) by adding 30µl of pepsin at a concentration of 2mg/ml. The mixture was then incubated for 60 minutes at 37 °C under mixing conditions. *In vitro* pepsin digestion was terminated by the addition of 10% (final concentration) trichloroacetic acid. Samples were then centrifuged at 13000 *x g* for 15 min, and the hydrolyzed peptide content in the supernatant was measured at 280 nm. After pepsin treatment, sample pH was adjusted to 8.0 with TRIS 1 M, and 0.12 ml (5mg/ml in sodium phosphate buffer) pancreatin from porcine pancreas (EC 232-468-9, ref P1625, Sigma) was added. Pancreatin digestion was stopped by the addition of 10% (final concentration) trichloroacetic acid after 60, 120, and 180 min. Samples were then centrifuged at 13000 *x g* for 15 min and the hydrolyzed peptide content in the supernatant was measured at 280 nm.

3.8 Colour Determination

The colour of fermented rice-based baby food samples were measured using a colorimeter (Model:Minolta CR-300, Japan) where L* is lightness value, a* is redness/greenness value and b* is yellowness/blueness value. The L value gives a measure of the lightness of the product colour from 100 representing pure white to zero representing black, as would be assessed by the human eye. A standard white calibration plate was used to calibrate the instrument. Five replicates were obtained for each sample and the averages were calculated: **Y=94.55; x= .3131 y= .3195**. The total colour difference 'ΔE' was also calculated as follows:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

3.9 Pasting Properties

The Micro-Visco Amylograph, (MVA) (Brabender OHG, Duisburg, Germany) was used in the determination of the pasting characteristics of the fermented rice-based baby food samples according to Mariotti *et al*. (2011). The moisture content of the samples was corrected to 14% and then a slurry was prepared with approximately 11.0g to about 100ml of deionized water. The slurry was first heated to 30°C where the temperature was held for 5 minutes then further increased to 95°C, held for 30 min at that temperature then dropped back to cool at 30°C while holding for another 5 minutes. The parameters determined included the gelatinization temperature; peak viscosity; breakdown; final viscosity and the setback.

3.10 Thiol determination

3.10.1 Accessible Thiols

In this experiment, about 0.150g of samples were measured into sample tubes in pairs as described by Iametti *et al.* (2006) and then about 5ml of Buffer A (NaCl (100mM) + $NaH_2PO_4.2H_2O$(50mM)) was added. Except for the blank, according to Ellman (1959), about 0.2mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) was added to each buffer before adding to the samples. The resulting solutions were then stirred for an hour at room temperature. They were then subjected to centrifugation at 3000*g for half an hour and the absorbance of the supernatant of each sample was measured at a wavelength of 412nm. The results obtained were expressed as mmol accessible thiols/g protein after deductions from total thiols results.

3.10.2 Total Thiols

Here, also about 0.150g of samples were measured into sample tubes in pairs and then about 5ml of Buffer B (NaCl (100mM) + $NaH_2PO_4.2H_2O$(50mM) + Urea (6M)) containing 0.2mM 5,50-dithiobis-(2-nitrobenzoate) (DTNB) was added. For the blank, no DTNB was added to the buffer before adding to the sample. The following steps are similar to the accessible thiols measurements. However, the results obtained were expressed as mmol total thiols/g protein.

3.11 Water Absorption Capacity and Water Solubility Index

Water absorption capacity index (WAI) and water solubility index (WSI) was carried out according to Anderson *et al.* (1969). About 2.5 g of each sample was suspended in 25 ml of deionized water at 30 ˚Cin a previously weighed 50 ml centrifuge tube, stirred vigorously for 30 minutes and then centrifuged at 3000 g for 10 min. Afterwards, the residue was weighed and the Water Absorption capacity was calculated as follows:

WAI (% d.b.) = (weight of sediments /weight of dry solids) χ 100.

The supernatant of each sample was then weighed into a tarred evaporating dish. This was then evaporated until gel formation and oven dried at 130 ˚C for 16 hours. The water soluble index was thus calculated as:

WSI (% d.b.) = (weight of solublesolidsin supernatant/weight of dry solids) χ 100.

3.12 pH and Titratable Acidity

The pH and titratable acidity values were measured using samples suspensions of the fermented dough and the final product, according to Lonner & Preve-Akesson (1988) with slight modifications. About 10 g of each sample was weighed (n=3) and 100 ml of distilled water was added to each sample. The resulting suspensions were stirred using a magnetic stirrer for 30 minutes. After centrifuging at 5000* g for 20 minutes at 18 ˚C, 40 ml of the supernatant was used in the assay. The pH of the supernatant of each sample was then measured by a previously calibrated Crison GPL 22 pH meter.

The titratable acidity was determined by titrating 40 ml of supernatant with 0.5M NaOH until a pH of 8.5 was reached. Values obtained were expressed as the ml of 0.1M NaOH needed to titrate 10g of products.

3.13 Starch Digestibility

Starch digestibility was estimated according to Englyst *et al.* (1992) by determining the glycaemic index, digestible starch (rapidly or readily digestible starch and slowly digestible starch) and free sugars content.

Free glucose was measured by dissolving about 1g of each sample in 2M KOH and bringing to boil at 100 ˚C for 30 min. The solution was then subjected to invertase action (0.2 ml, Boehringer Mannheim) at 37˚Cfor 30 minutes. This was vortexed; 0.5ml was transferred into 10ml of ethanol

and centrifuged. Glucose content of the supernatant was measured using the glucose oxidase-peroxidase kit (Boehringer, Cat Number 166391).

To evaluate the total starch, about 1g of each sample was placed in 50ml centrifuge tubes and an internal standard (5ml arabinose) was added. This was then subjected to pepsin treatment at 37 ^{o}C for 30min. Sodium acetate and an enzyme mixture (pancreatin, amyloglucosidase, invertase) was added with glass beads and placed in a shaking incubator at 37 ˚C. At the 20 and 120 min after hydrolysis was initiated, aliquots of samples were taken from the incubation mixture, centrifuged and 70 µl of supernatant was transferred into vials containing 1ml of water. The glucose released at 20 and 120 min was measured as rapidly available glucose or starch (RAG) and slowly available glucose or starch (SAG) respectively. The remainder sample solution was vortex-mixed and heated to 100 ˚C for 30 min, then cooled to 0 ˚C. 10ml of 7M KOH was then added, vortex-mixed again and placed in a shaking incubator at 0 ˚C for 30 min and vortex-mixed. An aliquot of 0.2 ml was transferred into I ml of 1M acetic acid and 40 µl of amyloglucosidase was added. This was then warmed to 70 ˚C for 30 min and heated to 100 ˚C for another 10 min. Then 12ml of ethanol was added and the resulting solution was centrifuged. About 200 µl of each sample supernatant was transferred into vials containing 1ml of deionized water and vortexed. The total glucose released was then measured by high-performance liquid chromatography (HPLC).

The resistant starch is calculated as the difference between total glucose (TS) and slowly available glucose (SAG). Digestible starch was also calculated as the difference between total starch and resistance starch.

3.14 Sensory Evaluation of Fermented Rice-Based Products

Sensory evaluation of samples was carried out subjectively using quantitative descriptive analysis (QDA) while the electronic nose and electronic tongue gave objective results. For the QDA, eight panelists made up of graduates and post-graduates from the Nutrition and Food Science Department, University of Ghana were used. Electronic nose was used to evaluate the aroma of the final products, while electronic tongue gave the flavour attributes of products.

3.14.1 Electronic Nose Measurements

The electronic nose is an innovative technological procedure that was developed in order to imitate the way the olfactory system of humans operate (Balwin *et al.*, 2011) with the odour/flavor perceived as a global fingerprint. A Portable Electronic Nose (PEN2) from Win Muster Airsense (WMA) Analytics Inc. (Schwerin, Germany) was used in this experiment. The electronic nose had essentially a sample delivery section where the volatiles that were collected in the headspace were injected into the detection system which contained the sensors (in this case, a metal oxide semiconductor) and the array of signals was interpreted on the computer with installed software.

About 0.5g of lyophilized samples was dissolved with 500 µL of water and placed in a 40 mL airtight glass vial fitted with a pierceable Silicon/Teflon disk in the cap. After a 1 hour headspace equilibration at room temperature to stabilize the odours in the food sample and allow the volatiles to be collected in the headspace, the measurement process was carried out. Samples were evaluated in duplicates. The conditions of operation were: flow rate 300mL/min, injection time 60

min, flush time 180 min, during which the surface of the sensors was cleaned with air filtered through active carbon. Sensor responses were recorded for all samples and subsequently multivariate analysis was used to determine the difference between samples statistically.

3.14.2 Electronic Tongue Measurements

Instrumental (objective) sensory analysis was executed using the Electronic Tongue (ET), a taste-sensing System SA 402B (Intelligent Sensor Technology Co. Ltd, Japan). About 15g each of lyophilized samples was weighed into falcon tubes containing 200 mL of deionized water. Solutions were vortexed for about 5 min and centrifuged at 5000*g for 10 min at 20°C. After centrifugation, the supernatants were filtered and analyzed in triplicate. For the preparation of the taste sensors,a total of 5 detecting sensors and 2 reference electrodes were used, separated in two arrays according to membrane charge: hybrid (CT0; CA0;AAE) and positive (C00, AE1). The detecting sensors and reference electrodes were first dipped into the reference solution (30 mM potassium chloride and 0.3mM tartaric acid) and the electric potential measured for each sensor was defined as Vr. Then the sensors were dipped for 30 seconds into the sample solution. For each sensor the measured potential was defined as Vs. For each sensor the “relative value” (Rv) was represented by the difference (Vs-Vr) between the potential of the sample and the reference solution. Sensors were rinsed with fresh reference solution for 6 seconds and then dipped into the reference solution again. The new potential of the reference solution was defined as Vr’. For each sensor, the difference (Vr’-Vr) between the potential of the reference solution before and after sample measurement was the CPA value (Change of Membrane Potential caused by Absorption) (CPAv) and corresponded to the “aftertastes” of the electronic tongue device. Before each new

measurement cycle started, electrodes were rinsed for 90 s with a washing solution and then for 180 seconds with the reference solution.

Each sample was evaluated in duplicate and the means of the sensor outputs were converted to taste information. The "taste values" were calculated by multiplying sensor outputs for appropriate coefficients based on Weber–Fechner law, which gives the intensity of sensation considering the sensor properties for tastes. Specifically, the "taste values" were estimated as:

Sourness = 0.3316 × Rv(CA0)

Saltiness = −0.252 × Rv(CT0)

Bitterness = −0.140 × Rv(C00) + 0.084 × Rv(CT0)

Aftertaste-bitterness = −0.210 × CPAv(C00)

Astringency = 0.1575 × Rv(AE1) + 0.1575 × Rv(CT0)

Aftertaste-astringency = −0.252 × CPAv(AE1)

Umami = -0.1575 ×Rv(AAE)

Richness = -0.420 × CPAv(AAE)

3.14.3 Quantitative Descriptive Analysis (QDA)

The Quantitative Descriptive Analysis (QDA) was used to assess the fermented rice-based baby food products according to Stone & Sidel (2004). A descriptive trained panel (n=8) developed

lexicons that defined each of the eight fermented rice-based baby food samples (milled and parboiled rice fermented at times 0hr, 12hr, 24hr, 48hr) in duplicate using a 15-cm line scale. Inherent product attributes such as appearance, aroma, flavor, texture (Moscowitz, 1995) and after-taste were scrutinized and a final list of lexicons were developed by the descriptive panel with the guidance of the panel leader as a facilitator after seven sessions (two screening sessions and five training sessions) that lasted about two and a half hours per session.

3.14.3.1 Panel Selection and Training

Twenty panelists were recruited from Nutrition and Food Science Department, University of Ghana. Selection was based on interest to participate, good health conditions, time availability and absence of food allergies. Panelists were prescreened on their ability to distinguish basic tastes in solution, detect basic aromas and flavours and also describe basic attributes associated with fermented and baby food products.

Training sessions began with a brief insight into descriptive sensory analysis of food products. Each sensory session lasted up to two and half hours. During this period, panelists were presented with four products which they were required to evaluate individually. Each session was concluded with discussions among panelists where a common list of descriptors and their respective definitions were developed for each attribute. Panelists were also introduced to diverse reference samples that enabled the elimination of redundant descriptors until a final list of clearly defined descriptive terms was drawn up and their intensities rated on a 15cm scale after five training sessions.

3.14.3.2 Product Evaluation

Products were presented in no particular order to the eight panelists in individual booths in a clean well lit sensory laboratory. The panelists evaluated the products in two forms: the powdered form and then in a reconstituted form (as would be consumed by baby) in 1 part of product: 5 parts of water. Samples were presented in 30ml small plastic cups placed in a white tray and each one of the trained panelists performed the evaluation using the developed list of descriptors and their definitions for each sample. The evaluation sessions were repeated twice to increase precision. Panelists were made to rinse their palettes with clean water and unsalted crackers between samples during taste sessions. Intensities of each of the attributes were rated on the 15-cm scale, where 'none'indicated the nonexistence of intensity, and fifteen tallied the most 'extreme' intensity. References were included where necessary.

3.15 Statistical Analysis

All data were analyzed using MINITAB V. 17. Two-way analysis of variance (ANOVA) was performed using the General Linear Model (GLM) test and Tukey's test was used to compare the differences between sample means. Statistical differences were observed as significant at p-value ≤ 0.05.Microsoft Excel was used to plot column and line radial graphs. Quantitative descriptive analysis results of sensory attributes were elaborated with radial graphs also plotted using Microsoft Excel. The Principal Component Analysis (PCA) was performed with correlation matrix on the means of all the sensory data to obtain the main variation in the descriptive sensory profiles of aroma and flavour attributes and represented in scores and loadings plots.

Pearson correlation analysis was performed betweencertain parameters and selected sensory attributes such as colour, aroma and flavour; total colour difference (ΔE), L*; a*; b*;pH and titratable or total acidity respectively. All treatments and analyses were conducted in duplicates and the mean values and standard deviations were stated.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Physicochemical Properties of the Drum-dried Fermented Baby Food

4.1.1 Colour

Colour is an important measure of quality in product development because it plays a crucial role in consumer acceptability. Colour measurements were expressed as CIE L*, a*,b*measurement, where L*, a* and b* represented the lightness (against a white tile scale of 100 and a black tile scale of 0), redness (+)/greenness(-) and yellowness (+)/blueness (-)values respectively. The L* values of both milled and parboiled rice tended to increase with fermentation time indicating that they became lighter in colour (Table 2). The effects of fermentation time and rice treatment showed significant interactions (p=0.00). Even though Onyango *et al*. (2004) made a contrasting observation where fermentation decreased the lightness value, this study agreed well with Lu *et al*. (2005) who reported that fermentation improved the whiteness index of rice-based flours which could be attributed to starch purification during fermentation.

As expected, overall, low redness values (a* values) were recorded for all the fermented rice-based baby food products (Table 2). Parboiled samples declined in redness values as fermentation time increased. Fermentation time and rice treatment interactions were significant ($p \leq 0.05$).

The b* values obtained indicated yellowness in samples (Table 2).There were significant interactions observed with respect to fermentation times and rice treatment type. It was found that there was a gradual increase in yellowness with fermentation time in milled rice samples from

18.90 to 22.87; however a drip to 17.36 was observed after 48 hours of fermentation (Table 2). In contrast, parboiled rice products recorded a decrease from 17.63 to 15.41(Table 2). The differences observed may be due to the presence of available sugars in the milled rice until a period of 48 hours of fermentation by which time most of the sugars were converted into acids indicating effect of acidic environment on colour. A p-value of 0.00 ($p \leq 0.05$) showed that all values recorded for the L*, a*, b* were significantly different with respect to fermentation time and treatment.

The negative **ΔL*** values obtained for all samples (Table 2) except these: 24-hour fermented parboiled product (**ΔL*** =1.52) and 48-hour fermented parboiled product (**ΔL*** =2.10), showed that milled samples were darker than parboiled samples as compared to control samples (raw milled and parboiled rice grains). It was also observed that as fermentation time increased, samples became lighter in colour as compared to raw rice. Positive Δa*values (Table 2) indicated more redness in all samples comparative to raw milled and parboiled rice respectively which could be attributed to fermentation since treatment effects were not significant in this case ($p \geq 0.05$). These values declined with increasing fermentation times except for an observed discrepancy shown in the 24-hour milled product (1.95). Values for Δb* were positive, an indication of observed yellowness in all samples relative to raw rice samples (Table 2b). The Δb* values decreased with increasing fermentation time in parboiled samples while in milled samples there was an increasing trend until the 48-hour fermentation time recorded a fall in value (9.85). This could be ascribed to the presence of increasing available sugars in milled samples until at 48-hour fermentation time when these sugars were converted to acids.

Delta E (ΔE) is the total colour difference taking into consideration the three tristimulus difference of ΔL*, Δ a* and Δb*. The higher the delta E, the greater the colour difference between the product and the raw material. Lower delta E values recorded by parboiled products showed they were similar to the colour of their raw material (lighter) as compared with milled samples. Fermentation time interactions with regard to treatment significantly contributed to the decline in obtaining darker products correspondingly. Discrepancies observed in these samples: the 24-hour fermented milled product (**ΔE**=16.51) and the 24-hour parboiled product (**ΔE**=2.35) may have been caused by processing factors.

Table 2a Estimated means ± standard deviations of the tristimulus colour values. p-value≤0.05

Rice Type	Fermentation time/hr	L*	a*	b*
Milled	0	83.80±0.53^{a}	1.61±0.08^{c}	18.90±0.18^{d}
	12	86.06±0.24^{b}	1.29±0.13^{b}	19.77±0.32^{d}
	24	86.40±0.29^{b}	1.99±0.28^{d}	22.87±0.69^{e}
	48	86.62±0 .42^{c}	0.39±0.17^{a}	17.36±0.92^{a}
Raw rice (milled)	-	92.15±0.26^{e}	0.04±0.03^{a}	7.51±0.15^{f}
Parboiled	0	83.48±0.24^{a}	1.73±0.15^{d}	17.63±0.19^{a}
	12	83.68±0.41^{a}	1.63±0.08^{c}	16.55±0.39^{a}
	24	86.73±0.36^{c}	1.24±0.10^{b}	15.54±0.41^{a}
	48	87.31±0.51^{d}	1.21±0.05^{b}	15.41±0.24^{a}
Raw rice (parboiled)	-	85.21±1.39^{b}	0.29±0.07^{a}	14.01±0.45^{a}

Table 2b Estimated means ± standard deviations of the tristimulus and total colour difference. p-value≤0.05

Rice Type	Fermentation time/hr	ΔL*	Δa*	Δb*	ΔE
Milled	0	-8.35±0.71^{a}	1.57±0.11^{a}	11.39±0.21^{f}	14.21±0.75^{d}
	12	-6.09±1.43^{a}	1.25±0.15^{a}	12.26±0.45^{g}	13.75±1.50^{b}
	24	-5.75±0.29^{a}	1.95±0.28^{a}	15.36±0.69^{h}	16.51±0.80^{e}
	48	-5.53±0.42^{a}	0.35±0.17^{a}	9.85±0.92^{e}	11.31±1.03^{a}
Parboiled	0	-1.73±1.29^{d}	1.44±0.19^{a}	3.62±0.35^{a}	4.27±1.35^{b,c}
	12	-1.53±0.41^{d}	1.34±0.08^{a}	2.54±0.39^{a}	3.26±0.57^{b,c}
	24	1.52±0.36^{e}	0.95±0.10^{a}	1.53±0.41^{a}	2.35±0.55^{a}
	48	2.10±0.51^{f}	0.92±0.051^{a}	1.40±0.24^{a}	2.69±0.57^{a}

4.1.2 pH and Total (or Titratable) Acidity

pH is the logarithmic measure of the concentration of hydrogen (H^+) ions in aqueous solution. Titratable acidity measurements are usually coupled with pH measurements because the former is a better measure of acidity since it gives the total acidity of the products by measuring acidy due to concentration of free H^+ ions as well as the H^+ions bound to the weak or organic acid (in this case, lactic acid). Overall, while the pH decreased, both milled and parboiled samples displayed an escalating pattern in total titratable acidity values in reference to increasing fermentation times showing an inverse relationship between the two parameters. Figure 7 shows the inverse relationship between pH values and titratable acidity measured as percent lactic acid in the baby food products. Parboiled products recorded lower pH values and corresponding higher titratable

acidity values in all samples as fermentation times increased with the highest value of (0.150±0.003) % lactic acid in the 48-hour fermented baby food product. This is because gelatinized starch products are more susceptible to fermentation than non-gelatinized starch products (Singh & Soni, 2001).The drop in pH values as fermentation time increased was more gradual with the milled product than with the parboiled fermented products (Figure 7). In general, decreased pH values and corresponding acidity increases observed is a common property for most fermented products (Blandino *et al.*, 2003). This impedes the growth of food-related pathogens in fermented products and thus renders them safe (Blandino *et al.*, 2003).

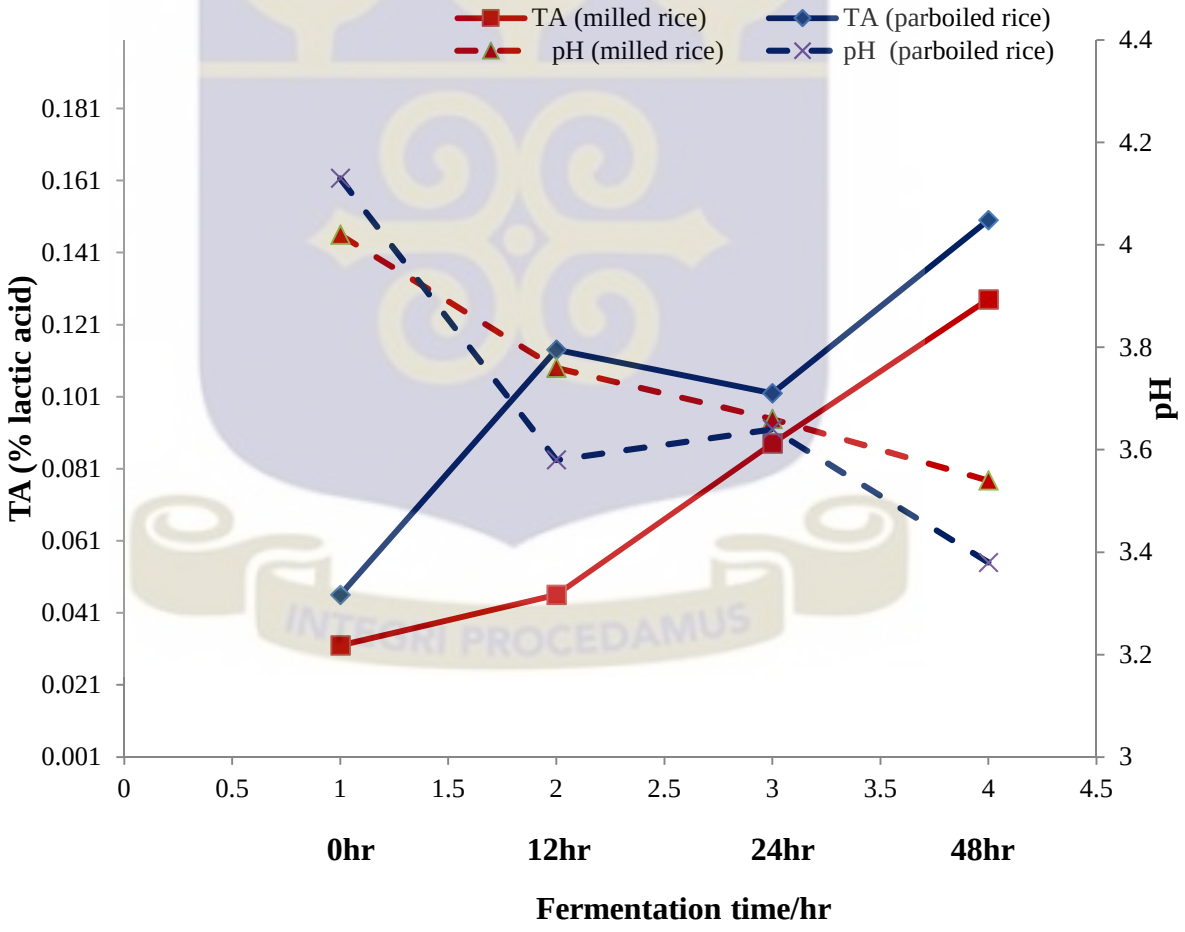

Figure 7 pH and titratable acidity (%TA as lactic acid) of milled and parboiled fermented rice-based baby food products as a function of fermentation ($p \leq 0.05$).

