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KNUST

**DEVELOPMENT AND EVALUATION OF NUTRITIOUS RICE-BASED
PASTA FROM LOW GRADE MILLED RICE**

BY

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**A DISSERTATION PRESENTED TO THE BOARD OF POST GRADUATE
STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF PHILOSOPHY IN HUMAN NUTRITION AND
DIETETICS**

AUGUST, 2016

DECLARATION

This research project is my original work except where sources have been acknowledged. The work has never been submitted nor would it ever be, to another university for the award of a degree.

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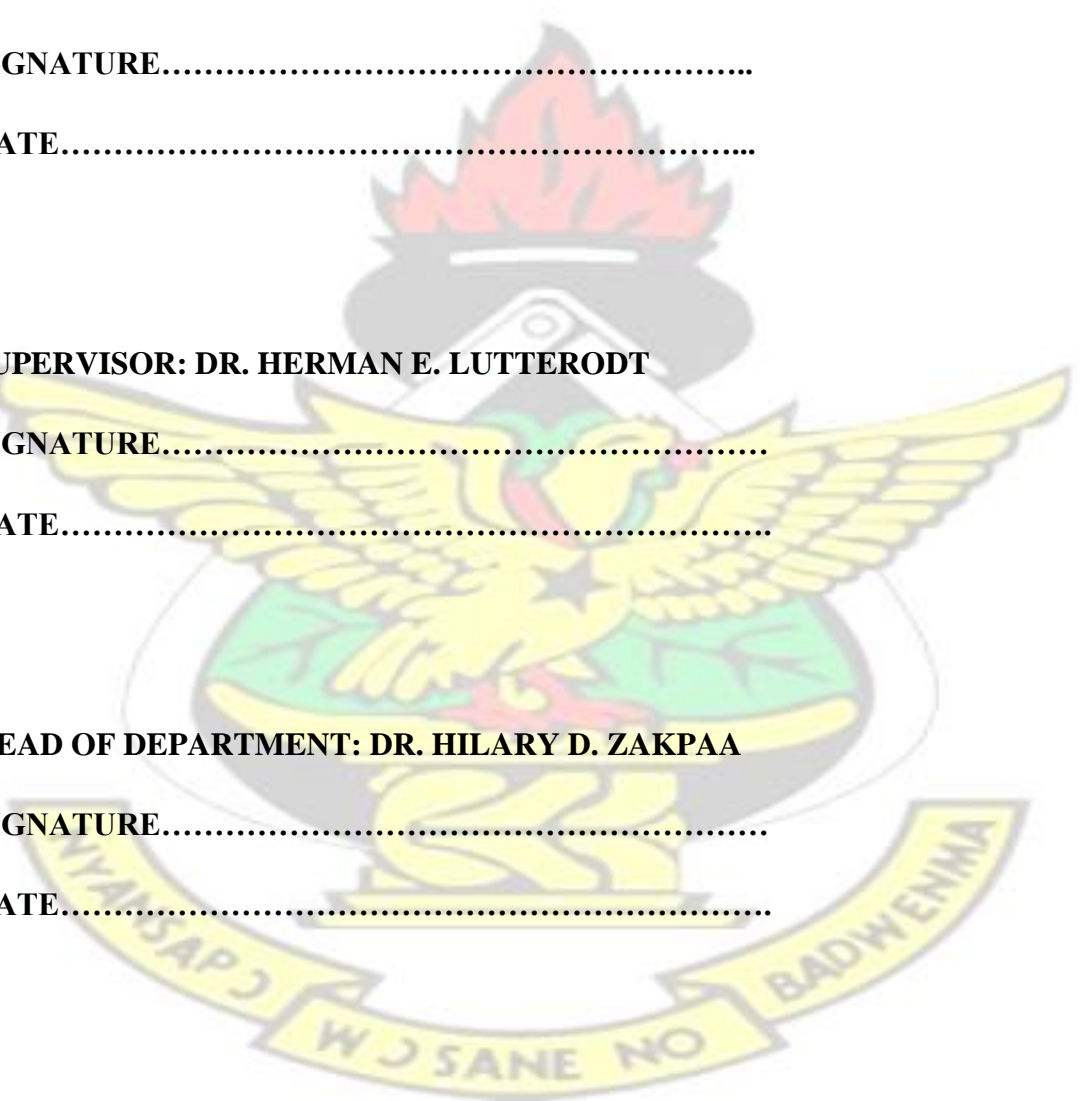
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DEDICATION

I dedicate this work to the Almighty God who made divine provision for me towards the completion of this Masters program. He through this training taught me one useful lesson which I have resolved to live by; to trust in God only and not any man. I also dedicate this work to Professor (Mrs.) Ibok N. Oduro who became an instrument God used to help me secure the opportunity to have this research work carried out at the Department of Food, Environment and Nutritional Sciences of the University of Milan. Another dedication goes to my academic mentor Rev. Joseph Adubofuor of the Department of Food Science and Technology, KNUST. Your training, guidance and confidence in me is taking me far. Daddy, I owe you a lot and promise to make you proud in my academic endeavors. Lastly, I dedicate this work to my wonderful parents Mr. John Kofi Amoah and Miss. Faustina Ghartey and my siblings Rita Amoah, Matilda Amoah, Christina Amoah, Josephine Amoah, Godwin Amoah and Freda Nancy Amoah as well as my wonderful friend Kellie Twumwaa Twum for their love, constant encouragement, understanding and moral supports.

