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PROCESS DEVELOPMENT AND PRODUCT CHARACTERISTICS OF EXTRUDED RICE-SOYBEAN SNACK

BY

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DECLARATION

This is to certify that this thesis is the result of research work undertaken by Hannah Fosua Akoto towards the award of Master of Philosophy in Food Science in the Department of Nutrition and Food Science, University of Ghana.

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ABSTRACT

The eating patterns of Ghanaians are changing due to urbanization, globalization, economic trends, as well as changes in social structure as a result of the increasing number of women working outside the home. These changes in eating patterns include higher consumption of snack foods by all age groups. A snack food is often smaller than a regular meal and normally consumed between meal times. Among cereal-based snacks, wheat and corn are the most popularly used cereals as compared to rice.

Locally grown and milled rice tends to be poorly patronized by consumers in Ghana due to quality defects, so incorporating this low grade rice into a ready to eat snack would provide an avenue for using the commodity which may otherwise be underutilized. This study therefore sought to develop a rice-soybean snack from low grade rice (parboiled or raw form) using extrusion-cooking technology. Rice and partially defatted soy flour blends of composition (75:25-90:10) were obtained based on ratios that were determined using constrained mixture designs for two components. In all twenty formulations were obtained and extruded using an intermeshing co-rotating twin screw extruder at constant screw speed of 1000rpm, barrel temperature of 200^{o}C and a circular die diameter of 4mm. Evaluation of consumer sensory preference was carried out using a 9 point hedonic scale to obtain five most preferred extrudates with high percentage of rice (TMF3 (90% raw Togo Marshall (TM), 10% partially defatted soybean (PDS)); TMF4 (75% raw TM, 25% PDS); TMF5 (82.5% raw TM, 17.5% PDS); PTMF3 (90% parboiled TM, 10% PDS); PTMF4 (75% parboiled TM, 25% PDS)) for further analysis. Physicochemical analysis on the five extrudates showed that increasing amount of partially defatted soybean (PDS) and rice parboiling treatment generally decreased expansion ratio, lightness and increased hardness.

For characterization of proteins, amount of PDS and rice parboiling treatment had a substantial effect on accessible thiols, protein solubility and electrophoretic patterns. Extrudate made using 75% raw rice and 25% PDS (TMF4) showed the least protein digestibility suggesting the presence of antinutritional factor such as trypsin inhibitors. Principal Component Analysis (PCA) was used to discriminate extruded snacks based on odour and taste from data obtained from electronic nose and tongue analysis. Taste characteristics were discriminated based on umami, saltiness, bitterness, sourness and astringency. It was concluded that amount of PDS and rice treatment has a significant effect on the physicochemical properties of the extrudates.

DEDICATION

This academic piece is dedicated to my parents Mr. and Mrs. Akoto, my siblings and Mr. Victor Essel. Thank you all for the love, support and encouragement throughout my stay in school.

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1.0 INTRODUCTION

1.1 Background

Changes in eating patterns are occurring at an increasing rate due to urbanization, globalization, economic trends, and changing demographics. This is further fuelled by changes in our social structure as a result of increase in the number of mothers working outside the home. These changes in eating patterns include an increase in the consumption of snack foods in all age groups (Noor Aziah, 2012; Piernas and Popkin, 2010). The increase in the consumption rate of snacks is also ascribed to changes in life styles and trends in consumer demands for attractive appearance and convenience foods (Harris *et al*., 2007; Omwamba *et al*. 2014). A snack food is often smaller than a regular meal and normally consumed between meal times (Savige *et al*., 2007). Snack foods comprise a very large variety of items including biscuit, popcorn, crackers, nuts and extruded snacks among others. Presently, high snack consumption in Ghana is manifested by their widespread presence in open markets, supermarkets, petty trading, and restaurants, in both urban and rural areas.

Techniques for manufacturing snack foods include drying, frying, roasting, baking, extrusion and many others (Okafor, 2014). Among several processing techniques, extrusion cooking has gained widespread application in the cereal based snack food industry, because it is considered an efficient manufacturing process (White, 1994; Raiz, 2001; Mezreb *et al*., 2003, Ibanoglu *et al*., 2006). Furthermore, extrusion processing completely eliminates the trypsin inhibitor activity of the extrudates thereby improving protein digestibility (Otegbayo *et al.* 2002).

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Cereal-based snack products provide an important part of the daily nutrient and calorie intake for many consumers (Bhattacharyya, 1997; Rhee *et al.* 2004). Among the cereals, wheat and corn are most popularly used. Rice (*Oryza sativa*) based snacks including extruded rice products are less common as compared to snack products from wheat and corn. However, rice has become an attractive ingredient in the extrusion industry as a result of its bland taste, hypoallergenicity and high digestibility (Kadan *et al*., 2003). Rice (*Oryza sativa*) is a staple food for approximately half of the world's population (Zhou *et al*., 2002). According to Itani *et al.* (2002), rice is ranked as the world's number one food crop. Tomlins *et al*. (2005) observed that rice has become a staple in Ghana and much of West Africa where it serves as an important convenience food for urban dwellers. Rice has become the second most important food staple after maize in Ghana (Ministry of Food and Agriculture, 2011a) and its intake continues to increase as a result of urbanization, population growth and change in consumer habits.

Even though rice forms a major part of the Ghanaian diet, preference is for imported rice over locally produced rice. Estimates by Amanor-Boadu (2012) showed that imported rice comprises about 70% of the quantity consumed in Ghana. This is because locally grown rice is low in quality and inconsistent in terms of taste, cooking quality, cooking time and aroma (Tetteh Anang *et al*., 2011). Moreover, the excessive chalkiness and high breakage percentage of local milled rice lowers the quality and reduces milling recovery. According to Gayin *et al.* (2009), these quality defects are influenced by inappropriate post-harvest handling, poor quality planting materials and poor agronomic practices.

1.1 Rationale

Even though locally grown and milled rice is poorly patronized by consumers in Ghana due to quality defects, incorporating this low grade rice into a ready to eat snack would provide an avenue for using commodity which may otherwise be underutilized. The growing consumer demand for convenience foods including snacks can be exploited by developing a nutritious and healthy snack using locally milled low grade rice.

Increasing the protein content of snack foods may further improve their consumer appeal and acceptance. Soybean (Glycine max) is an excellent source of protein (Liu, 1999) but it is underutilized in Ghana. Like most legumes, soybean protein is limiting in methionine, tryptophan and cysteine (Wang *et al*., 2006, Shewery, 2007). On the other hand, rice which is a typical cereal has high amounts of the limiting amino acids of soybeans (Tsai *et al*., 1975, Iqbal *et al*., 2006, Shewery, 2007). Furthermore rice is also deficient in the essential amino acids lysine and threonine (Tsai *et al*., 1975, Shewery, 2007) which are high in soy proteins. Therefore blending rice and soybeans in the right ratios provides a good protein source, since they mutually complement each other. According to Nkama *et al.* (1995), protein-energy malnutrition has been identified as one of the most important problems in Africa including Ghana. This is generally because some people can hardly afford high protein foods such as animal foods. There is therefore the need for developing nutritious snacks of high protein and energy using cereal-legume combinations. This work seeks to use High temperature, short time (HTST) extrusion cooking technology to produce ready to eat and consumer acceptable snack from locally milled rice and decorticated and partially defatted soybean. The development of a ready to eat snack will diversify the usage of locally grown rice and also maximize its utilization. This could lead to the provision of

ready market for locally grown rice. It could also lead to the strengthening of food security and sustainable livelihoods among rice processors and consumers in Ghana.

1.2 Main objective

The objective of this work was to develop a rice-soybean snack using extrusion-cooking technology.

1.3 Specific objectives

- To characterize two low grade rice varieties in Ghana.
- To evaluate the physicochemical properties of the raw materials and rice-soybean flour formulations.
- To determine an acceptable formulation for the production of extruded rice-soybean snack.
- To determine the physicochemical, functional, aroma and taste characteristics of the extrudates.

2.0 LITERATURE REVIEW

2.1 Rice Agronomy

Rice (genus *Oryza*) is a semi-aquatic grass crop that grows more easily in the tropics (Hof, 2007). However, it is tolerant to hot, humid, flooded, dry, and cool conditions and grows in saline, alkaline, and acidic soils. It also grows faster and most vigorously in wet and warm conditions (Hof, 2007). It is a member of the grass family (Gramineae) and belongs to the genus *Oryza* under tribe Oryzeae (Chang, 1985).The genus *Oryza* includes 20 wild species and 2 cultivated or domesticated species or cultigens (Chang, 1985). The wild species are widely distributed in the humid tropics and subtropics of Africa, Asia, Central and South America, and Australia (Chang 1985). The domesticated rice comprises two species of food crop in the Poaceae ("true grass") family: *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice) (Owens, 2001, Linscombe, 2006 as stated in Tokpah, 2010). These plants are native to Tropical and Subtropical Southern Asia and South eastern Africa, respectively (Linares, 2002).

Among the several *Oryza* species, *Oryza sativa* is the one most commonly cultivated in the humid tropics of Asia, where it originated. Asian cultivated rice has evolved into three eco-geographic subgroups: *Japonica* (broad thick grains; round-grain), *Javanica* (roundish grains; medium-grain) and *Indica* (slender, somewhat flat grains; long-grain). *Indica* varieties account for 80% of cultivated rice and feed about 3 billion people, mainly in developing countries (UNCTAD, 2005). Even though, *Oryza sativa* was originally known as Asian rice, it is now commercially grown in 112 countries, covering six continents except Antarctica, extending from 50° north latitude to 40° south latitude and from sea level to an altitude of 3000 m (Chang 1985). At the present time, *O. glaberrima* is being

replaced everywhere in West Africa by the higher yielding *O. sativa* varieties, introduced into the continent by the Portuguese as early as the middle of the 16th century (Linares, 2002). The long history of cultivation and selection under diverse environments has caused *O. sativa* to acquire a broad range of adaptability and tolerance enabling it to be grown in a wide range of water/soil regimens from deeply flooded land to dry hilly slopes (Lu and Chang, 1980).

The rice plant develops a main stem and many tillers, which arch into many clusters of flowers bearing the grains. It is normally grown as an annual plant, although in tropical areas it can survive as a perennial plant. The rice plant develops grain clusters called panicles; the plants are cut and threshed to release the grains. The edible seed is a grain (caryopsis) 5–12 mm (0.20–0.47 in) long and 2–3 mm (0.079–0.12 in) thick (Boumas, 1985). The intact grain has an abrasive, silicaceous seed coat or husk and known as paddy or rough rice as shown in Figure 2.1.

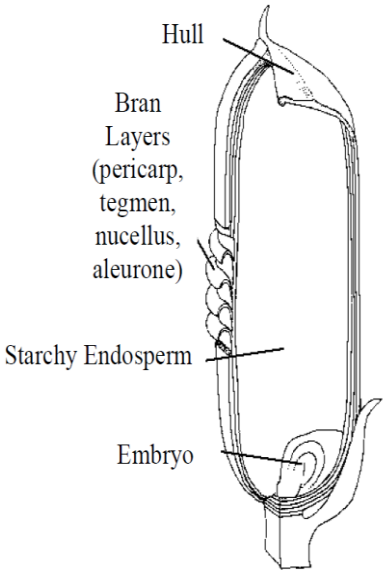


Figure 2.1: A Paddy Kernel, Showing the Major Parts

(Source: Bond, 2004)

The common forms of rice are referred to as *rough rice, brown rice,* and *milled rice.* Rough rice (paddy rice) is composed of 20% hull (the outermost layer), 10% bran and germ, and 70% starchy endosperm (Figure 2.1).

2.1.1 Origin and Distribution of Rice

The geographical site of the origin of rice domestication is not yet definitely known. According to Juliano (1993), the general consensus is that *O. sativa* rice domestication occurred independently in China, India and Indonesia, thereby giving rise to three races of rice: Sinica also known as Japonica (round-grain), Indica (long-grain) and Javanica (medium-grain). There are indications that rice was cultivated in India between 1500 and 2000 B.C. and in Indonesia around 1648 B.C (Chang, 1983 as stated in Juliano, 1993). Archaeological findings have shown that tropical or Indica rice was being cultivated in Ho-mu-tu, Chekiang Province, China at least 7000 years ago (Chang, 1983 as stated in Juliano, 1993).

Oryza glaberrima, commonly known as African rice, is a domesticated rice species. African rice is believed to have been domesticated 2,000-3,000 years ago in the inland delta of the Upper Niger River, in what is now Mali (Linares, 2002). Its wild ancestor, which still grows wild in Africa, is *Oryza barthii* (Linares, 2002).

2.1.2 Differences Between *O. glaberrima* and *O. sativa*

According to Richards (1996), minor morphological differences separate the two species of rice, making it difficult to differentiate them in the field. *O. glaberrima* has small grains

that are pear-shaped and have red bran and an olive-to-black seed coat, straight panicles that are simply branched, and short, rounded ligules. However, some *O. sativa* types also have pear-shaped grains with red bran. Other ecological differences are that African *O. glaberrima* varieties have certain negative features with respect to the Asian *O. sativa*: the seed scatters easily, the grain is brittle and difficult to mill, and most importantly its yields are lower. Nevertheless the *O. glaberrima* varieties also offer distinct advantages: the plants have luxurious wide leaves that shade out weeds and the species is more resistant than *O. sativa* to diseases and pests. African rice is more tolerant of fluctuations in water depth, iron toxicity, infertile soils, severe climates, and human neglect. However, despite the popularity of Asian rice (as a result of their higher yields), they are poorly adapted to many of the African environments where rice is grown (Somado *et al*., 2008). According to Linares (2002), these characteristics have made it worthwhile to attempt to cross both species. The new varieties, named ‘‘New Rice for Africa’’ (hence NERICA) developed in 2004 by Africa Rice Centre (WARDA, 2005), are a cross between *O. glaberrima* and *O. sativa*. They combine the hardiness of the African species with the productivity of the Asian species. Scientists at the West African Rice Development Association (WARDA) succeeded in crossing the two species by employing embryo rescue techniques that ensure the crosses are fertile and mature successfully due to high levels of hybrid vigour. Examples of NERICA rice varieties in Ghana are NERICA 1 (SAR-RICE 5) and NERICA 2 (SAR-RICE 6) (Ragasa *et al*., 2013).

2.1.3 Rice production and varietal distribution in Ghana

Rice is important to Ghana's economy and agriculture, accounting for nearly 15% of the Gross Domestic Product (GDP) and it is the second most important cereal after maize in Ghana (MiDA, 2010; Osei-Asare, 2010). The rice producing area totals about 45% of the total area planted to cereals (Kranjac-Berisavljevic, 2000). Ghana's rice production estimates range from 200,000 to 300,000 MT of paddy or roughly and 120,000 to 180,000 MT of milled rice (JICA, 2007). Rainfall remains the greatest driver of production variance. According to Kranjac-Berisavljevic (2000), and JICA (2007), rice production in Ghana can be categorized into three primary cropping types or undertaken in three different ecologies: lowland rain-fed ecology, which includes rice planted in the receding waters of the Volta and other rivers accounts for 78% of total harvested area; upland rain-fed ecology (6%), and irrigated ecology (16%).

Mechanization levels in rice production are low throughout Ghana, although most farmers hire tractor services for ploughing and harrowing. In the Northern Regions, bullock-drawn ploughs are also common. All other production and post-harvest activities are done manually, especially by the smallholders. Other constraints to production include low land-levelling of paddy fields and lack of bunds to retain rain water; inadequate supply of certified seed, fertilizers and other agro-chemicals; and inadequate credit facilities to ensure investment in productivity-enhancing technologies.

2.2 Rice preference and its utilization

Rice has become the second most important food staple after maize in Ghana and its consumption keeps increasing as a result of population growth, urbanization and change in

consumer habits. According to a report by JICA (2007), rice is cultivated in Ghana both as a food crop and a cash crop. Rice cultivation serves to meet the financial needs of the household. It also serves to sustain the food security during the lean season. There is a wide variation in rice consumer preference in Ghana on the basis of grain characteristics. Various studies show that Ghanaian consumers have a higher preference for imported rice because of its perceived higher cooking and sensory characteristics and quality (Diako *et al*., 2011; Tomlins *et al*., 2005). Diako *et al*. (2010) stated that while the appearance of raw rice is critical to consumers' choice, taste and aroma determine consumer preference for cooked rice. The study by Tomlins and his colleagues found that 86% of consumers prefer imported rice which was influenced by the consumer's gender and location, while local rice was only appealing to a "niche segment" comprising 14% (Tomlins *et al.*, 2005). The reasons given for not purchasing locally cultivated rice were poor postharvest handling and the generally perceived poor quality (Diako *et al*. 2010). Consumers therefore prefer rice imported mainly from Thailand, Vietnam, Taiwan and USA (Kula *et al*., 2009). Even though, a study by Diako *et al.* (2011) confirms that local varieties have a higher mineral content than imported varieties only a certain niche segments of health-conscious consumers purchase local brown rice. Parboiled local rice is commonly patronized in the Northern regions of the country.

In Ghana, a very small fraction of the rice crop is used as an ingredient in processed foods but the bulk is consumed as cooked rice. Rice is used in a wide range of traditional staples dishes the most common include fried rice, jollof rice, rice balls, rice porridge and plain-cooked rice. The various rice products are fundamentally different in their appearance and characteristics: plain rice is boiled rice with each rice grain still whole and independent of

the other, while 'rice balls' and 'rice porridge' may have the rice grains completely or partially broken in the product.

2.2.1 Rice as a staple food and as a source of employment

The importance of rice lies in many spheres – as food, as a source of income and employment (economy), as well as in social development and culture (Maclean *et al*. 2002)**.** According to IRRI (2009a) as stated in Appiah *et al*. (2011), rice is second of the world's most consumed cereals after wheat and before maize. The world average annual per-capita consumption of milled rice was 57.8 kg per person (1997-1999 average). This average is second to wheat, which averaged 70.8 kg per person from 1997 - 1999 for the world average. Maize averaged 19.0 kg per person as the third most consumed cereal per-capita worldwide from 1997 - 1999 (Childs, 2004).

It is estimated that rice sustains the livelihood for 100 million people and its production has employed more than 20 million farmers in Africa (WARDA, 2005 as stated in Appiah *et al*., 2011). Mohanty *et al*. (2010) estimated that 2.3 billion farmers and their households depend on rice as their main source of livelihood. It has also been stated by Hui (2007) that rice grains sustain two-thirds of the world's population, although the contribution of rice is different in the developing and developed countries, and also the types of processing are quite different. It is primarily consumed in the milled form, but there are also a number of products where rice is added as an ingredient, conferring creaminess, crunchiness, firmness and more (Rosell, *et al*., 2006). According to Hui (2007), rice-based products have often been a solution for consumers with allergenic problems due to its hypoallergenicity.

Table 2.1: Top 10 Rice Producing and Consuming Countries.

Countries	Production (metric tonnes)	Countries	Human consumption (kg per capita per year)
World	575,105,490	World	85.9
China	176,342,195	**Myanmar**	306.9
India	113,580,000	**Vietnam**	253.3
Indonesia	51,579,100	**Bangladesh**	245.4
Vietnam	34,447,200	**Indonesia**	222.6
Thailand	25,610,900	**Philippines**	156.8
Myanmar	22,780,000	**Thailand**	153.8
Philippines	13,270,650	**Nepal**	152.8
Japan	11,111,000	**India**	125.0
Brazil	10,457,100	**China**	124.1

(Source: Hui, 2007).

2.2.2 Nutrition composition of rice

Several factors affect the nutritional value of rice, such as genotype, environmental conditions during growth, crop management, storage and post-harvest processes; especially the degree of milling is paramount in contributing to the nutritional value of rice (Malik *et al*., 2002). Mbatchou and Dawda (2013) reported on proximate composition of four rice varieties cultivated in Ghana. Other studies have also reported the proximate composition of other rice varieties grown in different parts of the world. These are presented in Table 2.2.

Table 2.2: Proximate Composition of some rice varieties

Sample	**Country**	**%Moisture**	**%Fat**	**%Crude protein**	**%Crude fibre**	**%Ash**	**%Carbo-hydrate**
IR12979-24-1	Ghana	8.50	1.20	6.01	0.16	0.86	83.27
JASMINE-85	Ghana	13.50	1.10	6.82	0.76	0.93	76.89
WITA-9	Ghana	22.00	0.80	7.08	0.53	2.48	67.11
ANDY-11	Ghana	13.00	1.20	7.42	0.11	1.37	76.04
SIPI	Nigeria	18.00	0.50	1.58	2.00	1.00	76.92
FARO 14	Nigeria	7.33	0.50	6.22	1.50	1.00	83.45
BAHNG GAWK (BG)	Thailand	11.55	2.86	9.21	3.63	1.33	75.04
HAEK YAH (HY)	Thailand	12.38	2.91	7.40	4.18	1.40	75.92

(Source: Mbatchou and Dawda, 2013; Oko and Ugwu, 2010; Sompong *et al*. 2011).

Work reported by Abbas *et al*. (2011) showed the effect of milling on vitamin and mineral content of rice (Table 2.3).

Table 2.3: Effect of milling on vitamin and mineral content of rice

Extraction Rate%	100 Rough	82 Brown rice	72 Milling rice
Mineral content			
Calcium (mg/g)	0.3	0.1	0.1
Phosphorus (mg/g)	3.1	3.2	1.5
Zinc (ppm)	2.4	3.3	18.0
Iron (ppm)	38.0	8.8	4.1
Copper (ppm)	2.8	2.7	2.2
Vitamin Content			
Thiamine (µg/g)	2.8	2.4	1.6
Riboflavin (µg/g)	0.5	0.3	0.2
Pyridoxine (µg/g)	5.1	5.1	1.9
Biotin (µg/g)	91.0	48.0	43.0

Source: Abbas *et al*. (2011).

Cereal such as rice is deficient in lysine and threonine but have sufficient sulphur containing amino acids which are limited in legumes (Wang and Daun, 2006; Iqbal *et al.*, 2006; Shewry, 2007) whereas legumes are rich in lysine and threonine. Therefore combining them in product development is highly beneficial, since there is an increase in both the quantity and quality of the protein mix and also in some minerals such as iron (Pastor Cavada *et al*., 2011).

2.3 Rice Processing

Rice harvested from the field is threshed to produce paddy rice or rough rice, where the kernel is still within the hull or husk. The paddy rice is dried, either mechanically or by open-air. It is then cleaned to eliminate all straw, stones and other foreign objects that are larger or smaller than the rice kernels. The cleaned paddy rice may be milled thus removing the husk or hull to obtain brown rice which may be consumed as it is. The brown colour of the rice is caused by the presence of the bran layers, which are rich in minerals and vitamins especially the B-complex (Hui, 2008). This brown rice can be further milled into white grain rice by stripping off the bran of the endosperm and separating out broken kernels and other altered kernels such as portions of damaged and chalky kernels (Wood, 2002). The milled rice economic value is dependent on the proportion of broken rice kernels in the bulk (Monsoor *et al*., 2004) leading to rice grading. The milled rice can then be processed into several products such as table rice, expanded rice, snacks and more. The bran obtained can be used for protein concentrate, rice oil and biodiesel (Ju *et al.*, 2005). According to Shaikh *et al*., (2013), the husks or hull is normally used for silica ash or as a source of energy. The broken kernels separated from the whole grain can further be milled into rice flour (Hui, 2007) which is normally used for rice porridge, bread, biscuit and more.

2.3.1 Parboiled rice

Parboiling of rice is a process that involves the hydrothermal treatment of paddy before milling. It is obtained by soaking paddy rice at a temperature below the gelatinization temperature of the starch, steaming with or without pressure for several minutes, and cooling and slowly drying to minimize the formation of cracks (Hui, 2007). The resulting

rice is slightly yellowish, although its colour intensity decreases after cooking. It has been established that parboiled rice presents a superior nutritional value in relation to milled rice, mainly due to the retention of minerals and water-soluble vitamins (Juliano, 1985, Pedersenet *et al.*, 1989). Parboiling has been found by Wolever *et al*., (1986) to reduce the glycaemic index of rice, particularly that of high and intermediate amylose rice. Work carried out by Sareepuang *et al*., (2008), showed that the parboiling process significantly ($p<0.05$) increased protein and ash contents. At least theoretically, the higher retention of micronutrients in parboiled rice has been attributed to their solubilization and migration to the centre of the grain during the starch gelatinization process (Juliano, 1985). According to Barbiroli *et al*. (2013), parboiling stiffens the protein network in rice and makes starch more accessible to hydrolysis.

Drying of the gelatinized grain leads to a clear and harder endosperm, more resistant to breaking during milling (Hoseney, 1994). Parboiled rice requires longer cooking time because the gelatinized starch is more resistant to water absorption and the cooked rice is firmer and less sticky (Sinha, 2007). It is also used as a raw material for the subsequent production of canned rice, and other processed rice products. Puffed-rice products are also obtained from parboiled rice subject to pressure steaming, leading to its higher volume expansion (Sinha, 2007).

According to Bhattacharya *et al*. (1985) and Larsen *et al*. (2000), the advantages of the parboiling process stems from the gelatinization of rice starch which leads to hardening of rice kernel. The preservation of parboiled paddy and milled rice is longer and better than in the raw state. Germination is no longer possible and the endosperm has a compact texture making it resistant to attack by insects and microorganism (Matz, 1991).

Tomlins *et al*., (2005) stated that parboiling rice is a very common practice in the northern part of Ghana. It also causes the rice starch to become gelatinized resulting in harder, glassier rice which reduces breakages during milling. Most urban consumers in Ghana do not prefer parboiled rice especially due to change in rice colour and so it is difficult for the product to penetrate the southern markets and compete effectively with imported rice.

2.4 Rice Quality

Although rice forms a major part of the Ghanaian diet, locally grown rice is not much patronized because it has variable quality characteristics. Several factors account for the variability in rice quality (Tomlins *et al*., 2005). Rice quality can be considered in terms of paddy or milled form and it is mainly graded based upon several criteria, among which are starch (amylose/amylopectin) content, flavour, degree of milling, kernel size, percentage of broken kernels in milled rice (United States Standards for Rice, 2005). Although broken and whole rice kernels have similar starch yields and protein contents, broken kernels have been reported to be more susceptible to lipid hydrolysis than whole kernels (Monsoor and Proctor, 2003; Wang *et al*., 2002).

2.4.1 Quality characteristics of Paddy Rice

The quality characteristics of paddy rice are determined by factors such as the environmental weather conditions during production, crop production practices, soil conditions, harvesting and postharvest practices (Brooker, 1992). Some characteristic which determine the quality of paddy rice are; Moisture content of paddy. Paddy is at its optimum milling potential at moisture content of approximately 12-14% wet weight basis.