4.1.3 Proximate composition comparing physico-chemical properties of dough with final products

The pH and total titratable acidity of the baby food products significantly varied between treatments and fermentation times for both fermented dough and final products ($p \leq 0.05$) (Table 3). As expected, pH values showed a decreasing trend with increasing fermentation times in all samples (both dough preparations and final products) due to the formation of organic acids during fermentation (Blandino *et al.* 2003). From Table 3, dough samples showed lower pH values with corresponding higher total acidity values. Variations in pH and TA values for dough and final product when compared showed significant difference respectively ($p \leq 0.05$).

Moisture content of dough samples ranged from 41.15 to 42.49% for milled rice and 51.33 to 57.55% for parboiled rice (Table 3). These results are ascribable to the different amounts of water added during dough preparation to the rice grists of the two products; milled and parboiled rice. The drum-drying cooking process significantly (p-value=0.00) reduced the moisture content to (5.88 – 7.84) % and (5.12-11.22) % for milled and parboiled rice respectively (Table 3). Generally, milled rice had lower moisture content than parboiled rice (Table 3). As fermentation increased, the moisture content decreased for parboiled samples; however a vice versa situation occurred for the milled samples, but then differences between the final products for both milled and parboiled samples were statistically insignificant ($p \geq 0.05$).When the moisture content values of dough and final products were compared, significant differences were observed for both milled and parboiled rice ($p \leq 0.05$).

Water activity and moisture content are important preservation indices of dehydrated food products (Al-Muhtaseb *et al.*, 2002). When these two parameters are controlled, they could affect the storage stability of food products. From figures 8a and 8b, it was observed that as fermentation increased, moisture content increased while water activity increased slightly in milled samples. Parboiled samples, however, showed a decreasing trend in moisture content and water activity as fermentation proceeded. Parboiled samples had an initial higher value of water activity which drastically reduced with increasing fermentation time. The trend observed in parboiled samples confirms that as fermentation proceeded sugars were constantly used up by microorganisms. The increases in moisture content and water activity recorded by milled samples showed that glucose concentrations in these samples were higher at 12-hour fermentation in milled rice-based samples (Figures 8a and 8b). As water activity increased, glucose in the food products tended to absorb water from the atmosphere with the application of high temperatures which led to an increase in moisture content (Kapsalis, 1987). Fermentation time and treatment interactions on moisture content were not significant. Conversely, fermentation significantly influenced water activity of the fermented milled and parboiled rice-based baby food products ($p \leq 0.05$).

Table 3 Proximate composition table comparing fermented dough and final baby food products from milled and parboiled rice.

Rice Treatment		**Milled Rice**			
Fermentation time		**0hr**	**12hr**	**24hr**	**48hr**
pH	**Dough**	3.85±0.01^{d}	3.66±0.01^{c}	3.52±0.00^{b}	3.42±0.01^{a}
	Final Product	4.02±0.01^{d}	3.76±0.02^{b}	3.66±0.04^{c}	3.54.±0.04^{a}
TA	**Dough**	0.060±0.005^{a}	0.084±0.005^{a}	0.093±0.005^{a}	0.123±0.010^{b}
(%lactic acid)	**Final Product**	0.032±0.01^{a}	0.048±0.040^{a}	0.088±0.001^{b}	0.128±0.001^{c}
Moisture	**Dough**	42.49±0.01^{a}	41.65±0.01^{a}	41.90±0.21^{a}	41.15±0.74^{a}
Content (%)	**Final Product**	5.88±1.08^{a}	6.71±0.56^{a}	7.84±0.15^{a}	7.12±0.26^{a}
Rice Treatment		**Parboiled Rice**			
Fermentation time		**0hr**	**12hr**	**24hr**	**48hr**
pH	**Dough**	3.88±0.01^{d}	3.62±0.01^{c}	3.49±0.00^{a}	3.41±0.01^{a}
	Final Product	4.13±0.01^{d}	3.58±0.02^{a}	3.64±0.04$^{b,\,c}$	3.38±0.04^{a}
TA	**Dough**	0.135±0.001^{b}	0.168±0.005^{d}	0.198±0.009^{d}	0.228±0.005^{e}
(%lactic acid)	**Final Product**	0.046±0.001^{a}	0.114±0.001^{c}	0.102±0.002^{c}	0.150±0.003^{d}
Moisture	**Dough**	57.55±0.08	53.05±2.79	51.33±1.66	54.34±1.28
Content (%)	**Final Product**	11.22±0.25^{a}	8.72±0.59^{a}	5.12±0.15^{a}	5.22±0.16^{a}

*Values followed by same lower-case letters in the same row are not significantly different (p≥0.05).

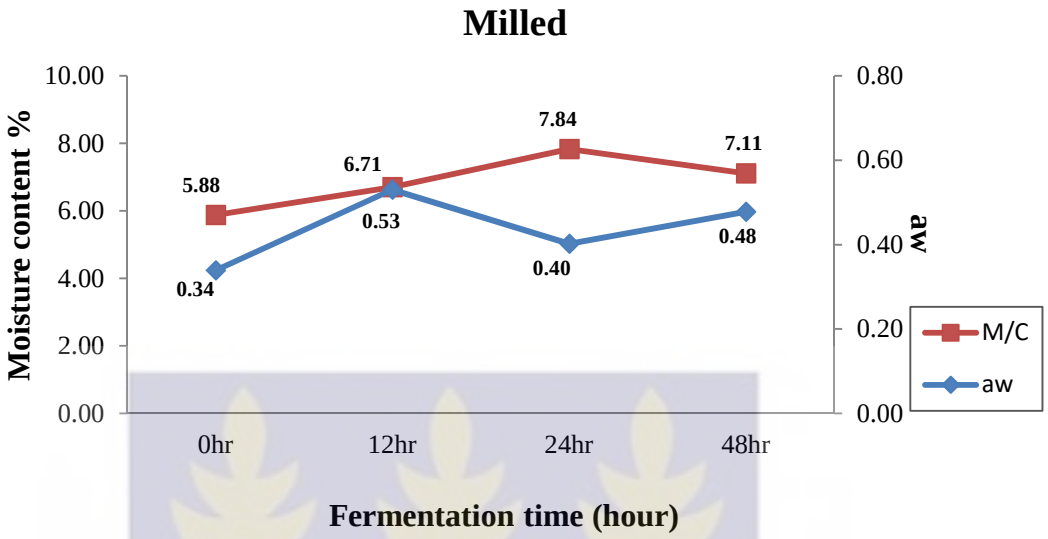

Figure 8a Moisture content and water activity of milled fermented rice-based baby food products as a function of fermentation ($p \leq 0.05$).

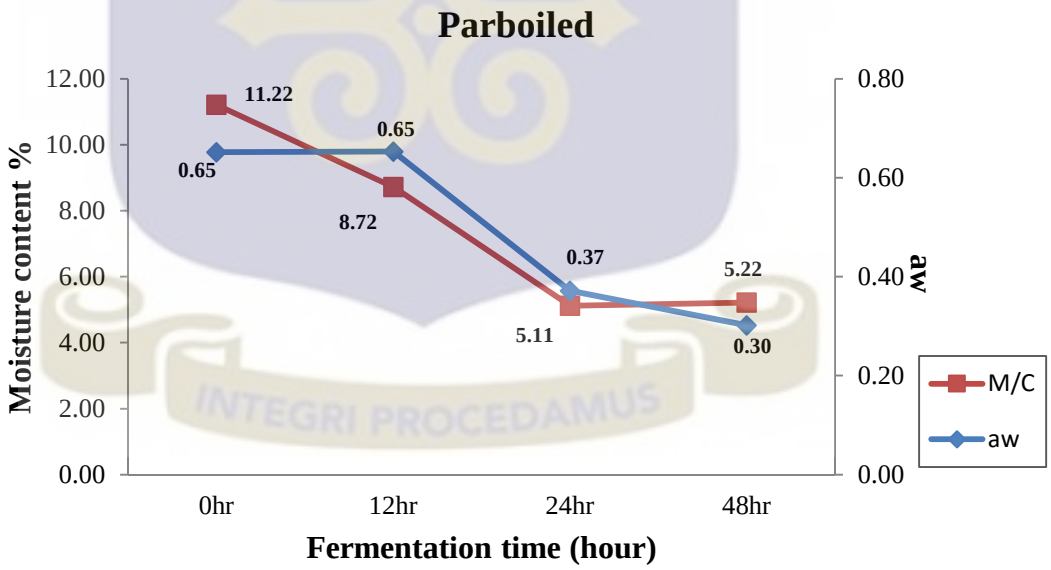

Figure 8b Moisture content and water activity of parboiled fermented rice-based baby food products as a function of fermentation ($p \leq 0.05$).

4.2 Functional Properties of Baby Food Products

4.2.1 Hydration properties:

4.2.1.1 Water Absorption Index (WAI)

A fundamental property of pre-cooked ready-to-eat foods is their rehydration characteristics. Pre-cooked and baby food cereals should have excellent rehydration properties in which large volumes of water are absorbed. There were significant differences observed between values for water absorption index in all fermented parboiled and milled samples. Higher WAI values were obtained for milled samples (Figure 9a) as fermentation time progressed from the 12 hour fermentation period through to the 48th hour. On the other hand, parboiled samples which recorded lower absorption values showed only a slight decrease in values with respect to an increasing fermentation period (Figure 9a). Treatment and fermentation interaction was significant among products ($p \leq 0.05$). Parboiling treatment caused significant differences among samples while fermentation time was statistically insignificant ($p \geq 0.05$).Water absorption index is a measure of the amount of water available for swelling, followed by gelatinization; often related to the behavior of starch granules in the presence of water (Elkhalifa *et al.*, 2006). The lower WAI values recorded for parboiled samples were probably due to the incidence of degraded starch granules which restricted the swelling power of the starch resulting in a decreased capacity for water absorption. The period of fermentation from the 12th hour contributed to a lower resistance to swelling as observed in milled samples (Figure 9a) due to the presence of excess available starch. This suggests that fermented parboiled samples will yield thinner gruels; this is similar to results reported by Elkhalifa *et al.* (2006) in a study based on effect of fermentation on the functional properties of sorghum flour. It is possible that the reduction in the WAI values during fermentation of parboiled samples was as a result of the presence of greater amounts of broken

down starch granules which further reduced the swelling capacity. Parboiling and the subsequent drum drying process contributed to the breakdown of starch molecules. This agrees with a study by Onyango *et al.* (2004) where decreasing WAI values were obtained for extruded fermented maize–finger millet blends used in the production of 'uji'.

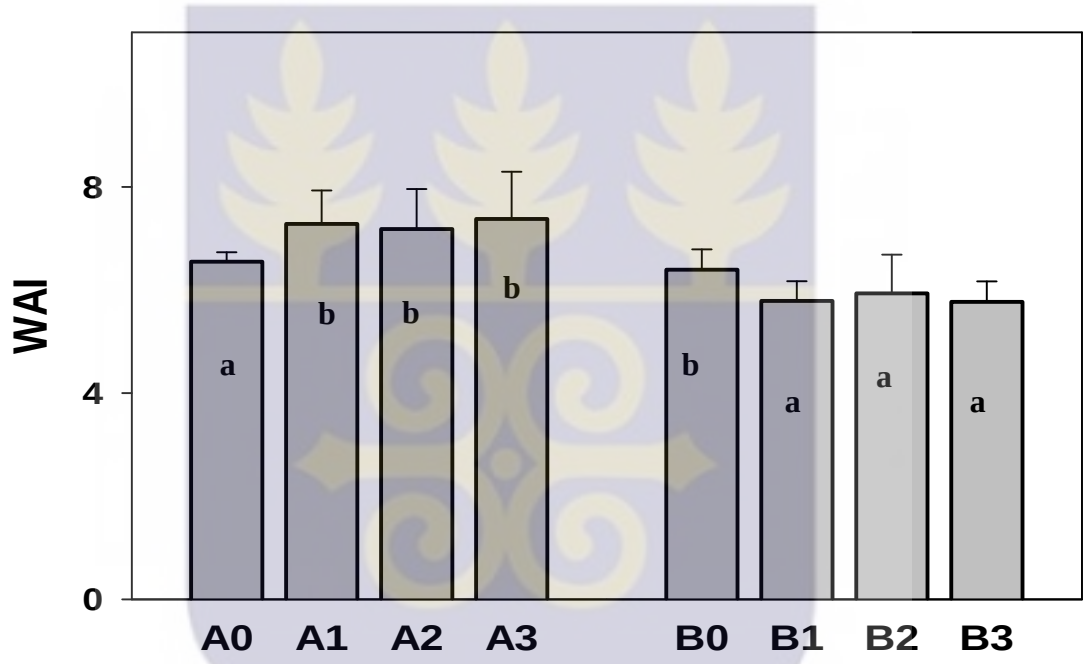

Figure 9a Effect of fermentation time and parboiling treatment on water absorption index (WAI). **A**: milled rice; **B**: parboiled rice. Similar lower-case letters shows differences are insignificant ($p \geq 0.05$).

0-zero hour fermentation
1-12 hour fermentation
2-24 hour fermentation
3-48 hour fermentation

4.2.1.2 Water Solubility Index (WSI)

Water solubility is a measure of the degree of solubility when higher-molecular starch materials are broken down to lower polysaccharide units. Usually, solubility increases after starch degradation has occurred. The water solubility values for milled samples decreased with increasing fermentation time (Figure 9b). The degree of degradation in parboiled samples and a possibly more efficient amylase activity in this case, explained the increasing WSI values recorded (Figure 9b) since the parboiling treatment was significant ($p \leq 0.05$) for all samples. Studies by Kirby *et al.* (1988) and Makowska *et al.* (2013) also accredited the observation of higher WSI results to starch degradation of extrudates but the latter acknowledged the contribution of processing conditions (extrusion). According to Singh *et al.* (2010), foods with high WSI values are easily digestible; a desired parameter of raw material for baby food processing. Fermentation time effects were significant ($p \leq 0.05$) and corresponded negatively with water solubility in the milled samples. This is because as fermentation time increased the fermentable sugars present served as an energy source for microorganisms and therefore was unavailable for water absorption and also there was an excess of starch that had not been broken down. However, Lu *et al.* (2003) found that fermented rice samples produced higher water solubility values in comparison with a control.

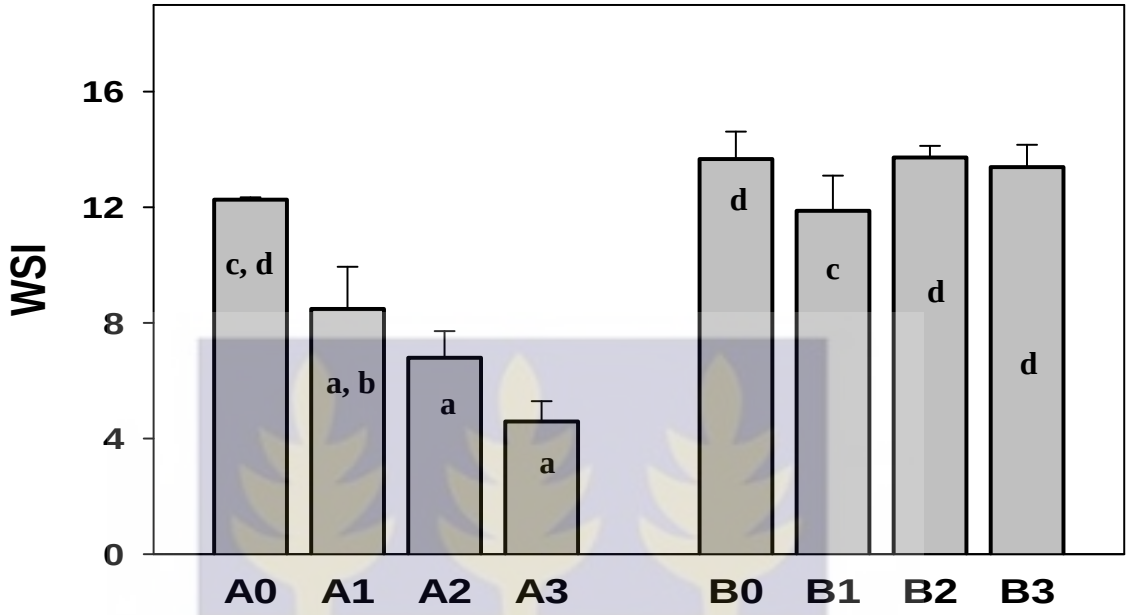

Figure 9b Effect of fermentation time and parboiling treatment on water solubility index**. A**: milled rice; **B**: parboiled rice. Similar lower-case letters shows differences are insignificant (p≥0.05).

0-zero hour fermentation
1-12 hour fermentation
2-24 hour fermentation
3-48 hour fermentation

4.2.2 Pasting Properties

Native starch does not interact much with cold water. But then as the water is heated to a specific temperature characteristic of the starch source, the starch granule swells and absorbs water several times the weight of the starch with a consequent increase in viscosity. This phenomenon is called gelatinization or pasting and the temperature at which this occurs is referred to as the gelatinization or pasting temperature. The starch pasting characteristics obtained for the fermented rice-based samples is shown in (Table 4).

Peak viscosity (PV) is the highest measure of viscidness during gelatinization of starch granules when heat is applied to slurry of a starch-based product. Some studies have suggested that peak viscosity is influenced by the starch source and overall starch concentration (Sanstedt & Abbot, 1961; El-Dash *et al.*, 1983). Peak viscosity values displayed by milled samples (Table 4) were greater in magnitude (184-381 BU) than in parboiled samples (77-98 BU) because of the presence of gelatinized starches in parboiled samples (Table 4). PV values increased with increasing fermentation time in milled samples while in parboiled samples there was a declining trend. The higher peak viscosity values observed in milled samples reflect a higher swelling potential that increased with fermentation time. A similar observation has been hitherto reported by Saalia *et al.* (2012). On the other hand, parboiled samples exhibited low PV values attributable to the presence of gelatinized starch which increased susceptibility to degradation by the alpha amylase enzyme added during processing of the fermented rice-based baby food product.

There was an overall increase in viscosity at 50 degrees hold for thirty minutes (trough viscosity) and final viscosity at 95 ˚C at the end of the heating cycle in all samples with respect to fermentation time (Table 4). Parboiled rice samples as compared with milled samples showed relatively low viscosity at 50 degrees hold (trough viscosity) and final viscosity at 95 ˚C because of the presence of pre-gelatinized starch granules. As fermentation time increased, both the trough and final viscosities of milled samples increased and this agrees well to the study of Saalia *et al.* (2012). Since trough viscosity and final viscosity indicates hot paste stability and paste consistency respectively, the results obtained confirmed a better stability of milled rice starch pastes with increasing fermentation time.

When pasting temperatures are high, there is an increased resistance to swelling (Sandhu & Singh; 2007). Generally, all samples recorded relatively low pasting temperatures (39-45°C) (Table 4). Pasting/gelatinization temperatures were higher for parboiled samples than for milled samples (Table 4) indicating that starch hydrolysis and solute concentration increases occurred at a faster rate in the former as fermentation increased. Samples obtained from the milled rice generally had lower pasting temperatures than those obtained for parboiled rice. For both groups of samples, fermentation increased the pasting temperature (Table 4). Parboiled samples had higher gelatinization temperatures as compared to milled samples because the amylose that leached out during the parboiling treatment recrystallized upon cooling and resulted in the formation of firmer structure. Generally, low gelatinization temperatures observed in all samples was favoured by fermentation and the drum-drying cooking process.

Breakdown values recorded for milled samples were relatively high and increased with fermentation time (Table 4). The breakdown values for parboiled rice samples were generally lower but also increased with fermentation time at lower rates. Breakdown measures the hot paste stability under shear during cooking (Chiang & Yeh, 2002). Since the milled rice starches had higher breakdown values, they may disintegrate more extensively than parboiled rice samples. Similarly, since fermentation resulted in increases in the breakdown values of the starches, fermented rice paste will be more easily disintegrated than non-fermented paste under shear during cooking. The influence of fermentation on the shear stability of cooked paste could be attributed to starch transformation by lactic acid bacteria (LAB).

Setback viscosity (SV) is the degree of synaeresis of cooked starch paste upon cooling. Milled samples recorded higher setback values (Table 4). This means that parboiled samples had a lower tendency to undergo retrogradation. Miles *et al.* (1985) suggested that this could be as a result of accumulation of amylose molecules due to retrogradation. The 24-hour fermented milled rice had the highest setback value of 201 BU. Research carried out by Wang *et al.* (2000) found that lower retrogradation values corresponded with lower pH values in the processing of fermented rice noodle. A similar trend was observed in the fermented baby food samples with discrepancies in the 24-hour fermented milled and parboiled samples.

Table 4 Pasting characteristics of fermented rice-based milled and parboiled products

Samples (fermented baby food)	Pasting/ Gelatini-zation temperature (°C)	Peak viscosity (BU)	Peak Tempera-ture (°C)	Trough viscosity (BU)	Final viscosity (BU)	Breakdown (BU)	Setback (BU)
A0	39.0	184.0	93.5	174.0	293.0	86.0	199.0
A1	39.0	315.0	93.4	292.0	306.0	192.0	185.0
A2	44.0	337.0	92.6	294.0	320.0	221.0	201.0
A3	44.0	381.0	93.5	364.0	301.0	260.0	181.0
B0	43.0	98.0	95.0	67.0	148.0	15.0	87.0
B1	43.0	88.0	94.4	88.0	150.0	22.0	84.0
B2	42.0	96.0	95.0	83.0	154.0	39.0	89.0
B3	45.0	77.0	94.9	76.0	139.0	21.0	84.0

0-zero hour fermentation time; **1**-12hr fermentation time; **2**-24hr fermentation time; **3**-48hr fermentation time; **A**-milled rice and **B**-parboiled rice.

4.2.3 Pasting Profile

The Microviscoamylograph curves (Figures 10 and 11) show the pasting profile of milled rice and parboiled rice respectively. Peak temperatures of all samples ranged between 93˚C and 95˚C. When sample slurries were held at 95˚C for about 30 minutes, initial high peaks (peak viscosity) were observed for milled samples while parboiled samples showed slight increases (Figure 10); however the holding time led to decline in viscosities of milled samples while parboiled samples kept constant viscosities at approximately 90 BU (Figure 11). The decrease in viscosities observed in milled samples was as a result of the breakdown of the molecular framework of starch and probably leaching of amylose during the holding time. This is an indication of hot paste stability of parboiled samples; comparatively, milled products are therefore more likely to give thinner pastes when boiling water is used for reconstitution. Lower viscosity increases were also observed for parboiled samples during the cooling period showing lower susceptibility to retrogradation.