ABSTRACT

Rice-based gluten-free pasta with albumen as a structure building ingredient has not only come to be used as an appropriate nutritional intervention for people with various forms of gluten intolerance, but has the potential to be used in the management of burns in children. The aim of the study was to develop and evaluate nutritious rice-based pasta from low-grade milled rice fortified with soy and orange-fleshed sweetpotato flour. Four different formulations based on pre-gelatinized rice flour and liquid egg albumen, and containing soybean and/or sweet potato (up to 20%) were prepared using a single screw extruder at 25°C and oven-dried for 17 hours at a temperature of 65°C. Biochemical characterisation (Bradford test, SDS-PAGE electrophoresis, in-vitro protein digestibility and accessible thiols), cooking indices (cooking time, cooking loss and water absorption capacity), as well as sensory evaluation using e-nose and tongue were carried out on the developed pasta. The results showed significant differences ($p < 0.05$) in the amount of proteins solubilised using the three different buffer treatments (saline, urea and urea/DTT buffer) for the Bradford test and thiol reactivity an indication of the presence of protein aggregates stabilized by hydrophobic interactions and disulphide bonds in all pasta samples, and a higher network-forming ability in soybean-enriched pasta, that

may explain the lower cooking losses of this specific sample. Pasta with rice and soy had the highest protein digestibility, followed by pasta with rice only. Soybean and sweet potato enrichment resulted in a decrease in the pasta consistency and in significant changes in the color of the resulting samples. In particular, soybean clearly affected pasta yellowness, whereas the addition of sweet potato resulted in a significant decrease in lightness and an increase of redness, most likely as a result of Maillard-type browning reactions. Also, significant differences ($p < 0.05$) existed in the cooking loss (9.07-10.50)g/100, water absorption (67.20-72.10)g/100g and firmness (233.61-535.87)N of the pasta samples produced. E-sensing approaches indicated that the sensory profile of the various pasta products strongly depends on the peculiar enrichment. Sweet potato increased the pasta astringency, whereas soybean enrichment resulted in a typical umami taste and a specific electronic nose response. The results from this study indicate that the different treatment/ingredient combinations significantly affect both nutritional and functional properties of the rice-based pasta. These findings will be useful in designing and producing gluten-free snacks that satisfy the consumers' expectations. The development of innovative enriched rice products is a promising way to exploit low-grade African rice varieties.



ACKNOWLEDGEMENT

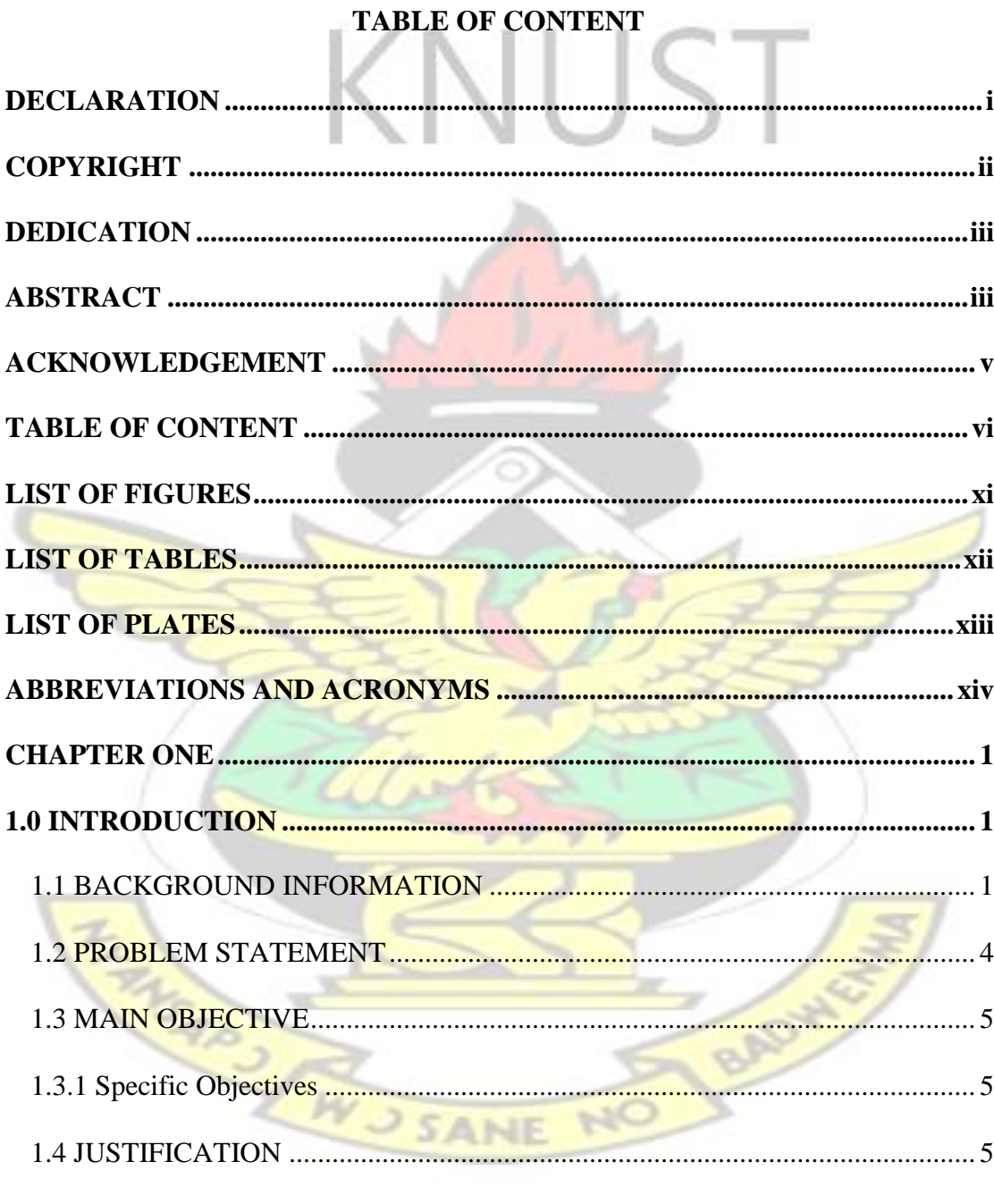
I am sincerely grateful to the almighty God for his favour and blessings throughout these years and for bringing me this far in life. Glory and honour be unto his holy name.