Grains with high moisture content are too soft to withstand hulling pressure which results in grain breakage and possibly pulverization of the grain (Badi, 2013). Grain that is too dry becomes brittle and has greater breakage. Therefore the optimal stage to harvest grain is at about 20-24% grain moisture or about 30 days after flowering (Farid *et al*., 2014). Purity degree refers to the presence of foreign materials such as chaff, stones, weed seeds, soil, rice straw, and stalks in the paddy (Badi, 2013). Unclean paddy increases the time taken to clean and process the grain. Foreign matter in the grain reduces milling recoveries and the quality of rice and increases the wear and tear on milling machinery. According to Sun and Siebenmorgen (1993), varietal impurity arising from a mixture of varieties results in different sizes and shape making it difficult to adjust hullers, whiteners and polisher to produce whole grains. These result in low initial husking efficiencies, a higher percentage of re-circulated paddy, non-uniform whitening, and lower grade of milled rice. Overexposure of mature paddy to fluctuating temperature and moisture conditions leads to development of cracks in individual kernel (Ram, 2009). Ram (2009) again stated that cracks in the kernel are the most important factor contributing to rice breakage during milling since it reduces head rice recovery. Another common defect is the presence of immature grains. The amount of immature paddy grains in a sample has a major effect on head rice yield and quality (Ram, 2009). According to Badi (2013), the immature rice kernels are very slender and chalky and this results in excessive production of bran, broken grains and brewer's rice.

2.4.2 Grain Quality

Rice is the only cereal crop cooked and consumed mainly as whole grains, and therefore quality considerations are very important (Hossain *et al*., 2009). According to Horna *et al*. (2005), grain quality is one of the important selection criteria by farmers and consumers of rice. Grain quality is not just dependent on the variety of rice, but also depends on the crop production environment, harvesting, processing and milling systems (Gayin *et al*., 2009). Consequently, the quality of rice grain is a complex character composed of many components such as physical, chemical and nutritional. Quality also depends on the consumer and the intended end use for the grain.

2.4.2.1 Physical quality characteristics of rice grain

The physical quality of milled rice is characterized by a combination of desirable and measurable characteristics. In line with the market requirements, the characteristics used to classify rice into grades are:

Chalkiness: Rice is referred to be chalky when the milled rice kernel is opaque instead of being translucent. Chalkiness disappears upon cooking and has no effect on taste or aroma; however it downgrades the quality and reduces milling recovery of rice grain (Gummert, 2010).

Milling degree is the measure of percentage bran removed from the brown rice kernel (Tokpah, 2010). Milling degree influences the colour and also the cooking behaviour of rice as under milled rice absorbs water slowly and does not cook well (Mandal, 2012).

Head rice is the weight of head grain or whole kernels in the rice lot. It normally includes broken kernels that are 75-80% of the whole kernel. High head rice yield is one of the most important criteria for measuring milled rice quality.

Damaged grains are whole or broken grains showing damage due to moisture, pests and diseases. Grain whiteness is a combination of varietal and physical characteristics as well as the degree of milling (Mutters *et al*., 2009). Grain whiteness is measured by a colourimeter or as an index number from a whiteness meter. It is often used to determine milling degree. According to Gummert (2010), brown rice gives a reading of approximately 20 on the whiteness meter, whereas well-milled rice is close to 40.

Rice is marketed according to three grain sizes and shapes. Thus long grain (3 to 4 times as long as it is wide), medium grain (2 to 3 times as long as it is wide) and short grain (short and almost round) are recognized. Kernel dimensions are primary quality factors in most phases of processing, drying, handling equipment, breeding and grading (Owen, 2001). The marketing values of rice as an agricultural product depend on its physical qualities after processing. The percentage of whole grain is the most important parameter for the rice processing industry.

2.4.2.2 Chemical quality characteristics of rice grains

Gelatinization temperature can be used to determine the time required for cooking milled rice. It is affected by the temperature during ripening (Mujumdar, 2014). According to Mutters *et al*. (2009), a high ambient temperature during development results in starch with a higher gelatinization temperature. Gelatinization temperature of milled rice is determined by its alkali spreading value (Mutters *et al*., 2009). In many rice-growing countries, there is

a distinct preference for rice with intermediate gelatinization temperature. Based on gelatinization temperature, rice can be classified as low when the temperature range is between 55-69^{o}C with alkaline spreading value having a range of 6-7 (Anonymous 1, Quality; Ram, 2009). Intermediate has a temperature range of 70-74^{o}C and alkaline spreading value of 4-5 (Ram, 2009). Rice has a high gelatinization temperature when it has a temperature above 74^{o}C with an alkaline spreading value of 2-3 (Anonymous 1, Rice Quality).

Starch is a polymer of glucose and the starch granules in rice are very small 2-8µm and polygonal in shape (Wang *et al*., 2002). Ram in 2009 stated that the amylose content of rice starches usually ranges from 15 to 35%. High amylose content rice shows high volume expansion and high degree of flakiness (Mutters *et al*., 2009). High amylose grains cook dry, are less tender, and become hard upon cooling (Ram, 2009). In contrast, low-amylose rice cooks moist and sticky (Anonymous 1, Rice Quality). Intermediate amylose rice is preferred in most rice-growing areas of the world, except where low-amylose japonicas are grown (Mutters *et al*., 2009). Based on amylose content, milled rice is classified as waxy (1-2% amylose), very low amylose content (2-9% amylose), low amylose content (10-20% amylose), intermediate amylose content (20-25% amylose) and high amylose content (25-35% amylose) (Lawal *et al*., 2011). Mutter *et al*. (2009) stated that amylose content of milled rice can be determined by using the colorimetric iodine assay index method.

Gel consistency measures the tendency of the cooked rice to harden after cooling (Shinde *et al*., 2014). Within the same amylose group, varieties with a softer gel consistency are preferred, and the cooked rice has a higher degree of tenderness. Harder gel consistency is associated with harder cooked rice and this is evident in high-amylose rice. Hard cooked

rice also tends to be less sticky (Mandal, 2012). Gel consistency is determined by heating a small quantity of rice in a diluted alkali (Mutters *et al*., 2009). In terms of gel consistency, measurement ranges and category are as follows: soft rice has a consistency of 61-100mm, medium rice has a gel consistency of 41-60mm and hard rice has a gel consistency of 26-40mm (Mandal, 2012).

Table 2.4: Physical and chemical properties of ten local rice varieties in Ghana.

Variety	Chemical properties (%)					Physical properties		
	Amylose	Protein	Moisture	Ash	Fat	Grain classification		Colour
						Size	Shape	
Ex-Hohoe	22.70	5.60	11.60	0.70	0.70	Long	Slender	White
Marshall	19.30	5.93	12.20	0.60	0.70	Medium	Slender	White
Jasmine 85	20.20	5.81	11.60	0.60	0.50	Long	Slender	White
Viwonor	30.50	7.32	11.39	0.44	1.56	-	-	Red
Bouake 189	31.60	8.08	10.41	0.58	0.11	-	-	-
Wita 9	28.39	7.53	13.78	0.52	1.23	-	-	-
TOX 3107	27.75	7.98	10.75	0.46	1.25	-	-	-
Jet 3	-	-	-	-	-	-	-	-
Perfumed	25.60	8.55	12.91	0.47	0.36	-	-	-
Emo korkor	-	-	-	-	-	-	-	Red

Source: Awudi, (2013) (MPhil thesis).

Table 2.5: Amylose content and colour of four rice varieties grown in Ghana.

Variety	L*	a*	b*	Amylose (%)
Ex-Baika	73.98 ± 0.03^{c}	1.46 ± 0.04^{c}	8.36 ± 0.15^{a}	17.5 ± 0.91^{b}
Ex-hohoe	69.04 ± 0.19^{a}	6.43 ± 0.05^{e}	10.54 ± 0.06^{c}	22.7 ± 0.06^{d}
Jasmine 85	72.50 ± 0.38^{b}	1.91 ± 0.10^{d}	9.00 ± 0.06^{b}	20.2 ± 0.02^{c}
Togo marshall	74.22 ± 0.42^{c}	1.16 ± 0.10^{b}	9.08 ± 0.10^{b}	19.3 ± 0.01^{c}

Source: Diako *et al*. (2011).

2.5 Soybean

2.5.1 General information on soybean

Legumes such as beans, peas, nuts, and soybeans play an important role in the traditional diets of many regions throughout the world. Soybeans (Glycine max) belonging to the family leguminosae is one of the oldest cultivated crops of the tropics and sub-tropical regions, and one of the world's most important sources of protein and oil (Arogundade *et al*., 2009). In general, soybeans comprise approximately 8% hull, 90% cotyledon, 2% hypocotyl axis (Cheftel *et al*., 1985 as stated in Eshun, 2009). The soy cotyledons contain the highest percentage of both protein and oil, whereas the hull has the lowest values of these components (Oomah *et al*., 1996). The seeds vary in shape and colour depending on the cultivar. In shape, they can be spherical to flatten while the colour varies from white, yellow and brown to black. It is the richest sources of protein among the plant foods. Soy protein provides several functionalities such as water-holding, binding, and emulsifying properties (Arrese *et al*., 1991; Liu, 1997). Soy protein lowers blood serum cholesterol in high cholesterol individuals and decreases the risk of coronary heart disease (Anderson *et al*., 1995; Messina, 1999, 2001). Work reported by Kennedy (1995); Gallagher *et al*., (2000) and Trock *et al*., (2000) show that soy protein can also decrease the incidences of breast and prostate cancer and inhibit bone resorption, partially because of the presence of isoflavones. Soy oils contain a significant amount of unsaturated acids: α -linolenic acid, known as omega-3 acid, linoleic, γ-linolenic and arachidonic acid, known as omega-6, oleic acid known as omega-9 acid, are important in the human nutrition (Nikolic *et al*., 2009; Olguin *et al*., 2003). Such health benefits have increased the interest in incorporating soy into food products.

2.5.2 Uses of Soybean

Soybeans in the form of full fat flour, partially defatted, concentrate, isolate and texturized have been used in a wide range of food products. According to Tunde-Akintunde (2000), soybean can be processed into soy milk, a valuable protein supplement in infant feeding, soy curds and cheese. It is also used in the production of soy sauce, tempeh, miso, natto, making of candies and ice cream and soybean flour which could be mixed with wheat flour to produce a wide variety of baked goods such as bread and biscuits (Onwueme *et al*., 1999). In Ghana, soybean is becoming an important crop due to its high protein and oil content. It is used in the production of soy khebab, soy paste, cooking oil, weanimix, and non-dairy milk especially for lactose intolerant consumers and the more.

2.5.3 Anti-nutritional factors

According to Liener (1994) and Liu (1997), the anti-nutritional factors (ANFs) in soybean are often associated with the low acceptance of soybean products as they also inhibit protein digestibility. These mainly consist of heat labile trypsin inhibitors, lectins, goitrogens, and phytates. In order for the nutritional value of soybean meal to be maximised, the anti-nutritional factors need to be inactivated or minimised. Research by Akpapunan *et al*. (1979) also shows that low-molecular weight oligosaccharides, primarily raffinose, stachyose and verbascose present in most legume seeds of which soybean is not of exception are linked to flatulence. According to Ndubuaku *et al*. (1989), these can be reduced through fermentation, removal of seed coat (decorticating), soaking in water, germination and cooking with a mixture of sodium carbonate and bicarbonate.

2.5.4 Soybean quality

Protein quality of soybean depends on two parameters: protein digestibility, and the amount of anti-nutritional factors (Parsons *et al.,* 1991). Temperature is critical in the production of soybean in order to deactivate the anti-nutritional factors naturally occurring in the raw soybeans. Inadequate heating fails to completely destroy the anti-nutritional factors. An experiment carried out by Caprita *et al*., (2010) revealed that urease index (UI) is useful to determine whether soybean meal has been heated enough to reduce the anti-nutritional factors.

The routine determination of protein digestibility and anti-nutritional factors is difficult especially in the daily production of soybean product and can therefore be replaced with indirect tests such as urease index (UI), protein dispersibility index (PDI), nitrogen solubility index (NSI) and KOH protein solubility (PS).

2.5.5 Soybean and its application as a fortificant

One of the cheapest and less complicated way to curb the Protein Energy Malnutrition (PEM) in the developing world including Ghana is through food fortification of plant origin.

Soybean has great potential as food because of its high levels of good quality protein and oil. It contains all the macro nutrients required for good nutrition, complete protein (40 g/100 g), soluble carbohydrate (18 g/100 g), dietary fiber (15 g/100 g) and fat (18 g/100 g) as well as vitamins and minerals (Liu, 1997; Singh *et al*., 2009). Soybeans supply all nine essential amino acids and have cholesterol reducing and anti-carcinogenic properties (Riaz, 1999). According to Caprita *et al*., (2010), soybean is the only vegetable food that contains

complete protein. Its high level of threonine and the high digestible lysine content complements the lysine deficiency of rice grains used in food products.

2.6 Snack intake

Snacks are small portions of food normally consumed in between meal times. Over the past few years in Ghana, there has been a shift in the food consumption patterns from traditional meal habits to processed foods like snacks (Steiner-Asiedu *et al*. 2012). The consumption of snacks has considerably increased because of changes in life styles and based on consumers demand for convenience foods (Harris *et al*., 2007, Omwamba *et al*. 2014).

2.6.1 Types of snacks

Snack processors use specific unit operations and different technologies to produce and classify snacks. According to Huber *et al*., (1990), snacks can be classified as follows: (i) first generation snacks: snacks in this category include all the natural products used for snacking, nuts, potato chips and popcorn. It is also obtained from whole grains combined with moisture content, cooking temperature and drying (Huber *et al*., 1990); (ii) second generation snack: majority of the snacks fall in this category. All the single ingredients snacks, simple shaped products like corn tortilla chips and puffed corn curls and all directly expanded snacks are included in this category; (iii) third generation snacks: these are not expanded through extrusion process and are therefore known as pellets or half cooked products (Serna-Saldivar, 2012). They normally expand through a process of deep-frying or hot air, or with the use of microwaves, just before consumption. Although they have an

additional process for expansion, these products present great advantages in transport and storage (Huber *et al*., 1990).

Out of these extruded snacks are important part of many consumers' daily nutrient and calories intake (Teltweiler, 1991).

2.7 Extrusion processing

Extrusion combines several unit operations such as mixing, kneading, cooking, shearing, shaping and forming (Akdogan, 1999). In extrusion cooking, the extruder transforms the starchy ingredients into a dough like melt under pressure (Serrano, 1997). This allows the cooked mass to be forced through small die openings in order to form a shape and expands to its final shape. A cutting device reduces the continuous stretch into a biting size (Moore, 1994). Extrusion cooking of starchy materials has become a very common technique to obtain a wide range of products, such as snacks, breakfast cereals, baby foods, confectionery, bakery products, pastas, pet food and meat analogues products (Bouzaza *et al*., 1996; Pansawat *et al*., 2008). It is not only a high temperature short time process, but also a versatile one especially with respect to ingredient selection to obtain a wide range of snack products such as direct expanded snacks, co-extruded snacks and indirect expanded snack products. The production of snacks through extrusion represents a great achievement for the Food Technology area as it efficiently converts crude flours into products with different shapes, flavours and long shelf-life. The advantages of this cooking process are based mainly on the fact that it is a high temperature short time (HTST) process, which minimises the degradation of food nutrients by heat while improving digestibility by gelatinising starch, denaturing protein and deactivating undesirable compounds, such as

enzymes and non-nutritional factors (Alonso *et al*., 2000a; Shimelis *et al*., 2007). A high temperature, short time (HTST) procedure is one which uses short residence time, high temperature, high pressure, large shear forces and intensive mixing during the process (Zheng *et al*., 1994). Extrusion cooking has become one of the most popular technologies worldwide for processing a number of food products due to its versatility, high productivity, low operating costs, energy efficiency and shorter cooking times (Frame, 1994; Harper, 1981; Smith *et al*., 1992). Cereals have excellent expansion properties because of their high starch content and are well suited to thermal extrusion (Singh *et al*., 1994). According to Rosell and Marco (2008) and Bryant *et al*. (2001), the unique properties of rice such as its hypoallergenicity, ease of digestion and bland taste make it a very desirable grain for new extruded food product development.

2.7.1 Characteristics of extruded products and their quality indices

Despite increased use of extrusion processing due to its advantages especially low cost, it is still a complicated process that has yet to be mastered (Desrumaux *et al*., 1999). Small variations in processing conditions affect process variables as well as product quality such as crispness, hardness and more. Product quality can vary considerably depending on the extruder type, screw configuration, feed moisture content, residence time, screw speed, feed rate and temperature profile in the barrel session (Harper, 1981; Baike *et al*., 2004; Ding *et al*., 2005).

The effect of extrusion variables on the properties of extruded cereals has been studied extensively (González *et al*., 2000; Kokini *et al*., 1992; Mason *et al*., 1986). The texture of expanded cereal based snacks is determined mainly by extrusion conditions such as

temperature profile in the barrel session, screw speed and feed moisture content (Ding *et al*., 2006). It is well known that the addition of legumes to cereals produces an increase in both the amount and quality of the protein mix (Young, 1991). This addition represents an economic way to improve the protein value of cereal-based foods (Messina, 1999). Starch is the main component of directly expanded products and the extent of starch transformation plays an important role in the functional properties of the final product. Extrusion conditions, characteristics of the starch granule and presence of other components such as protein, fibers and sugars directly affect the degree of transformation (Chanvrier *et al*., 2007). For example, molecules that readily hydrate (such as sugars and salts) may restrict water available to starch and reduce the degree of gelatinization (Tester *et al*., 2003). Variation in water addition is one processing parameter known to change the degree of transformation of the matrix, leading to differences in starch digestibility and in microstructure (Karkle *et al*., 2010; Yagci *et al*., 2010). Chanvrier *et al*. (2007) have suggested that extrudate microstructure may be used to control starch susceptibility to enzymatic action, however systematic studies on the relation between these two are lacking. Work carried out by Chaiyakul *et al*. (2008) showed that increasing of protein content significantly increased hardness and crispness intensity but less sticky mouth feel coating. Increasing feed moisture content resulted in increased final extrudate hardness, crispness and brittleness but reduced sticky mouth coating and colour.

2.7.2 Physicochemical Characteristics of extruded products

2.7.2.1 Colour

Colour is an important visual characteristic of food, and it also affects consumer preference and purchase decisions. According to Tiwari *et al*. (2008) and Esteve *et al*. (2005), colour correlates well with other physical, chemical and sensory quality indicators in food. Thus it plays a major role in the assessment of internal quality in the food industry and in food engineering (Alcicek *et al*., 2012; Mancini *et al*., 2005).

According to a work carried out by Hagenimana *et al*. (2006), changes in colour in relation to extruded product are mostly dependent on temperature and moisture content. The higher the feed moisture content, the brighter was the colour of the extrudates which were characterized by a high L* value and low a* value. Decreasing moisture reduced the lightness due to different competing effects during the process. High temperatures in combination with low water content are known to favour the Maillard reaction between reducing sugars and free amino groups. Chaiyakul *et al*. (2008) showed that increasing protein content or barrel temperature, or decreasing feed moisture resulted in decreasing of L*, while a* and b* values were increased.

2.7.2.2 Water absorption index (WAI)

Anderson *et al*. (1969) stated that the WAI measures the amount of water absorbed by starch and can be used as an index of gelatinisation. Altan *et al*. (2008), observed that the WAI measures the volume occupied by the granule or starch polymer after swelling in excess water. Work carried out by Hagenimana, *et al*., (2006) showed that the highest values of WAI of an extruded snack was obtained at 19–22% moisture content. This was

because moisture, acting as a plasticizer during extrusion cooking, reduced the degradation of starch granules and this resulted in an increased capacity for water absorption. However, at lower moisture content (16%) and an increase in extrusion temperature of 160^{o}C decreased WAI and this was probably due to an increase in starch degradation or starch decomposition as confirmed by Pelembe *et al*. (2002). Ding *et al*. (2006) also stated that the WAI decreases with increasing temperature if starch melting or dextrinization prevails over the gelatinization phenomenon.

2.7.2.3 Water solubility index (WSI)

WSI measures the amount of soluble components released from the starch after extrusion cooking (Ding *et al*., 2006) and it is often used as an indicator of degradation of molecular components (Kirby *et al*., 1988). WSI is also reported to be related to the presence of soluble molecules that have sometimes been attributed to dextrinization (Colonna *et al*., 1989). Work carried out by Hagenimana, *et al*. (2006) showed that low moisture content in extrusion cooking caused an increase in the amount of degraded starch granules resulting in an increased formation of water-soluble products. This phenomenon is caused by greater shear fragmentation of the starch during extrusion at low moisture contents. Nevertheless, extrusion of non-waxy, high amylose rice has been reported to result in low WSI (Pan *et al*., 1992). Furthermore, results from work carried out by Altan *et al*. (2008) showed that WSI increased significantly ($P < 0.05$) with increasing screw speed. A direct comparison of WSI values in the literature is difficult due to the difference in processing conditions and raw material used.

2.7.2.4 Expansion ratio

According to Guy and Horne (1988) and Harper (1981), when starch is extrusion-cooked, expansion is dependent on the formation of a starch matrix that entraps the water vapour, resulting in formation of bubbles. Padmanabhan and Bhattacharya (1989) explained that there are two dominant forces that causes expansion of extrudates. One is the elastic force and the other is the bubble growth force due to water vapour pressure. The dough moisture flashes off as steam at the die exit and causes expansion of extrudates on rapid extrusion at high temperatures. Ding *et al*. (2006) in the work also stated that the feed moisture content was found to have the greatest effect on the expansion of the extrudate. Increased feed moisture content during extrusion may reduce the elasticity of the dough through plasticization of the melt, resulting in reduced specific mechanical energy (SME) and therefore reduced gelatinization, decreasing the expansion ratio of the extrudate (Ding *et al*., 2006). Furthermore, Liu *et al*. (2000) explained that the increased feed moisture content reduced friction between the feed material, screw and barrel and also had a negative impact on the starch gelatinisation and reduce the product expansion. Moreover, Suksomboon *et al.* (2011) in the work stated that increased barrel temperature led to a sharp increase in expansion ratio value at all moisture content and screw speed ($p < 0.05$). Screw speed was also observed to have a slight effect on the expansion ratio of snacks (Suksomboon *et al.* 2011). Thus increased screw speed caused a slight decrease in expansion ratio. Launay *et al*. in 1983 explained this to be that the higher shear resulting from the higher screw speed reduces the melt viscosity of the feed material resulting in decreased expansion ratio.

2.7.2.5 Bulk density (BD)

The bulk density (BD) is an index of the extent of puffing. According to Ding *et al*. (2005), feed moisture and protein content have been found to be the main factor affecting the bulk density of extrudates. Previous work carried out by Hagenimana, *et al.*, (2006) showed that the lowest BD value were obtained when rice flour was extruded at a lower moisture contents and higher temperatures, whereas the highest value was obtained at higher moisture contents and lower temperatures. On the contrary, Suksomboon *et al.* (2011) in the work stated that the bulk density of snacks increased directly with feed moisture at all barrel temperatures and screw speeds ($P < 0.05$). This is because feed moisture had an influence on the reduction of elasticity characteristics and gelatinisation of the starch-based materials (Fletcher *et al*., 1985). Suksomboon *et al.* (2011) again stated that high barrel temperature led to a decrease in bulk density of extrudates ($P < 0.05$). This is because an increase in barrel temperature would increase the degree of superheating of water promoting bubble formation and also a decrease in melt viscosity leading to increased expansion that caused a decrease in density of extrudates. Bulk density values were found to decrease with an increase with extrusion temperature and screw speed and this is probably due to starch gelatinization. According to Case *et al*. (1992), increase in gelatinization leads to an increment in the volume of an extruded products while decreasing the bulk density of the extruded products.

2.7.2.6 Texture

Mechanical properties of cereal (starch-based) extrudates are perceived by the final consumer as criteria of quality. Texture quality has an influence on taste sensory

evaluation, and thus on the acceptability of the product. Characteristics that have great influence on acceptability are crispness, elasticity, hardness and softness. Chaiyakul *et al*. (2008) found that an increase of protein content from 20 to 30%, resulted in significant ($p \leq 0.05$) increase in hardness, crispness and noise intensity. These results were similar to the instrumental measurement, for which breaking strength index increased as protein content increased. Similarly Ding *et al* in 2005 concluded that extrudates from wheat had a higher breaking strength as compared to extrudates from rice and this was due to the presences of the gluten protein in wheat (Harper, 1981).

Increasing feed moisture content (>30%) led to an increment in breaking strength index (Ding *et al.,* 2005). This was due to the collapsing of the expanded product as a result of high moisture content thereby becoming harder. According to work carried out by Faller and Heymann (1996), it was noted that low moisture (19%) potato extrudates were harder and crispier than high feed moisture (25%) samples. Murray (2001) also found that brittleness of maize based extrudates increased progressively with increased feed moisture content. Singh *et al*. (2007) noted that the hardness of rice-pea grits extrudates decreased with the increase in feed moisture content from 18 to 24 %. Furthermore, Chaiyakul *et al*. (2008) noted that decrease in barrel temperature resulted in an increase in breaking strength index.

2.7.2.7 Microstructure

Density, porosity and pore size distribution, are very useful properties for food process design, since they characterize the texture and quality of foods such as snacks. Moraru *et al*. (2003) stated that macro/microstructure formation in extrusion processes is the

consequence of several overlapping events including biopolymer structural transformations (starch gelatinization and/or protein denaturation), nucleation, die-swell, cell growth, and cell collapse. The microstructure and morphology of extruded foods and their quality, is significantly determined by extrusion variables such as screw configuration, feed moisture, temperature profile in the barrel session, residence time, screw speed and feed rate as well as the ingredient selection. According to Wang *et al*. (2005), water acts both as a plasticizer for melt formation and as a blowing agent for expansion during conventional steam-based extrusion cooking. When the melt passes through the extruder die to the outside, it undergoes a sudden pressure drop resulting in water vapour nuclei generation in the melt. These cells grow in size as additional water vapor flashes off. Research work by Winoto (2005) showed that supercritical CO_2 (SC-CO_2) and die diameter had effect on product morphology of the extrudates. The work showed that as the die diameter decreases from 5.9 to 2.9 mm, the cross sectional expansion and the number of cells increased whereas the average cell size of the extrudate decreased.