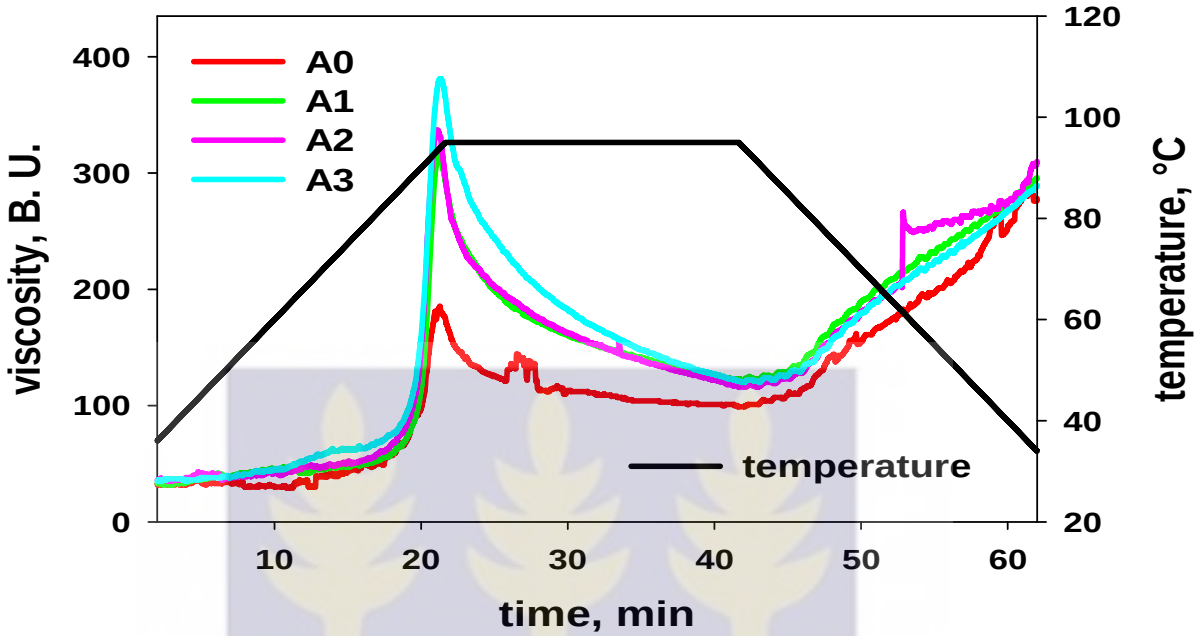

Figure 10 Microviscoamylograph showing pasting profile of fermented milled rice-based product. 0-zero hour fermentation time; 1-12hr fermentation time; 2-24hr fermentation time; 3-48 hr fermentation time; b-parboiled rice.

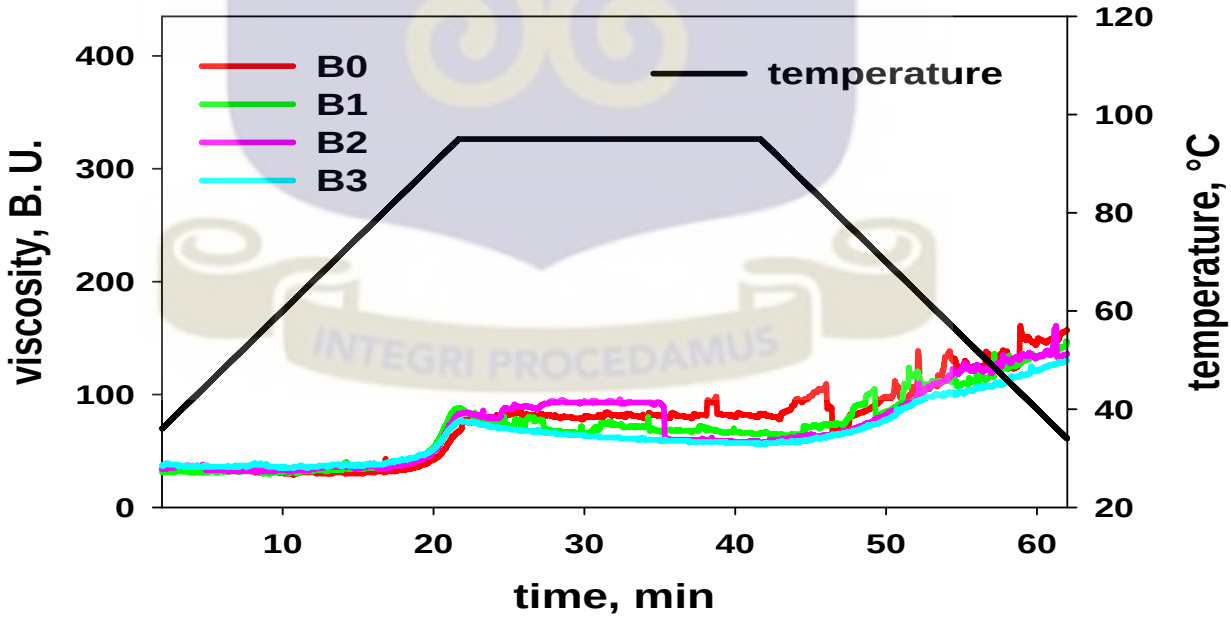

Figure 11 Microviscoamylograph showing pasting profile of fermented parboiled rice-based products. 0-zero hour fermentation time; 1-12hr fermentation time; 2-24hr fermentation time; 3-48hr fermentation time; B-parboiled rice.

Protein Functionality of the Drum-Dried Fermented Rice-Based Baby Food Products

4.3.1 Protein Solubility

Rice proteins include: albumins, globulins, prolamins and glutelins (Juliano, 1994). Because these proteins are soluble in different solvents, their solubilities in diverse buffer systems can be measured (Iametti *et al.*, 2006). Table 5 shows the solubility of proteins from the drum-dried products in different buffer systems. Generally, in all the three buffer systems, protein solubility increased with fermentation time until twelve hours. Thereafter it decreased through to 48 hours fermentation time. Milled rice proteins were more soluble than those in parboiled rice probably because of heat denaturation of the proteins in parboiled rice. The observed decrease in solubility after 12 hours corresponded to decreases in the pH of the medium suggesting that fermentation produced acids that precipitated proteins out of solution. In the saline buffer, some proteins dissolved at zero fermentation time (Figures 12 and 13).These are most probably proteins that are soluble in neutral salt conditions, such as globulins (deMan, 1999). From Figures 12 and 13, it was observed that solubility of proteins in saline buffer was very low in milled samples and even much lower in the case of parboiled samples. This trend was observed in all three buffer systems (Figures 12 and 13). Urea, a denaturing solvent, was added in order to solubilize protein aggregates. In the urea-saline buffer, it was observed that rice proteins turned out to be about three times more soluble for milled rice and about five times more soluble for parboiled rice (Table 5). This was because the urea-saline buffer was able to disrupt the structured protein network in all products stabilized by hydrophobic interactions. This is comparable to a study by Cabrera-Chávez *et al.* (2012) involving amaranth-enriched, gluten-free rice pasta where similarly, the addition of urea increased the protein solubility. The third buffer which contained dithrethiol (DTT) in addition to urea and salts gave the highest protein solubility values in all products

(Table 5, Figures 12 and 13). Essentially, in certain cereals such as corn and rice, proteins present are rich in sulfurous amino acids namely, methionine and cysteine (deMan 1999, Fennema, 1996). Consequently, DTT cleaved the disulphide bonds in the rice proteins and converted them into sulfhydryl groups, removing the last traces of the quaternary or tertiary protein structure of the proteins. Formation of disulphide bonds between the peptides and the extraction medium instigated the protein solubility increases. This agrees with a study by Mariotti *et al.* (2011).

In all three buffers, a decrease in protein solubilities was observed at fermentation times 24 and 48 hours respectively (Table 5, Figures 12 and 13). This means that after twelve hours of fermentation, proteins precipitated out of solution due to increasing acidity as a result of fermentation. It is possible that the isoelectric point of the rice proteins present was close to pH after 12hours of fermentation (pH~3.6). The isoelectric point (pI) of rice proteins is reported to be approximately pH 4 (Nehete *et al.*, 2013). When DTT was added, however, the protein solubility increases observed confirmed that interprotein disulphide bonds were formed in addition to hydrophobic interprotein interactions in samples that underwent parboiling treatment. Barbiroli *et al.* (2013) obtained similar results in his study involving the use of parboiled rice flour and untreated rice flour as raw materials for making pasta. Fermentation and treatment effects were significant for all buffer systems except for the Buffer C system which was not significantly affected by parboiling treatment ($p \geq 0.05$).

Table 5 Protein solubility of drum-dried fermented rice-based baby food samples.

Rice Type	Fermentation time	Saline Buffer	+Urea	+Urea/DTT
Milled Rice	0	1.9±0.3^{b}	6.5±0.3^{a,b}	27.7±1.0^{b}
	12	6.2±0.5^{d}	16.2±1.0^{d}	64.6±3.5^{d}
	24	5.0±0.2^{c}	11.0±0.5^{c}	29.1±21.8^{c}
	48	4.3±1.1^{c}	9.9±1.6^{b}	16.0±3.5^{a}
Parboiled	0	0.8±0.1^{a}	4.1±0.2^{a}	24.4±1.0^{b,c}
Rice	12	1.6±0.1^{a}	8.3±0.3^{b}	60.4±1.8^{d}
	24	0.6±0.1^{a}	4.6±0.3^{a}	19.0±0.6^{a}
	48	0.8±0.1^{a}	5.0±0.1^{a}	10.9±1.8^{a}

*Values with same lower case letters are not significantly different ($p \geq 0.05$)

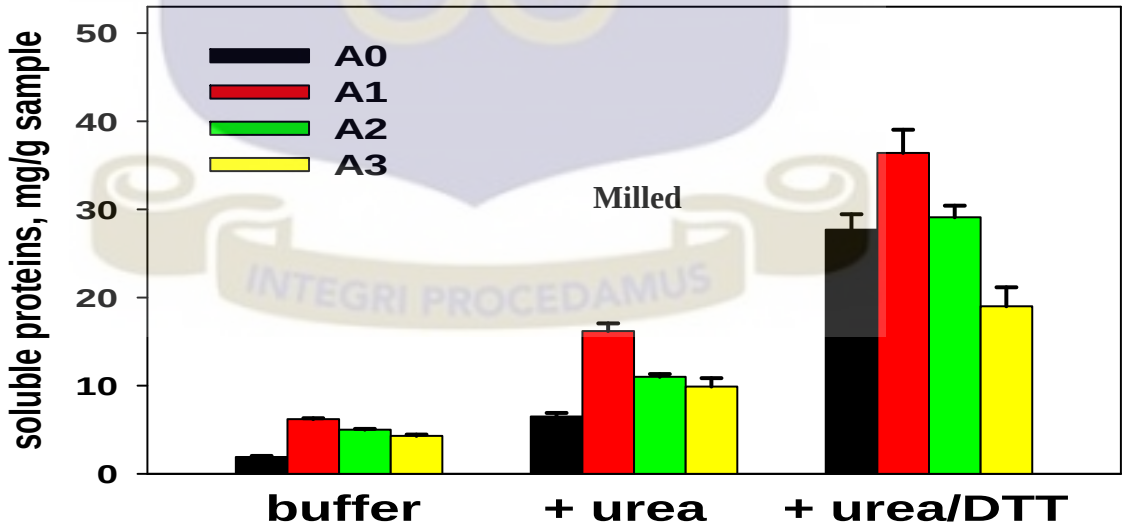

Figure 12 Protein solubility in different buffer systems for drum-dried milled fermented rice samples. Values with same lower case letters are not significantly different ($p \geq 0.05$).

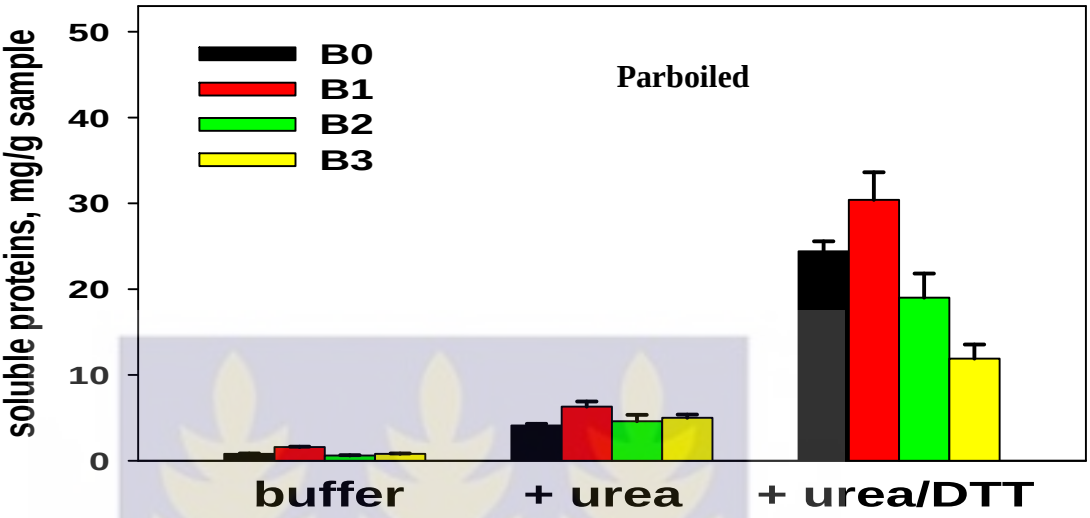

Figure 13 Protein solubility in different buffer systems for drum-dried parboiled fermented rice samples. Values with same lower case letters are not significantly different ($p \geq 0.05$).

4.3.2 Thiol Accessibility Studies

Thiol accessibility was carried out to find out the total amount of reactive protein thiols (measured under denaturing conditions on both the soluble and insoluble fraction) and the change in thiol accessibility owing to denaturation. This experiment was carried out to ascertain the extent of the effect of processing procedures on the proteins in cereal-based products. Similar experiments have been reported by Cabrera-Chávez *et al.* (2012). Elkhalifa *et al.* (2006) and Mariotti *et al.* (2011). As illustrated in Figures 14 and 15, parboiled rice-based products showed a slightly higher amount of accessible thiols than milled rice samples both in the presence and absence of the chaotrope (urea). However, Barbiroli *et al.* (2013) observed lower amounts of accessible thiols both in the absence and presence of urea in pasta products made with parboiled rice flour. The variation in the study results could be attributed to the role of fermentation time. During

fermentation, the proteins in the milled and parboiled rice-based samples were subjected to hydrolysis by the microbial proteases apart from the alpha amylase enzyme that was added in the formulation. Even though parboiling treatment denatured proteins, because this rice-type was more susceptible to hydrolysis, increasing fermentation time provided an acidic environment with the release of more H^+ (hydrogen) ions and thus more –SH bonds were formed and this explains accessible thiol increases in parboiled samples which increased slightly on the addition of urea.

In the case of milled samples, there was a decline in the amount of accessible thiols after 12 hours of fermentation in the presence and absence of urea (Figure 16). The lower pH environment created after twelve hours of fermentation may have caused rice proteins to precipitate out of solution indicating that the pH of samples at fermentation times 24hr and 48hr are close to the isoelectric point of some of the rice proteins. Fermentation time and parboiling interactions were significant (p-value≤0.05) for the saline buffer. The high-temperature processing technique, that is, drum-drying, in addition to parboiling treatment did not create a rigid structure of protein aggregates otherwise expected demonstrating that over here the fermentation process played a very significant role irrespective of heat denaturation of proteins (p≤0.05). Slight increases in accessible thiols due to addition of urea, a denaturing agent was also observed.

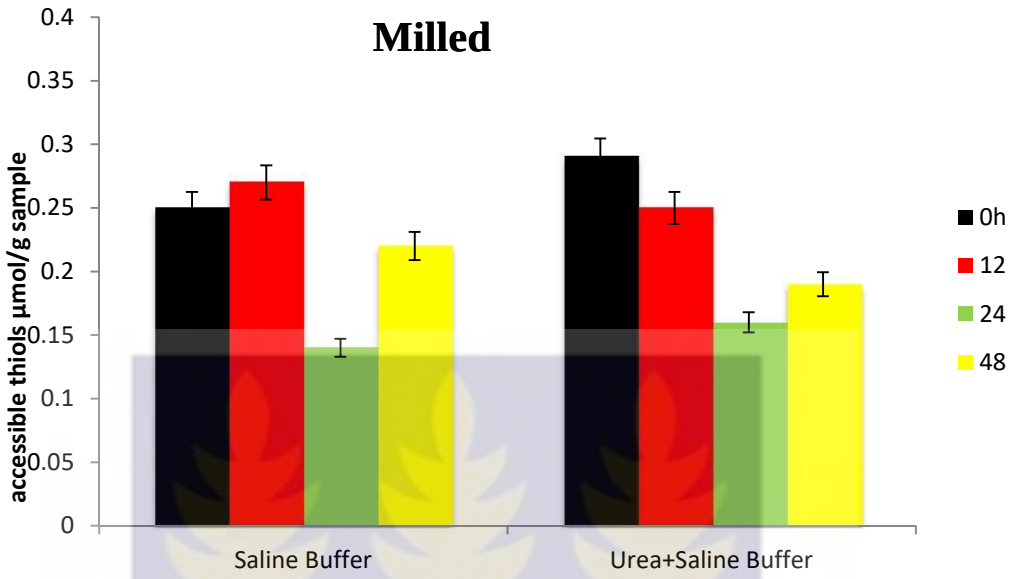

Figure 14 Accessible thiols in proteins of fermented milled rice-based baby food products.

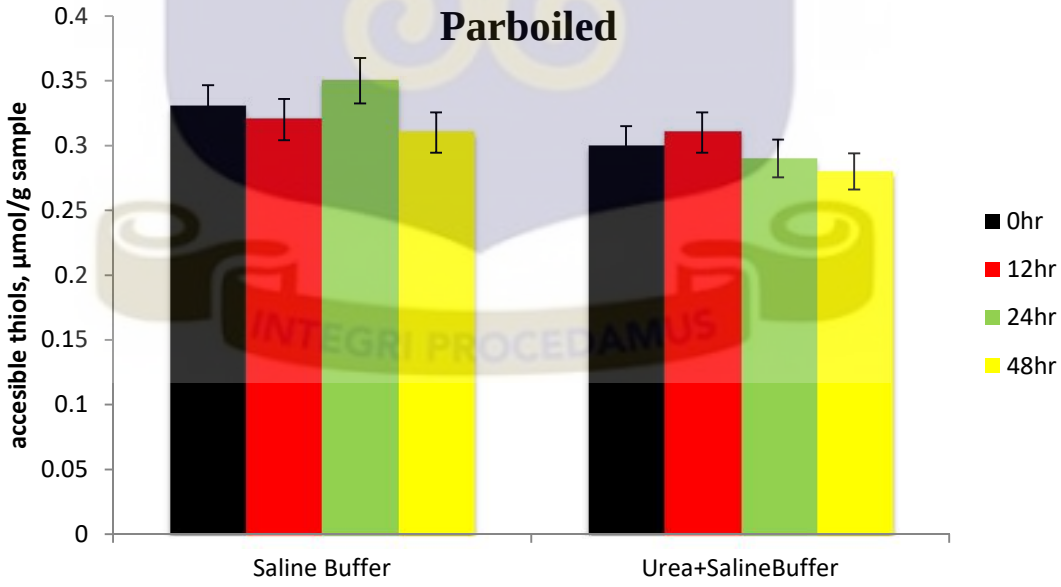

Figure 15 Accessible thiols in proteins of fermented parboiled rice-based baby food products.

4.3.3Sodium Dodecyl Sulfate Polyacrylamide-Gel Electrophoresis (SDS-PAGE) Patterns of Rice Proteins

The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) patterns of proteins obtained from the rice-based products were compared with bands that corresponded to standard molecular weights of proteins. SDS-PAGE investigated the formation of disulfide-linked soluble rice protein aggregates and provided information on the molecular characterization of specific protein fractions in different solvent extracts of the fermented rice samples in the presence of 2-mercaptoethanol. There was a general observation of three bands corresponding to proteins of molecular weights 14kDa, 30kDa and 45kDa respectively in both milled and parboiled rice samples (Figure 16 and 17). It was observed that most of the proteins in milled samples were hydrolyzed by the 12th hour of fermentation. Parboiled rice proteins took a longer fermentation time to hydrolyze because of the formation of more aggregates in this sample (Figure 17). Predictably, the presence of a chaotrope (urea) and a reducing agent resulted in the formation of aggregates which diminished with increasing fermentation time. In all samples, proteins at lower molecular weights (<45kDa) were made visible after disulphide-linkage protein aggregation in Buffer C (i.e. sodium phosphate-saline buffer with urea and dithrethiol), (Figures 16 and 17) confirming the presence of globulins (M~12-45kDa) (Sulieman *et al.*, 2008). It was also noticed that similar proteins were solubilized from all samples irrespective of the rice treatment (parboiled or non-parboiled) (Figures 16 and 17). A low molecular protein (M~14kDa) band was seen to constantly appear in saline-urea buffer (Buffer B) for milled samples (Figure 16) but was however not seen in parboiled samples (Figure 17). This could be an indication of the presence of basic proteins such as glutelins (deMan, 1999). Presumably, the parboiling treatment produced more digested proteins which were apparently involved in disulphide bonds as seen in Buffer C

(sodium phosphate-saline buffer, urea and dithrethiol) .Similarly, Mariotti *et al.* (2011) explained that urea-soluble proteins extracted from gluten-free rice and maize pasta take part in forming disulfide-linked aggregates. For the milled samples, the clear appearance of the three protein bands at 14kDa, 30kDa and 45kDa respectively in Buffer C at 48hour fermentation time (Figure17) was also as a result of more disulphide bridges due to the proliferation of sulfhydryl residues in the increasing acidic environment during fermentation. As illustrated in Figure 17, parboiled samples, showed clearly visible protein bands in buffer C (sodium phosphate-saline buffer, urea and dithrethiol). Because parboiled samples were more susceptible to digestion by enzymes, most of proteins were digested. As fermentation proceeded, increasing acidic environment also aided denaturation and formation of aggregates with corresponding disulphide linkages. By 48hours fermentation time, most of the proteins had been broken down to polypeptide units with very low molecular weights that could not be retained by the gel.

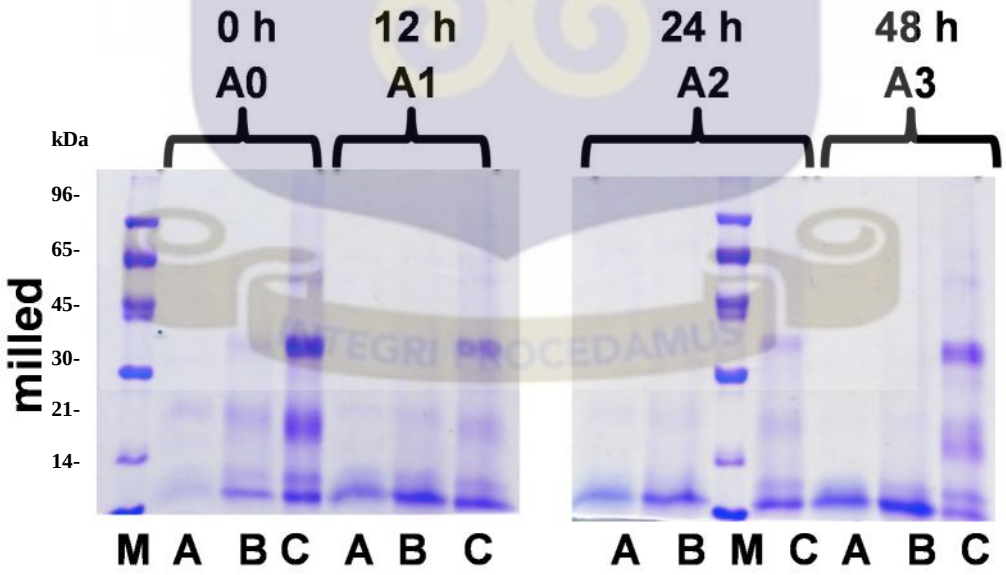

Figure 16 SDS-PAGE patterns of proteins in milled fermented rice-based baby food products. M: Marker. A: sodium phosphate-saline buffer, B: sodium phosphate-saline buffer and urea, C: sodium phosphate-saline buffer, urea and dithrethiol. Denaturation of samples were carried out in the presence of 1% (v/v) 2-mercaptoethanol.