I am most grateful to my guides; Dr. Erick Herman Lutterodt and Prof. Paa-Nii Torgbor Johnson for their time and guidance that has enabled the successful completion of this thesis. My appreciation also goes to the Global Rice Science

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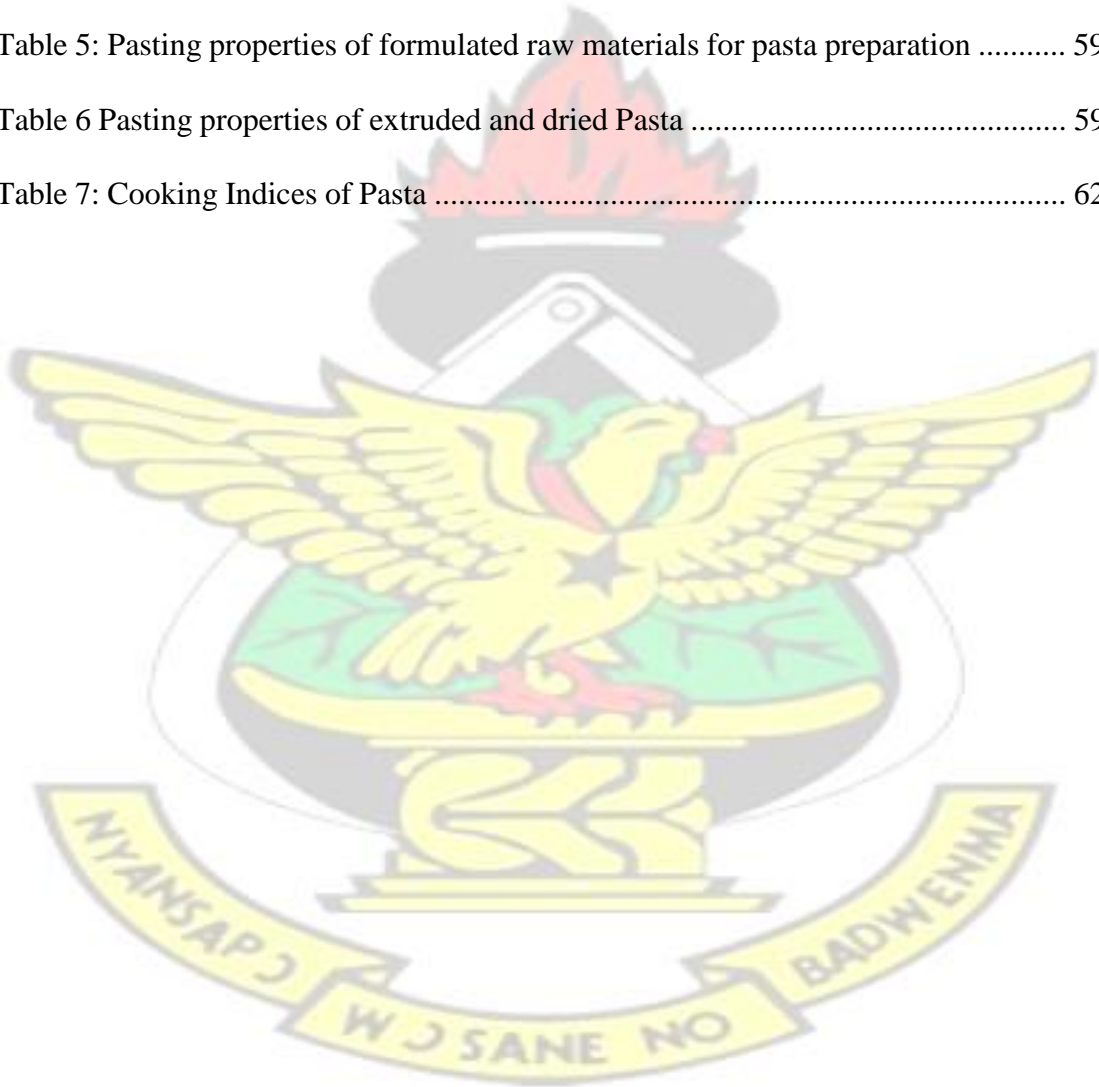
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ABBREVIATIONS AND ACRONYMS

AACC	American Association of Cereal Chemists
DEN (-)	Denaturing buffer (0.125M Tris-HCl, pH 6.8; 50% w/v glycerol, 1.7% w/v SDS; 0.01% w/v Bromophenol Blue) without β -mercaptoethanol
DEN (+)	Denaturing buffer (0.125M Tris-HCl, pH 6.8; 50% w/v glycerol, 1.7% w/v SDS; 0.01% w/v Bromophenol Blue) with β -mercaptoethanol
DTT	Dithiothreitol
FAO	Food Agriculture Organization
g	Gram
GF	Gluten-free
GIT	Gastro-Intestinal Tract
mL	Millilitres
MS	Mass spectrometer
OFSP	Orange-fleshed sweet potato
PC	Principal Component
PCA	Principal Component Analysis
R	Rice
RP	Rice and Potato
RPS	Rice, Potato and Soy
RS	Rice and Soy
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate– Polyacrylamide Gel Electrophoresis
SPSS	Statistical Package for Social Sciences
WAC	Water Absorption Capacity
WHO	World Health Organization
WARDA	West Africa Rice Development Association