2.7.2.8 Protein digestibility

The utilization of legumes such as soybean is limited due to the presence of certain heat labile and heat-stable antinutritional factors (ANF) that exhibit undesirable physiological effects (Pusztai *et al*., 2004). Among these are phytates, polyphenols, enzyme inhibitors (trypsin, chymotrypsin, and α-amylase) and hemagglutinins. On the other hand, Shahidi (1997) reported that some antinutrients might exert beneficial health effects at low concentration. Therefore several attempts have been made using different food processing methods such as soaking, germination, decortication, fermentation, autoclaving, radiation,

roasting, supplementation with various chemicals and enzymes and extrusion cooking in order to improve upon their digestibility and nutritive value (Fernandez *et al*., 1997; Alonso *et al*., 1998; Alonso *et al*., 2000a, Ramakrishna *et al*., 2006). The presence of antinutritional factors in legumes is shown to be reduced at varying degrees based upon the food preparation method. Kalpanadevi *et al*., (2013) in their work noted that the combination of germination followed by autoclaving completely eliminated the total free phenolics, tannins, hydrogen cyanide, phytic acid, trypsin inhibitor, oligosaccharides and phytoheamagglutinating activity of *Vigna unguiculata* subsp. *unguiculata* thereby increasing its protein digestibility and improving its protein quality. Also, Abd El-Hady, *et al*., (2003) indicated that soaking and extrusion significantly decreased antinutrients such as phytic acid, tannins, phenols, α-amylase and trypsin inhibitors. Martín-Cabrejas *et al*. (2009) in their work showed that cooking after soaking of some legumes promoted higher protein digestibility (from 7% to 12% improvement).

2.7.2.9 Odour and taste characteristics

Flavour/odour and taste are mostly used as quality parameters by consumers. Traditionally, sensory evaluation has been used for taste analysis. Human sensory panels and chromatographic techniques such as gas chromatography–mass spectrometry (GC–MS), high performance liquid chromatography (HPLC) and ion chromatography (IC) have been the traditional methods used for odour analysis and regulation in the food industry. According to François *et al*., (2003), GC–MS, HPLC and IC provide very detailed information regarding the contents of the odourous compounds. Although chromatographic techniques such as GC–MS can separate, identify and quantify individual volatile

chemicals, it is very hard to correlate the data with those by human sensory evaluation. Since odours are usually composed of different volatiles, the chromatographic techniques are not practical and handy for persons' daily use. Human sensory evaluation is a powerful method for odour and taste analysis since it provides immediate aroma and taste profile. However, it possesses some disadvantages in areas such as the standardization of measurements, correctness of training, reproducibility, high cost and taste saturation of the panelist (Beullens *et al*., 2008). Lau *et al*., (2000) noted that this method is highly subjective and illness or other factors can influence their performance and the final results of human sensory evaluation.

In view of this, the electronic nose (e-nose) and electronic tongue (e-tongue), which works by mimicking the human olfactory system and taste buds, respectively, have proved to be good alternatives for traditional techniques in perceiving odour and taste of food (Escuder-Gilabert *et al*, 2010). They can also be used in situations where human panelists could be exposed to potentially hazardous materials. E-noses and tongues are analytical systems that provide global information about the sample instead of information on particular components. However, if the data matrix obtained by such multisensory systems is analyzed with adequate chemometric processing tools, descriptive or predictive information of particular parameters could be extracted (Oliveri *et al*., 2010). Electronic nose and tongue can be considered as a qualitative and quantitative device for the analysis of complex samples. The objectives of qualitative analysis consists of discrimination, classification or identification of different samples (Vlasov *et al*., 2005).

In the area of foods, e-nose has been successful in studying flavour release relationships in low moisture bakery products (Piazza *et al*., 2008); monitoring the aroma of wines (García

et al., 2006) and various fruit juices (Gobbi *et al*., 2010; Karlshøj *et al*., 2007; Reinhard *et al*., 2008). Also, Feng *et al*. (2011) analysed volatile compounds of Mesona Blumes gum/rice extrudates via GC–MS and electronic nose. The application of metal oxide microbalance array sensor for volatile and smell analysis has been reported in literature (Wyszynski *et al*., 2005; Strike *et al*., 1999). Electronic nose with the function of chemical imaging (Weimar *et al*., 1998; Gopel, 1998) and multiparameter sensor systems (Yu *et al*., 2007) have been described. The sensing system can be an array of different sensing elements (chemical sensors), where each element measures a different property of the sensed chemical. Generally, result of an electronic nose is based on its evaluation of the sum of all the detected volatile compounds (Yu *et al*., 2008). The volatile compounds presenting in the headspace interact with the array of non-selective sensor and produce a chemical fingerprint or pattern characteristic to the odour or volatile compounds (Yu *et al*., 2009) and the pattern recognition software system is able to distinguish and recognize the odours.

Electronic tongue has also been used in evaluating the taste of food covering the area of freshness evaluation and shelf-life investigation (Gomez *et al*., 2008), process monitoring (Turner *et al*., 2003), authenticity assessment (Parra *et al*., 2006), foodstuff recognition (Legin *et al*., 1997), quantitative analysis and other quality control studies (Beullens *et al*., 2006). The basic idea behind electronic tongue technology is the application of an array of non-specific chemical sensors with a high cross-sensitivity, thus a wide selectivity towards a range of inorganic and organic substances in solutions as possible (Vlasov *et al*., 2005). It can provide the corresponding food material with a digital fingerprint, and classify the samples according to their quality by combining various chemometric data-processing

tools. The measuring principle for the E-tongue is based on the measurement of potential changes of several working electrodes against a reference electrode in zero-current conditions. The potential of the working electrode is a function of activity of ionic species across an ion sensitive membrane by diffusion in a sample solution; the potentiometric response depends on the distribution of ions on the sensor membrane (Ciosek *et al*., 2007).

It is evident that the e-nose and e-tongue sensor systems do not look at the same features when applied to the same sample. The e-nose sensors come into contact with its headspace while for the e-tongue, electrodes are immersed in the sample (Di Natale *et al*., 2000). The advantages of electronic nose and electronic tongue include rapid, real-time detection, lower operation costs, higher automation and easy-to-handle measurement set-up.

2.8 Conclusion

From the preceding literature review it is clear that there have been research studies on rice in Ghana that have focused on the physicochemical characteristics and sensory quality of some local varieties. Several other studies have also been conducted to compare locally grown rice to imported rice as a basis for the improvement of local rice and enhancement of its market competitiveness (Gayin *et al*., 2009; Tomlins *et al*., 2007; Adu-Kwarteng *et al*., 2003; Amissah *et al*., 2003; Manful *et al*., 1996). Research work has also been reported on the quality and sensory characteristics of varieties that are on the verge of being released in Ghana (Adu-Kwarteng *et al*., 2003). However, very few works have been carried out on diversifying the use of the less preferred rice varieties. Such uses would include development of other food products such as snacks. There is, therefore the need to conduct research on other uses of local rice varieties which are not preferred for cooking. Different food products such as snacks can be obtained by combining the rice with local staples presently available in the country. Combination of cereals such as local rice varieties with inexpensive plant protein sources such as legumes can provide the ingredient base for development of new food products. Cereals are deficient in lysine but have sufficient sulphur containing amino acids which are limited in legumes (Tsai *et al*., 1975; Wang and Daun, 2006; Iqbal *et al*., 2006; Shewry, 2007) whereas legumes are rich in lysine. The effects of the supplementation are highly beneficial, since nutritive value of the product is also improved. This project studied the combination of local and readily available raw materials which are inexpensive to develop a ready to eat extruded snack with long keeping quality and overall consumer acceptability.

3.0 MATERIALS AND METHODS

3.1 Raw materials

The milled and paddy forms of two widely grown local rice varieties namely *Viwonor (*high amylose content) and *Togo Marshall* (low amylose content) were obtained from the agents of Ghana Rice Inter-Professional Body (GRIB) in Ghana. Soybean (*Nangbaar* variety) was obtained from the Crop Research Institute of the Council for Scientific and Industrial Research (CSIR) located in Kumasi.

3.2 Methods

3.2.1 Determination of physicochemical properties of rice grains

The physicochemical properties of the rice grains, such as chalkiness, grain dimension, grain hardness and Alkaline spreading value (ASV) were determined using standard procedures.

3.2.1.1 Chalkiness and grain dimension determination.

Grain dimensions and chalkiness were estimated using the S21 Rice Statistics Analyzer, (LKL Technologia, Brazil). The equipment was run with a Classficador S21 version 4.05 Software. The S21 was calibrated using a reference sample supplied by the manufacturer. Approximately 50g of whole grains were weighed and emptied into the sample receiver of the S21 Rice Statistics Analyzer. The ‘’long white’’ classification set up was opened in the capture mode software. The equipment was then switched on to vibrate and cause the release of individual grains from the receiver to slide on a blue tile background and pass

beneath the attached camera that captured images of the grains. When all the grains had exited the receiver; the image capturing mode was stopped.

The grain dimensions were determined by processing the captured images and applying the ‘’advanced filter length distribution’’ on the software. The grain length and width were then recorded and the length/width ratio calculated.

To determine chalkiness, the ‘basic filter-chalky distribution’ was applied. The percentage total chalky area for the samples were recorded and reported as the percentage chalkiness of the samples.

Chalkiness for ‘Togo Marshall’ and grain dimension for both rice varieties (*‘Togo Marshall’* and *‘Viwonor’*) were determined in triplicate and duplicate. Chalkiness was not determined on the *Viwonor* rice variety due to the natural reddish pericarp.

3.2.1.2 Raw grain hardness

Grain hardness was measured using a grain hardness tester (Fujihara Seisakusho LDT, Japan) as described by Fofana *et al*., (2011).

Ten grains were used in the determination of hardness for each rice sample. The handle of the equipment was initially turned anti-clockwise to make room to place a grain on the sample table. The handle was then turned clockwise until a cracking sound was heard. At this time, the black pointer returned to the zero point and the “mother pointer” (red) remained. The reading of the “mother pointer” (kg) indicated the hardness of the grain. This measurement was carried out in duplicate.

3.2.1.3 Alkaline spreading value (ASV)

Alkaline spreading value (ASV) was determined using the method developed by Little *et al.* (1958) which involved visual observation of the degree of dispersion of grains of the milled rice after their immersion in 1.7% potassium hydroxide solution (KOH).

Approximately 10ml of 1.7% KOH solution was poured on 6 rice grains placed in a transparent petri dish and incubated at room temperature for 23 hours. ASV values were then evaluated by comparing it to a standard 7 point numerical scale chart (Jennings *et al*., 1979). Grains that were unaffected were given ASV of 1 and grains that were dispersed and disappeared completely were given a score of 7. Based on the alkaline spreading value, the gelatinization temperature (GT) of the rice samples was directly determined. Analysis was carried out in duplicate.

3.2.1.4 Milling recovery

This was determined from an already milled rice. The brown rice was polished in a Ricepal 32 (Yamamoto Co., Japan) rice polisher. The milled rice was separated into whole and broken grains using a Test rice grader. The milling recoveries were then estimated using the following equations:

$$\text{Head rice yield (\%)} = \frac{\text{weight of whole grains}}{\text{weight of brown rice}}$$

3.2.1.5 Cooking time

This was carried out according to Fofana *et al*. (2011). Five grams of milled rice was poured into 135ml of vigorously boiling distilled water in a 400ml beaker and covered with a watch glass. After 10min of further boiling, 10 grains were taken out every minute with a perforated ladle. The 10grains were pressed between two petri dishes and the grains were considered cooked when at least 9 out of the 10 grains no longer had opaque centers. The time was then recorded. This analysis was carried out in duplicate.

3.2.2 Parboiling of Rice

Parboiling of rice was carried out using a local process in a locally designed utensil in which both soaking and steaming is done. |The flow chart for the process is shown in Figure 3.1.

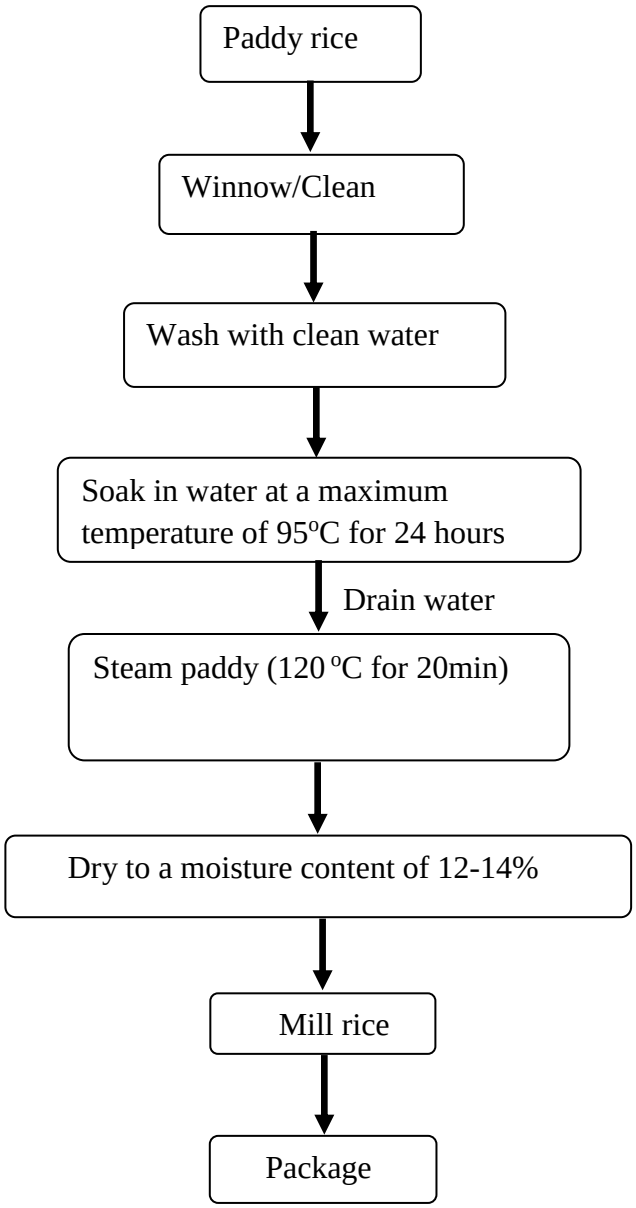


Figure 3.1: Process flow for parboiling rice

(Anonymous 2).

3.2.3 Flour Preparation from milled and parboiled rice

Rice flour was prepared according to the flow chart in Figure 3.2.

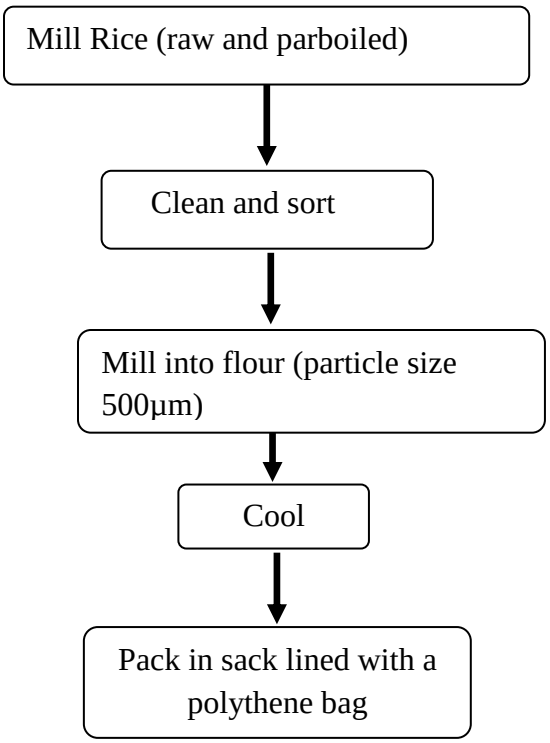


Figure 3.2: Process flow chart for rice flour preparation

3.2.4 Determination of physicochemical properties of rice flour

The parameters determined were colour, apparent amylose content, protein solubility and electrophoretic pattern of the extracted proteins

3.2.4.1 Colour of rice flour

The colour parameters of flour from raw and parboiled rice were placed in a plastic petri dish and was gently pressed to avoid space between them. L* (brightness/darkness index), a* (red/green index), b* (yellow/blue index) were determined using a Colorimeter (Minolta CR-300, Japan). The equipment was calibrated using the white calibration porcelain. The

protocol for calibration was adhered to. The positive and negative L* indicates brightness/darkness, positive and negative a* value indicates redness and greenness, respectively, and b* indicates yellowness for positive value and blueness for negative value. The Colorimeter was calibrated against a standard white calibration porcelain. For each sample, 5 measurements were taken at several points.

3.2.4.2 Apparent Amylose content

The cleaned milled grains (Raw *Togo Marshall*, Parboiled *Togo Marshall*, Raw *Viwonor* and Parboiled *Viwonor*) were ground in an Udy Cyclone Mill (UdyCorp., Fort Collins, USA) with a 1.0 mm mesh screen.

Amylose content was measured using the standard iodine colourimetric method ISO 6647-2-2011. Ethanol (1 ml, 95%) and 1M sodium hydroxide (9ml) were added to rice flour (100 mg) and heated in a boiling water bath for 10min to enable starch gelatinisation. After cooling, 1M acetic acid (1ml) and 2 ml iodine solution (prepared by dissolving 0.2g iodine and 2.0g potassium iodide in 100ml distilled water) were added and the volume made up to 100ml with distilled water. Absorbance of the mixture was measured using an Auto Analyzer 3 (Seal Analytical, Germany) at 600 nm. Amylose content was quantified from a standard curve generated from absorbance values of four well-known standard rice varieties (IR65, IR24, IR64 and IR8). For each sample four replicate measurements were taken.

3.2.4.3 Protein solubility of rice

Protein solubility of the two rice varieties; raw and parboiled (flour from '*Togo Marshall*' and '*Viwonor*') were determined using three different buffers. Thus buffer A: 50mM sodium phosphate monobasic dihydrate and 100mM NaCl at pH 7.0; buffer B: 50mM sodium phosphate monobasic dihydrate, 100mM NaCl and 6M urea at pH 7.0; buffer C: 50mM sodium phosphate monobasic dihydrate, 100mM NaCl, 6M urea and 10mM DL-Dithiothreitol (DTT) at pH 7.0. Protein solubility was carried out by suspending 0.5 g of finely ground sample in buffer A and 0.25g of finely ground samples in buffer B and also in buffer C. Suspensions were vortexed and agitated on a rotary shaker for 1 hour at 22^{o}C. The samples were then centrifuged at 10,000 rpm, at a temperature of 20^{o}C for 30min. The supernatant containing the total soluble proteins after centrifugation was used to prepare samples for Bradford Protein Assay and SDS-PAGE. The amount of protein in the supernatant was determined by the dye-binding method (Bradford, 1976) using bovine serum albumin as a standard. Results are expressed as mg proteins/g sample.

3.2.4.4 Electrophoretic pattern of extracted proteins in rice

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the rice varieties; raw and parboiled was performed according to the method described by Iametti *et al*. (2006).

A fixed volume of the supernatant obtained after treatment with the various solubilising buffers was diluted 1/1 (v/v) with SDS-PAGE denaturing buffer (0.125M Tris-HCl, pH 6.8; 50% w/v glycerol, 1.7% w/v SDS; 0.01% w/v Bromophenol Blue). The protein samples were denatured by boiling at 100^{o}C for 5 min. SDS-PAGE was carried out on a

fixed porosity gel at a constant 16mA (per gel). Gels were stained with Coomassie Brilliant Blue R250 (Simply Blue Safestain, Invitrogen, Carlsbad, CA, USA). Molecular mass markers (Lyophilized protein (LMW), 100µl of buffer pH 6.8 (Tris HCl; 1.5M + 0.4% SDS), 100 µl protein denaturing solution) covered 96, 65, 45, 30, 21 and 14 kDa.

3.2.5 Partially Defatted Soybean (PDS) flour Preparation

Soybean was cleaned and sorted. It was then dried in an oven at a temperature of 50^{o}C for 45min. It was then decorticated using a disc attrition mill (model 4E, Straub Co, Philadelphia, PA, USA) and winnowed in the open air. The decorticated soybean was partially defatted using the screw press to obtain partially defatted soybean cake. The cake was then milled into a particle size of 850µm.

3.2.5.1 Determination of physicochemical properties of soybean flour

3.2.5.1.1 Fat content determination on raw and partially defatted soybean

Fat content was determined using the soxhlet procedure according to the AOAC Method no. 945.16 (AOAC, 2005) with some modifications. Determinations were in duplicates. 2g of pre-dried sample was weighed into an extraction thimble with porosity permitting a rapid flow of petroleum ether. Sample in the thimble was then covered with glass wool. Petroleum ether was then poured to fill two thirds of an already weighed pre-dried round bottom boiling flask. A condenser, soxhlet flask and extractor were then assembled. Fat was extracted into the soxhlet extractor at a rate of 5-6 drops per second condensation for three hours. Boiling flask with extracted fat was dried in an oven at 60^{o}C overnight. It was then cooled in a desiccator and weighed.

$$\% \text{ Fat} = \frac{\text{g of fat in sample}}{\text{g of sample}} \times 100$$

3.2.5.1.2 Colour determination of partially defatted soybean (PDS)

Flour from PDS was placed in a plastic petri dish and was gently pressed to avoid space between them. A colorimeter (CR-300, Minolta, Japan) was used to determine colour value of the partially defatted soybean flour in terms of lightness, redness and yellowness (CIE L*, a* and b* values). The colorimeter was calibrated against a standard white calibration porcelain (L*= 97.15, a*= -0.01, b*= +2.08). For each sample 5 measurements were taken from several points.

3.2.5.1.3 Protein solubility of PDS

Protein solubility of partially defatted soybean was determined using three different buffers. Thus buffer A: 50mM sodium phosphate monobasic dihydrate and 100mM NaCl at pH 7.0; buffer B: 500mM sodium phosphate monobasic dihydrate, 100mM NaCl and 6M urea at pH 7.0; buffer C: 500mM sodium phosphate monobasic dehydrate, 100mM NaCl, 6M urea and 10mM DL-Dithiothreitol (DTT) at pH 7.0. Protein solubility was carried out by suspending 0.5 g of finely ground sample in buffer A and 0.25g of finely ground samples in buffer B and also in buffer C. Suspensions were vortex and agitated on a rotary shaker for 1 hour at room temperature. The samples were then centrifuge at 10,000 rpm, at a temperature of 20^{o}C for 30min. The supernatant containing the total soluble proteins after centrifugation was used to prepare samples for Bradford Protein Assay and SDS-PAGE. The amount of protein in the supernatant was determined using the dye-binding method (Bradford, 1976) with a calibration curve from bovine serum albumin. Results are expressed as mg proteins/g sample.

3.2.5.1.4 Electrophoretic pattern of the extracted proteins

SDS-PAGE of the partially defatted soybean was performed according to the method described by Iametti *et al*. (2006). A fixed volume of the supernatant obtained after treatment with the various solubilising buffers was diluted 1/1 (v/v) with SDS-PAGE denaturing buffer (0.125M Tris-HCl, pH 6.8; 50% w/v glycerol, 1.7% w/v SDS; 0.01% w/v Bromophenol Blue). The protein samples were denatured by boiling at 100^{o}C for 5 min. SDS-PAGE was carried out on a fixed porosity gel at a constant 16mA (per gel). Gels were stained with Coomassie Brilliant Blue R250 (Simply Blue Safestain, Invitrogen, Carlsbad, CA, USA). Molecular mass markers (Lyophilized protein (LMW), 100µl of buffer pH 6.8 (Tris HCl; 1.5M + 0.4% SDS), 100 µl protein denaturing solution) covered 96, 65, 45, 30, 21 and 14 kDa.

3.2.6 Product Formulations

To obtain rice soy extruded product, rice flour was mixed with partially defatted soy flour based on ratios that were determined using constrained mixture designs (Cornell, 1983) for two components. In all, five formulations for each rice treatment were obtained. For the four different rice treatments, twenty product formulations were obtained as shown in Table 3.1.

Table 3.1: Mixture design formulations from different treatments of rice and partiallydefatted soybean flour

Sample	Composite (%)	
	Rice	**Partially defatted soybean**
Raw or milled Togo Marshall (TM)		
TMF1	67.5	32.5
TMF2	60	40
TMF3	90	10
TMF4	75	25
TMF5	82.5	17.5
Parboiled Togo Marshall (PTM)		
PTMF1	67.5	32.5
PTMF2	60	40
PTMF3	90	10
PTMF4	75	25
PTMF5	82.5	17.5
(Raw) Viwonor (V)		
VF1	67.5	32.5
VF2	60	40
VF3	90	10
VF4	75	25
VF5	82.5	17.5
Parboiled Viwonor (PV)		
PVF1	67.5	32.5
PVF2	60	40
PVF3	90	10
PVF4	75	25
PVF5	82.5	17.5

3.2.7 Physicochemical analysis on formulations

3.2.7.1 Moisture determination

Moisture content was determined with reference to AOAC Official Method no 925.10 (AOAC, 2000); air oven method. Determinations were in duplicates.

3.2.7.2 Apparent Amylose content

The formulations were further ground in an Udy CycloneMill (UdyCorp., Fort Collins, USA) with a 1.0 mm mesh screen. Amylose content was measured using the standard iodine colourimetric method ISO 6647-2-2011 (ISO, 2011). Ethanol (1 ml, 95%) and 1M sodium hydroxide (9ml) were added to rice flour (100 mg) and heated in a boiling water bath for 10min to enable starch gelatinisation. After cooling, 1M acetic acid (1ml) and 2 ml iodine solution (prepared by dissolving 0.2g iodine and 2.0g potassium iodide in 100ml distilled water) were added and the volume made up to 100ml with distilled water. Absorbance of the mixture was measured using an Auto Analyzer 3 (Seal Analytical, Germany) at 600 nm. Amylose content was quantified from a standard curve generated from absorbance values of four well-known standard rice varieties (IR65, IR24, IR64 and IR8). For each sample four measurements were taken.

3.2.7.3 Pasting properties

Pasting properties of formulations were determined according to the method of Mariotti *et al*. (2005). Briefly, the formulation (15 g, db) dispersed in 100 ml of deionized water was directly placed into a stainless steel measuring bowl of Brabender Micro Visco Amylo-Graph (MVAG) (Brabender OHG, Duisburg, Germany). It was then heated from 30^{o}C to 95 ^{o}C, held for 20 min at 95^{o}C and cooled to 30^{o}C and finally held for 1 min. Heating and cooling rates were 3^{o}C/min and -3^{o}C/min. The rotation speed and measuring range were 250 rpm and 300 cmg. The following indices were considered: beginning of gelatinization, pasting temperature (temperature at which an initial increase in viscosity occurs); maximum/peak viscosity achieved during the heating cycle; viscosity after holding time at

95 ^{o}C/hot paste viscosity; breakdown (decrease in viscosity during the holding period, corresponding to the peak viscosity minus the viscosity after the holding period at 95 ^{o}C); setback (viscosity increase during cooling). The viscosity was measured in arbitrary Brabender units (BU) and was determined in duplicate.