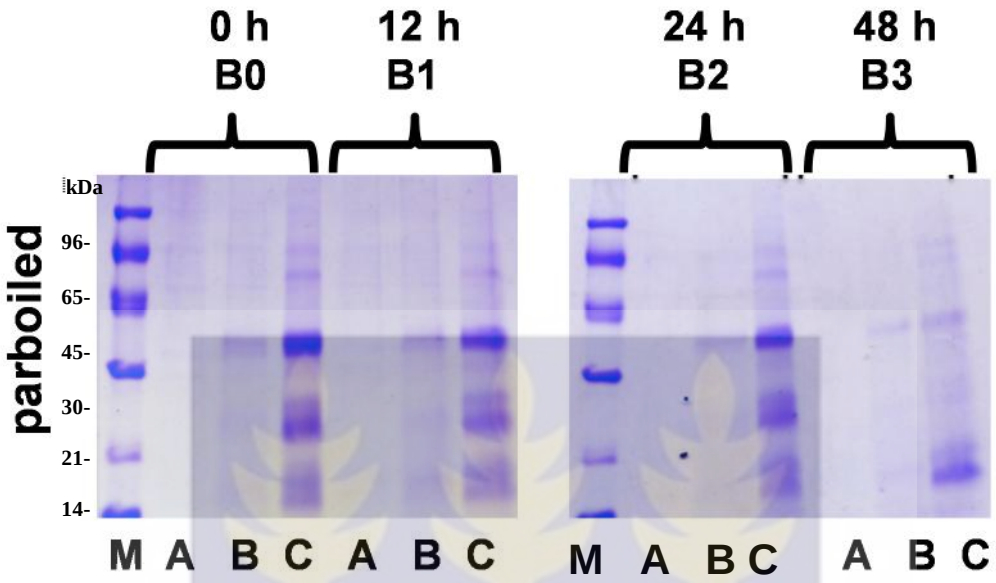

Figure 17 SDS-PAGE patterns of proteins in parboiled fermented rice-based baby food products. M: Marker; A: sodium phosphate-saline buffer, B: sodium phosphate-saline buffer and urea, C: sodium phosphate-saline buffer, urea and dithrethiol. Denaturation of samples were carried out in the presence of 1% (v/v) 2-mercaptoethanol.

4.4 *In vitro* Digestibility Studies

4.4.1 Protein Digestibility by pepsin and pancreatin

In vitro protein digestibility is a more convenient method to ascertain the *in vivo* digestibility of protein because recruitment of human subjects is not required and its measurement involves a relatively lesser period of time. Protein digestibility defines protein quality for the reason that it gives information on the amino acid availability profile (Hahn *et al*., 1982). Protein digestion

begins in the stomach where the enzyme pepsin which requires a low pH for activity plays a major role.

This experiment began within initial enzyme (pepsin) activity for an hour. After 60 minutes of pepsin activity, slight digestion of proteins was observed (Figures 18 and 19) but there were no statistical differences between all samples. Protein digestibility by pepsin was significantly slightly higher in milled than in parboiled samples with respect to significant increases in fermentation time ($p \leq 0.05$). Pancreatin was introduced after 60 minutes and there was a remarkable increase in rice protein hydrolysis. This agrees with a study carried out by Petitot *et al.* (2009) on wheat protein from dry pasta. Under the same conditions, fermentation showed significant influence on the hydrolysis of proteins by both pepsin and pancreatin. Increasing fermentation time for both milled and parboiled samples showed increasing susceptibility to protease hydrolysis of the rice proteins. On the whole, the 48 hour fermented rice samples showed higher protein digestibilities for all samples followed by the 24-hour fermentation period. Fermentation time significantly affected the protein digestibility in both milled and parboiled rice. Though the 12 hour fermented samples recorded the least protein digestibility values, it did not significantly differ from the 0-hour fermented samples ($p \geq 0.05$) at the initial stages of pancreatic activity (90 minutes). The highest peak of protein digestibility was recorded at time 180 minutes after pancreatic digestion where there were significant differences among samples except between the zero and twelve hour-fermented samples for both milled and parboiled samples (Figure 18 and 19). Digestibility of proteins have recently gained attention because it has been found to positively influence the rate of starch digestibility in rice-based products (Mujoo & Ali, 1998).

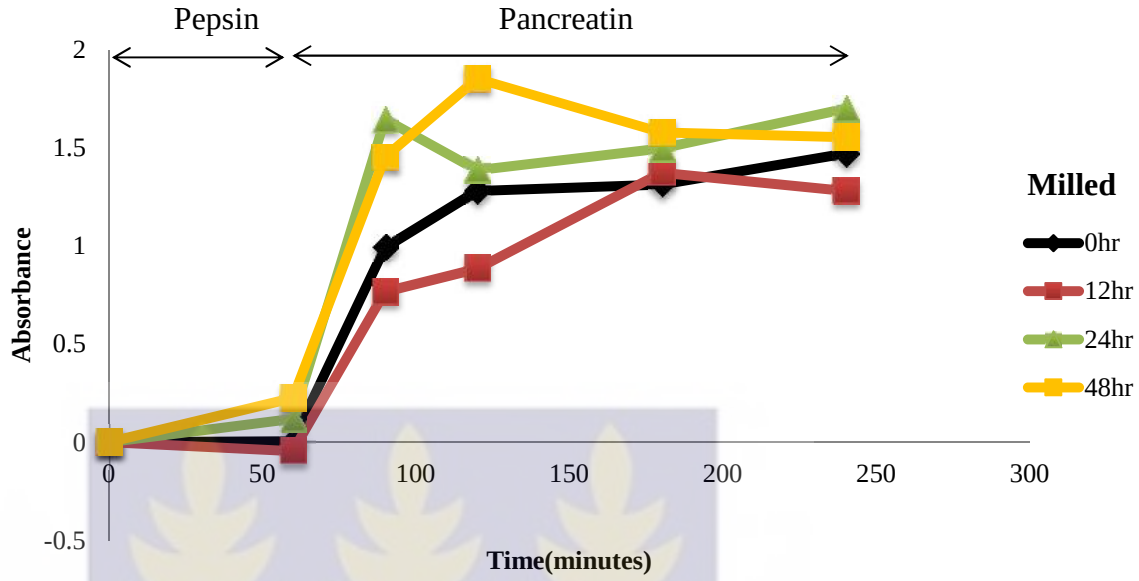

Figure 18 Effect of processing on the *in vitro* protein digestibility of drum-dried fermented parboiled rice-based baby food

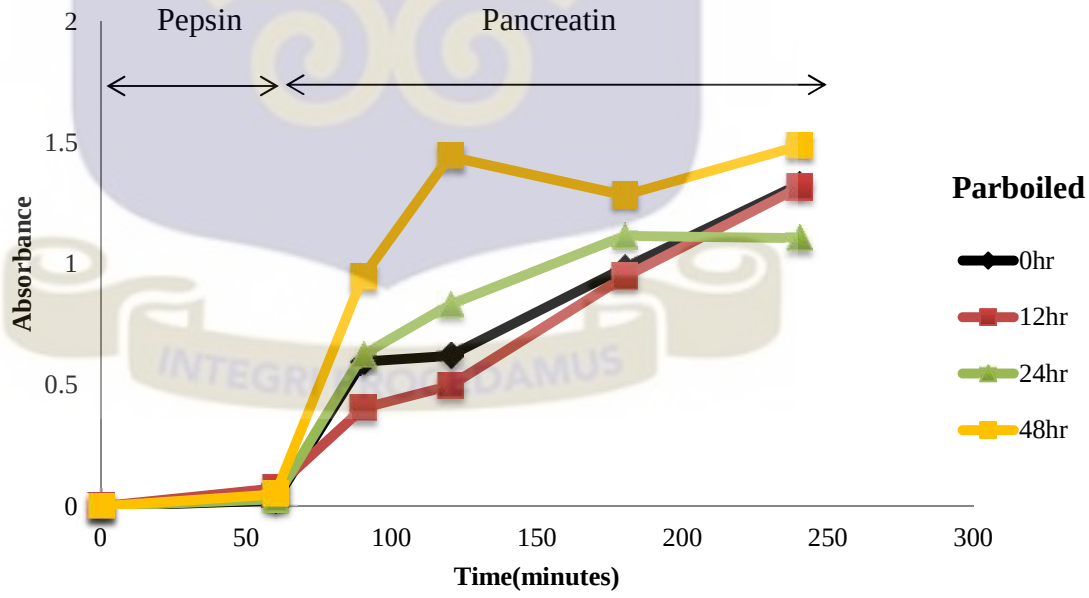

Figure 19 Effect of processing on the *in vitro* protein digestibility of drum-dried fermented parboiled rice-based baby food

4.4.2 Starch Digestibility and Free Sugars Determination

4.4.2.1 Rapidly/Readily Digestible Starch (RDS), Slowly Digestible Starch (SDS) and Glycaemic Index

Most cereal-based baby foods are starch-rich therefore starch metabolism following consumption is of grave interest. Starch digestibility of fermented rice-based products was estimated by measuring the rapidly/readily digestible starch (RDS), slowly digestible starch (SDS) and over all glycaemic index. From Table6, it was observed that readily digestible starch values were high while on the other hand, low levels of slowly digestible starch were obtained for both milled and parboiled rice. This confirms that rice is highly digestible. Recent findings have established the benefits of consuming foods high in slowly digestible starch because it allows for steady increase of plasma glucose and insulin levels after few hours' consumption (Jenkins *et al.*, 1982; Wolever & Mehling, 2002). However, very high or very low levels of non-digestible carbohydrate in infants have not been well studied though they impact the health of infants over a long period of time (Agett *et al.*, 2003). Overall, predicted glycaemic indices were high in all samples with the 48hr fermented samples of milled and parboiled rice recording the highest glycaemic values (Table 6). This confirms the relationship between the extent of breakdown of starch to glucose and the fermentation period even though high heat processing treatment such as drum drying has also been reported to increase starch digestibility (Sindhu & Khetarpaul, 2001). There were, however, no statistically differences between glycaemic indices of samples. Readily digestible starch values were relatively higher in milled samples than in parboiled samples. This means that there were still some sugars present in milled samples while those in the parboiled samples had already been used up by microorganisms. Reduction in slowly digestible starch as shown in the 48hr fermented milled and parboiled samples negatively corresponded to the higher glycaemic

indices observed in the 48-hour fermented milled and parboiled rice. Chung *et al.* (2011) reported that long grain rice recorded lower RDS and corresponding higher SDS. Thus, higher RDS and lower SDS values obtained could be attributed to the short, broken nature of rice grains used in this study.

Table 6 Estimated mean values for RDS, SDS and Predicted Glycaemic Index. A: milled, B: parboiled.

Sample	Fermentation time/hours	Ready digestible starch/ %	Slowly digestible starch/%	Predicted Glycaemic Index
A0	0	89.3 ±2.7[a]	2.4 ±0.4[a]	89.7
A1	12	85.3 ±0.2[a]	2.7 ±0.3[a]	89.3
A3	48	87.0 ±0.1[b]	1.5 ±0.3[b]	91.0
B0	0	83.9 ±1.0[a]	2.8 ±0.5[a]	89.2
B1	12	83.7 ±4.5[a]	2.7 ±0.4[a]	89.3
B3	48	80.9 ±0.7[b]	0.6 ±0.2[b]	92.0

RDS-readily or rapidly digestible starch; SDS, slowly digestible starch. A-milled rice; b- parboiled rice; 0-zero hour fermentation time; 1-12 hour fermentation time; 2-24 hour fermentation time; 3-48 hour fermentation time. Values followed by different superscripts in the same column are significantly different ($p \leq 0.05$).

4.4.2.2 Free Sugars in Rice

Starch digestibility is followed by the release of simple or free sugars which form is assimilated into the bloodstream. From Figures 20 and 21, it was observed that maltose and glucose sugars were present in both milled and parboiled rice. Lactose levels were negligible and was observed as a result of enzymes and solvents used in the analytical procedure. Higher levels of free sugars were observed in the milled rice samples as compared to the parboiled samples. Trace levels of maltose observed was as a result of the presence of residual dextrin in the samples which diminished after zero hour fermentation (Figures 20 and 21). In the milled samples, the 12-hour fermented sample recorded the highest glucose content followed by the zero-hour fermented sample. The reduction in glucose concentration observed in the 48-hour fermented milled rice (Figure 20) suggests that by that fermentation period, the microorganisms began to use up some of these sugars for their activities. This observation agrees well with Saalia *et al.* (2012).

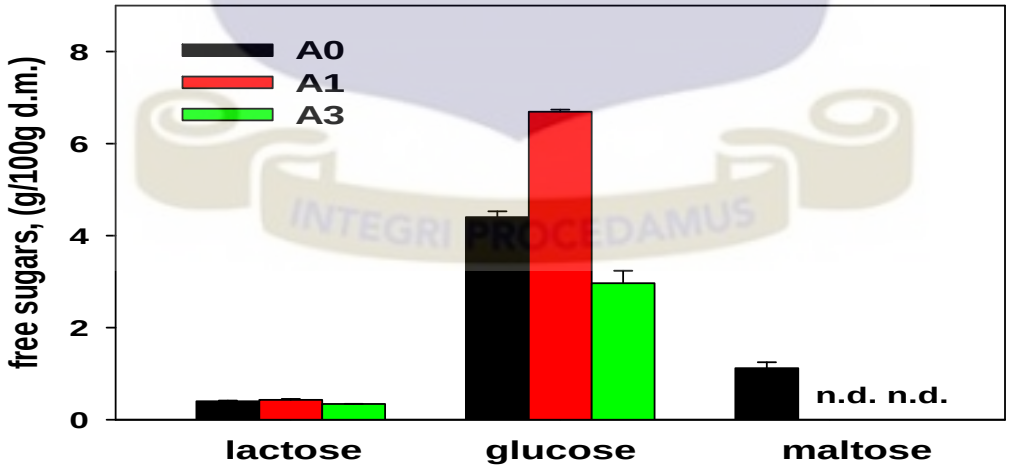

Figure 20 Free sugars present in fermentation milled rice-based product. A-milled rice 0-zero hour fermentation time; 1-12 hour fermentation time; 3-48 hour fermentation time.

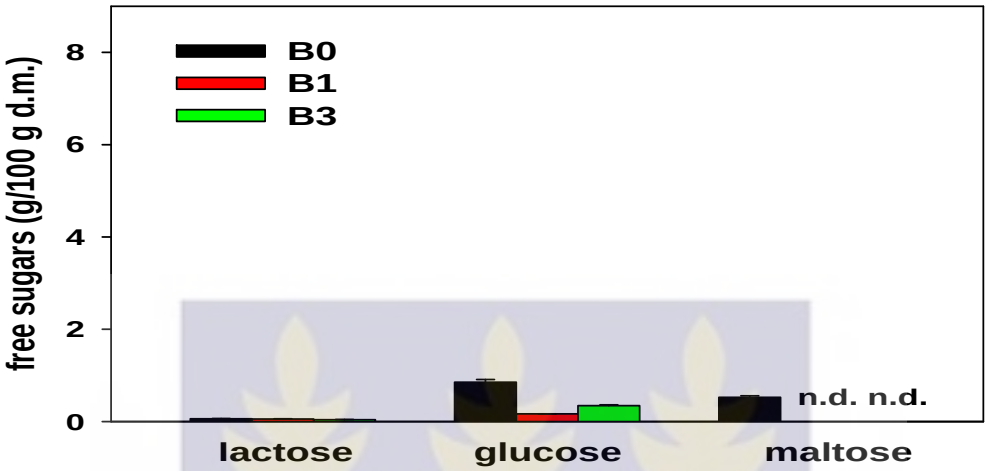

Figure 21 Free sugars present in fermentation milled rice-based product. B-parboiled rice 0-zero hour fermentation time; 1-12 hour fermentation time; 3-48 hour fermentation time.

4.5 Sensory Evaluation

When a product is developed in food processing, the qualitative features such as the appearance, aroma, flavour, texture and after-taste could be identified using a list of descriptors developed by a trained panel who in turn attempt to measure each perceived attribute on a scale, for example, a 15-cm scale. This is termed descriptive sensory evaluation and an example is using the quantitative descriptive analysis (QDA) technique (Stone *et al.*, 1974; Stone & Sidel, 2004). Recently, e-sensing instruments have also become useful in descriptive sensory evaluation and have reported use in several publications (Peris & Escuder-Gilabert, 2009; Escuder-Gilabert, 2010; Winquist *et al.*, 1998).In this study, descriptive sensory analysis was carried out using the quantitative descriptive analysis (QDA) process and the electronic nose and electronic tongue (instrumental e-sensors).

4.5.1 Quantitative Descriptive Analysis

Results from the quantitative descriptive analysis were illustrated by the sensory profiles described in the following figures (Figures 22 to 45).

4.5.1.1 Sensory profile description of fermented rice –based products (in powdery form)

Figure 22&23, 24&25, 26&27 and 28&29 show the sensory profile of milled and parboiled products in a spider-web graphical representation where the zero point of the descriptor scale is the mid-point, with the intensity increasing in the direction of the extremities. The mean value of each descriptive term was marked in the respective axis, and the sensory profile was drawn by the connection of the points. The intensities of attributes were rated by panelists using a 15cm scale from least (0cm) to extreme (15cm).

Both milled and parboiled samples were generally dry and creamy (Figures 22 and 23). The 24-hour fermented sample was the most creamy for milled rice but was most white in parboiled samples. Also while the zero hour fermented sample showed the most uneven and particulate surface appearance for parboiled samples, in the case of milled products, it was the 12 hour fermented sample (Figures 22 and 23). Significant differences observed between samples depended on whether products were milled or parboiled products for colour attributes (p-value=0.00) but fermentation time effects were not significant except between 0 and 12 hours of fermentation. Fermentation time did not affect surface dryness; however, it affected products' particulate surface appearance at a $p \leq 0.05$. Also, the parboiling treatment significantly affected dryness at$p \leq 0.05$but for the particulate descriptor, there were no significant differences. Almost all sample products were perceived as dry (Figures 22 and 23) with mean values range (10.5≤ x

≤12.2). There was also a significant positive correlation between the cream colour and fermented flavour (0.340*).

Aroma attributes are illustrated in Figures 24 and 25. It was observed that milled samples had more pronounced aroma intensities particularly the starchy attribute recorded by the 48hour fermented milled rice. Zero-hour fermented samples for both milled and parboiled rice had the most ricey aroma and the least fermented aroma. This explains why fermented aroma intensity corresponded negatively with ricey aroma in almost all products (-0.434*). The 24-hour fermented sample had the most fermented aroma for milled rice while 12-hour fermented sample had the most fermented aroma for parboiled rice. 24-hour sample had the most maize-like aroma and the least cardboardy aroma for both milled and parboiled rice. The effects of fermentation time on the intensities of ricey and cardboardy attributes were significant. There was a positive correlation between starchy and cardboard (0.405*) aromas and also between fermented aroma and fermented flavour (0.583*). The asterisks (*) is an indication significant of difference.

The most significantly pronounced flavour attributes were sour, fermented, sour-aftertaste and rich flavour recorded by the 48-hour fermented sample for milled rice and 12-hour fermented sample for parboiled rice (Figures 26 and 27). The parboiling treatment resulted in weaker intensities of the flavour attributes (Figure 27). As expected, fermentation time significantly augmented the fermented flavour and sourness thereby diminishing the sweet-aftertaste (p≤0.05). This explains the strong correlation between sour and fermented flavour (0.598*). The fermented flavour also significantly correlated well with the fermented aroma (0.583*). The 24 hour

fermented sample had the most maize/corn flavour for both milled and parboiled rice and this is analogous to the maize-like aroma obtained by the same sample.

Texture attributes were obtained using the mouth-feel and touch sensation for both milled and parboiled products. Differences between all texture attributes were significantly different between milled and parboiled samples except for the flaky texture. Parboiled products demonstrated lower intensities for almost all texture attributes (Figures 28 and 29). Also fermentation time did not significantly affect texture attributes, except for the particulate (touch sensation) and flaky qualities. Because these products were assessed by panelist in powdery form, it is not surprising that the dry texture was the most distinct texture attribute for both milled and parboiled products (Figures 28 and 29). There was also a strong positive correlation between gritty and particulate descriptors (0.561*).

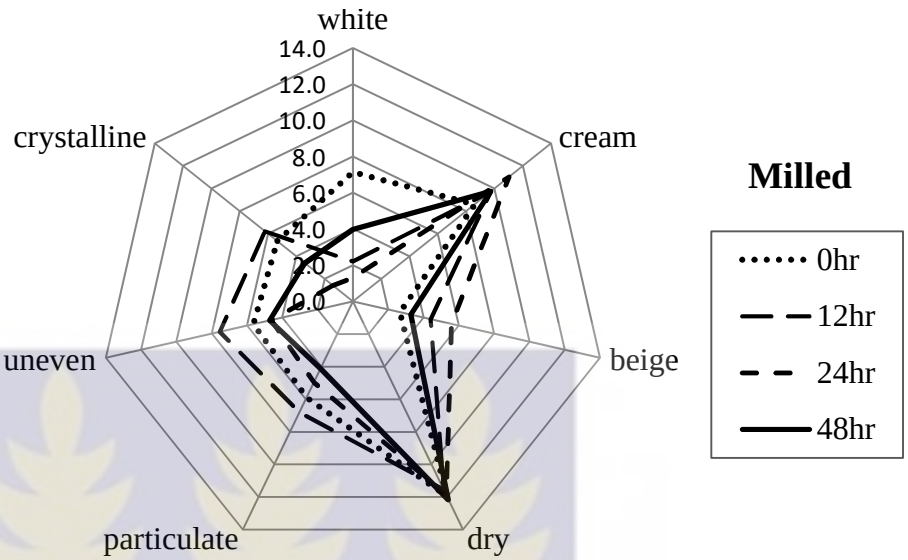

Figure 22 Sensory profile showing appearance attributes of fermented milled rice-based products (powdered form)

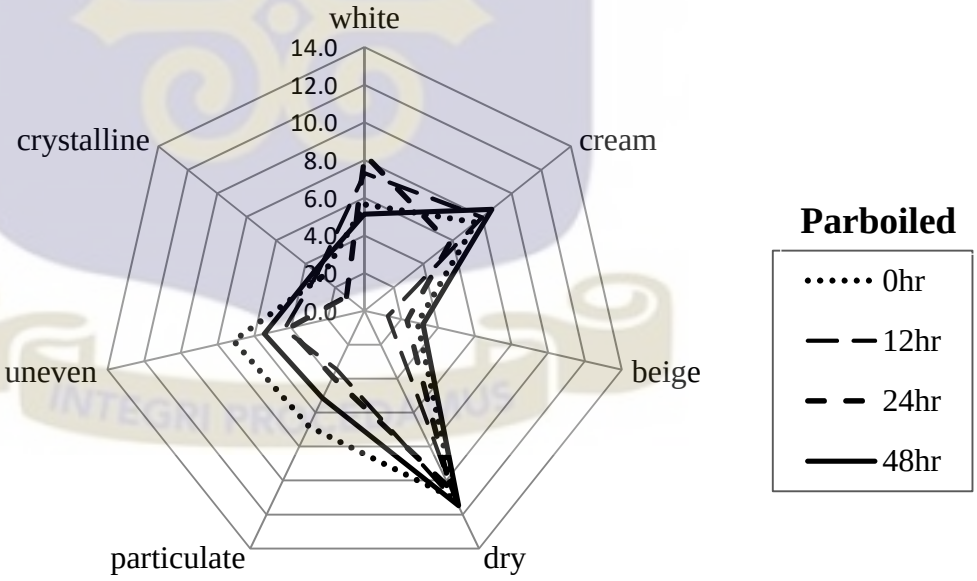

Figure 23 Sensory profile showing appearance attributes of fermented parboiled rice-based products (powdered form).