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND INFORMATION

Pasta is an important staple food which plays a crucial role in human nutrition that has existed for thousands of years (Sissons 2004; Fu 2008) and is associated worldwide with the “Italian way of life” (Iametti *et al.*, 2015). Pasta and noodles are both wheat-based products, but variations in the raw materials, formula ingredients, manufacturing as well as their consumption patterns makes them different (Hou, 2001). Durum wheat (*Triticum durum*) is reported to be the most ideal cereal for the production of high quality pasta (Feillet and Dexter, 1996). The flour of the wheat of (*Triticum aestivum*) is what is known for noodle production after the addition of water, salt or alkaline salts (Gulia *et al.*, 2014). There is an increase in the consumption of pasta and this has been attributed to factors including convenience, palatability, enhanced nutritional properties and its ability to stay on the shelf for long (Borneo and Aguirre, 2008; Boroski *et al.*, 2011).

Gluten-free pasta has gained world-wide consumption in developed countries especially by most people with various forms of gluten intolerance as seen in people suffering from celiac disease (Marti *et al.*, 2013). Celiac disease is a disease of inflammation of the small intestine that results from an immune-based reaction to dietary gluten in individuals with genetic predisposition and resolves with the exclusion of gluten from the diet (Rubio-Tapia *et al.*, 2013). The presence of gluten peptide molecules induces the thymus cells to produce cytokines that initiates the inflammatory and autoimmune reaction resulting in the stimulation of the plasma cells to produce antibodies to gliadin, transglutaminase and endomysium (Chand and

Mihas, 2006). The autoimmune inflammatory response results in villous atrophy, malabsorption, malnutrition and possibly malignancy (Mahan and Escott-stump, 2008). The prevalence of celiac disease is considered to be 1 in 133 persons in the United States of America (Fasano *et al.*, 2003), 1/200 in some European countries (Catassi and Fasano, 2008). In recent times, very high incidences of celiac disease have been reported in the general population (Catassi *et al.*, 1999; Ashabani *et al.*, 2001; Mankai *et al.*, 2006; Catassi *et al.*, 2004) and in at-risk group groups (Bouguerra *et al.*, 2005; Ashabani *et al.*, 2003) of Northern African populations specifically in the Maghreb which includes Morocco, Algeria, Tunisia, Libya and Egypt. The Sahawari population, a tribe in Northern Africa has been reported to have the highest celiac disease prevalence (5.6%) in the world today (Catassi *et al.*, 1999; Lionetti *et al.*, 1999).

Though some varieties of rice are popularly known for their high yields, they are not patronized by consumers for consumption at household level. This has been attributed to their poor cooking quality, coarse grain type and hence are rated as common or low grade rice varieties (Acharya, 2004; Bhashyam *et al.*, 2006). Low grade rice are sold at discounted prices by farmers which makes them run at a loss in Ghana since it is usually perceived to be of low quality. Gluten-free products are predominantly made from rice, corn and other gluten-free flour on the market. Rice flour has been used widely as a raw material in the preparation of gluten-free (GF) products and this has been attributed largely to its bland taste, white colour, high digestibility by enzymes as well as its hypoallergenic properties (Rosell and Marco, 2008). Along with these features, rice flour has low protein and a relatively poor ability to develop a cohesive network, which impairs its technological performance (Torbica *et al.*, 2012). It has been reported that several GF products tend to exhibit relatively poor cooking quality during cooking (Hager *et al.*, 2012), which is mostly due to the technological challenge that arises from replacement of gluten functionality in GF products (Marti *et al.*, 2013).

Thompson (2009) proposed the use of proteins as structural building agents for producing GF pasta, with the added benefit of improving the nutritional value of the product. Marti and co-workers (2013), investigated the effect of adding egg albumen and whey proteins on the structuring and texturing properties of GF pasta. In particular, egg albumen enhanced the appealing nature of pasta and gave a firmer pasta with minimal cooking loss as well as enhanced nutritional value.

The use of albumen as a building material in gluten-free pasta makes it have the potential to be used as an intervention in children with various forms of burns. Burns presents a major medical challenge accounting for a major portion of the workload for the Plastic Surgery and Burns Unit and it has been found that, 45% of burns patient are children (Thompson, 2011). Annually, 265,000 death cases attributed to Burns is reported in Ghana and children below 20 are the most affected (WHO, 2014). According to Prins (2009), there is an increase in protein requirements in burns patients due to the increased muscle breakdown, wound losses and tissue repair; necessitating the need for optimal protein intake. Development of rice-based pasta with egg albumen as the building body agent could be an effective intervention not only for people with celiac disease but also, burns patients mostly children in Ghana.

Several studies have recently emphasized the need to enhance the nutritional quality of cereal based GF products, by highlighting for example the general low content in essential micronutrients of GF products, when compared with their wheat-based counterparts (Marti *et al.*, 2013). This has made it necessary to find additional/alternate raw materials with high nutritional profile for the formulation of high quality gluten-free products such as pasta. In particular, GF products can be fortified with ingredients rich in high quality proteins (*e.g.* soy flour) and vitamins (*e.g.* orange-fleshed sweet potato flour) (Mastromatteo *et al.*, 2012).

In this present work, a nutritious and higher value rice-based pasta from low grade milled rice with the potential to be used as an intervention in patients with various forms of gluten intolerance and children with burns was developed.