3.2.8 Extrusion process

The 20 formulations (Table 3.1) with moisture content ranging from 10%-13% were extruded into snack products using an intermeshing co-rotating twin screw extruder (CLEXTRAL BC 21, Germany).

The extrusion conditions for all the 20 formulations were as follows:

- Constant screw speed of 1000rpm,
- Digi drive speed of 400 rpm,
- Barrel temperature of 200^{o}C
- A circular die diameter of 4mm was used.

3.2.9 Visual inspection of the twenty extrudates

Extrudates from the twenty different formulations were visually inspected for expansion, colour and texture. This was to visually access the effect of rice treatment (raw milled rice or parboiled) and the increment of PDS on expansion and other quality characteristics of final extrudates.

3.2.10 Sensory Evaluation of extruded snacks

Sensory panellists were recruited from the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Criteria for recruitment were that panellists were regular consumers of snacks. A balanced incomplete block design for 20 treatments (ie t=20), k = 5 (ie number of samples per panelists) as described by Cochran and Cox (1957) and Montgomery (2001) was used because an individual panelist would find it difficult to evaluate as many as 20 different products at one session. Extruded snacks prepared from raw of parboiled rice and partially defatted soybean were presented to consumers in disposable plates coded with three-digit random numbers. Water was provided for consumers to use during the test to minimize any residual effect between samples. Consumer preference test was carried out using the 9 point hedonic scale with 1 - dislike extremely, 5 – neither like nor dislike and 9 - like extremely (Peryam *et al*., 1957). Sensory attributes which were determined were aroma, colour, mouthfeel, aftertaste and overall acceptability.

3.2.11 Physicochemical analysis on the five selected extrudates

3.2.11.1 Moisture determination

Moisture content was determined according to the air oven method described in the AOAC Official Method no. 925.10 (AOAC, 2000). Determinations were in duplicates.

3.2.11.2 Colour

Flour from the extrudates were placed in a plastic petri dish and was gently pressed to avoid space between them. A colorimeter (CR-300, Minolta, Japan) was used to determine

colour value of the extrudates in terms of lightness, redness and yellowness (CIE L*, a* and b* values). The colorimeter was calibrated against a standard white calibration porcelain. For each sample, 5 measurements were taken from several points.

3.2.11.3 Aroma determination using electronic nose

Analyses were performed with a Portable Electronic Nose (PEN2) from Win Muster Airsense (WMA) Analytics Inc. (Schwerin, Germany). It consists of a sampling apparatus, an array of chemical gas sensors producing an array of signals when confronted with a gas, vapour or odour and an appropriate pattern-recognition software (Win Muster v.1.6) for data recording and elaboration as shown in Plate 3.1.

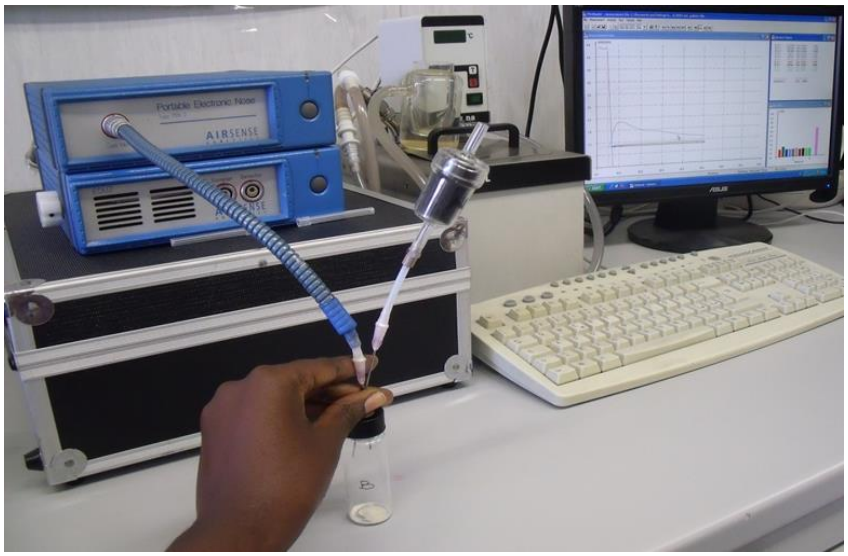


Plate 3.1: Electronic nose PEN2

The key principles involved in the electronic nose concept, is the transfer of the total headspace of a sample to a sensor array that detects the presence of volatile compounds in the headspace and a pattern of signals is provided that are dependent on the sensors selectivity and sensitivity and the characteristics of the volatile compounds in the headspace (Plate 3.1).

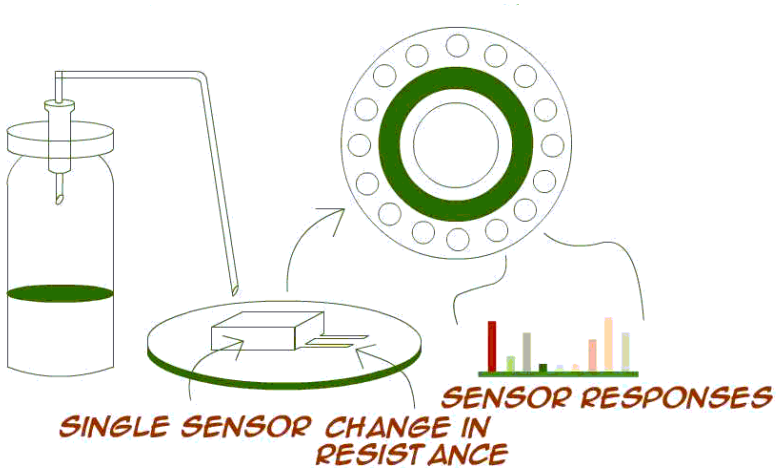


Plate 3.2: Basic elements of an electronic nose

By chemical interaction between odour compounds and the gas sensors the state of the sensors is altered giving rise to electrical signals which are registered by the instrument. In this way the signals from the individual sensors represent a pattern which is unique for the gas mixture measured and is interpreted by multivariate pattern recognition techniques. Samples with similar odourous generally give rise to similar sensor response patterns and samples with different odourous show differences in their patterns.

The sensor array of the electronic nose PEN2 is composed of 10 Metal Oxide Semiconductor (MOS) type chemical sensors as shown in Table 3.2. The sensor response is expressed as resistivity (Ω).

Table 3.2: List and characteristics of 10 Metal Oxide Semiconductors (MOS) of sensor array in electronic nose PEN2.

Number in array	Sensor name	General description
1	W1C	Aromatic compounds
2	W5S	Very sensitive, broad range sensitivity, react on nitrogen oxides, very sensitive with negative signal
3	W3C	Ammonia, used as sensor for aromatic compounds
4	W6S	Mainly hydrogen, selectively (breath gases)
5	W5C	Alkanes, aromatic compounds, less polar compounds
6	W1S	Sensitive to methane (environment) ca. 10 mg kg^{-1}. Broad range
7	W1W	Reacts on sulphur compounds, H_2S 0.1 mg kg^{-1}. Otherwise sensitive to many terpenes and sulphur organic compounds, which are important for smell, limonene, pyrazine
8	W2S	Detect alcohols, partially aromatic compounds, broad range
9	W2W	Aromatic compounds, sulphur organic compounds
10	W3S	Reacts on high concentrations > 100 mg kg^{-1}, sometime very selective (methane)

3.2.11.3.1 Operation Procedure

Lyophilized samples of 0.2g was placed in a 40 mL airtight glass vial fitted with a pierceable Silicon/Teflon disk in the cap. After an hour headspace equilibration at room temperature, the measurement sequence was started. Operating conditions 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. All samples were analysed twice and the average of the sensor responses was used for subsequent statistical analysis. The analysed samples are reported in Table 3.3.

Table 3.3: Five selected extrudates and their coding for electronic nose analysis.

Sample	Code	Composition (%)	
Raw Togo Marshall Rice Variety (TM)		Rice	Partially defatted soybean
TMF3	B	90	10
TMF4	C	75	25
TMF5	A	82.5	17.5
Parboiled Togo Marshall (PTM)			
PTMF3	D	90	10
PTMF4	E	75	25

3.2.11.4 Taste determination using electronic tongue

Analyses were performed with the Taste-Sensing System SA 402B (Intelligent Sensor Technology Co. Ltd, Japan) namely Electronic Tongue (ET). The ET is a liquid analytical device that mimics the taste-sensing mechanism of the gustatory system; it comprises two sensor arrays that are specific for liquid and are able to evaluate tastes: sourness, saltiness, bitterness, umami and astringency as shown in Plate 3.3.

Plate 3.3: Taste sensing system.

Source: Tahara and Toko, (2013)

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 3.4.

Table 3.4: 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

For this work 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 measurement principle of the electronic tongue (Plate 3.3) is based on the capability of taste substances to change the potential of detecting sensors through electrostatic or hydrophobic interaction with the hydrophilic and hydrophobic groups of the lipid membranes. The response of each sensor, recorded as the difference between the potential detected by the sensor and the potential of the reference electrode, is elaborated by a computer and processed via a pattern recognition system.

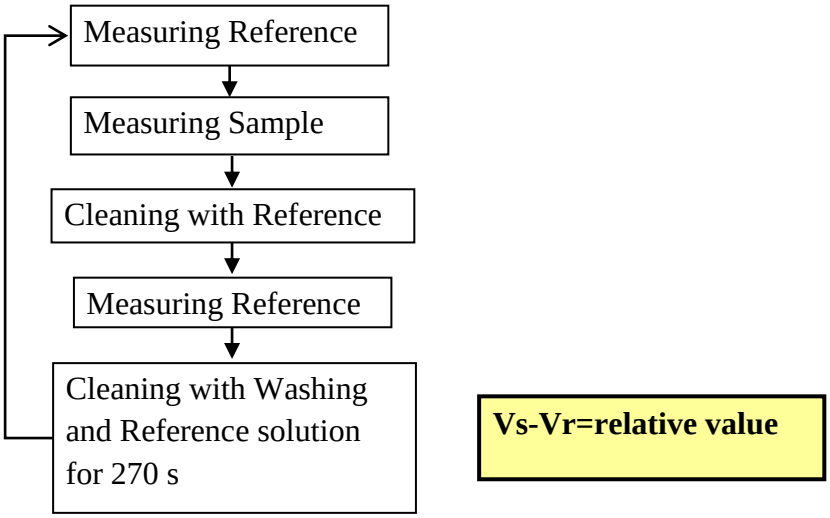


Figure 3.3: Electronic Tongue measuring process.

A sample solution was first and foremost prepared by adding 3 g of flour obtained from the extrudates to 30 ml of distilled water to form suspensions. Suspensions were vortexed for about 2 min and centrifuged at 5000 rpm for 5 min at room temperature. After centrifuging, the supernatants were filtered and diluted 1: 4 (w/w) with distilled water. The

detecting sensors and reference electrodes were then 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 30s 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 s 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 is the CPA value (Change of Membrane Potential caused by Absorption) (CPAv) and corresponds to the ET "aftertastes". Before a new measurement cycle started, electrodes were rinsed for 90 s with a washing solution and then for 180 s with the reference solution. Each sample was evaluated two times and the averages 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. In particular, the "taste values" were estimated as:

$$\text{Sourness} = 0.3316 \times \text{Rv(CA0)}$$

$$\text{Saltiness} = -0.252 \times \text{Rv(CT0)}$$

$$\text{Bitterness} = -0.140 \times \text{Rv(C00)} + 0.084 \times \text{Rv(CT0)}$$

$$\text{Aftertaste-bitterness} = -0.210 \times \text{CPAv(C00)}$$

$$\text{Astringency} = 0.1575 \times \text{Rv(AE1)} + 0.1575 \times \text{Rv(CT0)}$$

$$\text{Aftertaste-astringency} = -0.252 \times \text{CPAv(AE1)}$$

Data Analysis for electronic nose and tongue.

The data processing of the multivariate output data generated by the sensor array signals represents another essential part of the electronic nose and tongue concept. The statistical techniques used are based on commercial or specially designed software using pattern recognition routines like Principal Component Analysis (PCA). Principal Components Analysis (PCA) is a procedure that permits to extract useful information from the data, to explore the data structure, the relationship between objects, the relationship between objects and variables and the global correlation of the variables. It was used for explorative data analysis as it identifies orthogonal directions of maximum variance in the original data, in decreasing order, and projects the data into a lower-dimensionality space formed of a subset of the highest-variance components. The orthogonal directions are linear combinations (principal components PCs) of the original variables and each component explains in turns a part of the total variance of the data; in particular, the first significant component explains the largest percentage of the total variance, the second one, the second largest percentage, and so forth.

3.2.11.5 Bulk density

Bulk densities of the extrudates were determined using a seed displacement method by Bhatnagar *et al.*, (1995) with some modifications. The weight of each sample was weighed with an electronic balance. Sesame seeds were used as the displacement medium. A beaker was filled with sesame seeds to the brim and levelled with a straight edge. The seeds were poured out and the extrudate placed into the beaker; the sesame seeds were then poured back into the beaker and levelled with a straight edge. The displaced sesame seeds were

placed into a graduated cylinder (250 ml). The bulk density (g/cm^3) was calculated by dividing the weight of the extrudates by the volume displaced. Nine measurements were taken for each extrudates.

3.2.11.6 Water Absorption Index (WAI) and Water Solubility Index (WSI)

The WAI and WSI indices were measured using a technique developed for cereals (Anderson *et al*., 1969). In particular, 2.5 g of ground extrudate was suspended in 25 ml of water at room temperature for 30 min., gently stirred using a stirring rod and then centrifuged at 3000 g for 10 min. The supernatant was decanted into an evaporating dish of known weight. The WSI is the weight of dissolved solids in the supernatant expressed as a percentage of the original dry weight of sample. The WAI is the weight of gel obtained after removal of the supernatant per unit weight of original dry solids. Determinations were made in duplicate.

$$WAI = \frac{Weight\ of\ sediment}{Weight\ of\ dry\ solids}$$

$$WSI = \frac{Weight\ of\ dissolved\ solids\ in\ supernatant}{Weight\ of\ dry\ solids} \times 100$$

3.2.11.7 Expansion ratio

Expansion ratio was determined according to the method of Bisharat *et al*., (2013).Thus the ratio of the diameter of the extrudates to the diameter of the die:

$$\text{Exp.} = \frac{d}{d_o}$$

where d (cm) is the diameter of the extrudates and d_o (cm) is the diameter of the die. Ten replicates were used for the determination of expansion ratio.

3.2.11.8 Hardness analysis

Extruded products were punctured by using a Zwick Z005 testing machine equipped with a 100 kN load cell (Zwick GmbH & Co., Ulm, Germany) fitted with a 4-mm diameter cylindrical flat-faced probe. The speed of advance was 1 mm $^{s-1}$. The force-deformation curves were recorded from the surface of the extrudate and ended after the pin had reached the 50% of sample deformation. The work (Nmm) divided by the height of the sample (mm) was chosen to represent the textural properties of extrudates that had different diameters. 20 measurements were carried out for each sample.

3.2.11.9 Protein solubility

Protein solubility of extrudates (in the flour form) were determined using three different buffers. Thus buffer A: 50mM sodium phosphate monobasic dihydrate and 100mM NaCl at pH 7.0; buffer B: 500mM sodium phosphate monobasic dehydrate, 100mM NaCl and 6M urea at pH 7.0; buffer C: 500mM sodium phosphate monobasic dehydrate, 100mM NaCl, 6M urea and 10mM DL-Dithiothreitol (DTT) at pH 7.0. Protein solubility was carried out by suspending 0.5 g of finely ground sample in buffer A and 0.25g of finely ground samples in buffer B and also in buffer C. Suspensions were vortex and agitated on a rotary shaker for 1 hour at room temperature. The samples were then centrifuge at 10,000 rpm, at a temperature of 20^{o}C for 30min. The supernatant containing the total soluble proteins after centrifugation was used to prepare samples for Bradford Protein Assay and

SDS-PAGE. The amount of protein in the supernatant was determined by a dye-binding method (Bradford, 1976) using bovine serum albumin as a standard. Results are expressed as mg proteins/g sample.

3.2.11.10 Electrophoretic pattern of the extracted proteins

SDS-PAGE was performed according to Iametti *et al*. (2006). A fixed volume of the supernatant obtained after treatment with the various solubilising buffers was diluted 1/1 (v/v) with 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). The protein samples were denatured by boiling at 100^{o}C for 5 min. SDS-PAGE was carried out on a fixed porosity gel at a constant 16mA (per gel). Gels were stained with Coomassie Brilliant Blue R250 (Simply Blue Safestain, Invitrogen, Carlsbad, CA, USA). Molecular mass markers (Lyophilized protein (LMW), 100µl of buffer pH 6.8 (Tris HCl; 1.5M + 0.4% SDS), 100 µl protein denaturing solution) covered 96, 65, 45, 30, 21 and 14 kDa.

3.2.11.11 Accessible thiols

Accessible thiols (expressed as µmol thiols/g sample) were determined by using (Iametti *et al*., 2006) with some modifications. 0.25g of finely ground samples was suspended in 5ml of 50mM sodium phosphate, 0.1M NaCl, pH 7.0 in the presence or absence of 6M urea, containing 0.2mM of 5, 5'-dithiobis-(2-nitrobenzoate) (DTNB) (Ellman, 1959). After 1 h stirring at 25^{o}C, samples were centrifuged (13000 xg, 30 min, 25^{o}C). The blank was prepared by suspending 0.25g of samples in 5ml of 50mM sodium phosphate, 0.1M NaCl,

pH 7.0 in the presence or absence of 6M urea, without adding DTNB. The supernatant absorbance was read at 412 nm. The results were expressed as µmol thiols/g sample.

3.2.11.12 In vitro protein digestibility

This was carried out using two enzymes thus pepsin and pancreatin. For in vitro pepsin digestion, 1.0g of finely ground sample was weighed into polypropylene test tube. 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) thus by adding 30µl of pepsin at a concentration of 2mg/ml. The mixture was then incubated for 60min at 37 °C under mixing conditions. In vitro pepsin digestion was terminated by the addition of 10% (final concentration) trichloroacetic acid after 60 min. Samples were then centrifuged at 13000*rpm* for 15 min, and the hydrolyzed peptide content in the supernatant was measured at 280 nm.

Protein hydrolysis from pancreatin was preceded by pepsin digestion. Sample pH was adjusted to about 8.0 by adding TRIS 1 M. Proteins were hydrolyzed by pancreatic enzymes (pancreatin from porcine pancreas, EC 232-468-9, ref P1625, Sigma) by adding 120 µl of pancreatin for 60 min at 37 °C under mixing conditions. Pancreatin digestion was terminated by the addition of 10% (final concentration) trichloroacetic acid after 60min in the ratio 1:1. Samples were then centrifuged at 13000*rpm* for 15 min. This was again repeated for 120 and 180min. The hydrolyzed peptide content in the supernatant was measured at 280 nm.

4.0 RESULTS AND DISCUSSION

4.1 Selection of local rice varieties for the preparation of extruded snack.

There are several local rice varieties in Ghana which are considered low grade due to the poor sensory and physical qualities (Tomlins *et al*., 2005). Awudi (2013), characterized ten local rice varieties and Diako *et al*. (2011) also characterized four local rice varieties based on their physicochemical properties as shown in Table 2.4 and 2.5. The low amylose content value (19.30%) for *Togo marshall* rice as shown in Tables 2.4 and 2.5 is responsible for it becoming sticky after cooking (Juliano, 1985) and therefore makes it not to be preferred by most consumers in Ghana (Fofana *et al*. 2011). Similarly, grain colour has implications for consumer preference. In Ghana there is a high visual appeal for white milled rice since most consumers have the perception that white milled rice is superior (Adu-Kwarteng *et al*., 2003) and therefore has a high market value (Wadsworth, 1994). In view of this, *Viwonor* rice variety is least preferred due to its natural reddish colour. This therefore, creates the need to diversify these two varieties in the production of ready to eat snack products.

Furthermore, the availability of the local rice serves as an important criterion in the selection of rice variety for the production of extruded snack especially for purposes of commercialization. A preliminary survey and interactions with rice farmers, millers and distributors from three major areas (Ashiaman, Madina and Hohoe) of the Greater Accra and Volta region suggest that *Togo marshall* and *Viwonor* rice varieties were readily available in the markets throughout the year. This is because the two varieties are widely cultivated by farmers in the Greater Accra and Volta region in Ghana (Interview with Mr. Forson, Ghana Rice Inter-Professional Body (GRIB) 12/09/13).

4.2 Physicochemical characterization of two selected local rice varieties.

The physical and chemical properties of two local rice varieties recognized as low grade rice, thus *Togo marshall* and *Viwonor* rice (Plate 4.1) were determined.

A: *Togo marshall*

B: *Viwonor*

Plate 4.1: Two locally cultivated (low grade) rice varieties (Togo marshall and Viwonor).

4.2.1 Grain hardness

Hardness describes a product which displays substantial resistance to deformation or the "first bite" that would be perceived (Bourne, 2002). Rice grain hardness is an important quality criteria especially for the rice industry since it contributes to changes during storage, processing and as well as affecting the extent of grain resistance to pest and insect attacks (Fofana *et al*., 2011). According to Bienvenido (1972), grain hardness is related to

the packing density of the grain starch. *Viwonor* rice variety showed a relatively higher hardness value of 7.97kg as compared with *Togo marshall* rice variety with a hardness value of 7.46kg. However, the analysis of variance for the data of grain hardness showed that there was no significant differences between the rice varieties (Table 4.1). This means that the differences in the varieties especially in relation to the arrangement of the starch granules of the two varieties did not have an impact on their hardness (Bienvenido, 1972).

4.2.2 Milling recovery (%)

According to Adu-Kwarteng *et al*. (2003), milling recovery is a measure of milling quality and hence economic value. Thus 50% or less milling recovery is undesirable since it suggests that 50% of the rice is discarded as waste or not consumable after milling. The data for milling recovery show significant difference between the rice varieties. The *Viwonor* rice variety showed significantly higher milling recovery than *Togo marshall* rice variety as shown in Table 4.1. The result is in the same order as observed for hardness values shown in Table 4.1. It is possible that the higher hardness values for the *Viwonor* rice variety contributed to the relatively higher milling recovery of 52.29% as compared to *Togo marshall* rice variety with a milling recovery value of 45.90% (Correa *et al*., 2006).

4.2.3 Grain Dimension

Grain size and shape (length/width) is a varietal property that dictate the marketability and commercial viability of rice. According to Rani *et al*. (2006), preferences for rice grain size and shape vary from one group of consumers to another. Grain length is used for the classification of grain size thus extra-long, >7.50 mm; long, 6.61 to 7.50 mm; medium,

5.51 to 6.60 mm; and short, 5.50 to 4.50 mm (Juliano, 1993). Furthermore, length to width (L/W) ratios are used in the classification of grain shape, with a higher ratio indicating slender shapes, >3.0; and a lower value indicating medium, 2.1-3.0; bold, 1.1-2.0 or round shapes ≤ 1.0 (Juliano, 1993).

There was no significant difference in relation to both grain length and grain shape at $p \leq 0.05$ as shown in Table 4.1 for both *Togo marshall* and *Viwonor* rice varieties. Length of the *Togo marshall* rice variety and *Viwonor* rice variety were 6.68mm and 6.67mm respectively, indicating that they could be classified as long (Juliano, 1993). The length to width ratio for *Togo marshall* rice variety (2.97) and *Viwonor* rice variety (2.97) as shown in Table 4.1 falls within 2.1-3. This means that the two varieties can be classified as medium shape according to Juliano (1993). This was also in agreement with the finding by Diako *et al*. (2011) who concluded that *Togo marshall* together with some local rice varieties such as Ex-Baika, Ex-Hohoe were all medium-shaped grains based on FAO (1975) categorization. In Ghana, studies have shown a higher demand for long-grain rice than for short, round grain types (GLG-SOFRENCO, 1997).

4.2.4 Grain Chalkiness

Grain appearance is mainly determined by the endosperm opacity. Opacity is as a result of chalky texture caused by interruption of final filling of the grain. According to Adu-Kwarteng *et al.* (2003), the presence of chalkiness in rice grain is described as a "defect'' that affects milling, marketing and storage properties of rice. Even though, chalkiness disappears upon cooking and has no direct effect on cooking and eating qualities, excessive chalkiness downgrades the quality and reduces milling recovery (USDA, 2005).

On the basis of Standard Evaluation Scale (SES) categorization (USDA, 2005), *Togo marshall* rice variety with percentage chalkiness of 4.93% (Table 4.1) had a chalky score of 1. The Codex Alimentarius Commission (1990) acceptable tolerance defect for chalkiness is 11.0%, therefore, it can be concluded that the percentage chalkiness value for the *Togo marshall* sample falls within the acceptable tolerance level for defect in terms of chalkiness. Chalkiness could not be detected in *Viwonor* rice variety due to its natural reddish-brown pigmentation.

4.2.5 Cooking time

Cooking time of rice refers to the time taken by the rice starch to gelatinize (Bienvenido, 1972) when subjected to heating in excess water. At this point the starch granules take in water as the temperature is increased to the gelatinization temperature. The starch granules also loose their crystalline nature and this change is irreversible. Cooking time is an important factor to consumers and it is influenced by the variety, cultivation and postharvest practices, degree of milling, and cooking methods (Park *et al*., 2001). Falade *et al*. in 2014 observed that cooking and eating characteristics of rice such as the cooking time are influenced by the ratio of amylose and amylopectin in the rice grain.

Table 4.1 shows that *Viwonor* rice variety had a significantly longer cooking time (21.00min) than the *Togo marshall* rice variety (16.00min). The longer cooking time of *Viwonor* rice variety may be due to its relatively higher amylose content shown in Table 4.2 as suggested by Bahmaniar and Ranjbar (2007) that amylose content directly influence the cooking and eating qualities of milled rice. Based on the result, it can be concluded that

Togo marshall rice variety will cook faster than *Viwonor* rice variety and would therefore be suitable for products which require shorter cooking time.

Table 4.1: Physical properties of Togo marshall and Viwonor rice varieties.