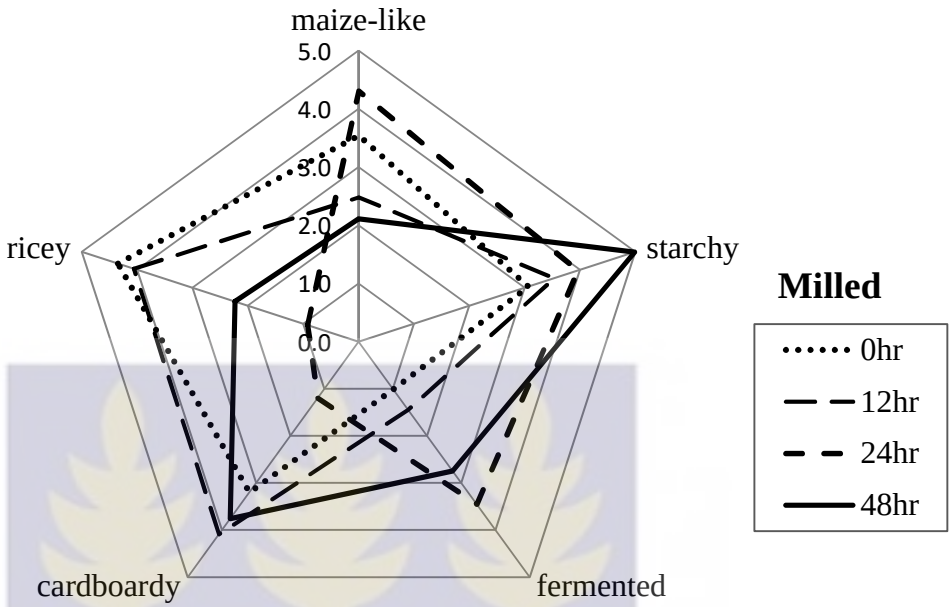

Figure 24 Sensory profile showing aroma attributes of milled fermented rice-based products (powdered form)

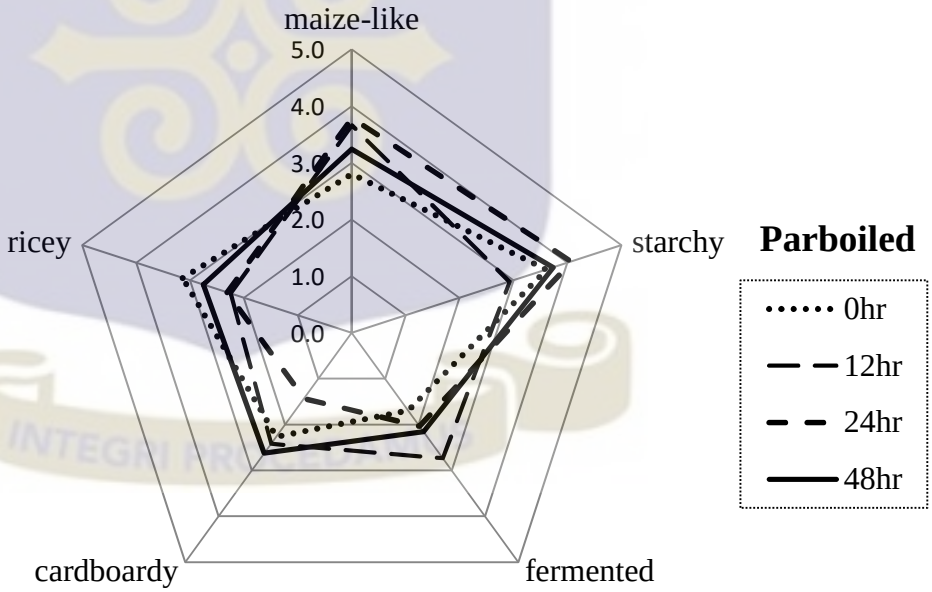

Figure 25 Sensory profile showing aroma attributes of fermented parboiled rice-based products (powdered form)

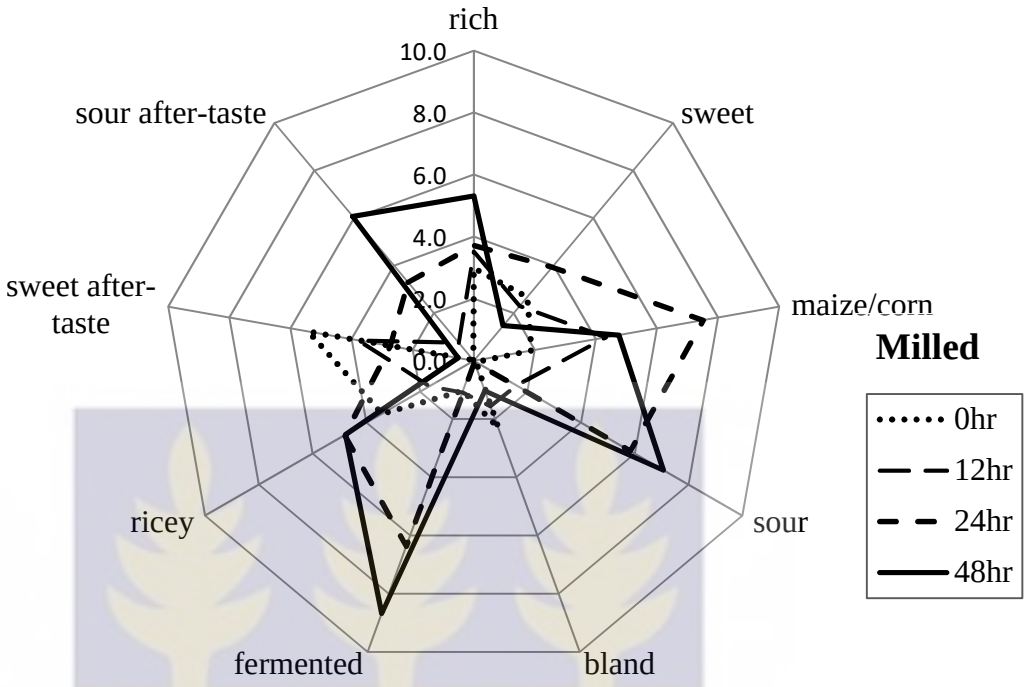

Figure 26 Sensory profile showing flavour attributes of fermented milled rice-based products (powdered form)

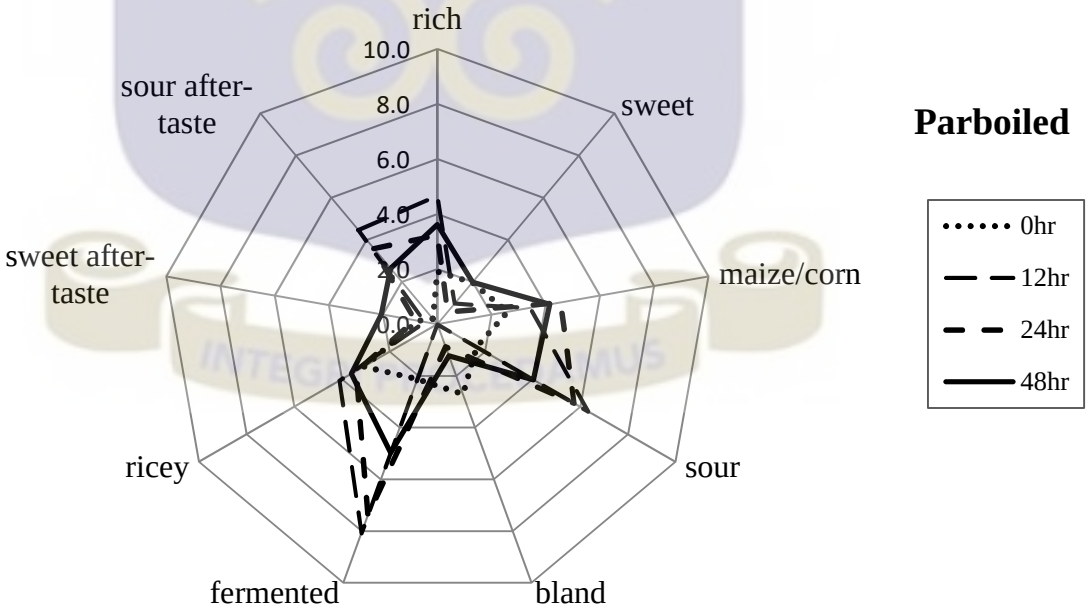

Figure 27 Sensory profile showing flavour attributes of fermented parboiled rice-based products (powdered form)

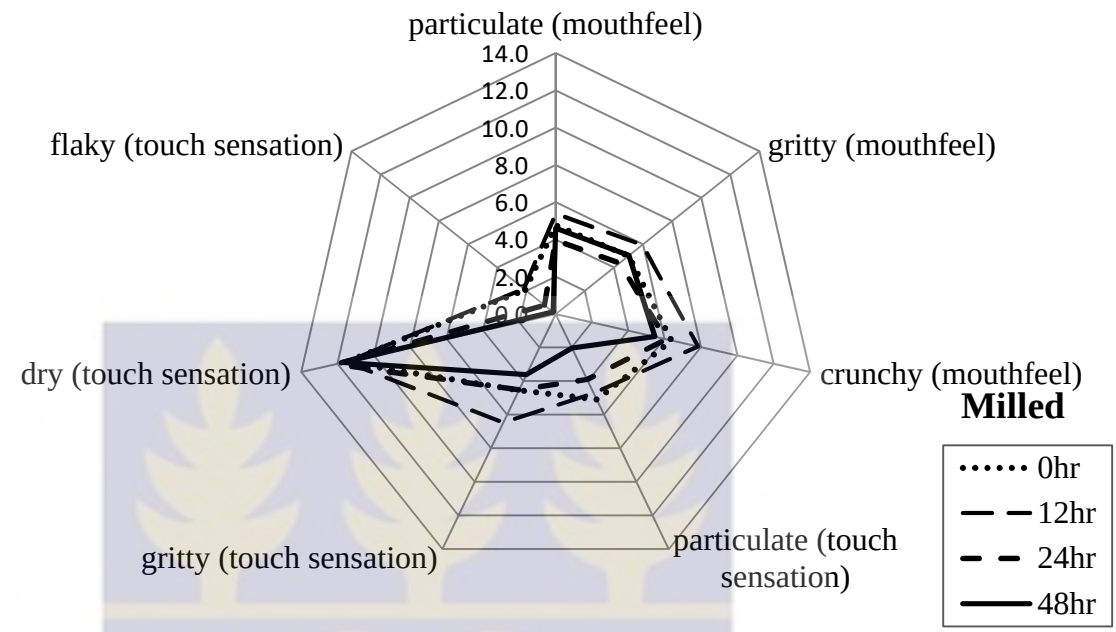

Figure 28 Sensory profile showing texture attributes of fermented milled rice-based products (powdered form).

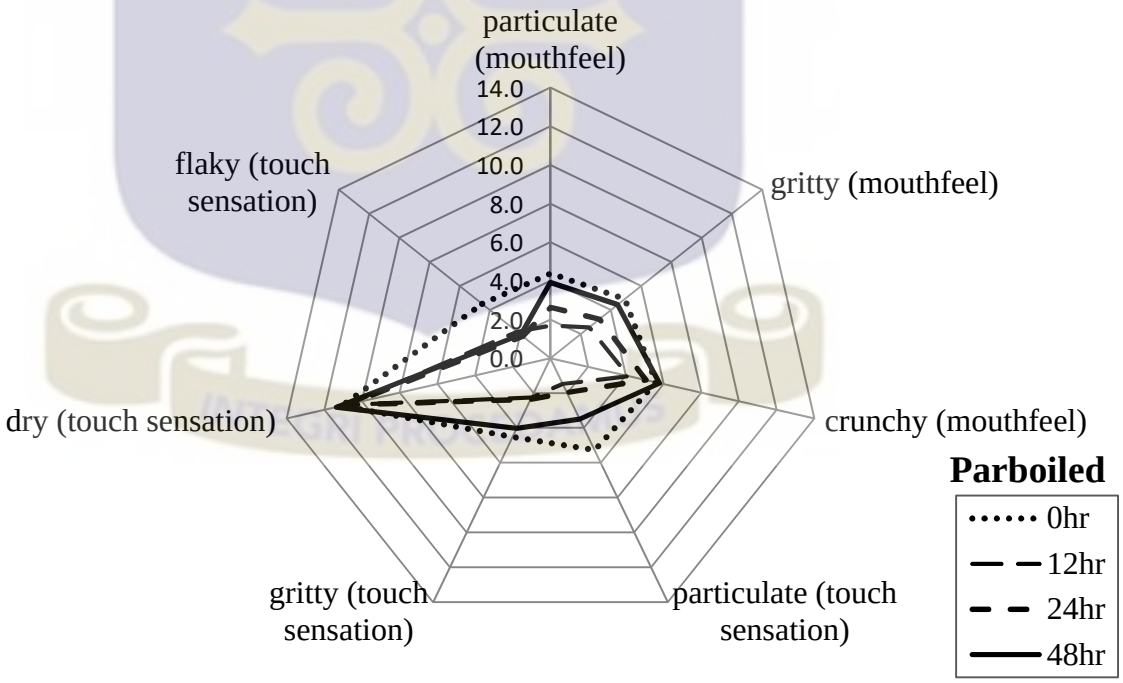

Figure 29 Sensory profile showing texture attributes of fermented parboiled rice-based products (powdered form).

Table 7 List of sensory descriptors and definitions for fermented rice-based products (powdery form)

MODALITIES	Descriptors	Definition
1. **APPEARANCE**		
a. **Colour**	White	Colour related to white corn grits ('eko egbemi')
		REF: white corn grits
	Cream	Colour exhibited in cheese (laughing cow cheese)
		REF: laughing cow cheese
	Beige	Pale yellowish-brown colour
		REF: Heinz salad cream
b. **Surface texture**	Particulate	Discrete/minute separate powdery particles
		REF: milled raw rice
	Crystalline	Relating to the reflectiveness/glassy surface of the particles
		REF: broken glass pieces
	Uneven	A mixture of non-uniform particles with rough ridges
		REF: sand particles
2. AROMA	Maize-like	Unsweetened corn porridge
		REF: corn porridge without sugar
	Starchy	Distinct uncooked cereal starch
		REF: corn dough in cold water
	Fermented	Related to cooked sour corn dough
		REF: cooked 'kenkey' and 'banku'

	Cardboardy	Related to a stack of stored packed cartons/boxes REF: stack of stored boxes made of cardboard
	Ricey	Uncooked milled non-perfumed rice REF: uncooked raw rice
3. FLAVOUR	Rich	Strong intense full flavour cassava grits (Gari) without sugar
	Ricey	Uncooked milled non-perfumed rice REF: uncooked raw rice
	Sour	Related to citrus acids REF: lemon juice
	Maize/corn	Oven- roasted maize flour REF: roasted maize flour (tom-brown)
	Fermented	Related to cooked sour dough REF: mixed flavour from kenkey and banku
	Sweet	Relating to sugar REF: granulated sugar
	Bland	Related to tasteless flavour (like clean drinking water) REF: clean lukewarm water
4. **AFTERTASTE**	Sour	Biting taste after swallowing REF: lemon juice
	Sweet	Relating to sugar

		REF: granulated sugar solution
5. **a. TEXTURE**		
(mouth feel)	Particulate	Discrete/minute separate powdery particles
		REF: milled uncooked raw rice
	Crunchy	Snaps when bitten into
		REF: cream cracker biscuit
	Gritty	Feels sandy in the mouth with uniform ridges as related to cooked corn grits
		REF: cooked corn grits
b.TEXTURE		
(touch sensation)	Gritty	Particles with sharp ridges when rubbed with the fingers
		REF: uncooked corn grits
	Particulate	Discrete/minute separate powdery particles
		REF: uncooked raw rice
	Dry	Has very low moisture content
		REF: dry powder
	Flaky	Small flat-disked thin pieces
		REF: broken corn flakes pieces

REF: Reference food

4.5.1.2 Sensory profile description of fermented rice –based products (in re-constituted form)

Upon reconstitution with water, both milled and parboiled became smooth and pasty (Figures 30 and 31). The intensities of the white colour appearance attribute was also enhanced after reconstitution with water. However this colour decreased significantly with fermentation time but significantly increased after 24 hour fermentation period ($p \leq 0.05$). The 48-hour fermented sample recorded the highest value for white colour in parboiled samples while for the milled rice, 0-hour fermented sample had the highest value for white colour followed by 48-hour fermented sample. Milled samples had a more creamy colour than parboiled samples. Parboiling and fermentation resulting in obtaining a smoother surface appearance (Figure 31). 48-hour fermented sample was smooth in parboiled rice but pasty in milled rice while the 24-hour fermented sample was least smooth in milled and least pasty in parboiled. The same sample was also the most pulpy in milled and least pulpy in parboiled samples. Overall, parboiling treatment effects was not significant for all descriptors used for re-constituted products ($p \geq 0.05$). Obviously, all samples recorded higher intensities for pasty and smooth appearance but differences among samples were not statistically significant ($p \geq 0.05$).

Reconstitution with water significantly reduced starchy, ricey and cardboardy aroma notes and enhanced maize-like and fermented aroma notes in both milled and parboiled sample (Figures 32 and 33). The fermented aroma significantly increased as fermentation proceeded. Products that recorded high intensities for fermented aroma presented corresponding low ricey aroma intensities (Figures 32 and 33). This explains why ricey aroma decreased significantly with increasing fermentation time ($p \geq 0.05$). The 48-hour fermented sample showed the most fermented

aroma for milled rice while 24 –hour fermented sample showed the most fermented aroma for parboiled samples.

The 48hr fermented samples recorded higher intensities for fermented, sour, rich, maize-like flavours and sour after-taste for both milled and parboiled samples (Figures 34 and 35). The fermented flavour significantly increased with increasing fermentation time. An observed strong positive correlation (0.267*) between fermented flavour and fermented aroma was statistically significant. This observation was similar to sour and sour-aftertaste flavour attributes (0.247*). As observed with aroma attributes; ricey flavour significantly decreased with fermentation time increases (Figures 34 and 35) and was significantly negatively correlated with the fermented flavour (-0.136*). The zero-hour fermented sample had the most ricey flavour for both milled and parboiled rice. Rich flavour also correlated well with fermented (0.614*) and starchy flavours (0.412*) ($p \leq 0.05$). The asterisks (*) is an indication of significance of difference.

With the texture of products, the 48hr fermented milled rice product presented higher intensities for all texture attributes (Figure 36 and 37) while for parboiled samples, differences between texture attributes owing to the mouth feel was not clearly defined (Figure 37). Apart from the differences clearly shown for the pasty owing to touch sensation attribute among products for both milled and parboiled rice which significantly increased with fermentation time ($p \leq 0.05$); the rest of the texture attributes were not significantly different from each other. There was a significant strong positive correlation between sticky texture (touch sensation) and fermented aroma, fermented, rich, starchy and sour flavours ($p \leq 0.05$). The 12-hour fermented sample was

soft owing to touch sensation for both milled and parboiled samples. The 48-hour fermented sample showed the most gritty texture owing to mouth-feel for milled samples while the zero-hour fermented sample showed the most gritty texture owing to mouth-feel for parboiled rice.