1.2 PROBLEM STATEMENT

Research and development interventions in the African rice sector in the past focused mainly on increasing farm-level production with little attention paid to post-harvest handling, processing and quality enhancement issues, which play major roles in the final retail price and is an incentive for rice value chain actors. Quantitative losses in rice are relatively low when compared to other commodities such as fruits and vegetables. However, due to the rudimentary handling methods used in many SubSaharan African countries, losses can still be significant. It is estimated that, on average, quantitative post-production losses for rice in Africa are in the order of 15–20%.

Some varieties of rice are popularly known for their high yields, but they are not patronized by consumers for consumption at household level. This has been attributed to their poor cooking quality, coarse grain type and hence are rated as common or low grade rice varieties (Acharya, 2004; Bhashyam *et al.*, 2006). Low grade rice are sold at discounted prices by farmers which makes them run at a loss in Ghana since it is usually perceived to be of low quality. Because of this challenge, rice value chain actors in Africa do not receive adequate compensation for their investment and this is a potential threat to the very long term survival of the African rice industry (Global Rice Science Partnership (GRiSP), 2011).

Also, because of its properties, low grade milled rice does not fit into existing food products and hence under-utilized. The under-utilization of low-grade rice is a serious threat to food insecurity. This is because, food insecurity seriously prevents the

accessibility of nutritious diets that have adequate and balanced micronutrient and macronutrients. This has resulted in an increase in malnutrition cases recorded in most African countries with it attendant increase in infant mortality rate in Africa.

1.3 MAIN OBJECTIVE

The main objective was to develop and evaluate nutritious and higher value rice-based pasta from lower grade milled rice.

1.3.1 Specific Objectives

1. To develop gluten-free pasta from low grade milled rice fortified with soy and orange-fleshed sweet potato flour
2. To compare the biochemical, functional and sensory properties of the developed gluten-free pasta made from low grade rice, soy and sweet potato flour.

1.4 JUSTIFICATION

The project may contribute to the improvement of the livelihoods of rice value chain actors through the development and dissemination of nutritious and higher value ricebased pasta using locally grown lower value milled rice which would otherwise be sold at discounted prices on the local market. This could be an effective poverty reduction tool amongst rice growers in the villages.

Development of nutritious and high value rice-based pasta from locally grown lower grade milled rice, orange-fleshed sweet potato and soy flour will contribute to increased food security through the transformation of low value low grade milled rice into value added food pasta. This will promote reduction in food loss and waste through food product development with the tendency of becoming one of the leading global strategies

for achieving a sustainable food future. Food diversity amongst consumers will also be promoted.

Due to globalization, Ghana stands the chance of benefitting from the exportation of rice-based pasta to most developed countries with high incidences of celiac disease.

This will enhance a boost in the country's economy with a transformation of our Agricultural sector. Rice product value and overall rice productivity in many African countries can significantly increase through diversified and improved processing practices and technologies.

Development of nutritionally enhanced rice-based pasta enriched with egg albumen could be an appropriate nutritional intervention for people with various form of gluten-enteropathy as well as children who present various forms of burns. This will reduce the burden on governments' expenditure on importing foreign products to be used as interventions in people with such conditions.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 PASTA

Pasta is a wheat-based food product, produced from durum wheat (*Triticum durum*) mainly by mixing wheat semolina with water and is usually extruded. Consumption of pasta can be done usually fresh after processing or it could also be dried (Sozer, 2009).

Though pasta and noodles are both wheat-based products, variations in the country of origin, raw materials, formula ingredients, manufacturing as well as their consumption patterns makes them different (Hou, 2001). The increase in patronage and consumption of pasta has been attributed to its convenience, long shelf-life, palatability and its enhanced nutritional properties (Marti *et al.*, 2013). Intrinsically, quality attributes of

pasta are found to be influenced primarily by the properties of the protein and starch fractions as well as other factors pertaining to the semolina wheat origin (Wood *et al.*, 2001) and the processes involved in the pasta production such as mixing, extrusion and drying conditions (Debbouz and Doetkott, 1996). The quality and quantity of protein has been found to be one of the critical factors affecting pasta properties (D'Egidio *et al.*, 1990). Pasta with high protein content and a strong gluten network has a better performance in terms of its cooking properties (D'Egidio *et al.*, 1990 and Feillet and Dexter, 1996).

2.1.1 Gluten-free pasta

In recent decades, there has been the introduction of a third group of pasta products commonly referred to as gluten free. Gluten free pasta has not only gained a lot of attention by people with celiac disease but also the vast number of individuals who would want to exclude gluten in their diet due to health concerns. In recent times, there has been the introduction of gluten free products for people with celiac disease made from rice, maize and other gluten free flour (Marti and Pagani, 2013). However, gluten free products have been found to exhibit low cooking qualities when compared to their wheat counterpart (Hager *et al.*, 2012; Lucisano *et al.*, 2012). This has mainly been attributed to the lack of gluten responsible for structure formation in gluten free products.

2.1.1.1 Replacing the structural role played by gluten in gluten-free pasta

Gluten enhances the formation of structure in pasta during pasta processing and has been implicated in imparting a good cooking quality to pasta (Fernandes *et al.*, 2013). Despite the fact that, the demand for GF products with good sensorial, cooking behaviour and healthier properties tends to offer to the food manufacturer a great market opportunity, the replacement of the functional role played by gluten in GF products

pose a major technological problem to the food manufacturing industry. The difficulty in producing GF is related to the functional role played by gluten in the food systems (Marti and Pagani, 2013). To overcome the technological challenge that comes with replacing the functional role played by gluten in GF products, two main methods have been proposed (Marti *et al.*, 2013). The first approach is based on choosing appropriate processing conditions that has the ability to create an efficient arrangement of starch components in the final products (Marti *et al.*, 2011). The other approach is based on choosing ingredients and additives (mainly hydrocolloids and emulsifiers) appropriately and with these ingredients and additives having the ability to induce a cohesive structure that forms a network to give the pasta a strong texture.