Parameter	Varieties		P-value
	Togo marshall	*Viwonor*	
Hardness (Kg)	7.46±0.59[a]	7.97±0.37[a]	0.3755
Milling recovery (%)	45.90±0.75[a]	52.29±0.47[b]	0.0019
Length (L) mm	6.68±0.02[a]	6.67±0.01[a]	0.2948
Width (W) mm	2.25±0.01[a]	2.25±0.02[a]	0.6366
L/W	2.97±0.01[a]	2.97±0.02[a]	0.8982
Chalkiness (%)	4.93±1.23	-	-
Cooking time (min)	16.00±0.00[a]	21.00±0.00[b]	< 0.05

4.2.6 Alkaline spreading value (ASV)

Alkali spreading value is the degree of spreading of individual milled rice kernel in a weak alkali solution (1.7% KOH) at room temperature (32±2℃) (Oko *et al*., 2012). It is used as an indicator of gelatinization temperature of milled rice starch granules **(**Delwiche *et al*., 1996**).** According to Falade *et al.* (2014), gelatinization temperature is the temperature at which 90% of rice starch granules swells in hot water and loose its crystalline structure and birefringence or gets swollen irreversibly in hot water. There was a significant difference for the *Togo marshall* rice variety and *Viwonor* rice variety in their alkaline spreading values at $p \leq 0.05$ as shown in Table 4.2. The *Togo marshall* had a significantly higher ASV than *Viwonor* rice variety. The ASV of *Togo marshall* rice variety (6.67) signifies a

gelatinization temperature range of 55-69.5^{o}C (low) (Juliano, 1993). Furthermore, the ASV for *Viwonor* rice variety (5.88) also signifies a gelatinization temperature range of 70-74^{o}C (intermediate) (Juliano, 1993). This result is in agreement with the long and short cooking time for *Viwonor* and *Togo marshall* rice varieties respectively as shown in Table 4.1.

4.2.7 Apparent amylose content (AAC)

Starch (amylose and amylopectin) constitute about 90% of the dry matter content of milled rice. Rice varieties differ widely in the cooking and eating properties, depending on the ratio of the starch fractions, amylose and amylopectin. Amylose is the linear fraction of starch, and amylopectin, the branched fraction (Oko *et al*., 2012). Amylose content is the major influence on the eating and cooking qualities of rice. According to Juliano (1985), rice with high amylose content cook dry and fluffy and become harder upon cooling while low amylose (below 20%) varieties cook moist and sticky. In this study the *Viwonor* rice variety had a significantly higher apparent amylose content (20.62%) than that of *Togo marshall* rice variety (17.45%) as shown in Table 4.2. According to Juliano (1993), amylose content in milled rice samples are categorized as follows: waxy (1-2%), very low amylose (2-12%), low amylose (12-20%), intermediate (20-25%) and high (25-33%). Based on this classification, *Viwonor* rice variety can be classify as intermediate whiles *Togo marshall* rice variety as low amylose rice. The notable differences in the apparent amylose content between the two rice varieties may be due to different factors such as genotype, environmental conditions, and agricultural practice (Kim and Wiesenborn, 1995). According to Fofana *et al*. (2011), the intermediate amylose varieties are generally most preferred in West Africa because they cook dry and fluffy retaining their soft texture

even after cooling. This suggests that the *Viwonor* rice variety would be most preferred to *Togo marshall* rice variety for cooking in West Africa.

Table 4.2: Chemical properties of Togo marshall and Viwonor rice varieties

Parameter	Varieties		P-value
	Togo marshall	*Viwonor*	
Alkaline spreading value (ASV)	6.67±0.30[a]	5.88±0.18[b]	0.0467
Amylose %	17.45±0.36[a]	20.62±0.26[b]	0.0000

4.3 Physical and chemical analysis of the raw materials used for extruded products

4.3.1 Colour analysis

Colour determination was carried out on the raw materials used in the extrusion process. These were partially defatted soybean (PDS), Raw *Togo marshall* (RTM) rice variety, Parboiled *Togo marshall* (PTM) rice variety, Raw *Viwonor* (RV), Parboiled *Viwonor* (PV) rice variety.

Data were expressed as L*, a* and b* values, where L* (luminosity) values varied from black (0) to white (100), chroma a* values varied from green (-60) to red (+60), and chroma b* values varied from blue (-60) to yellow (+60). There were significant differences among the values for the raw materials (PDS, RTM, PTM, RV and PV) in terms of L*, a* and b* indices at $P \leq 0.05$ as shown in Table 4.3. The significantly high L* value (93.18) for Raw *Togo marshall* (RTM) rice variety was because it was polished rice and contains about 90% starch which is naturally white in colour. Furthermore, the significantly low whiteness (L*) value (79.63) observed for RV was as a result of its naturally reddish pericarp (Diako *et al*., 2011). The decreased L* values (84.69 and 77.10)

for Parboiled *Togo marshall* (PTM) and Parboiled *Viwonor* rice varieties as compared to their raw form might be as a result of the parboiling treatment of the rice grain which might have led to diffusion of husk pigments into the endosperm especially during the soaking period of the parboiling treatment. This was also confirmed by Dutta, *et al.* (2012) who reported a decrease in the lightness value of parboiled rice. Furthermore, the least L* value (83.89) for Partially Defatted Soybean (PDS) as compared to RTM and PTM may be due to its relative darker colour as a result of the high temperature application during the defatting process thereby favouring Maillard reaction between reducing sugars and free amino groups. The high a* values for RV (4.11) as compared to RTM (-0.31) confirms the presence of naturally reddish pericarp in RV rice variety. The negative a* value (-0.31) for RTM as compared to PTM (-0.12) suggest the presence of some green pigment in the RTM rice variety which was significantly reduced after parboiling as observed in Table 4.3.

Lastly, the high positive b* value (22.76) for PDS is related to the original yellowish colour as a result of some carotenoids pigments in the soybean. The relatively higher b* values for parboiled rice (PTM; 15.54 and PV; 12.83) as compared to their raw form (RTM; 6.58 and RV; 12.04) suggest that the parboiling process which is a hydrothermal treatment significantly improved the yellowish nature of the rice grain as a result of the diffusion of yellowish pigment from the rice husk. It can therefore be concluded that parboiling treatment significantly affected the L*, a* and the b* indexes of the colour. Grain colour is one of the important factors for visual appeal and most consumers are of the perception that white rice is superior (Adu-Kwarteng *et al*., 2003). Therefore based on colour the *Togo marshall* rice variety will be preferred to *Viwonor* rice variety.

Table 4.3: Colour measurement of raw materials

Raw materials	L*	a*	b*
PDS	83.89±0.73[a]	0.54±0.18[a]	22.76±0.83[a]
RTM	93.18±0.36[b]	-0.31±0.07[b]	6.58±0.22[b]
PTM	84.69±0.16[c]	-0.12±0.10[c]	15.54±0.32[c]
RV	79.63±0.26[d]	4.11±0.07[d]	12.04±0.18[d]
PV	77.10±0.51[e]	3.10±0.10[e]	12.83±0.53[e]
P value	<0.0001	<0.0001	<0.0001

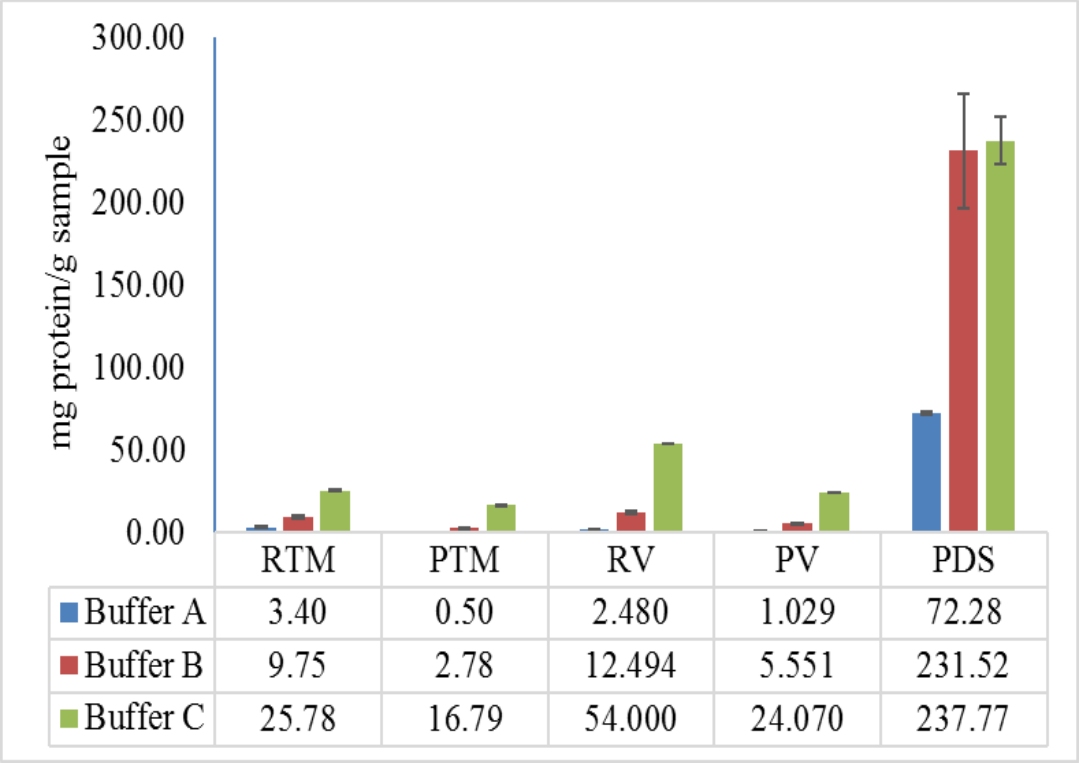
[a, b, c, d, e]. Means and standard deviation with the same superscript in a column are not significantly different at $p \leq 0.05$.
Key: PDS: Partially Defatted Soybean, RTM: Raw *Togo marshall* rice variety, PTM: Parboiled *Togo marshall* rice variety, RV: Raw *Viwonor*, PV: Parboiled *Viwonor* rice variety.

4.3.2 Protein solubility

Protein solubility properties of raw and parboiled forms of both rice varieties (*Togo marshall* and *Viwonor*) as well as partially defatted soybean (PDS) in three different extraction buffers were evaluated.

Generally for all samples, it was observed that protein solubilzation in buffer A (50mM sodium phosphate monobasic dihydrate and 100mM NaCl) was less than that in buffer B (buffer A and 6M urea) which was also less than the protein solubilzation in buffer C (buffer B and 10mM DL-Dithiothreitol (DTT)). This is because, according to Bhattacharya (2004), buffer A which is a saline buffer solubilised water and salt soluble proteins such as albumins and globulins. The addition of a denaturing agent (urea) to the saline buffer (buffer A) enabled the dissociation of protein aggregates stabilized by hydrophobic

interactions there by increasing the quantity of solubilized protein. Furthermore, buffer C which involved the addition of dithiothreitol (DTT) led to an increased solubilisation of the proteins since it also ensured the dissociation of aggregates stabilized by disulphide linkages (Bonomi *et al*., 2012) as shown in Figure 4.1. The high level of solubilized proteins from partially defatted soy (PDS) in all buffers as compared to the various rice varieties confirms the high protein content in PDS (Shewery, 2007) as compared to rice. The protein solubility for the raw rice (of both *Togo marshall* and *Viwonor*) in all the buffers was higher than their counterparts parboiled rice (PTM and PV). Thus the parboiling process impacted negatively on the solubility of proteins in the rice endosperm. Parboiling of rice causes physicochemical transformations such as starch gelatinization and retrogradation, leaching of amylose as well as denaturation and aggregation of proteins. This confirms the study by Reza *et al*. (2005) where they stated that many of the peptides found in raw rice were absent in the parboiled rice. Harris and Juliano (1977) also noticed that the protein bodies in raw rice changed drastically during parboiling process of rice. They also observed that the protein fraction were less extracted from parboiled rice. It can therefore be concluded that the rice proteins were greatly modified by the parboiling treatment.



	RTM	PTM	RV	PV	PDS
Buffer A	3.40	0.50	2.480	1.029	72.28
Buffer B	9.75	2.78	12.494	5.551	231.52
Buffer C	25.78	16.79	54.000	24.070	237.77

Figure 4.1: Amount of solubilized protein from raw materials.

(RTM; Raw *Togo marshall* rice varieties, PTM; Parboiled *Togo marshall* rice variety, RV; Raw *Viwonor* rice variety, PV; Parboiled *Viwonor* rice variety and PDS; Partially defatted soybean) in different buffers systems. Buffer A: (50mM sodium phosphate monobasic dihydrate and 100mM NaCl). Buffer B: (500mM sodium phosphate monobasic dehydrate, 100mM NaCl and 6M urea) and Buffer C (500mM sodium phosphate dehydrate, 100mM NaCl, 6M urea and 10mM DL-Dithiothreitol (DTT).

4.3.3 Protein electrophoretic patterns

Electrophoretic patterns shown in Figure 4.2 confirm the solubility data presented in Figure 4.1 and indicate that the peptides bands from proteins solubilized by urea and DL-Dithiothreitol (DTT) are more than those soluble in their absence (Mariotti *et al*., 2011). This generally confirms the presence of protein aggregates stabilized by hydrophobic interactions and disulfide bonds in both rice varieties and partially defatted soybean (PDS).

The high number of peptide bands observed for PDS as compared to both rice varieties confirms that cereals and legumes do not have the same types of proteins, hence the differences in their peptides bands. Furthermore, the protein electrophoretic patterns of raw rice were different from their parboiled rice counterparts. The electrophoretic patterns of raw rice (RTM and RV) had a higher number and more intense peptide bands than that of their parboiled counterpart (PTM and PV) rice suggesting that parboiled rice gave a poor protein extraction characteristics in all buffers as was also confirmed by Reza *et al.* (2005). This clearly shows that parboiling treatment of the rice led to the diffusion of water soluble proteins such as albumin from the rice endosperm to the surrounding water especially during the soaking stage of the parboiling treatment. Furthermore, the heat treatment during the parboiling process might have also led to protein denaturation thereby reducing its solubility properties thus confirming the protein solubility results in Figure 4.2.

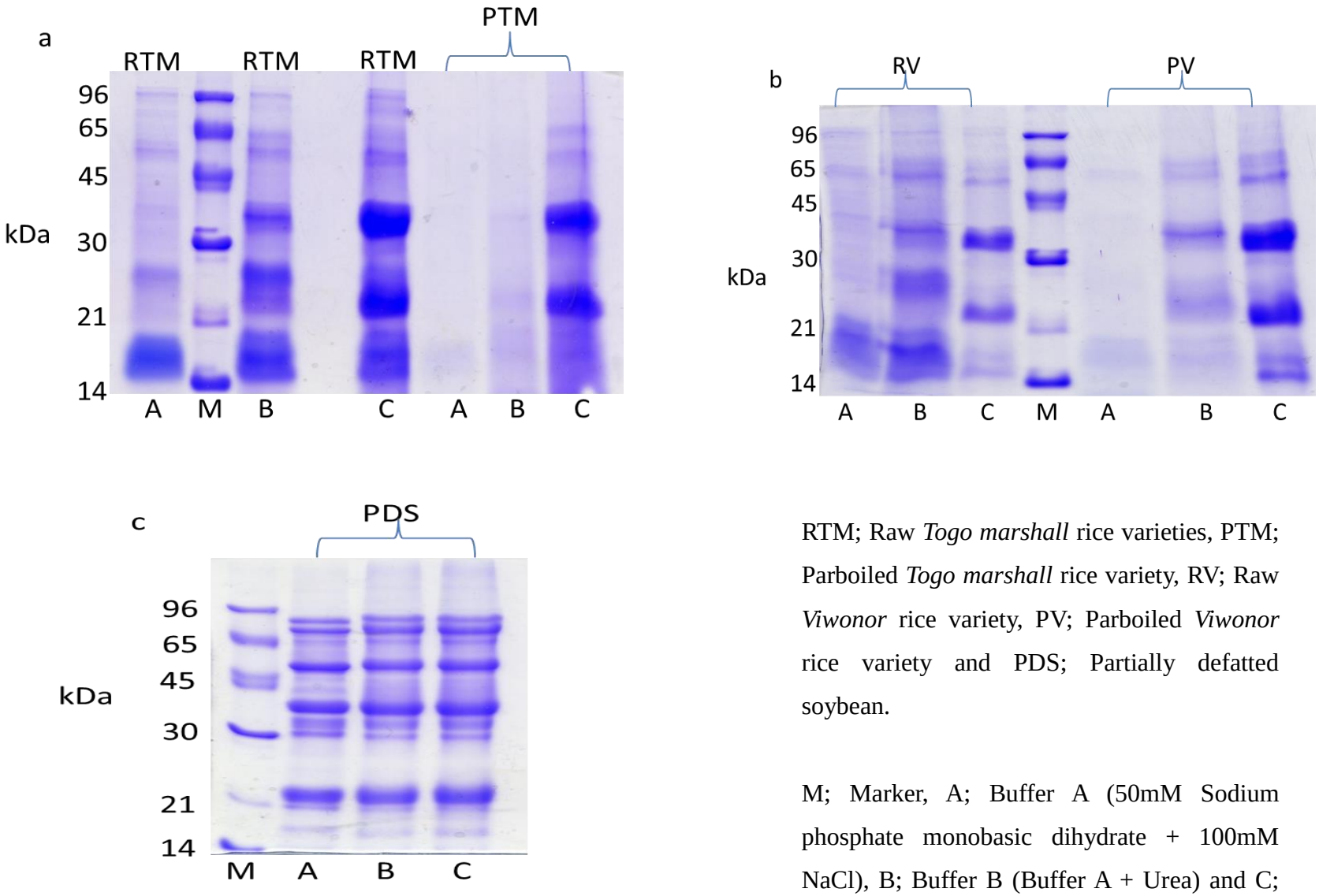


Figure 4.2: Electrophoretic patterns of proteins in the raw materials

RTM; Raw *Togo marshall* rice varieties, PTM; Parboiled *Togo marshall* rice variety, RV; Raw *Viwonor* rice variety, PV; Parboiled *Viwonor* rice variety and PDS; Partially defatted soybean.

M; Marker, A; Buffer A (50mM Sodium phosphate monobasic dihydrate + 100mM NaCl), B; Buffer B (Buffer A + Urea) and C; Buffer C (Buffer B + DTT).

4.4 Chemical analysis of soybean

4.4.1 Changes in fat content due to defatting

Raw soybean had a fat content of 21.01%, this was substantially reduced to 16.94% (Figure 4.3) by partially defatting it using a screw press. The partially defatting process was to reduce the percentage fat content during the inclusion of soybean content in the formulation in order to ensure high performance of the extruder (Riaz, 2000).

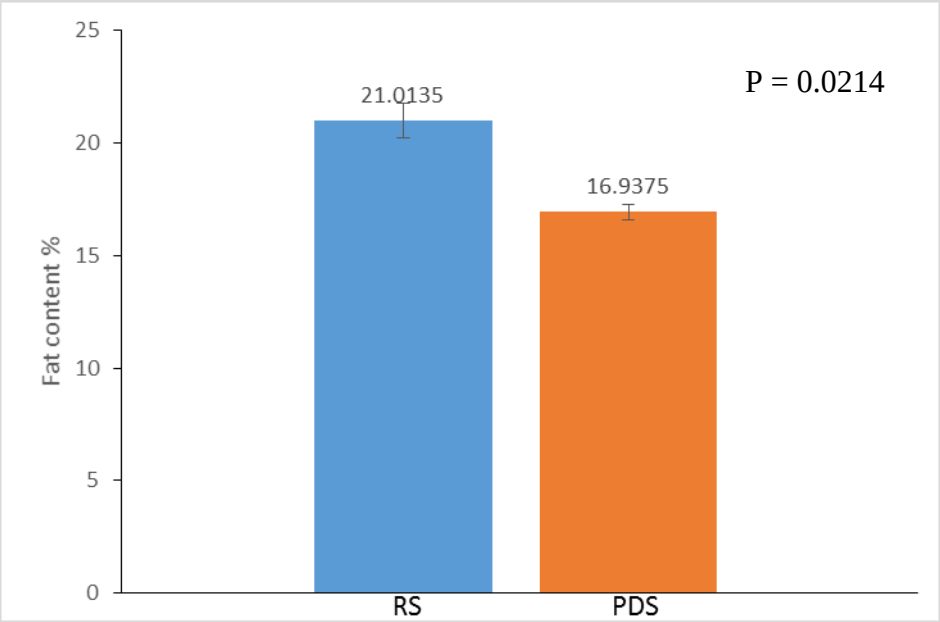


Figure 4.3: Changes in fat content. RS: Raw soybean (full fat soybean); PDS: Partially defatted Soybean.

4.5 Evaluation of extrudate quality through visual examination

In all twenty different formulations were obtained and all extruded. From the visual inspection of rice-soybean extrudates variable expansion was noted. The degree of expansion decreased gradually as the quantity of partially defatted soybean (PDS) increased as observed in Plate 4.2, 4.3, 4.4 and 4.5. This confirms the fact that the addition

of protein to starchy raw materials in extrusion cooking reduced the expansion of the products by reducing the extensibility of the starch polymer during its extrusion at the die exit (Chaiyakul *et al.*, 2009). Moreover, it can be observed that the addition of 40% of PDS drastically reduced the expansion of the extrudates made from raw rice in both rice varieties (shown in Plate 4.2 and 4.4) from foamy expanded like extrudate to crispy biscuit like extrudate. This was actually irrespective of their differences in amylose content; thus *Togo marshall* rice variety being classified as low amylose content and *Viwonor* rice variety as intermediate amylose content as shown in Table 4.2. This might be that the starch matrix of the pre-gelatinized starch with the addition of 40% PDS was able to better entrap water vapour during the extrusion cooking as compared with formulation from raw rice and 40% PDS. According to Baladran-Quintana *et al*. (1998) as stated in Asare *et al.* (2012) expansion is the swelling of cells (starch granules) upon the imbibition of water, thus the water molecules enter the cell granules causing solvation of the molecules, occupying capillary and intramolecular spaces of the molecules.

In relation to colour, it can be observed that there was no much notable differences with respect to extrudates from *Viwonor* rice variety in both raw of parboiled form. This is because the natural red pericarp of the rice variety musk the colour even after extrusion cooking as observed in Plate 4.2 and 4.3. With respect to extrudates from raw *Togo marshall* rice variety, there was an observation of yellowish bright colour which was gradually changed to a darker colour as the quantity of PDS increased as observed in Plate 4.4 and 4.5. The extrudates from parboiled *Togo marshall* gave a notable difference in colour as compared to extrudates from its raw form as shown in Plate 4.5 and 4.4. Thus the colour changed during the parboiling process had an impact even after extrusion cooking.

Plate 4.2: Extrudates from Raw ‘Viwonor’ rice variety. (Rice: Soybean)

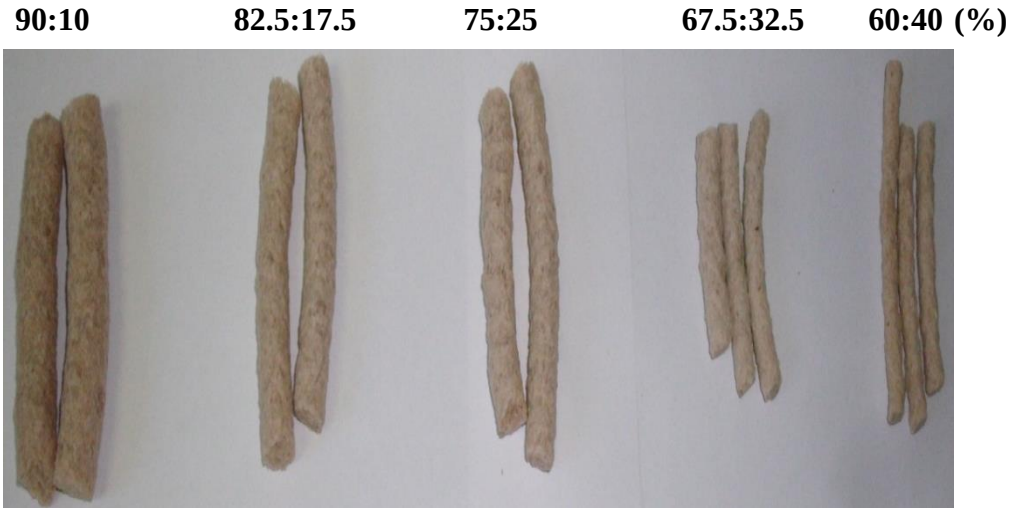


Plate 4.3: Extrudates from Parboiled ‘Viwonor’ rice variety. (Rice: Soybean)

Plate 4.4: Extrudates from Raw ‘Togo marshall’ rice variety. (Rice: Soybean)

Plate 4.5: Extrudates from Parboiled ‘Togo marshall’ rice variety. (Rice: Soybean)

4.6 Consumer acceptability of twenty extruded snacks

According to Martinez *et al.,* (2007), sensory evaluation is the nearest to a consumer's estimation and still remains the most reliable test for the especially since it allows the characteristics of products to be evaluated. Consumer acceptance of extruded foods especially snacks is mainly due to sensory attributes such as aroma, mouthfeel, colour, aftertaste and more (Majumdar *et al*. 2014). According to Jin-Soo *et al*. (1994), several extrusion processing variables such as feed moisture, die diameter and differences in ingredients composition accounts for the quality of finished products and finally influence the success of the product. Pamies *et al*. (2000) also stated that the success or failure of a new extruded snack food product is directly related to sensory attributes, among which mouthfeel plays a major role.

The sensory results of the twenty extrudates show that there were no significant differences among the values for aroma with TMF2 (thus having 60% *Togo marshall* rice variety and 40% PDS) having the highest aroma score value of 6.6±1.07 as shown in Table 4.4. This might be as a result of the strong beany flavor of the extrudate which was generated during the extrusion cooking due to the high percentage of partially defatted soybean. The score values in terms for aroma ranged between 6.6-5 shows that the extrudates were liked slightly to neither liked nor disliked.

According to Nisha *et al.* (2004), colour in many cases acts as a trigger for acceptance of products by the consumers, it plays an important role in visual recognition and assessment of the surface and the subsurface properties of the food. There was a significant difference among the values for colour at $p \leq 0.05$ as can be seen in Table 4.4. Extrudate TMF3 (90% *Togo marshall* rice and 10% PDS) had the highest colour preference value of 7.6 which is

interpreted on the hedonic scale to be like moderately. This might be as a result of the bright colour due to the high percentage of *Togo marshall* rice variety which is naturally white in colour. Extrudate PVF2 (60% Parboiled *Viwonor* rice variety and 40% PDS) had a significant least value of 4.6±2.12 (Dislike Slightly) for colour as can be seen in Table 4.4. This might be due to the *Viwonor* rice variety which naturally had a reddish pericarp and the high quantity of PDS. Also the parboiling treatment of the rice further darkened its colour. Consumers' dislike of the colour for PVF2 was not unusual since probably they might not be familiar with such darker coloured extruded snack especially since such products are not common on the local markets.