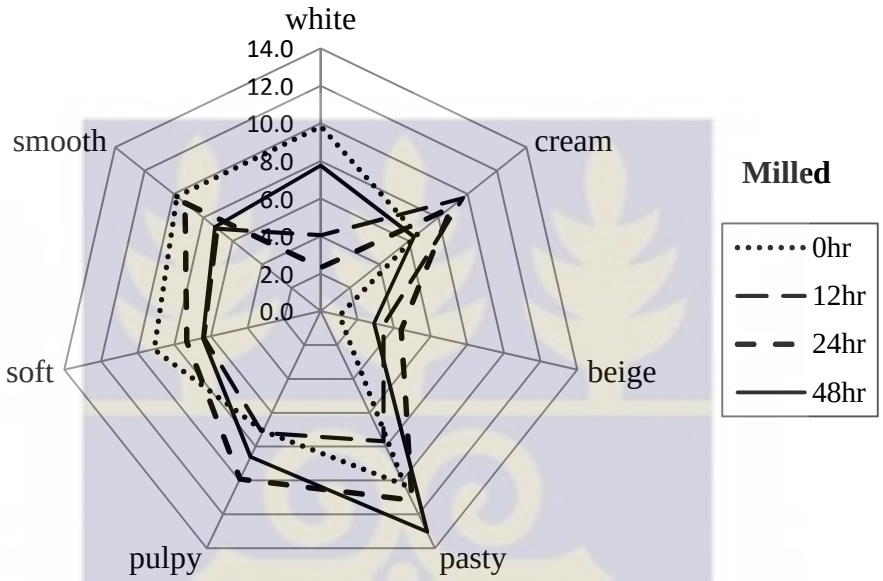

Figure 30 Sensory profile showing appearance attributes of fermented milled rice-based products (re-constituted form)

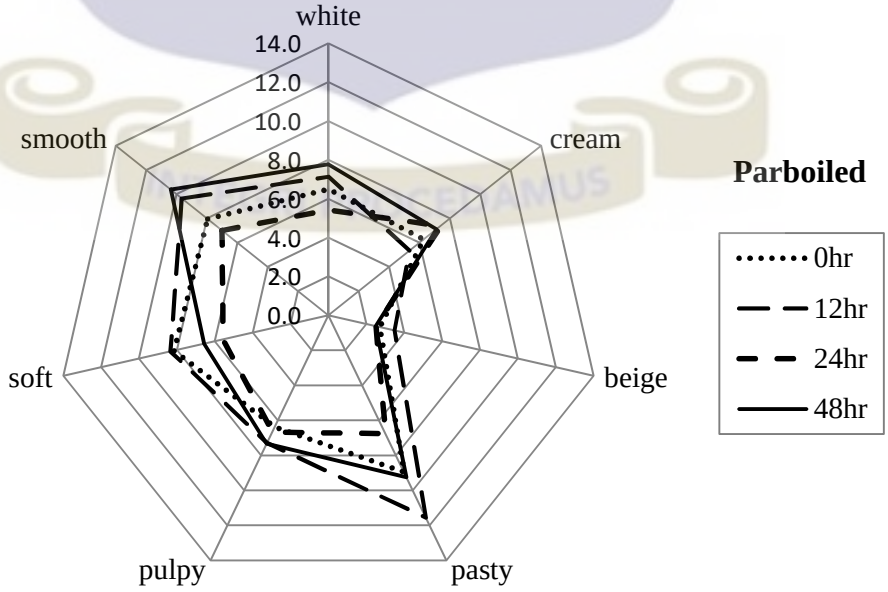

Figure 31 Sensory profile showing appearance attributes of fermented parboiled rice-based products (re-constituted form)

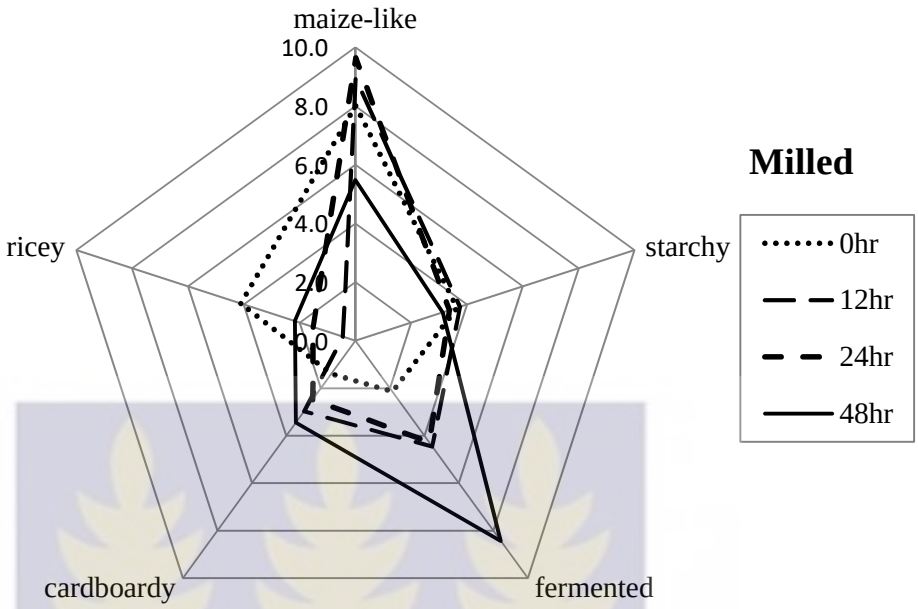

Figure 32 Sensory profile showing aroma attributes of fermented milled rice-based products (re-constituted form)

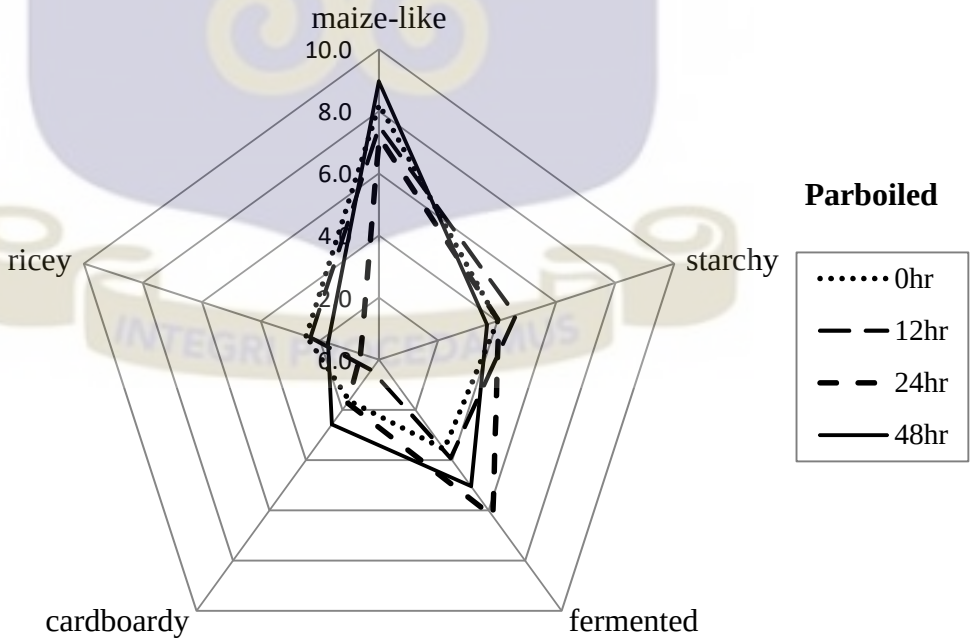

Figure 33 Sensory profile showing aroma attributes of fermented parboiled rice-based products (re-constituted form)

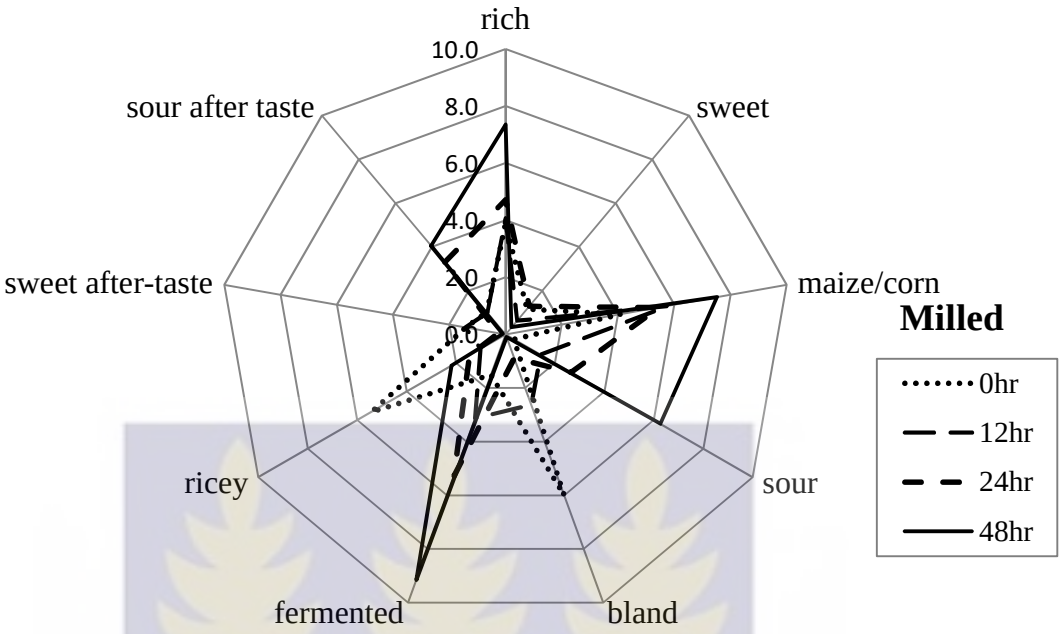

Figure 34 Sensory profile showing flavour attributes of fermented milled rice-based products (re-constituted form)

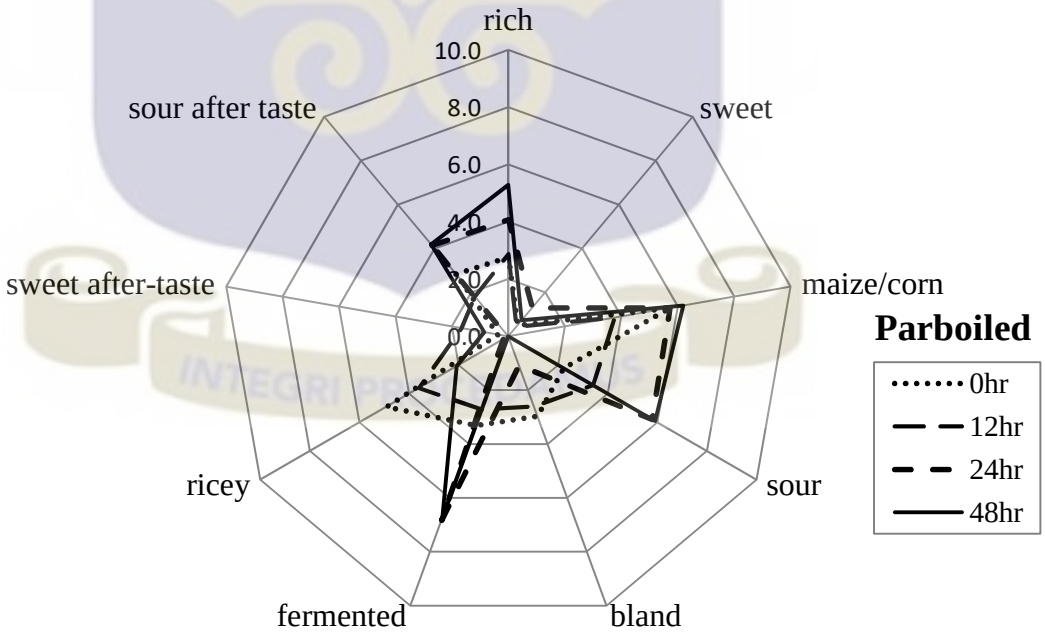

Figure 35 Sensory profile showing flavour attributes of fermented parboiled rice-based products (re-constituted form)

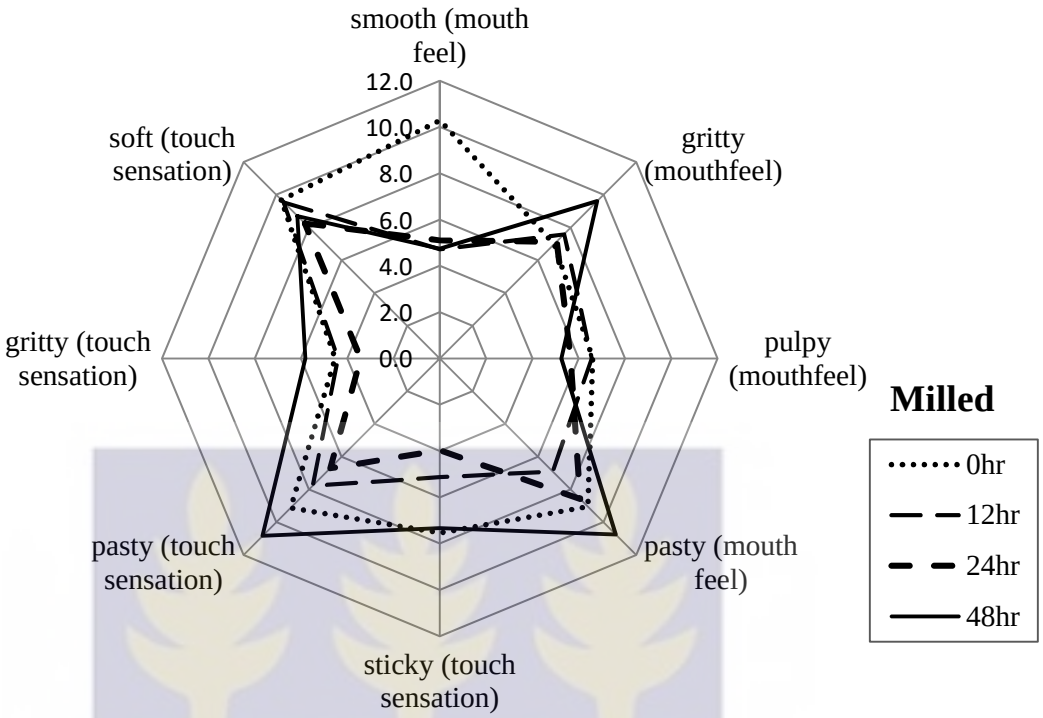

Figure 36 Sensory profile showing texture attributes of fermented milled rice-based products (re-constituted form)

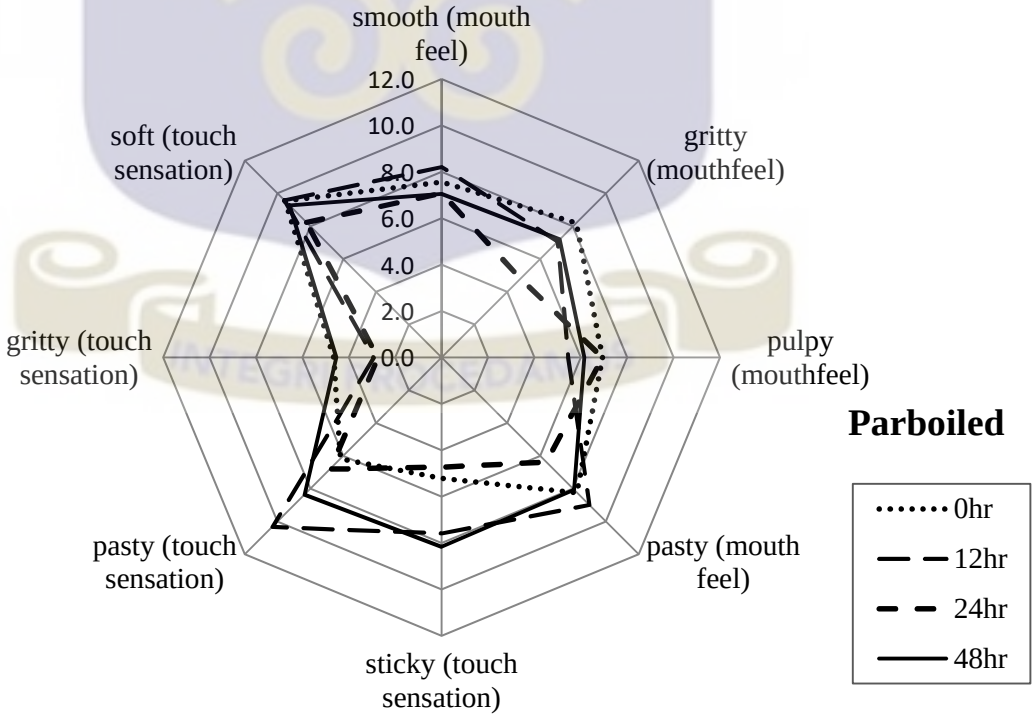

Figure 37 Sensory profile showing texture attributes of fermented parboiled rice-based products (re-constituted form)

Table 8 List of sensory descriptors and definitions for fermented rice-based products (re-constituted).

MODALITIES	Descriptors	Meaning/Definition
1. APPEARANCE	White	Colour related to white corn grits(‘eko egbemi’) REF: white corn grits
c. **Colour**	Cream	Colour exhibited in cheese REF: laughing cow cheese
	Beige	Pale yellowish-brown colour REF: Heinz salad cream
d. **Surface texture**	Pasty	Molten like cooked white corn grits porridge REF: cooked white corn grits porridge
	Smooth	Uniform consistency REF: smooth paste of Bird’s custard
	Soft	Texture relating to blended Ga kenkey (‘ice kenkey’) paste REF: Blended Ga kenkey (‘ice kenkey’) paste

	Pulpy	Mushy, semi-solid consistency REF: a fruit smoothie (blended banana)
2. AROMA	Maize-like	Unsweetened corn porridge REF: cooked corn porridge without sugar
	Starchy	Distinct uncooked cereal starch REF: fresh milled corn dough
	Fermented	Related to cooked sour corn dough REF: fermented corn dough
	Cardboardy	Related to a stack of stored packed cartons/boxes REF: packed boxes of cardboard
	Ricey	Uncooked milled non-perfumed rice REF: milled raw rice
3. FLAVOUR	Rich	Strong intense full flavour REF: cassava grits without sugar

	Ricey	Uncooked milled non-perfumed rice REF: milled raw rice
3. FLAVOUR	Sour	Related to citrus acids REF: lemon juice
	Maize/corn	Oven- roasted maize flour REF: Oven- roasted maize flour
	Fermented	Related to cooked sour dough REF: mixed flavour of Ga 'kenkey' and 'banku'
	Sweet	Relating to sugar REF: Sugar
	Bland	Related to tasteless flavour REF: Clean lukewarm water
4. **AFTERTASTE**	Sour	Biting taste after swallowing REF: lemon juice
	Sweet	Relating to sugar

			REF: granulated sugar solution
5.	**a. TEXTURE (mouth feel)**	Smooth	Uniform consistency Uncooked smooth paste of Bird's custard
		Gritty	Feels like sandy paste in the mouth with uniform ridges as related to cooked corn grits REF: dry cassava grits
		Pulpy	Mushy, semi-solid consistency as related to a fruit smoothie REF: blended banana
		Pasty	Molten in the mouth like cooked white corn grits porridge REF: cooked white corn grits porridge
	b.TEXTURE (touch)	Sticky	Adhesive to touch with sticky surface REF: cooked corn grits
		Pasty	Molten to touch like cooked white corn grits porridge

		REF: cooked white corn grits porridge
	Soft	Texture relating to blended Ga kenkey (‘ice kenkey’) paste REF: blended Ga kenkey (‘ice kenkey’) paste
	Gritty	Feels like sandy paste to touch with uniform ridges as related to cooked corn grits REF: cooked corn grits

4.5.2 Sensory evaluation using E-sensors (electronic nose and electronic tongue)

Instrumental sensory evaluation of aroma attributes were carried out using the electronic nose and the electronic tongue was used for the flavour attributes respectively in the step-wise procedure described in Figure 38.

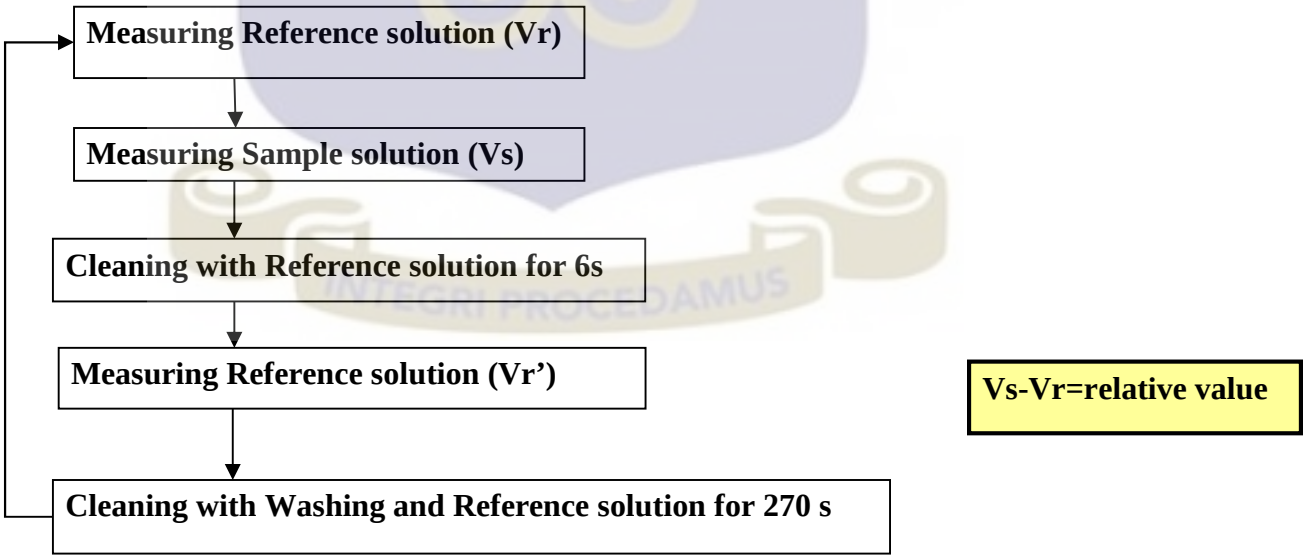

Figure 38 Step-wise procedure for electronic tongue sensory evaluation

4.5.3 Principal Component Analysis (PCA)

The Principal Component Analysis (PCA) was used to establish the variation and correlation between attributes for aroma and flavour descriptors using data obtained from the quantitative descriptive analysis and e-sensing (electronic nose and electronic tongue) measurements. Four principal components were generated (PC1, PC2, PC3, PC4) in the scree plot and eingen values obtained. One-way analysis of variance (ANOVA) was used to identify the significant principal components: principal component 1 (PC1) and principal component two (PC2)which were used for illustration and evaluation of the scores and loadings plot (Figures 38-45). The model was based on 50.9%variation among the aroma attributes and 58.7% variation among the flavour attributes for QDA respectively and 84.7% and 75.8% of the total variance for the electronic nose and tongue respectively. Inthe PCA plots attributes and/or samples close to each other in the same quadrant are correlated. The loadings plotusually helps to elaborate the score plot. Discrimination of products along the second principal component is relevant to elaboration of PCA results.

The negative loadings which were mostly made up of parboiled samples in the lower left quadrant of the score plot (Figure 39a) exhibited the fermented and maize-like aroma. The fermented and maize-like aromas were therefore positively correlated for powdered samples as observed in the loadings plot (Figure 38b). Scores obtained in figure 38a indicated that more of the fermented milled rice exhibited the starchy, ricey and cardboardy and these aromas are negatively correlated with fermented and maize-like aroma for the powdery fermented rice-based products.

For flavour attributes of powdered products, the ricey, bland, sweet flavours and sweet after-taste was presented by mostly milled rice samples particularly, the 12-hour fermented (A1) and the

zero-hour fermented (A0) milled rice–based products (figures 40a and 40b). These attributes negatively correlated with the rich, sour, fermented, maize/corn flavours and sour-aftertaste observed in the negative lower quadrant of the loadings plot. Rich flavour was mostly exhibited by the 24-hour fermented milled rice-based product (A2) while the sour, fermented flavours and sour after-taste were observed mostly in the parboiled samples as well as the 48-hour fermented milled rice-based products.

Re-constitution of samples with water changed the distribution of the aroma attributes in the loading and score plots (Figures 41a and 41b). The maize-like aroma was segregated from the rest of the attributes in the re-constituted samples (Figure 41a). Fermented and starchy aromas were exhibited mostly by parboiled samples and negatively correlated with the ricey and cardboardy aromas which were observed in the milled fermented re-constituted products are illustrated in Figures 41a and 40b.

Upon reconstitution of samples with water, there was a variation established between the rich flavour and fermented, sour, maize-like and sour-aftertaste (Figures 42a and 42b). The rich flavour was exhibited mostly by the 24-hour fermented milled (A2) as well as the 12-hour parboiled (B0) rice-based products (Figures 42a and 42b). The bland attribute was mostly observed in the zero-hour fermented milled (A0) rice-based product. Ricey, sweet and sweet after-taste was observed to be closely correlated flavour attributes of re-constituted rice-based product and showed by the zero-hour(A0) and 12-hour(A1) fermented milled rice-based products. The loading plot of the lyophilized samples evaluated with the electronic nose constituted the involvement of almost all the WC sensors of the electronic nose (Figure 43a). On the left side of

loadings plot, WS and WW sensors showed that the aroma profile of the 48-hour fermented milled rice-based product (A3) was very different from the rest of the products. Similar sensors were also relevant for the discrimination of the 24-hour fermented parboiled rice-based product. The score plot demonstrated that samples were grouped along the second principal component in the top and lower portions of the left portion of plot. There was a clear separation of the 48-hour fermented milled rice-based sample (A3) from the other samples (Figure 43b).

Considering the score plot of the flavour attributes of samples measured with the electronic tongue (Figure 44b), a clear difference was observed between the milled samples (A) in the positive or upper quadrants and the parboiled samples (B) in the negative or lower quadrants along the second Principal Component(PC2) (Figure 44a). The zero-hour (B0) fermented parboiled rice-based samples which were located in the positive part of the PC2, was perceived as more astringent, rich and they also had some bitter notes in the loadings plot (Figure 44a) with respect to milled samples (Figure 44b). The 'umami' taste was expressed by the zero-hour fermented milled rice-based sample in the loadings plot (Figure 44a) and was perceived to be different from the 12-hour, 24-hour and 48-hour fermented milled rice-based samples located in the negative part of PC2 and which did not exhibit clear taste attributes. The parboiled samples: (12-hour fermented) B1, (24-hour fermented) B2, (48-hour fermented) B3 located in the positive part of PC2 were characterised by saltiness and sour flavour attributes (Figures 44a and 44b). After-taste A and after-taste B were not clearly defined but there was evidence of their relevance in the loadings plot (Figure 44a).