2.1.1.2 The role of egg albumen in gluten-free pasta

Albumen are water soluble proteins and have been reported to have a nutritionally balanced better amino acid compositions due to the higher levels of lysine and methionine present in it (Lasztity 1984). It is found to be composed of non-glutelin proteins (mainly monomeric) and found to be soluble in water and dilute buffers (Goesart *et al.*, 2005). Approximately, 60% of egg protein is located in the egg white (Vaclavik and Christian, 2014). Recently, the use of protein as structuring building ingredients has been proposed in addition to the role they play in enhancing the nutritional value of the products (Thompson, 2009). Marti *et al.* (2013) investigated the effect of adding egg albumen and whey proteins on the structuring and texturing behaviour of GF pasta. In their study, they observed that, egg albumen gave an appealing appearance to pasta and gave a product with minimal cooking loss, enhanced nutritional value and firmer than others. The appearance of small starch granules homogeneously surrounded by a protein network was found and therefore concluded

that, formulating pasta ingredients with 15% addition of liquid albumen to parboiled rice resulted in enhanced textural and structural features of rice-based gluten-free pasta.

2.1.1.3 The role of starch in gluten-free pasta

Corn, wheat and rice as well as root tubers including potato and cassava are the most important sources of starch (Betancur-Ancona, 2001). Textural properties of various foods are greatly imparted by starch (Singh *et al.*, 2003). The two main components of starch are amylose and amylopectin molecules and occur in molar ratios of 15-25% and 75-85% respectively (Tako *et al.*, 2014). The structure of amylose is linear and is made up of 1, 4-linked α -D-glucopyranosyl residues (Takeda *et al.*, 1993). Amylopectin, a branched macromolecule on the other hand is made up of 1, 4(94%-96%) and 1, 6-linked (4%-6%) α -D-glucopyranosyl residues (Hizukuri, 1986). Promoting new and efficacious starch organisation through effective processing method has been deemed a potent approach in replacing the functional role played by gluten in a gluten-free pasta (Mestres *et al.*, 1993). Once starch molecules are dissolved on heating, a high viscous solution is produced which then changes into strong gelling (retrogradation) state when stored for long times (Tako *et al.*, 2014). The structural role played by starch in dictating the properties of gluten-free products is as a result of its ability to re-associate and interact after gelatinization, producing products with newly organized structures that retard further starch swelling and solubilisation during cooking (Marti and Pagani, 2013).

2.2 RICE

Rice (*Oryza sativa*) is a domesticated tropical cereal from the wild grass *Oryza rufipogon* some 10000 to 14,000 years ago. It is one of the world's crop plants to be domesticated and constituted the nutritional basis for the great early civilizations in

East and South Asia. Indica (which thrives well in tropical regions) and japonica (grown predominantly in the subtropical and temperate regions of East Asia) are the two main subspecies of rice believed to have been derived from independent domestication events. *Oryza glaberrima* is another cultivar of rice species which was domesticated much later in West Africa. Rice is a special diverse crop-genetically, in the way it is grown and how it is used by humans (GRiSP, 2010). In terms of nutrient content, rice contains 2.2% fat, 7.3% protein, 64.3% available carbohydrate, 0.8% fiber and 1.4% ash content approximately (Zhou *et al.*, 2002).

2.2.1 Rice production and International trade

In terms of world production of rice, South-east Asia leads when it comes to the continent with the highest rice production making Asia account for 90% of the total world rice cultivation. Its cultivation is seen widely in the tropics; since the ability to control flood in South Asia is very efficient making them reap high yields of rice during the harvesting season. A lot of the imported rice found on the West African market have been reported to come from South-east Asia. West Africa is reported to be the leading consumer (61.9%) and producer (64.2%) of rice in Sub-Saharan Africa (WARDA, 1996). The economic value of rice has steadily increased over the past two decades. The quantity of rice imported into Africa has more than doubled and this has mainly been attributed to a nutrition transition in the nutritional habits of many Africans especially those in the rural areas who have shifted from consuming their usual traditional foods like millet, sorghum and cassava to the intake of rice and wheat. This has resulted in an increase in the per capita consumption of rice in Africa from 14.8kg/year to 16.3 kg/year for the period 1980-1992 (FAO, 1995).

2.2.2 Rice Production and Consumption in Ghana

Rice, the second most consumed cereal crop in Ghana and next to maize, is rapidly gaining prominence as a cash crop for many farmers (MiDA, 2010; Osei-Asare, 2010). In Ghana, there are two planting seasons been the main which runs from May to June and the off season which runs from January to February. The harvesting seasons also has the main and the off seasons with the main running from October to November whilst the off runs from May to June (www.ricepedia.org/republic-ofGhana). Plans and strategies such as the Food and Agricultural Sector Development Policy (FASDEP) I and II, Ghana Poverty Reduction Strategy (GPRS I), Growth and Poverty Reduction Strategy (GPRS II), Accelerated Agricultural Growth and Development Strategy and Medium Term Agricultural Sector Investment Plan (METASIP) have prioritised rice as one of the food security crops in Ghana (Ragasa *et al.*, 2013). Local rice production in Ghana has been found to be very dominant in the Northern Region (37%), Upper East Region (27%) and Volta Region (15%). Generally, rice production in Ghana is increasing. The fast rate of rice production as evidenced since year 2007- a sign of positive growth - has been attributed the various initiatives put in place to develop the rice sector in Ghana including the national fertiliser subsidy program introduced in 2008, passage of National Rice Development Strategy (Ragasa *et al.*, 2013). Consumption of rice is increasing rapidly in terms of annual per capita, from 17.5 kilogram per annum between 1999-2001 to 22.6 kilogram per annum between 2002 and 2004 (Amanor-Boadu, 2012). WARDA (2007) reported that, this increase in per capita consumption of rice (*Oryza spp. L.*) reached 38 kilogram per annum and it projected to reach 63 kilogram per annum by 2015. The transformation of rice into Ghana's most important cereal crop after maize has been attributed to the increase in per capita consumption of rice (Amanor-Boadu,