There was a statistically significant difference in relation to mouthfeel, aftertaste and overall acceptability at $p \leq 0.05$. With regard to mouthfeel, TMF4 (75% *Togo marshall* rice and 25% PDS) was liked moderately with a high preference value of 7 while PVF3 was disliked very much with a preference value of 2.8±1.40 (Table 4.4). The addition of high percentage of PDS to starch-rich flour could have led to the production of "protein-type" extrudates which reduced the stickiness of the products (Obatolu *et al*., 2006). Extrudate TMF4 significantly had a higher aftertaste preference of 6.5±1.78 (like slightly) with extrudate PVF5 having the least value of 2.7±1.83 as shown in Table 4.4. The high aftertaste value for TMF4 may be as a result of the high PDS content (25%) which led to the generation of a soy taste (Shogren *et al*. 2006) after consumption. With respect to overall acceptability, there was no significant difference among the values for TMF1, TMF2, TMF3, TMF4, TMF5, PTMF2, PTMF3 and PTMF4 with their preference score ranging between 6.8-6.3 (like moderately) and these were significantly higher as compared

to the rest of the extrudate with PVF3 being least preferred with a preference value of 3±2 (dislike moderately) as observed in Table 4.4.

Based on the mean scores for the overall acceptability values, five extrudates thus TMF3 (90% raw Togo Marshall (TM), 10% Partially defatted soybean (PDS)); TMF4 (75% raw TM, 25% PDS); TMF5 (82.5% raw TM, 17.5% PDS); PTMF3 (90% parboiled Togo Marshall (TM), 10% PDS); PTMF4 (75% parboiled TM, 25% PDS) were selected from the lot not only because they had high preference scores but also because their formulations had a high percentage of rice (especially since the project focused on maximizing the utilization of low grade rice varieties in Ghana). (Further analyses were then carried out on the five selected extrudates and their formulations).

Table 4.4: Mean scores of sensory attributes of twenty extruded snacks from different rice varieties and partially defatted soybean.

Extrudates	Sensory attribute				
	Aroma	Colour	Mouthfeel	Aftertaste	Overall acceptability
TMF1	6.3±0.82^{a}	7.1±0.74^{ab}	5.6±1.58^{abc}	6.1±0.99^{bc}	6.5±0.71^{b}
TMF2	6.6±1.07^{a}	5.7±1.83^{ab}	5.5±2.59^{abc}	5.6±2.59^{abc}	6.8±1.55^{b}
TMF3	6.2±1.81^{a}	7.6±1.58^{b}	5.9±2.08^{bc}	5.7±2^{abc}	6.6±2.12^{b}
TMF4	6.5±1.18^{a}	6.4±1.71^{ab}	7±1.83^{c}	6.5±1.78^{c}	6.8±1.69^{b}
TMF5	6.4±1.07^{a}	6.4±1.65^{ab}	6.2±1.32^{bc}	5.9±1.60^{bc}	6.5±1.27^{b}
PTMF1	5.2±0.92^{a}	6.4±1.07^{ab}	5.1±1.45^{abc}	4.7±1.25^{abc}	5.4±0.84^{ab}
PTMF2	5.9±1.37^{a}	6.6±1.84^{ab}	5.6±1.78^{abc}	5±1.94^{abc}	6.3±0.95^{b}
PTMF3	6.2±1.40^{a}	6.7±1.49^{ab}	6±2^{bc}	5.6±2.5^{abc}	6.7±1.83^{b}
PTMF4	6.3±1.34^{a}	7.1±0.99^{ab}	5.4±1.51^{abc}	5.8±1.81^{abc}	6.3±1.25^{b}
PTMF5	5.9±1.73^{a}	5.5±1.84^{ab}	5.4±1.90^{abc}	4.7±2.11^{abc}	5.7±1.16^{ab}
VF1	5±1.05^{a}	5.6±1.96^{ab}	5.3±1.49^{abc}	4.8±1.81^{abc}	5.1±1.45^{ab}
VF2	6.5±1.43^{a}	5.1±2.28^{ab}	5.2±1.81^{abc}	6±2^{bc}	5.4±1.96^{ab}
VF3	5.5±2.07^{a}	5.9±1.52^{ab}	4.1±1.85^{abc}	3.9±2.18^{abc}	4.2±2.04^{ab}
VF4	6.2±1.03^{a}	6±1.15^{ab}	5.3±2.06^{abc}	4.8±2.2^{abc}	4.2±2.49^{ab}
VF5	5.5±1.96^{a}	6.3±1.34^{ab}	5.4±1.51^{abc}	5.1±1.60^{abc}	5.2±1.81^{ab}
PVF1	5±1.15^{a}	5.3±1.83^{ab}	4.6±2.01^{abc}	3.9±1.79^{abc}	4.6±1.78^{ab}
PVF2	5.1±1.45^{a}	4.6±2.12^{a}	4.5±1.96^{abc}	4±2^{abc}	4.7±2.31^{ab}
PVF3	5±2.11^{a}	5.7±1.06^{ab}	2.8±1.40^{a}	3±2.49^{ab}	3±2^{a}
PVF4	6.2±1.23^{a}	5.6±1.43^{ab}	3.9±1.79^{ab}	3.2±1.81^{ab}	4.6±2.63^{ab}
PVF5	5±2.40^{a}	5.3±2.67^{ab}	3.5±2.59^{ab}	2.7±1.83^{a}	3.2±1.87^{a}
P value	0.0641	0.0081	0.0002	0.0000	0.0000

$^{a, b, c:}$ Means and standard deviation with the same superscript in a column are not significantly different at $p<0.05$. Scale: 9 - like extremely, 8 - like very much, 7 - like moderately, 6 - like slightly, 5 - neither like nor dislike, 4 - dislike slightly, 3 - dislike moderately, 2 - dislike very much, 1 - dislike extremely.

KEY: TM (Raw Togo Marshall rice variety), PTM (Parboiled Togo Marshall rice variety), V (Raw Viwonor rice variety), PV (Parboiled Viwonor rice variety), F1 (Rice, 67.5%: Soybean, 32.5), F2 (Rice, 60%: Soybean, 40), F3 (Rice, 90%: Soybean, 10), F4 (Rice, 75%: Soybean, 25), F5 (Rice, 82.5%: Soybean, 17.5)

4.7 Chemical analysis of the formulations of the selected extrudates

4.7.1 Apparent amylose content (AAC)

According to Singh *et al*. (2006), amylose constitutes larger percentage of the amorphous component of the starch granules where water penetration into the granule is more pronounced. Furthermore, Hamaker and Griffin (1993) as cited in Adu-Kwarteng *et al.* (2003) concluded that amylose content is an important factor in determining the cooking and pasting behaviour of rice and its end use. Results show that, there was a significant difference in the values for amylose content for all formulations at $p \leq 0.05$ as shown in Figure 4.4. There was an obvious trend indicating that formulation with high rice content had high amylose content for both untreated (raw) rice and parboiled rice based formulations. This was as a result of the rice being the main source of amylose in the formulations. It was also noticed that the parboiled rice based formulations generally had higher amylose content than those of untreated rice (Figure 4.4). The parboiling treatment probably led to extensive gelatinization of the rice starches (thus the amylose leaching out) and retrogradation of the amylose molecules which tend to associate or bind strongly together upon cooling (Adu-Kwarteng *et al.*, 2003). This suggests that parboiling as a treatment affects the availability of the amylose content of rice products during extraction and analyses.

Noda *et al*. (2003) and Riley *et al*. (2004) stated that amylose content plays a key role in the digestion of starches, as starches with low amylose content digest more easily than those of high amylose content. Therefore based on this it can be concluded that products from TMF4 (Raw rice 75 % and PDS 25 %) formulation are likely to digest more easily as compared to products from the other formulations.

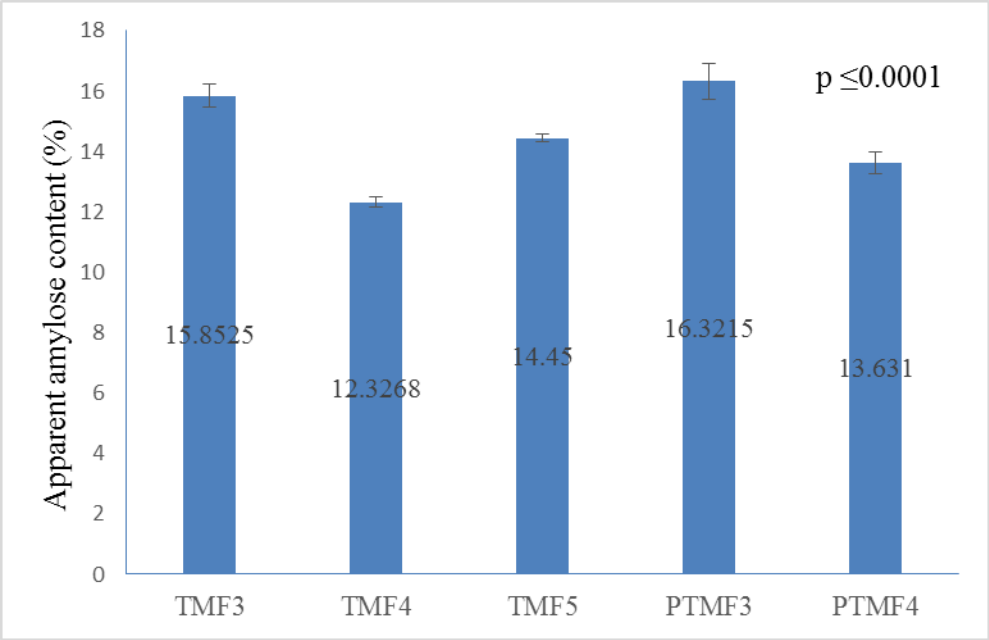


Figure 4.4: Apparent Amylose Content (AAC) for five formulations from Togo marshall rice variety and Partially Defatted Soybean.

TMF3 (90% raw Togo Marshall (TM) rice, 10% Partially defatted soybean (PDS)); TMF4 (75% raw TM rice, 25% PDS); TMF5 (82.5% raw TM rice, 17.5% PDS); PTMF3 (90% parboiled Togo Marshall (TM) rice, 10% PDS) and PTMF4 (75% parboiled TM rice, 25% PDS).

4.8 Functional properties of the formulations of the selected extrudates

4.8.1 Pasting properties of the five formulations

The Brabender viscoamylograph was used for the characterization of starches in the five different formulations. The viscosity of a paste depends on a large extent on the degree of gelatinization of the starch granules and the extent of their molecular breakdown (E-Dash *et al.*, 1983). Thus pasting viscosity allows one to determine the degree of modification of the starches or flours when subjected to heat treatment under moist conditions.

4.8.1.1 Beginning of gelatinization/Initial viscosity

Beginning of gelatinization/initial viscosity refers to the viscosity of the suspension in Brabender Unit (BU) at the start of heating. The initial viscosity of the flour in water at 30 ºC is called the cold viscosity. In the preparation of instant foods this property indicates the capacity of the flour to absorb water at room temperature to form a paste, gel or viscous liquid. In this work, it was noted that formulations were not significantly different from one another at $p \leq 0.05$ and that the rice treatment and the various formulations have no significant effects on the initial viscosity. Formulations from the parboiled rice had greater initial viscosity (thus before gelatinization) with viscosity values from 26.50-27.00 BU as against 16.50-20.00 BU for raw rice (Table 4.5). The high initial viscosity is typical of gelatinized starch products, which indicates a high level of polymer breakage with a consequent increased in water absorption even at room temperature. The TMF4 (Raw rice 75 % and PDS 25 %) had the lowest initial viscosity value (16.50±6.36) BU because it had a relatively high percentage of partially defatted soybean. Shimelis *et al*. (2006) concluded that legumes have a diluting effect by forming complexes with starch during gelatinization thereby restricting swelling of the suspension at the start of heating. The high initial viscosity values for the formulations with parboiled rice (PTMF4 and PTMF3) imply that they could be used directly in the preparation of foods with water or cold milk, since they show good ability to absorb water at room temperature as was also confirmed by Souza *et al*. (2012).

4.8.1.2 Maximum/peak viscosity

The maximum/peak viscosity which is the highest viscosity attained during the heating cycle before their physical breakdown was noted to be significantly different for the formulations at $p \leq 0.05$ as shown in Table 4.5. The formulation that was composed of 90% raw rice (*Togo marshall*) and 10% partially defatted soybean (TMF3) had a peak viscosity of 1036.50±6.36 BU. This formulation had the highest raw rice composition and the least amount of partially defatted soybean. Its peak viscosity was significantly higher than formulations with relatively lower amounts of raw rice, such as for TMF5 (82.5% raw rice; 17.5% PDS) (965.50±2.12) BU and TMF4 (739.00±35.36) BU. The increasing presence of partially defatted soybean in the formulation only served to dilute the starch content and consequently lowered the peak viscosity. Zaidul *et al.* (2007) observed that peak viscosity is influenced by starch granule swelling, amylose leaching, starch crystallinity, amylose content, branch chain length distribution of amylopectins as well as the content of other components such as proteins and lipids.

Parboiling of rice seemed to influence peak viscosity of the formulations. Even for formulations that had the same ratio of rice (90%) and PDS (10%), those with parboiled rice showed significantly lower peak viscosities than with raw rice. This is because the parboiling treatment of the rice which involved heating under moist condition led to the pre-gelatinization of the rice starch. According to Shimelis *et al*. (2006), peak viscosity is the maximum viscosity attained by gelatinized starch during heating in water, indicating the water binding capacity of the starch granule. Peak viscosity has also been closely linked with the degree of starch damage and that high starch damage results in high peak viscosity

(Sanni *et al*., 2001). Therefore the high peak viscosity of TMF3 (1036.50±6.36 BU) is likely to lead to its high starch damage.

4.8.1.3 Hot paste viscosity

The hot paste viscosity is the viscosity of the paste at the end of the heating phase at 95ºC. There were significant differences in the hot paste values of the different formulations (Table 4.5). High raw rice concentration led to higher hot paste viscosity in the formulations. The hot paste viscosities of the formulations made using parboiled rice showed significantly lower values. The values further decreased with increasing partially defatted soybean concentration. According to Bainbridge *et al*. (1996), high paste stability is a requirement for industrial use of starch and that starch with low paste stability will have a greater need for cross-linking than that with high value.

4.8.1.4 Breakdown

The hold period after the heating phase of 95ºC is often accompanied by a breakdown in viscosity. Breakdown viscosity according to Falade *et al.* (2014), measures the vulnerability or susceptibility of the cooked starch to disintegrate. Breakdown viscosities of the formulations were significantly influenced by rice treatment, and also by soybean content in the formulation. Formulations with raw rice showed tremendously high breakdown viscosities. On the other hand formulations with parboiled rice showed extremely low breakdown viscosity. According to Dutta *et al*. (2012) the decrease of breakdown viscosity might be due to the failure of complete pasting and swelling of starch granules induced by the reduction of water absorption of starch granules. For all

formulations however, increasing soybean content decreased the breakdown viscosity (Table 4.5). Adebowale *et al*. (2005) stated that the higher the breakdown in viscosity, the lower the ability of the starch sample to withstand heating and shear stress during cooking. Therefore, TMF3 (90% raw *Togo marshall* rice; 10% PDS) formulation showing higher breakdown viscosity value (624.50±6.36) will not be able to withstand more heating and shear stress compared to the other formulations.

4.8.1.5 Setback

According to Lee *et al*. (2004), setback values indicate the hardness of gel paste upon cooling and it is considered by Zaidul *et al*. (2003) to be an indirect measurement of retrogradation tendency or syneresis of starch upon cooling of cooked starch pastes. There were significant differences among the formulations in terms of setback viscosity. Formulations with raw rice, such as TMF3 had very high setback values (535.00±25.46), while formulations with parboiled rice such as PTMF4 had very low setback viscosities value (170.50±2.12) (Table 4.5). The low setback viscosity indicates a low tendency to retrograde and the low likelihood of syneresis taking place upon cooling. According to Adebowale *et al*. (2003), the lesser tendencies of starch to retrograde is an advantage in food products such as soups and sauces, which undergo loss of viscosity and precipitation as a result of retrogradation.

4.8.1.6 Pasting temperature

Shimelis *et al.* (2006) observed that pasting temperature is one of the properties that determines the temperature at which the viscosity begins to increase during the heating

process. This gives an indication of the minimum temperature required for sample cooking, as well as the energy cost involved and the stability of other components. There were no significant differences among the formulations in terms of pasting temperature. From Table 4.5, it can be observed that formulations from raw rice had higher pasting temperatures than formulations from parboiled rice. TMF5 had the highest pasting temperature of (68.60±0.14) ^{o}C followed by TMF3 (68.10±0.14) ^{o}C and TMF4 (57.05±17.75) ^{o}C while PTMF4 (51.40±0.00) ^{o}C and PTMF3 (47.85±1.34) ^{o}C had the lowest pasting temperature. This is because the parboiling process which required a heat treatment might have caused a partial starch degradation which led to the decrease in pasting temperature. This contradicts the findings by Swasdisevi *et al.* (2010) that parboiling treatment of rice causes the increase in pasting temperature. The contradiction could be due to the presence of proteins and lipids from PDS which interacted with the starch from rice as was observed with the initial viscosities as noted in Table 4.5. Starches with lower pasting temperatures are generally considered to be easier to cook. However, very low pasting temperatures are associated with low paste stability, which is usually considered to be an undesirable property (Aishat *et al*., 2007).

Table 4.5: Pasting parameters of five formulations (Rice and Partially Defatted Soybean (PDS)).

Samples	Parameters					
	Beginning of gelatinization [BU]	**Maximum viscosity [BU]**	**Hot Paste Viscosity [BU]**	**Breakdown [BU]**	**Setback [BU]**	**Pasting temperature[ºC]**
TMF3	**20.00**±1.41 ^{abc}	**1036.50**±6.36^{e}	**412.00**±0.00^{e}	**624.50**±6.36^{c}	**535.00**±25.46^{d}	**68.10**±0.14 ^{ab}
TMF4	**16.50**±6.36 ^{a}	**739.00**±35.36^{c}	**310.50**±9.19^{c}	**428.50**±26.16^{b}	**491.00**±5.66^{c}	**57.05**±17.75 ^{ab}
TMF5	**19.00**±0.00 ^{ab}	**965.50**±2.12^{d}	**360.00** ±12.73^{d}	**605.50** ±14.85^{c}	**529.50**±14.85 ^{d}	**68.60**±0.14 ^{b}
PTMF3	**26.50**±0.71 ^{bc}	**194.00**±0.00^{b}	**192.50**±0.71^{b}	**1.50** ±0.71^{a}	**251.50**±0.71^{b}	**47.85**±1.34 ^{a}
PTMF4	**27.00**±0.00 ^{c}	**130.50**±0.71^{a}	**130.00**±1.41^{a}	**0.50**±0.71^{a}	**170.50**±2.12^{a}	**51.40**±0.00 ^{ab}
p-value	**0.0511**	<**0.0001**	<**0.0001**	<**0.0001**	<**0.0001**	**0.1412**

* Values are means ± standard deviations. Different letters (a, b, c, d, e) in the same column for each parameter indicate significant differences between means ($p \leq 0.05$). BU= Brabender Unit. TMF3 (90% raw Togo Marshall (TM) rice, 10% Partially defatted soybean (PDS)); TMF4 (75% raw TM rice, 25% PDS); TMF5 (82.5% raw TM rice, 17.5% PDS); PTMF3 (90% parboiled Togo Marshall (TM) rice, 10% PDS) and PTMF4 (75% parboiled TM rice, 25% PDS).

4.9 Chemical analysis of the five formulation and their extrudates

4.9.1 Changes in moisture content for formulation and their extrudates

Moisture content is an important determinant of shelf stability of food products. The moisture content of the formulations ranged from 10.10-12.57% with formulation TMF5 having the highest moisture content of 12.57%. The highest moisture content for TMF5 led to the highest expansion ratio of 3.79 and the least bulk density of 0.07 when the formulation was extruded as shown in Table 4.7. There was also a substantial decrease in moisture content for all extudates after extrusion and this might be as a result of the pressure differences in and outside the extruder die enabling the release of water vapour to the outside of the die thereby forming vacuoles leading to product expansion. Ashworth and Draper in 1992 stated that high moisture products (>12%) usually have shorter shelf stability compared to lower moisture products (<12%). Therefore, the low moisture content of the extrudates ranging 6.04-8.77% will allow easy handling, storage and extended shelf life of the extrudates. This is because products with higher moisture content have high water activity, which enhances microbial activity leading to spoilage.

Table 4.6: Changes in moisture content

	Samples					
Moisture content %	TMF3	TMF4	TMF5	PTMF3	PTMF4	p-value
Formulation	11.95±0.13^{a}	11.67±0.35^{a}	12.57±1.94^{a}	11.03±0.47^{a}	10.1±0.14^{a}	0.2116
Extrudates	8.77±0.17^{c}	6.05±0.04^{a}	6.04±0.0^{a}	7.74±0.25^{b}	7.93±0.29^{b}	0.0001

Values are means ± standard deviations. Different letters (a, b, c) in the same row for each parameter indicate significant differences between means ($p \leq 0.05$). TMF3 (90% raw Togo Marshall (TM), 10% partially defatted soybean (PDS)); TMF4 (75% raw TM, 25% PDS); TMF5 (82.5% raw TM, 17.5% PDS); PTMF3 (90% parboiled Togo Marshall (TM), 10% PDS); PTMF4 (75% parboiled TM, 25% PDS).

4.10 Physical properties of the selected Rice extrudates

The physical properties determined included expansion ratio, bulk density, colour and hardness of extrudates.

4.10.1 Expansion ratio (ER)

When starch is extrusion-cooked, expansion is dependent on the formation of a starch matrix that entraps the water vapour, resulting in formation of bubbles (Guy *et al*., 1988). There was a significant difference among the extrudates in their expansion ratio (Table 4.7). Extrudates made with raw rice had significantly higher expansion ratios than those from parboiled rice. The expansion ratio value (2.31±0.11) for PTMF4 (Parboiled rice 75 % and PDS 25 %) was significantly low as compared to the others. Among all the formulations increasing amount of soybean content generally decreased the expansion ratio. This is a confirmation of Chaiyakul *et al.* (2009) study which found that the addition of protein to starchy raw materials in extrusion cooking reduced the expansion of the products by reducing the extensibility of the starch polymer during its extrusion at the die exit.

4.10.2 Bulk density (BD)

Bulk density (BD) is an index of the extent of puffing and it considers expansion in all directions. The bulk density values for the extrudates were in the range 0.07-0.13 g/cm^3. From Table 4.7, it can be observed that parboiled rice based samples had higher density than that of raw rice products (thus PTMF3 (0.13) and TMF3 (0.11) g/cm^3; PTMF4 (0.13) and TMF4 (0.08) g/cm^3), suggesting that the parboiling treatment modified starch matrix

leading to a more compact structure that is less able to organize under high temperature and pressure conditions.

4.10.3 Colour

Colour is one of the important quality factor directly related to the acceptability of food products and it is therefore an important physical property of extruded products. Altan *et al*. (2008) observed that colour changes can give information about the extent of browning reactions such as caramelization, Maillard reaction, degree of cooking and pigment degradation that take place during the extrusion process. There was a significant difference in the L* values of the extrudates as shown in Table 4.7. There was generally a decrease in L* values as the level of PDS increased. Obatolu *et al*. (2006) and Yagci *et al*. (2009) confirmed this by reporting that extrudates turn to be slightly darker when the quantity of a partially defatted legume in the formulation is increased. It was also observed that extrudates obtained from parboiled rice formulations had significantly lower L* values as compared to those obtained from raw rice formulations (Table 4.7). This suggest that the diffusion of husk pigments into the endosperm during the parboiling treatment significantly had an impact on the L* index.

There was also a significant difference among the values for a* of the extrudates at $p \leq 0.05$ as shown in Table 4.7. This implies that the parboiling treatment positively impacted on the redness of the extrudates.

Table 4.7 shows that b* index increased as the amount of PDS in the formulation increased, suggesting that the yellowness of the extrudates increased with increasing soybean content. This was in agreement with work by Obatolu *et al.*, (2006) that the incorporation of PDS

enhanced the b* of puffed extrudates. This can be attributed to the presence of carotenoids pigments in the soybean which was not totally degraded after the extrusion cooking. Furthermore, it was also observed that b* values for extrudates from parboiled rice were significantly higher than that from raw rice extrudates. This might be that the diffusion of colour pigments from the rice husk into the endosperm during the parboiling process significantly affected the b* thus the yellowness. It can therefore be concluded that soybean level in the formulation and the parboiling treatment has an impact on the colour of the extrudates.

Table 4.7: Expansion ratio, bulk density and colour characteristics of five extrudates

	Parameter				
			Colour		
Samples	**Expansion ratio**	**Bulk density (g/cm^3)**	L*	a*	b*
TMF3	**3.73**± 0.30^{c}	**0.11**±0.02^{b}	**87.37**±0.51^{a}	**-0.63**±0.06^{a}	**15.14**±0.24^{a}
TMF4	**3.30**± 0.16^{b}	**0.08**±0.00^{a}	**85.04**±0.61^{b}	**-0.04**±0.06^{b}	**20.42**±0.29^{b}
TMF5	**3.79**± 0.21^{c}	**0.07** ±0.00^{a}	**84.10**±0.24^{c}	**0.11**±0.02^{c}	**19.72**±0.19^{b}
PTMF3	**3.14**±0.15^{b}	**0.13**±0.01^{c}	**82.61**±0.56^{d}	**0.31**±0.06^{d}	**19.31**±0.35^{c}
PTMF4	**2.31**± 0.11^{a}	**0.13**±0.01^{c}	**81.74**±0.92^{e}	**0.62**±0.11^{e}	**21.25**±0.63^{d}
p-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Values are means ± standard deviations. Different letters (a, b, c, d, e) in the same column for each parameter indicate significant differences between means ($p \leq 0.05$). TMF3 (90% raw Togo Marshall (TM), 10% partially defatted soybean (PDS)); TMF4 (75% raw TM, 25% PDS); TMF5 (82.5% raw TM, 17.5% PDS); PTMF3 (90% parboiled Togo Marshall (TM), 10% PDS); PTMF4 (75% parboiled TM, 25% PDS).