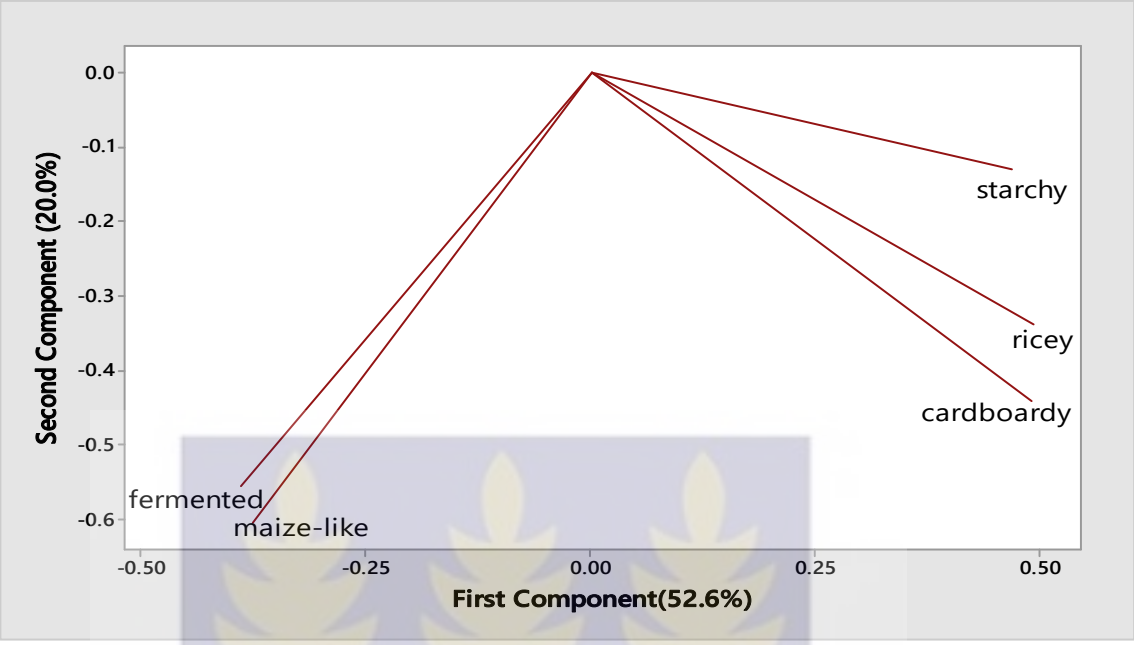

Figure 38a Loading plot of aroma attributes (powdered samples)

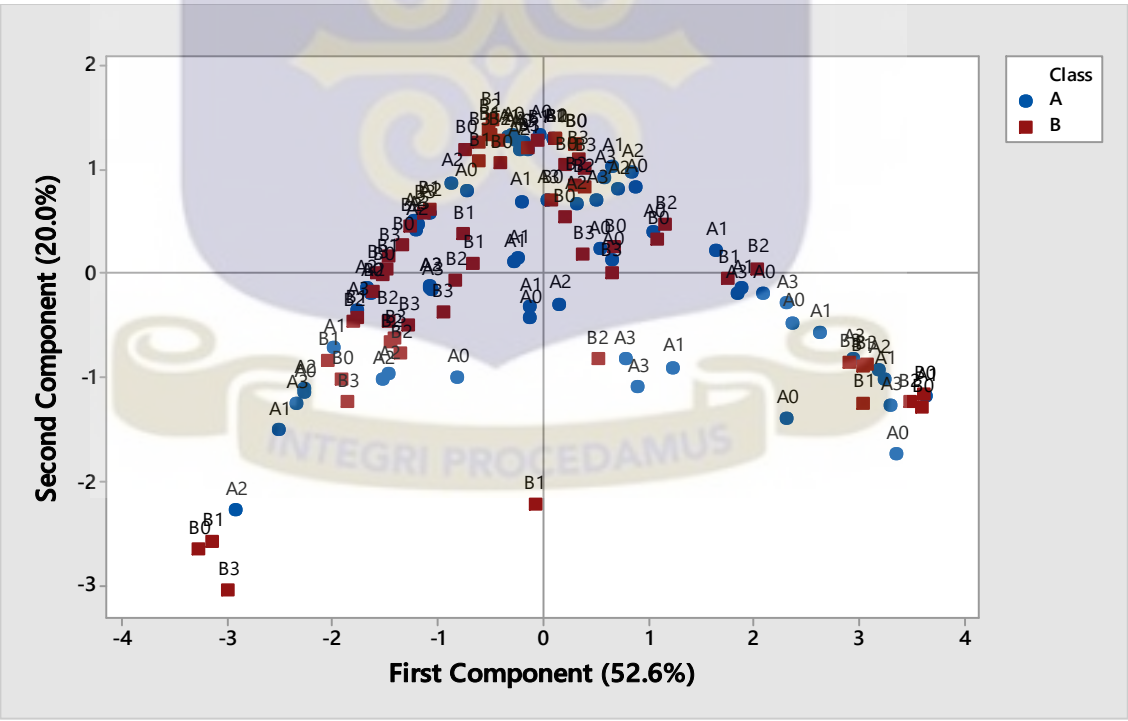

Figure 38b Score plot of aroma attributes (powdered samples)

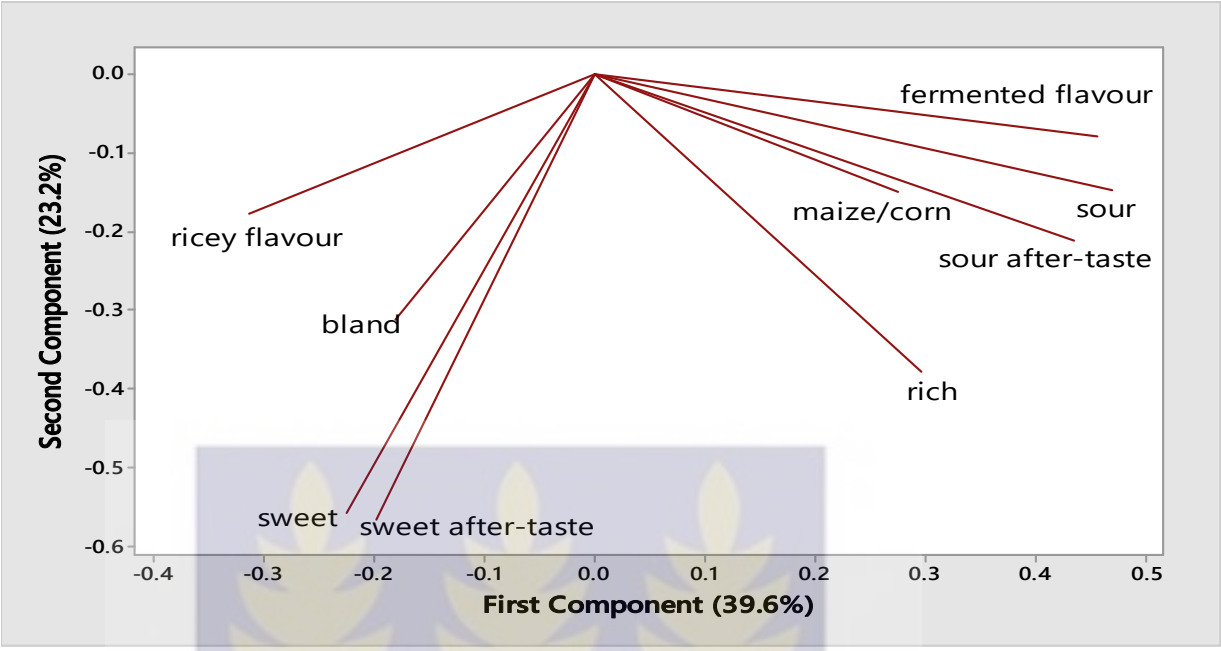

Figure 39a Loading plot of flavour attributes (powdered samples)

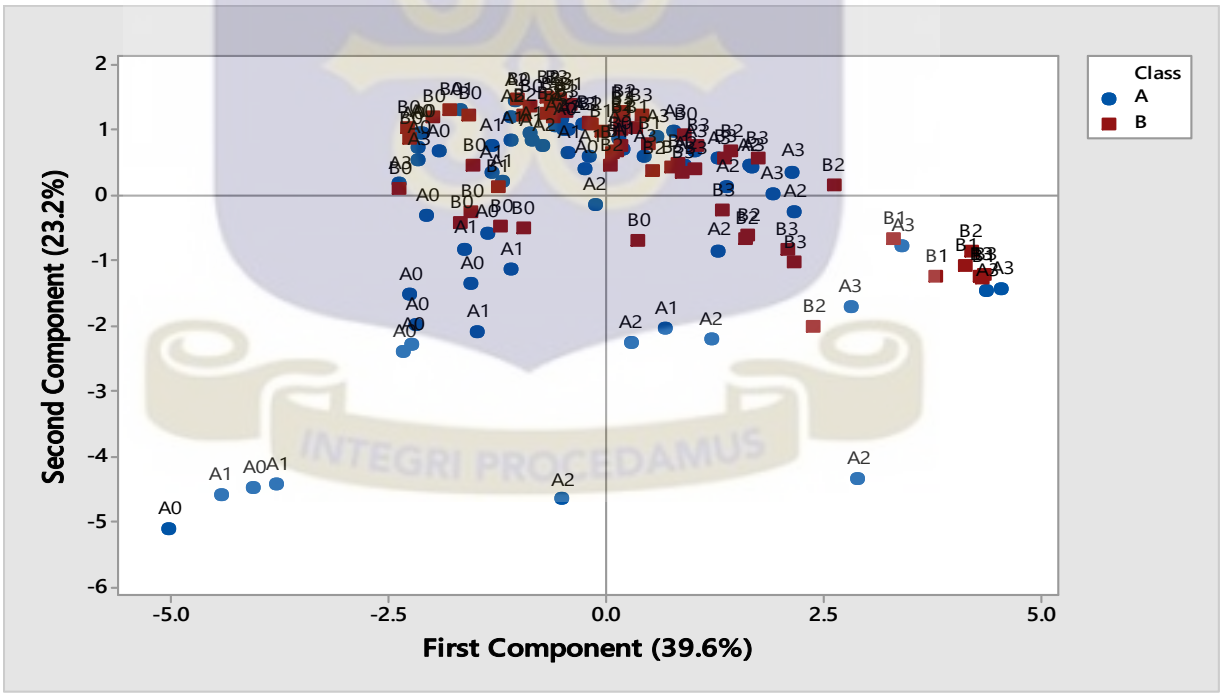

Figure 39b Score plot of flavour attributes (powdered samples)

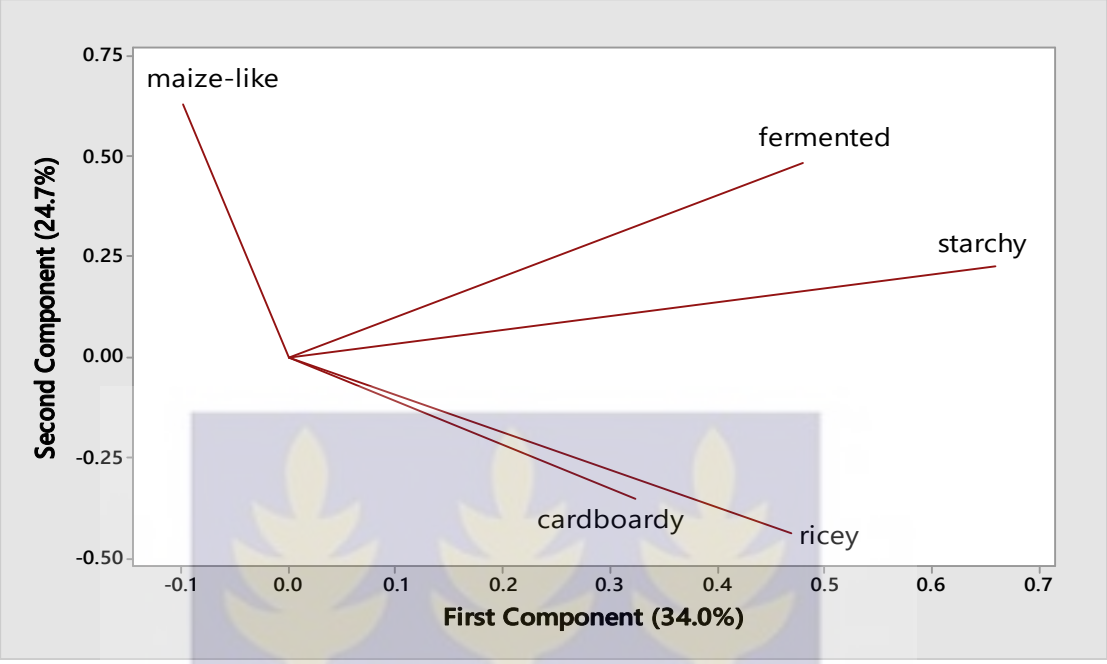

Figure 40a Loading plot of aroma attributes (re-constituted samples)

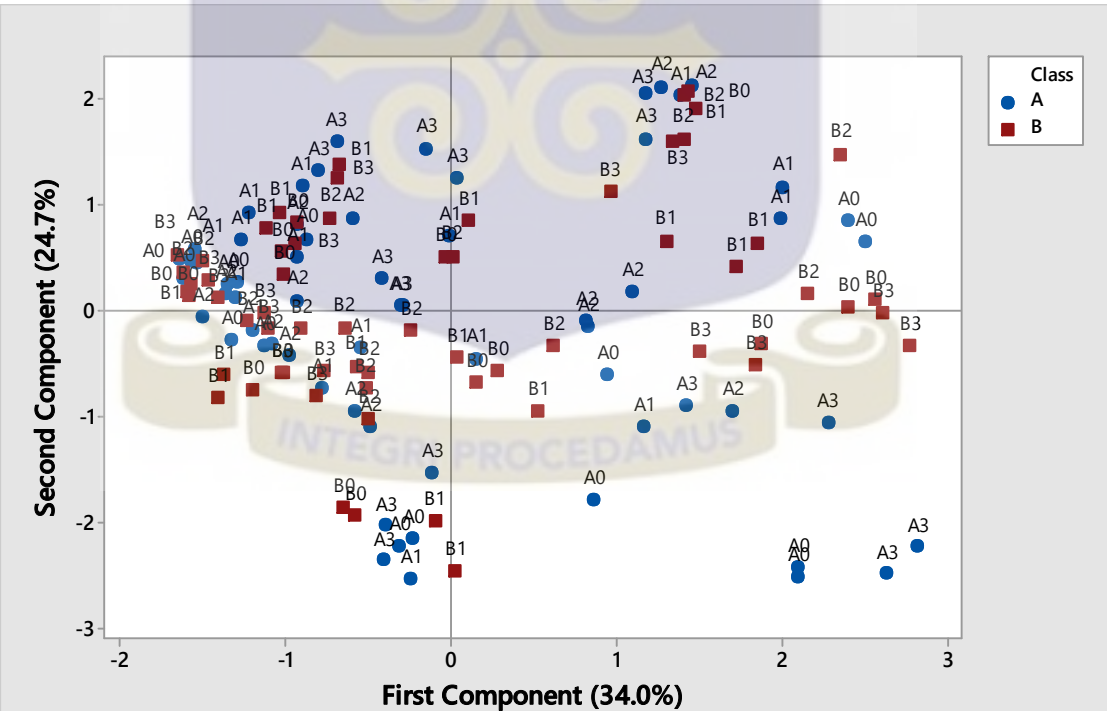

Figure 40b Score plot of aroma attributes (re-constituted samples)

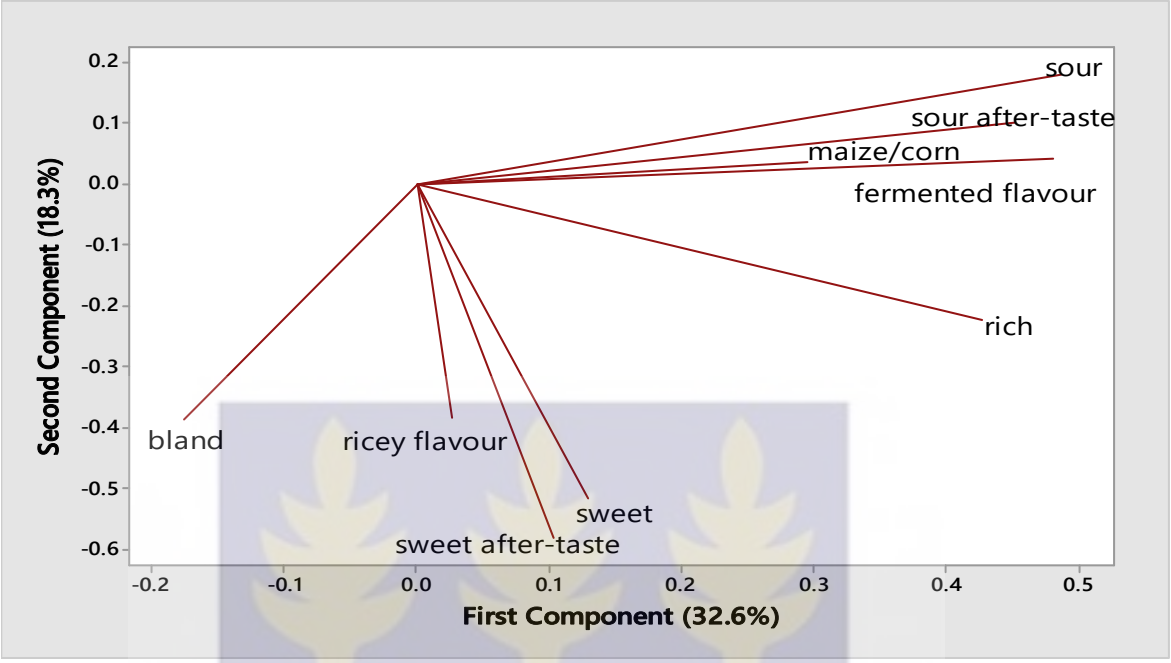

Figure 41a Loading plot of flavour attributes (re-constituted samples)

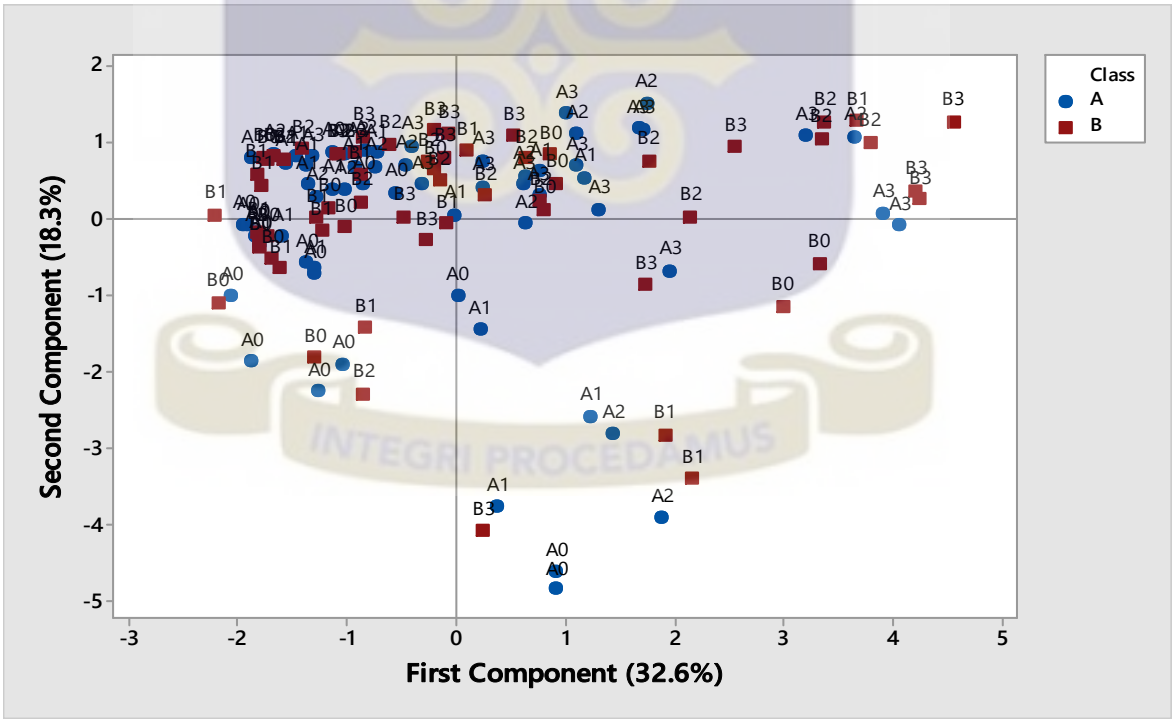

Figure 41b Score plot of flavour attributes (re-constituted samples)

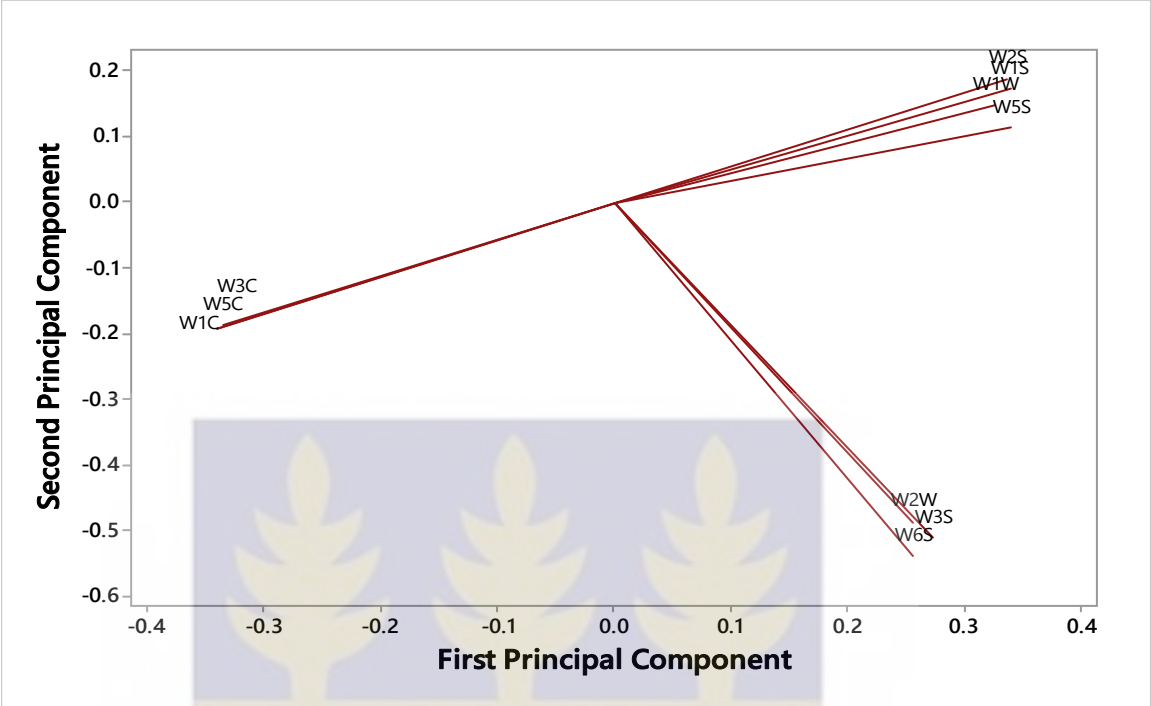

Figure 42a Loading plot for aroma attributes of samples using the electronic nose