2012). Demand for rice has been estimated to rise at a compound annual growth rate of 11.8 % in the medium term (MiDA, 2010). The high level of foreign rice imported into the Country has contributed to the putting of much pressure on foreign currency reserves and food security in Ghana. According to Amanor-Boadu (2012), imported rice contributes to about 70 percent of the overall quantity of rice consumed in Ghana.

2.2.3 Post-harvest losses of rice

Post-harvest losses in rice production has been explained to do with any reduction in the quantity of edible grain due to reduction of availability, edibility, wholesomeness or quality that results in preventing the rice grains from being consumed by people (Harris and Lindbald, 1978). Loss of rice grain occurs almost at every stage of the production cycle from the farm to the final stage of consumption. Harvest and postharvest losses account for 15 to 50% of the market value of the initial production which has been estimated to range between \$30 and \$75 per ton. Post-harvest losses destroy about 15 to 16% of the rice crop in developing countries (FAO, 2004). More, critical stages than others have been identified in the rice postharvest systems, and this often seen in tropical and subtropical areas where rice is more vulnerable to damage and more likely to suffer quantitative and qualitative losses. Drying and storage of rice are particularly important amongst the critical stages in the rice-postharvest system (Satin, 1997; FAO, 1997). Appiah *et al.* (2011) reported that the loss of rice during milling accounts for up to 30% when they interviewed farmers from the Ejisu Juabeng District of Ghana. The loss is attributed to the low grade milled rice produced during milling.

2.2.4 Rice flour

Variation in rice grain endosperm in terms of it been waxy (glutinous) or non-waxy

(non-glutinous) is dependent on the amylose and amylopectin content of the rice. Colour of flour, polyphenol oxidase and milling extraction rate affect directly the colour of final product (Baik *et al.*, 1995). Zhang *et al.* (2005) reported a negative correlation between the brightness of noodles and the ash content. The ability of the noodle to resist sheeting, resist tearing, breakage and shrinkage of dough sheet is dependent on adequate dough strength and extensibility. The amount and quality of protein tends to affect the behaviour of instant noodles such as fat absorption, colour and the texture quality together with the properties of dough such as its ability to absorb water and colour. A positive correlation is observed between flour protein content and cooked noodle firmness whereas a negative correlation occurs with noodle brightness (Wang *et al.*, 2004; Asentorfer *et al.*, 2010). The properties of starch is also a very critical factor in determining the quality of noodles due to the fact that noodle texture depends to a greater extent on gelatinized starch (Bushuk, 1998). Differences in the properties of starch have been identified to play the role of determining the softness and viscoelastic properties of noodle (Yun *et al.*, 1997). Pasting characteristics (Zhang *et al.*, 2005), peak viscosity (Bhattacharya and Corke, 1996), flour swelling volume (Crosbie, 1991), the ratio of amylose to amylopectin and starch damage (Oh *et al.*, 1985) influence the properties of noodles. There is a higher preference for flour with low gelatinization temperatures particularly due to their ability to hydrate with water quickly during cooking. A higher damaged starch is seen to result in producing noodle with poor colour, high cooking loss and higher surface swelling (Hatcher *et al.*, 2002). Good cooking noodle quality is seen in noodles with fine flour particle with lower starch damage. Flour swelling volume is an indication of the swelling/gelatinization potential of flour (Zeng *et al.*, 1997). Optimum amylose content of 22% has been observed for good Japanese noodles of higher quality.

2.2.4.1 Pre-gelatinised rice flour

Gelatinisation is a needed condition to produce the binding effect during drying of extruded pasta (Juliano and Sakurai, 1985). Raina *et al.* (2005) reported that, the textural quality of both uncooked and cooked pasta is significantly improved when pre-gelatinised rice flour is used. Pre-gelatinization of the starches in flour is a very important process in food processing since it enhances the formation of cold gel for native and/or modified starches. This makes pregelatinized starches have the ability to impart an increased viscosity to food products without the need to cook the products at high temperatures. Retention of the functional properties of the product is also enhanced by pregelatinized starches ([www. Cargillfoods.com](http://www.Cargillfoods.com)). During pregelatinization, which is a starch modification process, there is the heating of starch slurry to gelatinize it, dried instantly and then milled to obtain the desired granular requirement. With agitation, the product obtained can be spread in water to produce paste and this can be compared to that obtained from cooking raw starch (Corn Refiners Association, 2006). The proteins in rice is a little around 7 -12% and are unable to form a strong cohesive network like the role played by gluten. This necessitates the need to gelatinise the starches to make them provide a framework for the rice-based pasta product (Buhler, 2015).

2.3 PRODUCTION OF SOYBEAN SEEDS AND MEAL

During the 2009/2010 farming season the world soybean production was about 260.6mln ton (Rynek, 2010) with USA, Brazil, Argentine as well as China been the major producers produced close to 87% of the overall quantity of soybean seeds.