4.10.4 Hardness of five extrudates

Hardness was determined by puncturing the eatrudates. Ding *et al.* (2005) stated that hardness is the average force required for a probe to penetrate an extrudate. There were significant differences among the values for hardness as shown in Figure 4.5. Choudhury and Gautam (2003) as well as Onwulata *et al*. (2001) concluded that extrudate hardness was strongly influenced by protein content. Increased protein content in feed material produced a less expanded product and more rigid network, resulting in higher resistance to shear and thereby increasing extrudates hardness (Chaiyakul *et al.*, 2009). This was largely observed in extrudates obtained from formulations with parboiled rice. It was also noted that extrudates obtained from formulations with parboiled rice had higher hardness as compared to those obtained from raw rice formulations.This has been explained by Bhattacharya (2004) to be as a result of the more homogeneous and compact ultrastructure obtained as a result of starch gelatinization during the parboiling treatment. It can therefore be concluded that the parboiling treatment and amount of protein as determined by the level of PDS in the formulation significantly affected hardness.

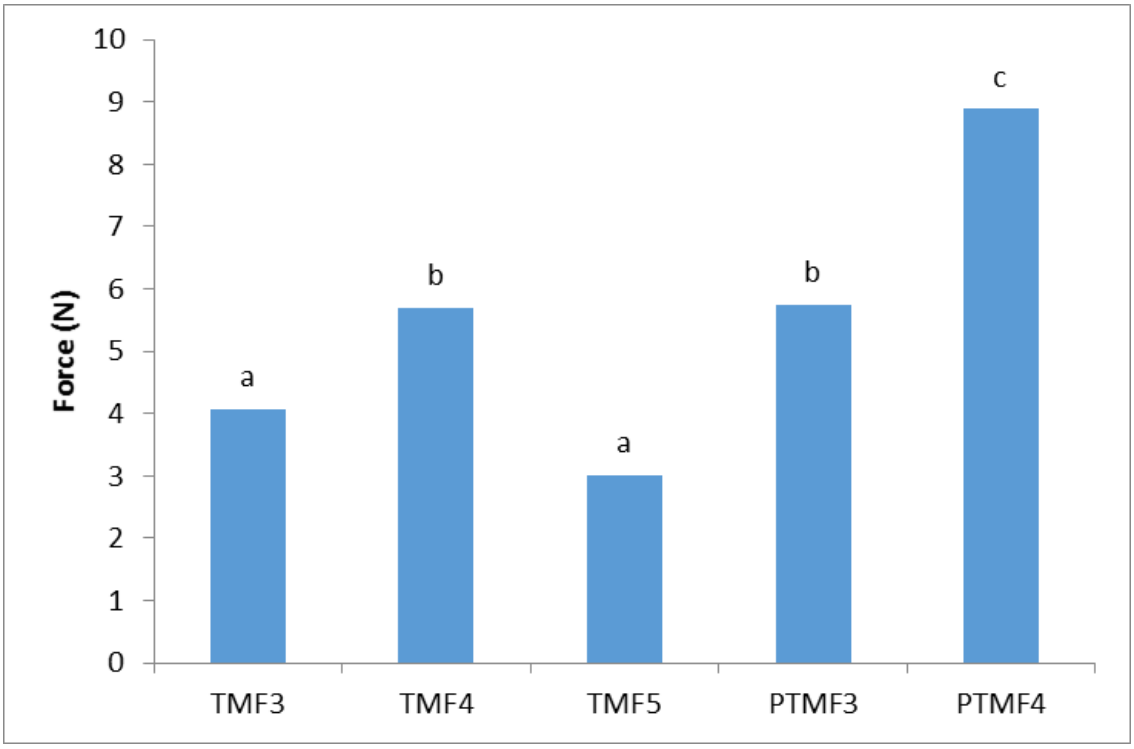


Figure 4.5: Hardness (N) of five extrudates.

Different letters (a, b, c) represent statistically significant differences between the samples ($p<0.05$).

TMF3 (90% raw Togo Marshall (TM), 10% partially defatted soybean (PDS)); TMF4 (75% raw TM, 25% PDS); TMF5 (82.5% raw TM, 17.5% PDS); PTMF3 (90% parboiled Togo Marshall (TM), 10% PDS); PTMF4 (75% parboiled TM, 25% PDS).

4.11 Functional properties of selected extrudates

The functional properties determined included water absorption index (WAI) and water solubility index (WSI).

4.11.1 Water absorption index (WAI)

Water absorption index, an indicator of the ability of flour to absorb water, depends on the availability of hydrophilic groups which bind water molecules and on the gel-forming capacity of macromolecules. Anderson *et al*. (1969) stated that WAI measures the amount of water absorbed by starch and can be used as an index of gelatinization. The order for WAI in this study is as follows TMF4<TMF5<PTMF4<PTMF3<TMF3 as shown in Table 4.8. There was a significant difference among the values for WAI at $p \leq 0.05$. According to Colonna *et al*. (1989) starch granules damaged or gelatinized during extrusion cooking absorb water and swell at room temperature unlike native starches which do not absorb water at room temperature. It can therefore be deduced that the significantly higher WAI value for TMF3 (90% raw Togo Marshall (TM), 10% partially defatted soybean (PDS)) after the extrusion cooking may be as a result of its great starch damage or degradation due to its high raw rice quantity.

4.11.2 Water solubility index (WSI)

According to Hernandez-Diaz *et al*. (2007), water solubility index (WSI) is used as an indicator of starch degradation and also measures the degree of starch conversion during extrusion cooking thus at lower WSI there is minimal degradation of starch and such condition leads to low amounts of soluble molecules of the extrudates. There were significance differences in the WSI values at $p \leq 0.05$ (Table 4.8). The significantly higher WSI value for extrudate TMF5 implies that the amount of free polysaccharide or soluble molecule released from the extrudate after addition of excess water was higher than that of the other extrudates (Yang *et al*., 2008).

Table 4.8: Water Absorption (WAI) and water solubility indexes (WSI) of five extrudates.

Samples	Parameters	
	WAI	**WSI**
TMF3	**6.10**±0.10^{d}	**19.62**±0.40^{b}
TMF4	**4.91**±0.05^{a}	**23.27**±0.61^{d}
TMF5	**5.02**±0.15^{a}	**25.27**±0.49^{e}
PTMF3	**5.63**±0.03^{c}	**21.57**±0.12^{c}
PTMF4	**5.33**±0.10^{b}	**18.02**±0.27^{a}
p-value	**0.0003**	**0.0001**

TMF3 (90% raw Togo Marshall (TM), 10% partially defatted soybean (PDS)); TMF4 (75% raw TM, 25% PDS); TMF5 (82.5% raw TM, 17.5% PDS); PTMF3 (90% parboiled TM, 10% PDS); PTMF4 (75% parboiled TM, 25% PDS).

4.12 Molecular characterization of protein from five extrudates

The protein molecular analysis determined included protein solubility, protein electrophoretic pattern, accessible protein thiols and in-vitro protein digestibility of extrudates.

4.12.1 Protein solubility

Structural features of proteins in rice-soybean extrudates were evaluated by extraction in three different buffers thus buffer A (50mM sodium phosphate monobasic dihydrate and 100mM NaCl), buffer B: (buffer A and 6M urea) and Buffer C (buffer B and 10mM DL-Dithiothreitol (DTT)) with different dissociating abilities towards covalent and non-covalent inter-protein bonds. In particular saline buffer (buffer A) enables the solubilisation of water and salt soluble proteins such as albumins and globulins (Bhattacharya, 2004). As shown in Figure 4.6, the amount of soluble proteins in buffer A appears very low and

comparable in all extrudates. Addition of a denaturing agent such as urea to the saline extractant resulted in an increase in protein solubility as shown in Figure 4.6. This is due to the fact that urea promoted the dissociation of aggregates stabilized by hydrophobic interactions (Moroni *et al*., 2009). A further increase in the amount of soluble proteins was evident when both urea and dithiothreitol (DTT) were present in the extractant (buffer C). This is because the addition of dithiothreitol (DTT) further reduced disulphide bonds thus by destabilizing some tertiary protein folding and breaking up of quaternary protein structure (Lukesh *et al*., 2012).

This was confirmed by Cabrera-Chávez *et al*. (2012) and Mariotti *et al*. (2011) who concluded that combining urea and DTT in the extraction medium further increased the amount of solubilized protein. Comparing extrudates from raw rice (ie TMF3, TMF4 and TMF5), it was obvious that the amount of soluble proteins in all buffers (A, B and C) increased as the percentage of partially defatted soybean (high protein source) increased.

In terms of TMF4 (raw *Togo marshall* (TM) 75% and partially defatted soybean (PDS) 25%) and PTMF4 (parboiled (TM) 75% and PDS 25%), the highest amount of solubilised proteins for PTMF4 as compared to TMF4 in all buffers suggests that both hydrophobic interactions and inter-protein disulphide bonds play a fundamental role in the overall stabilization of the proteins. This was similarly observed by Barbiroli *et al.* (2013) while addressing the effects of processing conditions on rice macromolecular structure and protein-protein and protein-macromolecules interactions in rice pasta. In relation to TMF3 and PTMF3 the same observation for TMF4 and PTMF4 was made during the solubilisation with buffer C but for buffer A and B, a vice versa observation was made (Figure 4.6). This may suggest the presence of strong aggregation phenomena especially

due to the high percentage of parboiled rice (90%) in PTMF3. Cabrera-Chávez *et al.* (2012) suggested that the sequential starch gelatinization/retrogradation cycles might have resulted in protein entrapment in an organized starch structure. The presence of a highly denatured network is more evident since a significant fraction of the total proteins is not solubilised in buffer A and B but unlike protein solubilisation using buffer C, the presence of disulphide-reducing agent, DL-Dithiothreitol (DTT), enabled an increase in protein solubilisation (Moroni *et al*., 2009). This also confirms the role of disulphide bonds in the stabilisation of insoluble protein aggregates (Bonomi *et al*., 2012). It can therefore be concluded that a structured protein network is present and is stabilized by inter-protein disulphide bonds. Furthermore, parboiling treatment and the quantity of PDS affects the proteins structural features in the rice-soybean extrudates.

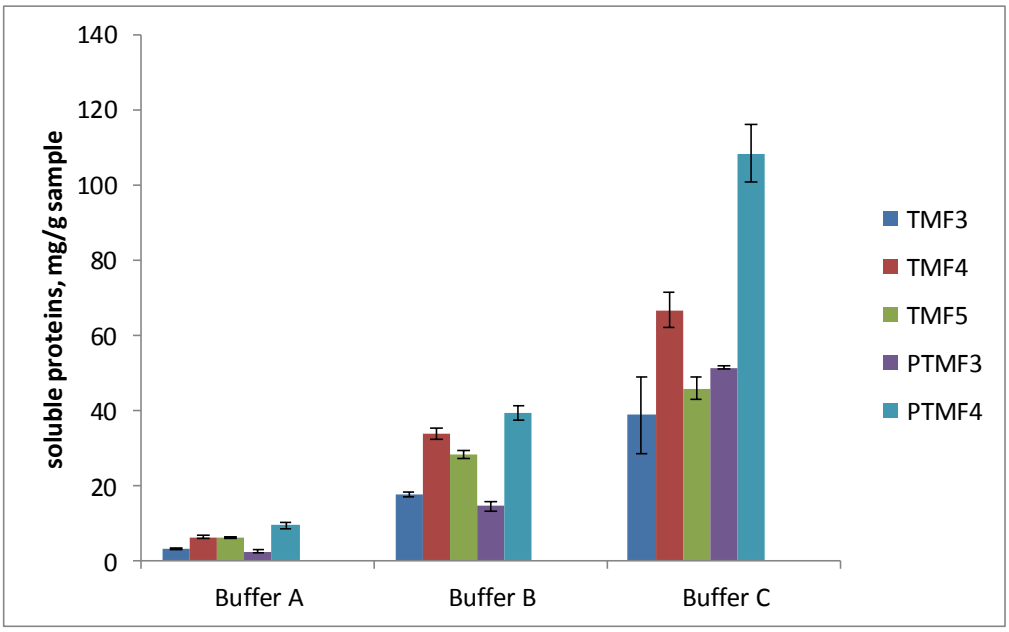


Figure 4.6: Amount of solubilized protein in different buffers systems.

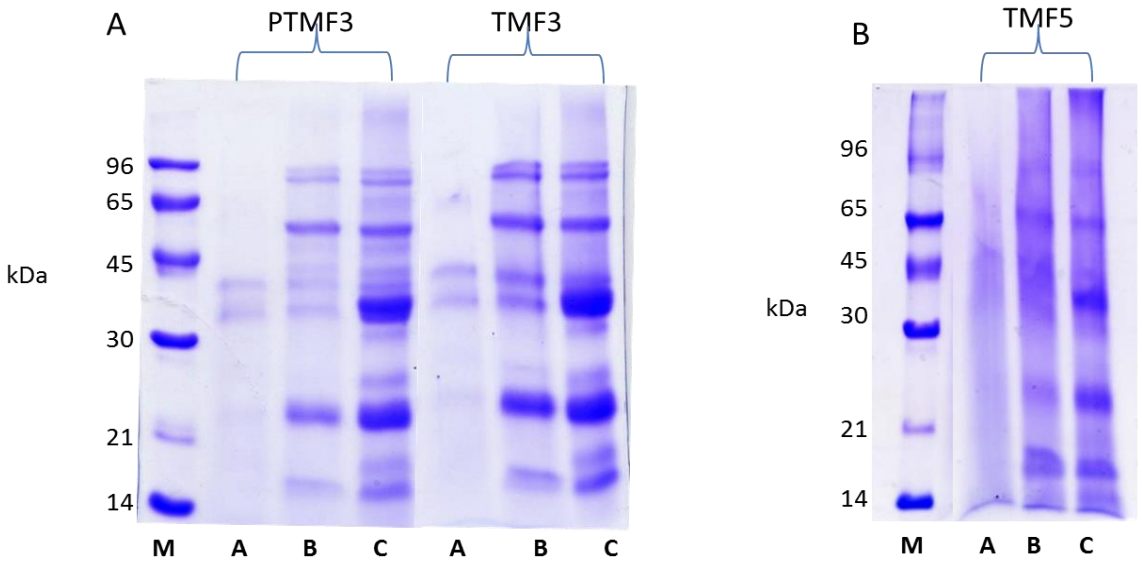
Buffer A: (50mM sodium phosphate monobasic dihydrate and 100mM NaCl). Buffer B: (Buffer A+ 6M urea) and Buffer C (Buffer B+ 10mM DL-Dithiothreitol (DTT)). TMF3 (90% raw Togo Marshall (TM), 10% partially defatted soybean (PDS)); TMF4 (75% raw TM, 25% PDS); TMF5 (82.5% raw TM, 17.5% PDS); PTMF3 (90% parboiled TM, 10% PDS); PTMF4 (75% parboiled TM, 25% PDS).

4.12.2 Electrophoretic patterns of extracted proteins from the selected extrudates

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) has been used as a qualitative tool for the separation and eventual molecular weight determination of proteins in foods sources (Luo *et al.*, 2004). Separation of proteins in the extrudates by SDS-PAGE allowed for a qualitative comparison among samples in terms of representative protein families and to estimate their molecular weight, as shown in Figure 4.7.

In relation to all five extrudates, it can be noted that the number and the intensity of peptide bands increased when urea (buffer B) or urea and DTT (buffer C) were used for protein extraction, thus confirming the presence of protein aggregates stabilized by hydrophobic interactions and disulfide bonds. The electrophoretic patterns of the

extrudates confirm the solubility data presented in Figure 4.6 and their tracings or peptide bands indicate that the proteins solubilized by urea and DTT (buffer C) are derived from disulfide-linked aggregates and are larger than those solubilized in the absence of chaotrope eg. urea and reductants such as DTT (Mariotti *et al*., 2011). Comparing all the five extrudates, those with high percentage of partially defatted soybean (PDS) gave more peptide bands and had higher molecular weight. This suggest that extrudates with more PDS percentages are much diversified in types of proteins as compared to those with lower percentage of PDS.



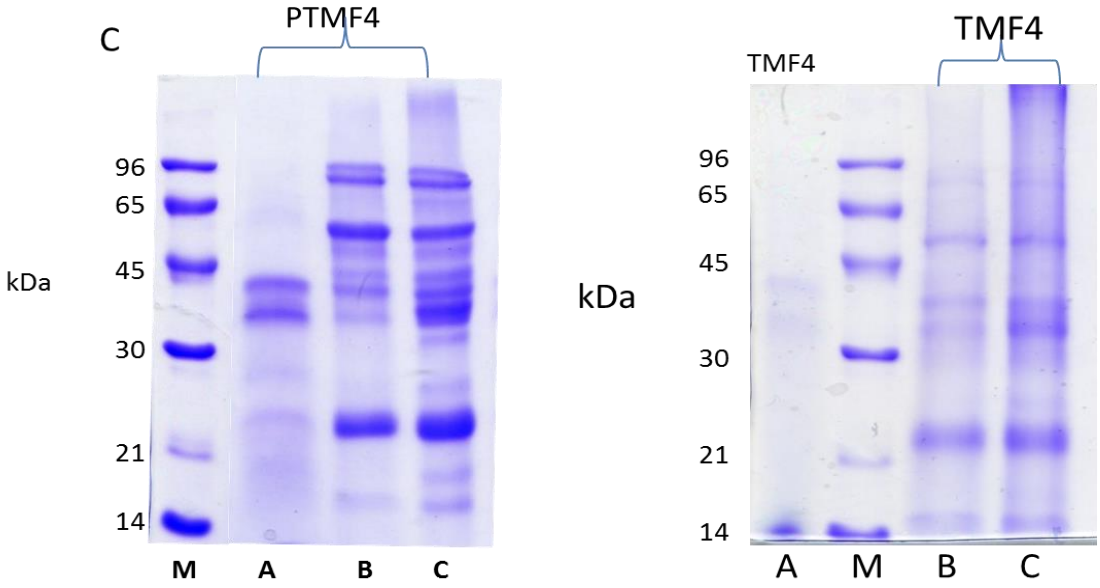


Figure 4.7: Electrophoretic patterns of proteins in five extrudates

(from raw/parboiled rice and partially defatted soybean) by using different extraction buffers.

Thus Buffer A: 50mM sodium phosphate monobasic dihydrate and 100mM NaCl; |Buffer B: buffer A + 6M urea; Buffer C: buffer B + 10mMDTT. M: Protein marker, TMF3 (90% raw Togo Marshall (TM), 10% partially defatted soybean (PDS)); TMF4 (75% raw TM, 25% PDS); TMF5 (82.5% raw TM, 17.5% PDS); PTMF3 (90% parboiled TM, 10% PDS); PTMF4 (75% parboiled TM, 25% PDS).

4.12.3 Accessible protein thiols from extrudates

According to Iametti *et al.* (2013), thiol–disulfide exchange reactions, are major contributors to the formation of a covalently-linked protein networks in many foods, where disulfides represent the most "natural" type of inter protein covalent bond. In this frame it may be pointed out that thiol–disulfide exchange reactions occur as a function of the accessibility of the involved thiols, which in turn depends on structural features of the proteins. The degree of structural "stiffness" of the protein network or the extent of

modification induced in the individual extrudates was evaluated through thiol accessibility studies in the presence and absence of urea. These studies were carried out independently of protein solubility and the measurements provide two separate parameters, namely the total content in reactive thiol (measured under denaturing conditions on both the soluble and insoluble fraction) and the increment in thiol accessibility due to denaturation (Iametti *et al*., 2006). Generally, the accessible thiols values for all extrudates suggest a loose protein network and this is as a result of the extrusion cooking due to the high temperature involved. It is also noted that the number of accessible thiols increased in the presence of urea for all extrudates as shown in Figure 4.8. This increase might be due to the fact that the hydrophobically stabilized compact structure of the protein matrix must be destabilized by the chaotrope enabling thiols to readily become accessible (Mariotti *et al*., 2011). The relatively lower values for extrudes obtained using parboiled rice formulations (PTMF4 (0.9559 µmol/g) and PTMF3 (0.9191µmol/g)) as compared to extrudates obtained from raw rice formulations (TMF4 (1.2647 µmol/g) and TMF3 (1.0662 µmol/g) in the presence of urea confirm the impact of parboiling treatment on the compactness of the protein matrix making thiols not easily accessible. It can therefore be concluded that rice parboiling had an effect on the overall protein organization of the extrudates.

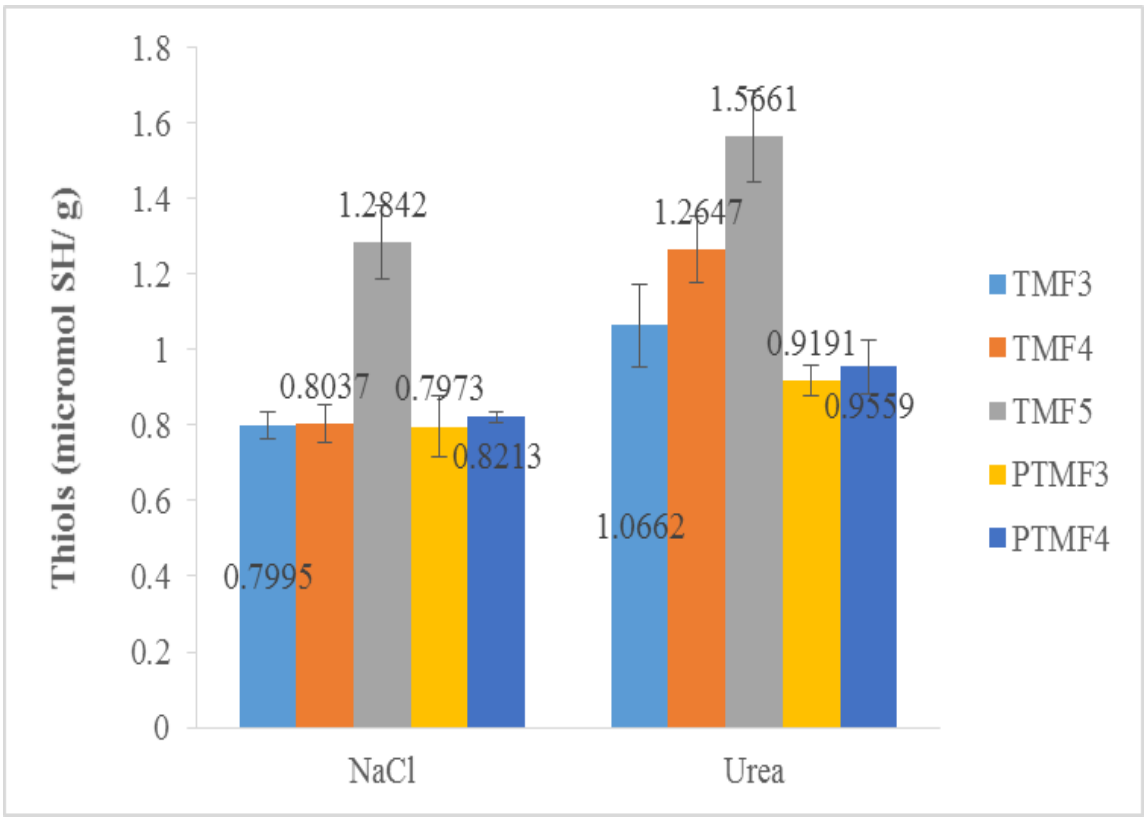


Figure 4.8: Accessible thiols of five extrudates from rice and partially defatted soybean.

TMF3 (90% raw Togo Marshall (TM), 10% partially defatted soybean (PDS)); TMF4 (75% raw TM, 25% PDS); TMF5 (82.5% raw TM, 17.5% PDS); PTMF3 (90% parboiled TM, 10% PDS); PTMF4 (75% parboiled TM, 25% PDS).

4.12.4 In vitro protein digestibility

Protein digestibility is one of the most important factors determining protein quality of raw materials and their products (Parsons *et al.*, 1991). The results show that with the exception of TMF5, all the other extrudates showed almost an equal digestibility by pepsin after 1 hour incubation time (Figure 4.9). All the extrudates were however hydrolyzed by pancreatin but at significantly different rates. Extrudates made from raw rice formulations were generally more digestible by pancreatin. However increasing amounts of the partially

defatted soybean in the formulation decreased the protein digestibility by pancreatin. Thus among the extrudates made with raw rice formulation, those with high percentage of PDS (25%) showed the least protein digestibility after the fourth hour. This might suggest the presence of quite a substantial amount of antinutritional factor such as trypsin inhibitors that bind to trypsin and thereby reducing the extent of protein-digestion (Stauffer, 1990). Petitot *et al*. (2009) and de Zorzi *et al*. (2007) also concluded that resistance of proteins to digestion could be attributed to the presence of highly aggregated proteins stabilized by covalent protein interactions, such as inter peptide cross-linking. Extrudates obtained from parboiled rice had low pancreatin digestibility, irrespective of the amount of soybean in the formulations. Starch modification induced by the parboiling treatment may have resulted in the decrease of protein digestibility by pancreatin. This could be related to the presence of the pre-gelatinized starch-rich rice matrix that could have changed the pattern and outcome of protein structural reorganization.

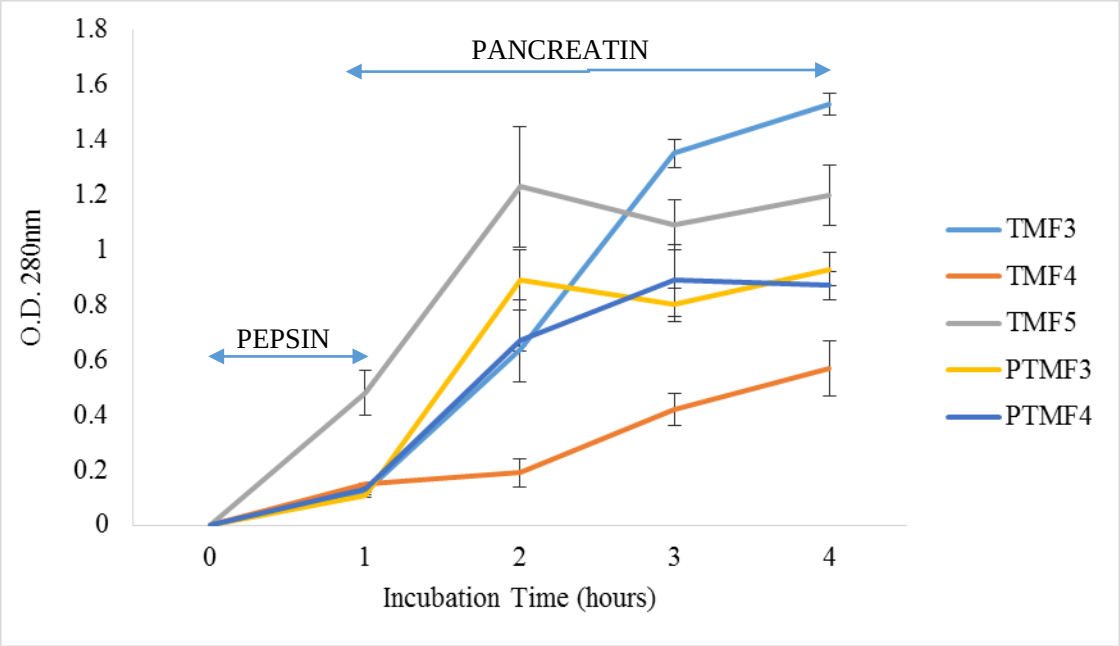


Figure 4.9: In-vitro protein digestibility of five extrudates from rice and partially defatted soybean.