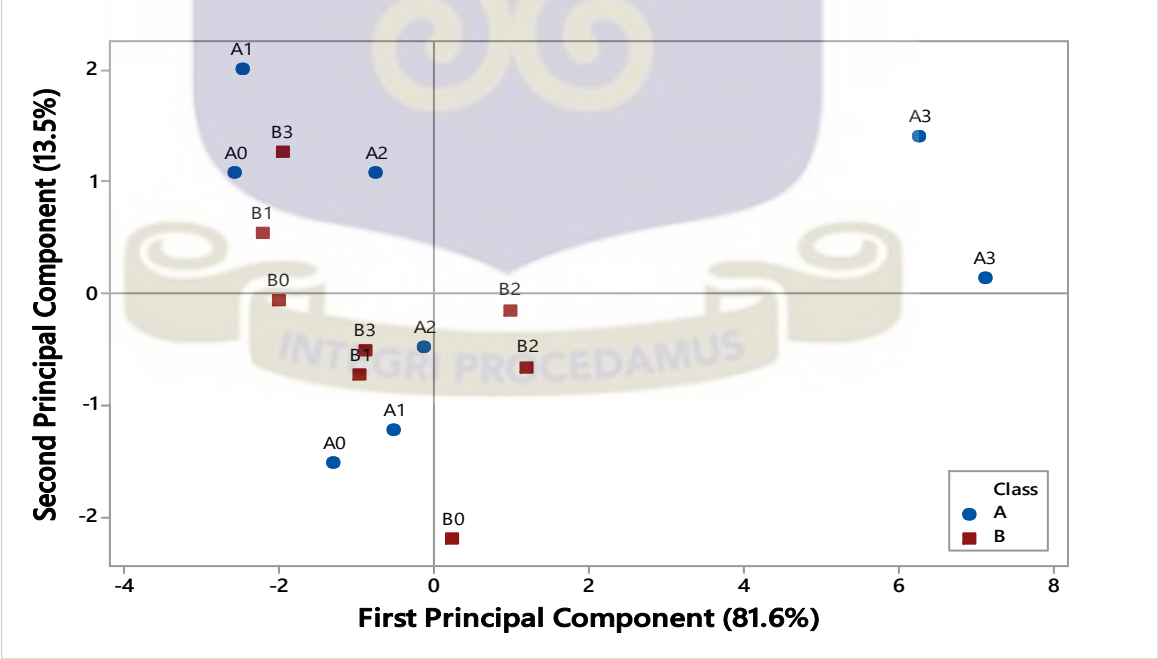

Figure 42b Score plot for aroma attributes of samples using the electronic nose

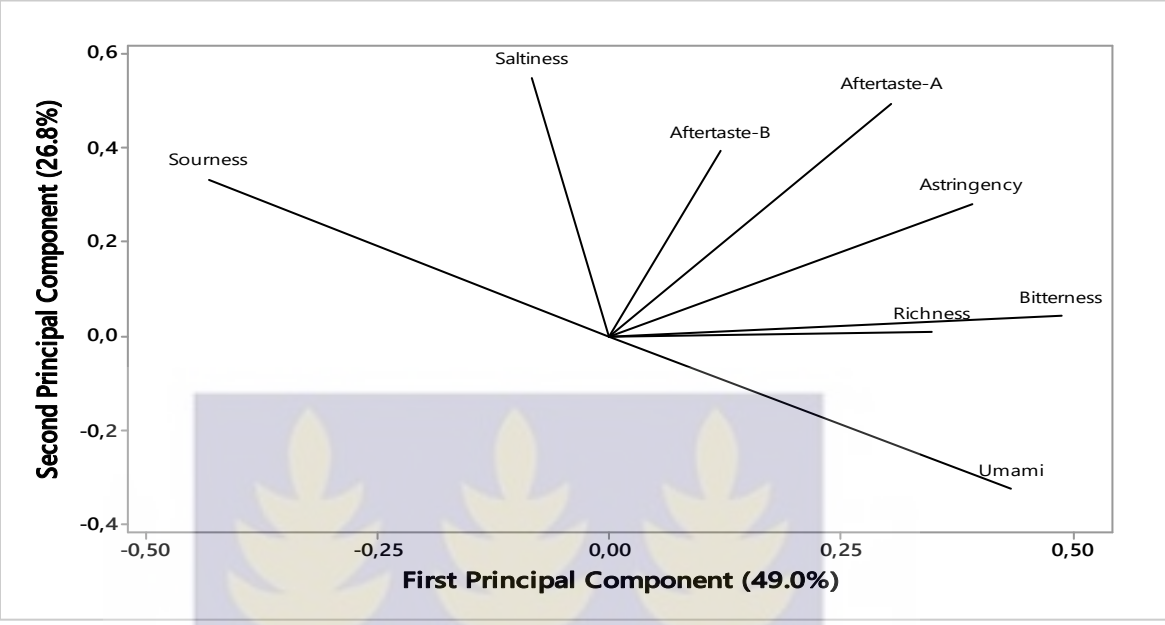

Figure 43a Loading lot for flavour attributes of samples using the electronic tongue

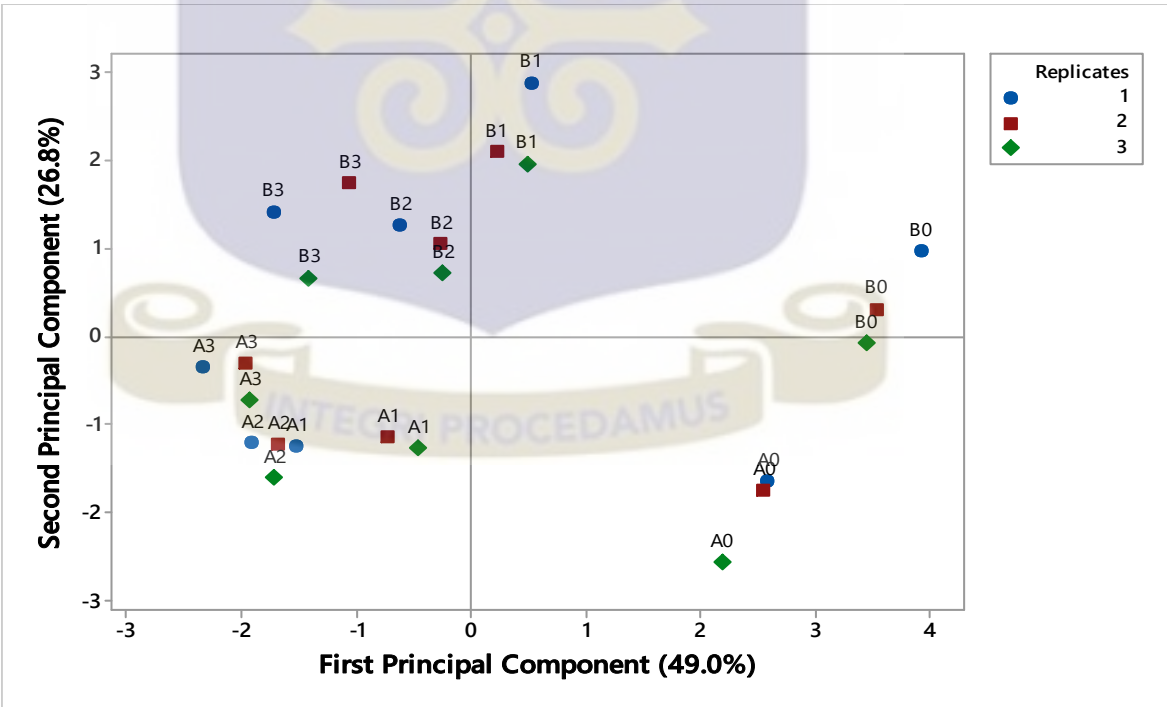

Figure 43b Score plot for flavour attributes of samples using the electronic tongue

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Local rice contains a large percentage of brokens and hence has a low market value. Parboiling as a pre-milling method produces rice that is nutrient dense but with harder texture, discolored grains with a strong characteristic flavour. Size reduction and fermentation which are simple food processing techniques were employed to transform and improve the physical, functional, and sensory properties while adding value to local rice in Ghana.

The fermentation time as well as the parboiling treatment had varied influences on ready-to-eat rice-based baby food products. Parboiling together with increasing fermentation time improved the lightness of the products Parboiling and increasing fermentation time also produced products that were more sour with lower pH values and corresponding higher titratable or total acidity values. Milled fermented rice-based products had higher water absorption indices but lower water solubility indices as fermentation increased. Milled fermented rice-based samples showed higher values for all pasting indicators: peak viscosity, breakdown, setback; except for gelatinization temperature and holding viscosities (trough and final viscosity) which were higher for fermented parboiled rice-based products as fermentation progressed. The pasting profiles of fermented milled rice-based samples indicated that they would produce thinner gruels when re-constituted with hot boiling water because of the low trough and final viscosity (hot paste stability) values. Protein solubility increased with fermentation time until after twelve hours when it decreased through to 48 hours fermentation time. Solubility of milled rice proteins was higher than those in

parboiled rice. The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) showed that most of the proteins in milled samples were hydrolyzed by the 12th hour of fermentation while parboiled rice proteins took a longer fermentation time to hydrolyze because of the formation of more aggregates in this sample group. Parboiled rice-based products showed higher amount of accessible thiols than milled rice samples with increasing fermentation time. Protein and starch digestibilities of all products were mostly influenced by fermentation time rather than the parboiling treatment.

Sensory evaluation showed that parboiled samples were described differently in relation to the aroma and flavour attributes perceived as being generally more sour, fermented and rich. Powdered forms of products were generally dry and gritty in texture while re-constituted forms were described as smooth, pasty and gritty.

5.2 Recommendation

It is recommended that further studies should be carried out to determine and classify the volatiles responsible for the flavour and aroma notes as described in the sensory results of this study using a Gas Chromatography-Mass Spectrophotometer (GC-MS). High-pressure liquid chromatography (HPLC) analysis should be employed to identify the specific organic acids and sugars that contributed to the pH and total acidity values observed in the study. Furthermore, microbiological

studies should also be carried out to ascertain the microorganisms present at the different fermentation periods during dough preparation and soaking time. The sorption characteristics and shelf-life studies of the products produced in this study should also be investigated. Other local rice varieties could also be investigated following similar processing procedures as was done in this study.

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APPENDIX A

BALLOT SHEET (POWDERED SAMPLES)

Panelist Code: …… Signature: ……..... Date: ……

Part One

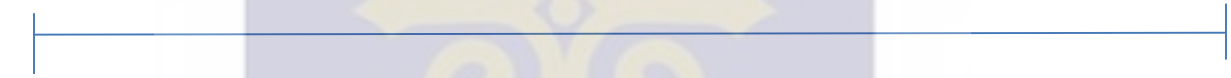
Four coded samples have been placed before you. Please rate the intensity of each attribute (**appearance, aroma, flavour**, **after-taste** and **texture** (a. mouthfeel and b.touch sensation)) using the intensity scale to assess each descriptor for each sample.

Sample code ……..

APPEARANCE

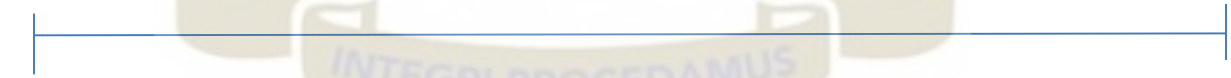
Observe each sample in terms of colour, size, shape and surface texture and rate each attribute on the respective intensity scale.

a. Yellow



none extreme

b. Cream

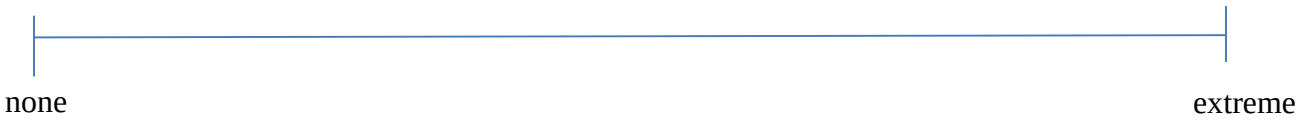


none extreme

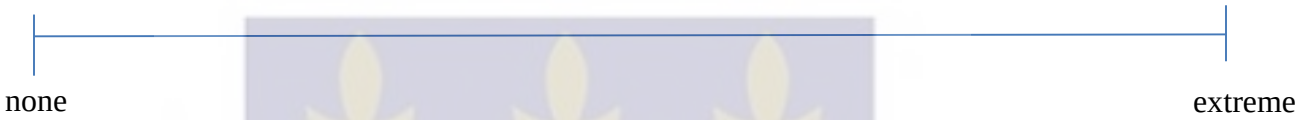
c. White

none extreme

d. Beige



e. Coarse



f. Fine

g. Dry

h. Crystalline

i. Fibrous

Sample code ……..

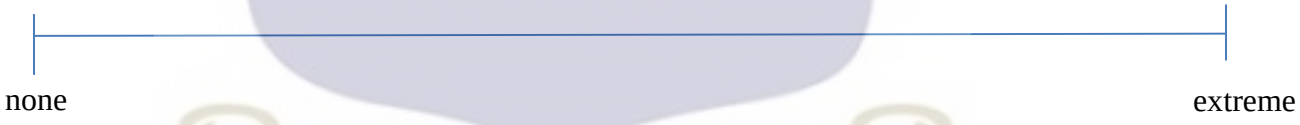
AROMA

Hold each sample close to your nose, smell and rate each attribute on the respective intensity scale.

a. Milky

b. Starchy

c. Vanilla



d. Cardboardy

e. Ricey

none extreme

f. Gari

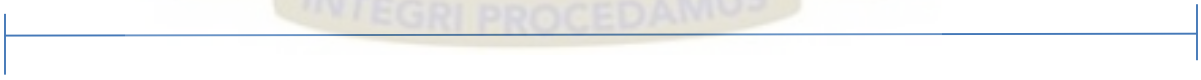
none extreme

FLAVOUR

Put about half a spoonful of each sample into your mouth while moving the sample around in your mouth to coat the whole oral cavity and all of your tongue while smelling. Then rate each attribute on the respective scale.

Note: **Take a bite of cracker and drink some water between samples at all times throughout the session.**

a. Milky



none extreme

b. Sweet

none extreme

c. Maize/Corn

d. Sour

e. Roasted corn-soy bean flour

AFTER-TASTE

a. Sweet

b. Sour

Sample code ……..

Place about half a teaspoonful of sample into your mouth, chew and cover your tongue and the whole of your oral cavity. Rate the intensity of each attribute on the respective intensity scale.

Note: **Take a bite of cracker and drink some water between samples at all times throughout the session.**

TEXTURE (a. mouth-feel)

a. Coarse

b. Fine

c. Gritty

d. Crunchy

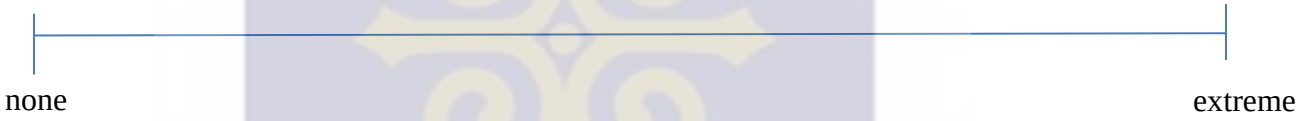
Sample code ……..

TEXTURE (b. touch sensation)

Place half a teaspoon of each sample into a tissue given you and rub the sample between your thumb, index and second fingers to feel the particles of each sample. Rate the intensity of each attribute using the respective intensity scale.

a. Coarse

b. Fine



c. Gritty

BALLOT SHEET (RECONSTITUTED SAMPLES)

Panelist Code: …… Signature: ……..... Date: ……

Part One

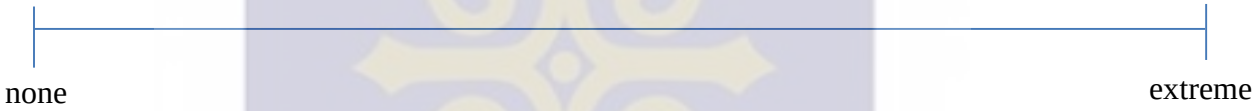
Eight coded samples have been placed before you. Please rate the intensity of each attribute (**appearance, aroma, flavour**, **after-taste** and **texture**(a. mouthfeel and b.touch sensation)) using the intensity scale to assess each descriptor for each sample.

Sample code ……..

APPEARANCE

Observe each sample in terms of colour, size, shape and surface texture and rate each attribute on the respective intensity scale.

j. White



k. Cream

l. Beige

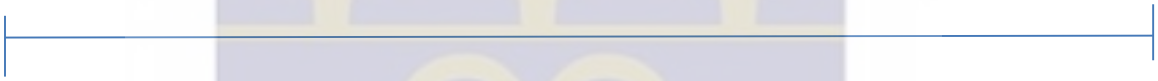
m. Particulate

fine coarse

n. Smooth

none extreme

o. Pasty



none extreme

AROMA

Hold each sample close to your nose, smell and rate each attribute on the respective intensity scale.

g. Maize-like

none extreme

h. Starchy

none extreme

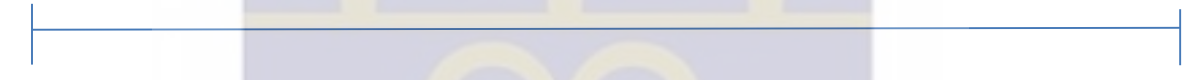
i. Fermented

none extreme

j. Cardboardy

none extreme

k. Ricey



none extreme

FLAVOUR

Put about half a spoonful of each sample into your mouth while moving the sample around in your mouth to coat the whole oral cavity and all of your tongue while smelling. Then rate each attribute on the respective scale.

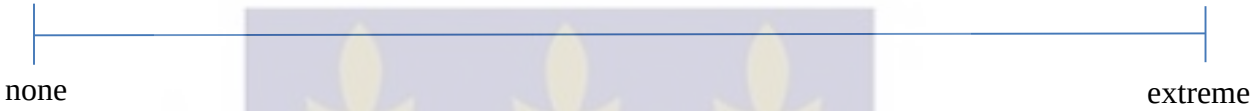
Note: **Take a bite of cracker and drink some water between samples at all times throughout the session.**

f. Rich

none extreme

g. Sweet

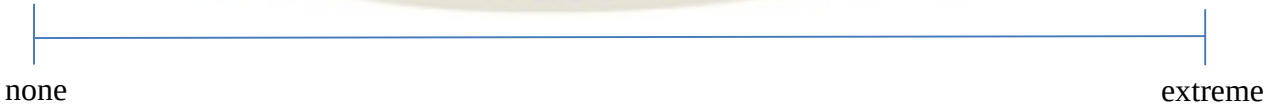
h. Maize/Corn



i. Sour

j. Bland

k. Fermented



l. Ricey

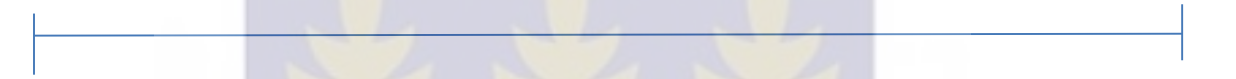
Sample code ……..

AFTER-TASTE

c. Sweet

none extreme

d. Sour



none extreme

Place about half a teaspoonful of sample into your mouth, chew and cover your tongue and the whole of your oral cavity. Rate the intensity of each attribute on the respective intensity scale.

Note: **Take a bite of cracker and drink some water between samples at all times throughout the session.**

TEXTURE (a. mouth-feel)

a. Particulate

fine coarse

b. Gritty

none extreme

c. Pasty

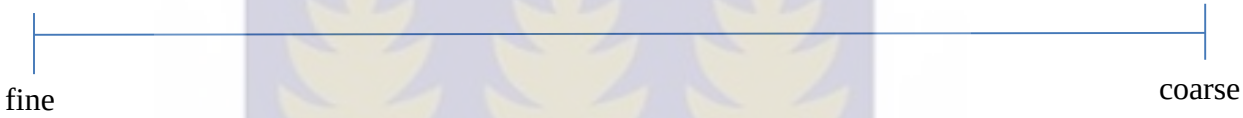
none extreme

Sample code ……..

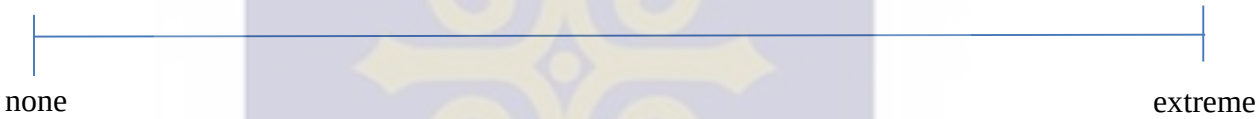
TEXTURE (b. touch sensation)

Place half a teaspoon of each sample into a tissue given you and rub the sample between your thumb, index and second fingers to feel the particles of each sample. Rate the intensity of each attribute using the respective intensity scale.

d. Particulate

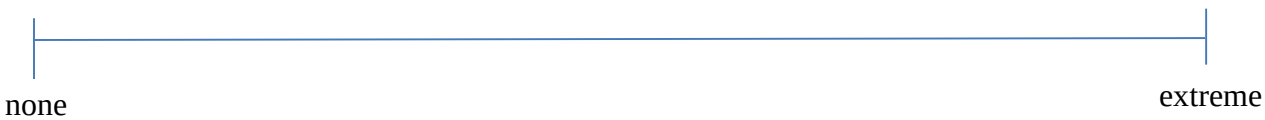


e. Gritty



f. Smooth

g. Pasty



APPENDIX B

ANALYSIS OF VARIANCE FOR pH

Source	df	Sum of squares	Mean square	F	P-value
Main Effects					
A: Fermentation Time	3	0.402	0.133912	14.64	0.027
B: Treatment	1	0.008	0.007812	0.85	0.424
Interactions					
A*B	3	0.027438	0.009146		
Error	0				
Total	7				

ANALYSIS OF VARIANCE FOR LIGHTNESS VALUE (L*)

Source	df	Sum of squares	Mean square	F	P-value
Main Effects					
A: Fermentation Time	3	14.2731	4.7577	5.06	0.108
B: Treatment	1	0.3528	0.3528	0.37	0.584
Interactions					
A*B	3	2.8231	2.8231	0.9410	
Error	0				
Total	7				

ANALYSIS OF VARIANCE FOR REDNESS VALUE (a*)

Source	df	Sum of squares	Mean square	F	P-value
Main Effects					
A: Fermentation Time	3	0.964	0.321	1.49	0.376
B: Treatment	1	0.035	0.035	0.16	0.714
Interactions					
A*B	3	0.647	0.216		
Error	0				
Total	7				

ANALYSIS OF VARIANCE FOR YELLOWNESS VALUE (b*)

Source	df	Sum of squares	Mean square	F	P-value
Main Effects					
A: Fermentation Time	3	8.312	2.771	0.75	0.590
B: Treatment	1	23.702	23.702	6.43	0.085
Interactions					
A*B	3	11.055	3.685		
Error	0				
Total	7				

ANALYSIS OF VARIANCE FOR WATER SOLUBILITY INDEX (WSI)

Source	df	Sum of squares	Mean square	F	P-value
Main Effects					
A: Fermentation Time	3	57.503	19.168	2.60	0.227
B: Treatment	1	4.968	4.968	0.67	0.472
Interactions					
A*B	3	22.115	7.372		
Error	0				
Total	7				

ANALYSIS OF VARIANCE FOR WATER ABSOPRTION INDEX (WAI)

Source	df	Sum of squares	Mean square	F	P-value
Main Effects					
A: Fermentation Time	3	2.712	0.904	1.48	0.378
B: Treatment	1	0.341	0.341	0.56	0.509
Interactions					
A*B	3	1.837	0.612		
Error	0				
Total	7				