TMF3 (90% raw Togo Marshall (TM), 10% partially defatted soybean (PDS)); TMF4 (75% raw TM, 25% PDS); TMF5 (82.5% raw TM, 17.5% PDS); PTMF3 (90% parboiled TM, 10% PDS); PTMF4 (75% parboiled TM, 25% PDS).

4.13 Aroma and taste determination using electronic nose and tongue

According to Baldwin *et al*. (2011), electronic noses (e-noses) and electronic tongues (e-tongues) mimic the human smell and taste, and their communication with the human brain.

4.13.1 Aroma analysis of the five extrudates

The electronic nose is the common name of electrochemical sensor systems responding to flavour/odour (volatiles) using an array of simple and non-specific gas sensors and a pattern recognition software system (Buratti *et al.*, 2004). Essentially, it is particularly useful for the analysis of headspace of liquid or solid food samples (Schaller *et al*. 1998)

and each odour leaves a characteristic pattern or fingerprint on the sensor array, and a pattern recognition is use to distinguish and recognize the odours.

The electronic nose was used to evaluate the aromatic profile of extrudates (Figure 4.10a) obtained from different formulations. For each sample the electronic nose responses were collected and analyzed using multivariate procedures (Principal Component Analysis (PCA)) performed in a correlation matrix to achieve a partial visualization of the data set in a reduced dimension. By examining the score plot (Figure 4.10a), the first two principal components (PCs) could explain 81.4% of total variance, and it can be seen that the extrudates are distributed along both PC1 and PC2 from right to the left part of the plots describing a trend related to changes in aroma compounds.

Considering the PC1 (thus assessing sample similarity in a vertical direction), it can be observed that extrudates were discriminated into two folds thus raw rice based extrudates with PDS content not less than 17.5% on the right and parboiled rice based extrudates; raw rice based extrudate with PDS content of 10% on the left. Generally on the loading plot (Figure 4.10b) which explains 45.5% of the total variation, it is noticed that only W1W (sulphur-organic) had the least magnitude and therefore had no substantial influence on PC1 while the others had a higher influence (W1C (aromatic), W3C (aromatic), W6S (hydrogen), W5C (arom-aliph), W1S (broad-methane), W2S (broad-alcohol), W2W (sulph-chlor) and W3S (methane-aliph) having negative PC scores while W5S (broad range) had a positive PC score.

Extrudate made using raw rice with at least 17.5% PDS (a and c) located in the positive part of the plot suggests similar odorous compounds due to the response of W5S (broad range) sensor on the loading plot. This might be as a result of oxidation of their relatively

high lipid content from the PDS as compared to the raw rice based extrudate with 10% PDS. This was confirmed by Choe *et al*. (2006) who stated that the unsaturated fatty acids present in food lipids such as oleic, linoleic, α and γ-linolenic can undergo thermally induced oxidation during cooking to generate volatile compounds which determine the aroma characteristics of the foods.

Extrudates obtained from parboiled rice based formulations with any amount of partially defatted soybean (d and e), are located to the left (or negative) half of PC1. Thus the parboiling treatment according to Sareepuang *et al.* (2008) which involves gelatinization and pigment transfers from the rice husks to the rice endosperm affects the organoleptic properties of cooked rice. Maarse (1991), Parnsakhorn and Noomhorm (2008) also concluded that parboiling treatment of the rice gave rise to some peculiar flavonoids thereby leading to their similar aromatic profile.

The second principal component (PC2) could explain 35.9% of the total variation in the sensory scores. The sensory scores for extrudates in the negative half of PC2 were influenced by WC sensor series (Figure 4.10b).The samples in this location were either extrudates made with raw rice with 25% PDS, or with any type of rice treatment (raw or parboiled) with low (10%) PDS.

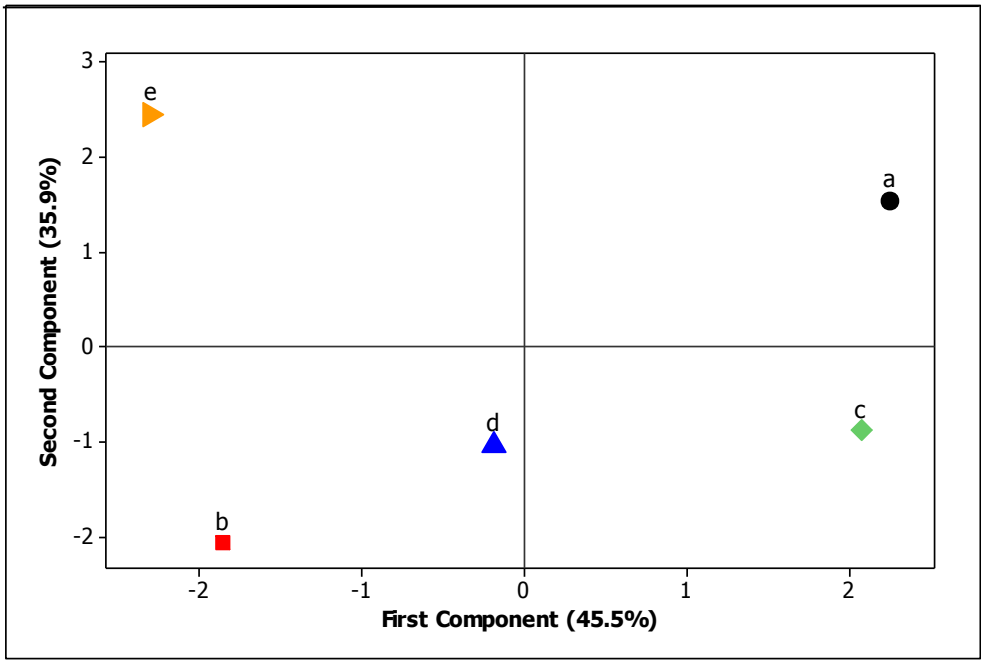


Figure 4. 10a: PCA score plot of five extrudates.

a: TMF5 (raw *'Togo marshall'* rice variety-82.5%, Partially defatted soybean (PDS)-17.5%); b: TMF3 (raw *'Togo marshall'* rice variety-90%, PDS-10%); c: TMF4 (raw *'Togo marshall'* rice variety-75%, PDS-25%); d: PTMF3 (parboiled *'Togo marshall'* rice variety-90%, PDS-10%); e: PTMF4 (parboiled *'Togo marshall'* rice variety-75%, PDS-25%).

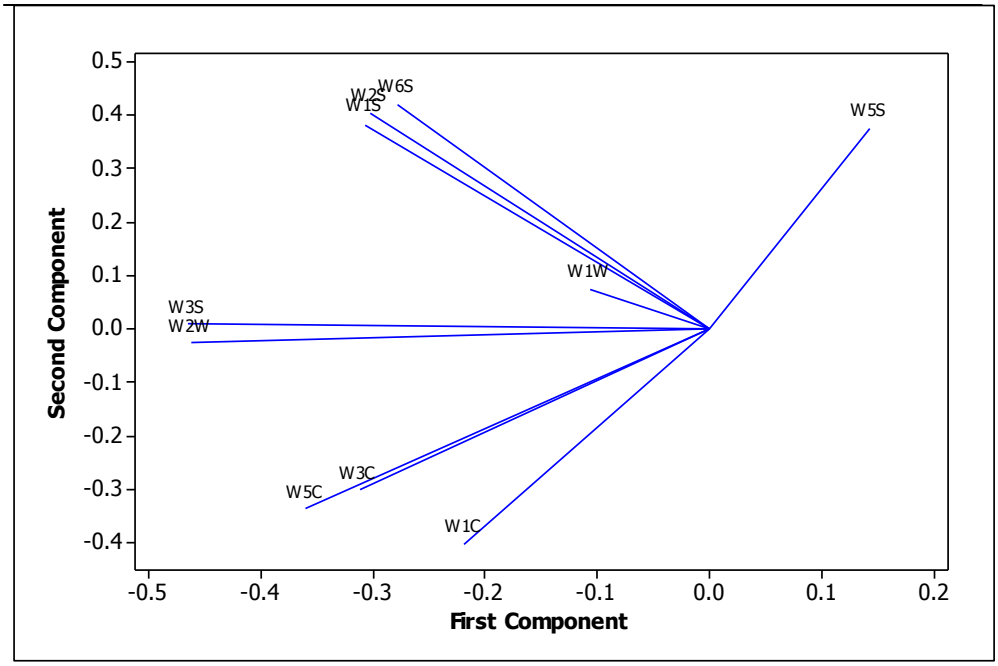


Figure 4.10b: PCA loading plot of five extrudates.

W1C (aromatic) W5S (broadrange) W3C (aromatic) W6S (hydrogen) W5C (arom-aliph) W1S (broad-methane) W1W (sulphur-organic) W2S (broad-alcohol) W2W (sulph-chlor) W3S (methane-aliph).

4.9.4.2 Taste analysis of the five extrudates

Taste analysis was conducted using the electronic tongue (ET). ET is a liquid analytical device that mimics the taste-sensing mechanism of the gustatory system; it comprises sensor arrays that are specific for liquid

Taste has an important role in the development of food products such as snacks. In relation to consumer acceptability and compliance to quality standards, taste is one of the prime factors determining the market penetration and commercial success of products (Jain *et al.* 2010). The taste values collected by ET were analysed using multivariate techniques, in particular Principal Component Analysis (PCA). Principal component analyses could

account for 96.5% of the total variation in the taste of the extrudates based on the data from e-tongue. PC1 alone accounted for 65.8% of the total variations in the taste of the extrudates, and separated the extrudates based on the percentage of PDS in the formulation and irrespective to the rice treatment (either raw or parboiled): extrudates that were obtained from either raw or parboiled rice but with high partially defatted soybeans (25%) (C and E) clustered to the left (negative half), while those that were obtained from low levels of soybean (10%) (B and D) were located to the right half (Figure 4.11a). The loadings plot (Figure 4.11b) shows that extrudates that were located to the left (negative) half of the plot were based on umami and salty taste. On the other hand, extrudates that were separated to the right based on low PDS content (10%) tasted bitter, sour and astringent. It can therefore be concluded that the amount of PDS in the formulation affected the taste properties of the extrudates.

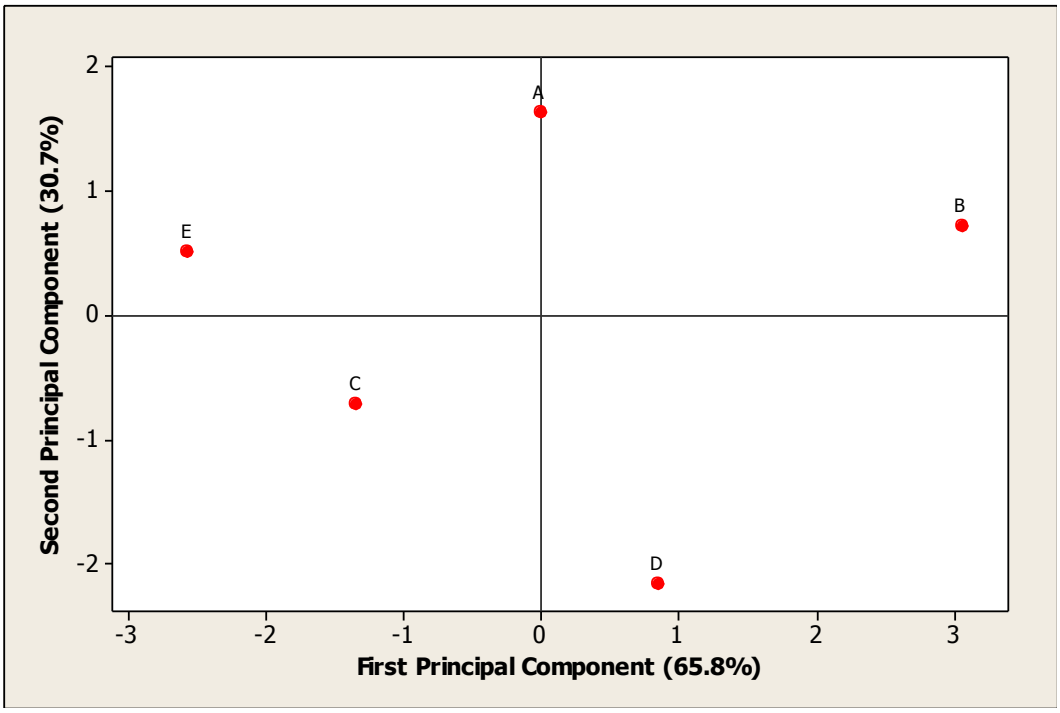


Figure 4.11a: Score plot of samples (A-E) in the plane defined by the first two principal component.

A: TMF5 (raw *'Togo marshall'* rice variety-82.5%, Partially defatted soybean (PDS)-17.5%); B: TMF3 (raw *'Togo marshall'* rice variety-90%, PDS-10%); C: TMF4 (raw *'Togo marshall'* rice variety-75%, PDS-25%); D: PTMF3 (parboiled *'Togo marshall'* rice variety-90%, PDS-10%); E: PTMF4 (parboiled *'Togo marshall'* rice variety-75%, PDS-25%).

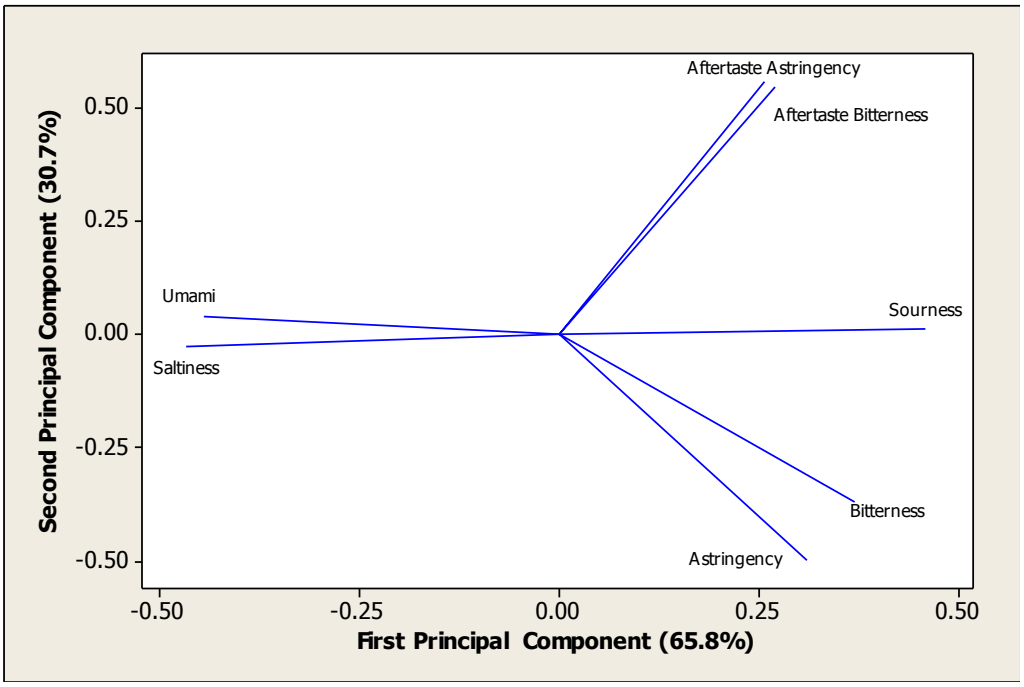


Figure 4.11b: Loading plot of samples (A-E) in the plane defined by the first two principal component

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

i. The *'Togo marshall'* rice and *'Viwonor'* rice differed based on physicochemical properties such as milling recovery, cooking time, alkaline spreading value and amylose content. This implies the milling recovery, alkaline spreading value and amylose content are parameters that can be used to characterize '*Togo marshall'* rice and *'Viwonor'* rice. '*Togo marshall'* rice variety can be characterized as rice with low milling recovery, lesser cooking time, low gelatization temperature and amylose content. *'Viwonor'* rice variety can also be characterized as rice having higher milling recovery, longer cooking time, intermediate gelatinization temperature and amylose content.

ii. There are differences in the raw materials (thus Partially Defatted Soybean: PDS, Raw *Togo marshall* rice variety: RTM, Parboiled *Togo marshall* rice variety: PTM, Raw *Viwonor:* RV and parboiled *Viwonor* rice variety: PV) in terms of colour, protein solubility and electrophoretic patterns. RTM was whiter whilst RV was more reddish and PDS more yellowish. PDS had a higher protein solubility and more peptides bands as compared to the other raw materials. This confirms that rice varieties and soybean do not have the same types and amount of proteins.

iii. Extruded rice-soybean snack from *'Togo Marshall'* formulations are more acceptable to consumers than those from *'Viwonor'* rice. This implies that *'Togo Marshall'* would make a better extruded rice-soybean snack.

iv. High levels of Partially Defatted Soybean in the formulation lowers the in vitro protein digestibility, and influences the flavour and taste of the extrudate.

v. Based on physical, chemical, functional and tastes properties, formulation with 82.5% raw *Togo marshall*; 17.5% PDS is suitable for the production of extruded rice-soybean snack.

5.2 Recommendation

i. Microbial analysis and shelf-life studies of the rice-soybean snack is recommended to assess the stability and microbial safety of the product.

ii. The application of gas chromatography mass spectrometry (GC-MS) is required to establish the exact flavour compounds present in the extrudates.

iii. Nutritional analysis of the extrudates is required to ascertain the effect of the extrusion cooking on major and micronutrients in the product.

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APPENDICES

APPENDIX I: SENSORY SCORE SHEET

SAMPLE CODING:

0 in the middle is sample 1 (Raw Togo Marshall)

Samples ending with 8 means sample 2 (Parboiled Togo Maeshall)

3 in the middle is sample 3 (Raw Viwonor)

6 in the middle is sample 4 (Parbioled Viwonor)

	Sample																			
Panelist	TMF1	TMF2	TMF3	TMF4	TMF5	PTMF1	PTMF2	PTMF3	PTMF4	PTMF5	VF1	VF2	VF3	VF4	VF5	PVF1	PVF2	PVF3	PVF4	PVF5
1	204					418					639					263	564			
2		103	506				228	318				736								
3									678				531	934				467	869	
4				307	406					748					835					361
5	204					418					639					263	564			
6		103	506				228	318				736								
7									67				53	93				46	86	

									8				1	4				7	9	
8				307	406					748					835					361
9	204					418					639					263	564			
10		103	506				228	318				736								
11									678				531	934				467	869	
12				307	406					748					835					361
13	204					418					639					263	564			
14		103	506				228	318				736								
15									678				531	934				467	869	
16				307	406					748					835					361
17				307	406					748					835					361
18									678				531	934				467	869	
19		10	50				22	31				7								

		3	6				8	8				36							
20	204					418					639					263	564		
21				307	406					748					835				361
22									678				531	934				467	869	
23		103	506				228	318				736								
24	204					418					639					263	564		
25				307	406					748					835				361
26									678				531	934				467	869	
27		103	506				228	318				736								
28	204					418					639					263	564		
29				307	406					748					835				361
30									678				531	934				467	869	

31		103	506				228	318				736							
32	204					418					639					263	564		
33	204					418					639					263	564		
34		103	506				228	318				736							
35									678				531	934				467	869	
36				307	406					748					835					361
37	204					418					639					263	564		
38		103	506				228	318				736							
39									678				531	934				467	869	
40				307	406					748					835					361

APPENDIX II: BALLOT SHEET FOR CONSUMER ACCEPTANCE OF EXTRUDED RICE-SOYBEAN SNACK

Date……

Panellist No. ……..

Instructions:
You have been provided with five samples of extruded rice-soybean snack; which you are to evaluate by indicating your degree of liking for each sample attribute of the sample.
Please clean your palates or rinse your mouth with water or cream crackers in between samples. Also allow a period of at least 2min interval before smelling the next sample.

Scale/Interpretation
9. Like Extremely
8. Like Very much
7. Like Moderately
6. Like Slightly
5. Nether Like nor Dislike
4. Dislike Slightly
3. Dislike Moderately
2. Dislike Very much
1.Dislike Extremely

Attributes					
(*pick and smell)*					
Aroma	----------	----------	----------	----------	---------
(*observe the product)*					

Colour ---------- ---------- ---------- ---------- ---------

(*take a bite)*

Mouthfeel ---------- ---------- ---------- ---------- ---------

(*take a bite*)

Aftertaste ---------- ---------- ---------- ---------- ---------

Overall -

acceptability ---------- ---------- ---------- ---------- ---------

Please turn over

Any additional comments?

……

……
……

……
……

……
……

……
……..

APPENDIX III: ANOVA TABLES

Beginning of gelatinization

ANOVA Table for Beginning of gelatin by Sample

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	176.6	4	44.15	5.13	0.0511
Within groups	43.0	5	8.6		
Total (Corr.)	219.6	9			

ANOVA Table for Breakdown [BU] by Sample

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	777586.	4	194397.	1026.92	0.0000
Within groups	946.5	5	189.3		
Total (Corr.)	778533.	9			

ANOVA Table for Maximum viscosity [B by Sample

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1.4557E6	4	363926.	1404.58	0.0000
Within groups	1295.5	5	259.1		
Total (Corr.)	1.457E6	9			

ANOVA Table for Pasting temperature by Sample

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	720.11	4	180.028	2.84	0.1412
Within groups	316.85	5	63.37		
Total (Corr.)	1036.96	9			

ANOVA Table for Setback [BU] by Sample

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	235795.	4	58948.8	325.50	0.0000
Within groups	905.5	5	181.1		
Total (Corr.)	236701.	9			

ANOVA Table for Start of cooling per by Sample

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	109811.	4	27452.8	551.26	0.0000
Within groups	249.0	5	49.8		
Total (Corr.)	110060.	9			

ANOVA Table for Start of holding per by Sample

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	523437.	4	130859.	1222.98	0.0000
Within groups	535.0	5	107.0		
Total (Corr.)	523972.	9			

ANOVA Table for L by Samples

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	788.887	5	157.777	465.37	0.0000
Within groups	8.1368	24	0.339033		
Total (Corr.)	797.024	29			

ANOVA Table for b by Samples

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	1179.72	5	235.943	766.64	0.0000
Within groups	7.38628	24	0.307762		
Total (Corr.)	1187.1	29			

ANOVA Table for a by Samples

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	116.325	5	23.2649	1655.47	0.0000
Within groups	0.33728	24	0.0140533		
Total (Corr.)	116.662	29			

APPENDIX IV: MULTIPLE RANGE TESTS

Multiple Range Tests for Beginning of gelatin by Sample

Method: 95.0 percent LSD

Sample	*Count*	*Mean*	*Homogeneous Groups*
TMF4	2	16.5	X
TMF5	2	19.0	XX
TMF3	2	20.0	XXX
PTMF3	2	26.5	XX
PTMF4	2	27.0	X

Multiple Range Tests for Breakdown [BU] by Sample

Method: 95.0 percent LSD

Sample	*Count*	*Mean*	*Homogeneous Groups*
PTMF4	2	0.5	X
PTMF3	2	1.5	X
TMF4	2	428.5	X
TMF5	2	605.5	X
TMF3	2	624.5	X

Multiple Range Tests for Maximum viscosity [B by Sample

Method: 95.0 percent LSD

Sample	*Count*	*Mean*	*Homogeneous Groups*
PTMF4	2	130.5	X
PTMF3	2	194.0	X
TMF4	2	739.0	X
TMF5	2	965.5	X
TMF3	2	1036.5	X

Multiple Range Tests for Pasting temperature by Sample

Method: 95.0 percent LSD

Sample	*Count*	*Mean*	*Homogeneous Groups*
PTMF3	2	47.85	X
PTMF4	2	51.4	XX
TMF4	2	57.05	XX
TMF3	2	68.1	XX
TMF5	2	68.6	X

* denotes a statistically significant difference.

Multiple Range Tests for Setback [BU] by Sample

Method: 95.0 percent LSD

Sample	*Count*	*Mean*	*Homogeneous Groups*
PTMF4	2	170.5	X
PTMF3	2	251.5	X
TMF4	2	491.0	X
TMF5	2	529.5	X
TMF3	2	535.0	X

* denotes a statistically significant difference.

Multiple Range Tests for Start of cooling per by Sample

Method: 95.0 percent LSD

Sample	*Count*	*Mean*	*Homogeneous Groups*
PTMF4	2	130.0	X
PTMF3	2	192.5	X
TMF4	2	310.5	X
TMF5	2	360.0	X
TMF3	2	412.0	X

* denotes a statistically significant difference.

Multiple Range Tests for Start of holding per by Sample

Method: 95.0 percent LSD

Sample	*Count*	*Mean*	*Homogeneous Groups*
PTMF4	2	85.0	X
PTMF3	2	133.5	X
TMF4	2	494.0	X
TMF5	2	577.5	X
TMF3	2	629.0	X

* denotes a statistically significant difference.

Multiple Range Tests for L by Samples

Method: 95.0 percent LSD

Samples	*Count*	*Mean*	*Homogeneous Groups*
PV	5	77.096	X
RV	5	79.63	X
PDS	5	83.888	X
PTM	5	84.692	X
RSB	5	86.55	X
RTM	5	93.176	X

Multiple Range Tests for b by Samples

Method: 95.0 percent LSD

Samples	*Count*	*Mean*	*Homogeneous Groups*
RTM	5	6.578	X
RV	5	12.036	X
PV	5	12.828	X
PTM	5	15.54	X
PDS	5	22.764	X
RSB	5	24.708	X