Major exporters of the seeds of soy include Argentine (about 11%), Brazil (about 33%) and USA (about 44%), with China (about 38%) been the main importer.

2.3.1 Soya bean production and varieties in Ghana

The past decade has seen an increased interest in the cultivation of Soybean in Ghana. This has been attributed to Government's policy geared towards the encouragement of the development, production and utilization of soya bean in the country and this is captured within the framework of the Medium Term Agricultural Development Program. As a result of this, soya bean is becoming a very important cash crop with over 8,000 to 10,000ha cultivated annually (Asafo-Adjei, personal communication). In Ghana, production of soybean is estimated to be about 50,000 metric tons per annum, with only about 15 metric tons are used (Dzogbefia *et al.*, 2007). Four varieties of soya beans are known to be released by SARI in Ghana. These include Salintuya1, Salintuya 2, Jenguma and Quarshie. Jenguma literally means "stay and wait for me" and has 40% protein and 20% oil. It is high yielding and has the ability to resist pod shattering as well as a killer to the striga weed that hinders crops performance and yields (www.modernghana.com).

2.3.2 Chemical composition and nutritional value of seeds and soybean meal

Protein from soybean meal is reported to have a higher nutritional value in terms of protein quantity and quality. Amongst the seeds belonging to the legume family, soybean seeds contain the highest crude protein with an excellent composition of amino acid. Soybean seeds contains up to 40% of crude protein with the fat content making up to over 20%. On a dry matter basis, the crude protein content of soy seeds ranges from 32-43.6%, fats constitute about 15.5-24.7%, water constitute about 5.611.5%, crude ash makes up to about 4.5-6.4%, neutral and acid detergent fibre constituting 10-14.9%, and 9-11.1% respectively with carbohydrates content forming about 31.7 - 31.85% (Ensminger *et al.*, 1990; NRC, 1998). Essential amino acids present in soybean seeds include leucine, isoleucine, lysine, methionine, cysteine, tyrosine, threonine,

tryptophan, histidine and valine despite the fact that, they are limited by two main amino acids which include methionine and tryptophan (Zarkadas *et al.*, 1993). However, protein from soy has low contents of sulphur-containing amino acids, with the most limiting amino acid been methionine and cystine and threonine following in that order (Eggum and Beames, 1983). Soybean flour contains very little amount of starch (4.66-7%) but contains a lot of hemicellulose and pectins.

2.3.3 Anti-nutritional factors inhibiting protein digestibility in soy-based products and methods of inactivating

2.3.3.1 Anti-nutritional factors inhibiting protein digestibility in soy

Soybean contains several anti-nutritional factors that militate against the digestion of soy proteins by protease enzymes (Liener, 1994). Anti-nutritional factors in soybean include protease inhibitors, phytates, stachyose and raffinose.

2.3.3.1.1 Protease inhibitors

Nutritional value of soy has been found to be limited by trypsin and chymotrypsin inhibitors. According to Winiarska-Mieczan (2007), Kunitz and Bowman-Birk inhibitors constitute the most important trypsin inhibitors which have been found to be very effective in reducing the activity of trypsin and chymotrypsin. Trypsin inhibitors interfere with protein digestion in soy-based food products.

2.3.3.1.2 Phytates

Phytates are noted for reducing the bioavailability of minerals such as magnesium, potassium, calcium, zinc and iron through the process of metal chelation. Phytates have also been reported to decrease the enzymatic activity of pepsin, trypsin, amylase and also, the availability of protein, amino acids, starch and energy (Sebastian *et al.*, 1998; Ravindran *et al.*, 2000).

2.3.3.1.3 Stachyose and raffinose

These are carbohydrates with low molecular weight. They are anti-nutritional compounds found in raw soybean seeds (Padgett *et al.*, 1996) and have also been found to reduce the digestibility of soy proteins by digestive enzymes.

2.3.3.2 Methods of inactivating anti-nutritional factors in soybeans

Several unit operations can be used in the processing of soybeans (Berk, 1992) and these include cleaning, soaking, smashing, conditioning, dehulling, flaking, boiling or toasting of soybean seeds. The presence of anti-nutritional factors in soybean has necessitated the need for adequate heat treatment in order to inactivate the antinutritional factors (Van Eys *et al.*, 2004). Heat treatment of soybean seeds has been found to be one of the effective ways of inactivating anti-nutritional factors in soy with the effectiveness depending on the temperature used as well as the duration of the heat treatment (Banaszkiewicz, 2011).

2.4 SWEET POTATO

Ipomoea batatas is the botanical name for sweet potato, with morning-glory as its family (Convolvulaceae) and is believed to have originated from Latin America. Despite the fact that it is still unclear as to when exactly sweet potato arrived on the shores of the African continent. It is also reported that, slave traders brought sweet potato to Africa, and its introduction has resulted in the displacement of the true yam in tropical Africa (Davidson, 1999). Sweet potato is known to be a perennial crop with the edible portion been the tuberous root, the shoots and young leaves (Woolfe, 1992). Cultivation of the crop is seen usually in the tropics as well as the subtropics (Scott, 1992). Sweet potato is been ranked as the seventh most consumed food crop worldwide (Tumwegamire, 2007). The shelf-life of sweet potato is short making it susceptible to deterioration (Teye, 2010) and is a major challenge to farmers in

developing countries like Ghana. In Ghana and most tropical developing countries, the roots of sweet potato can only be stored for up to 21 days only (Teye, 2010). Sweet potato has enormous economic prospects that could be obtained from its marketing and production (Teye *et al.*, 2011) but its highly perishable nature necessitates the need for its utilization in food product development. The perishable nature of sweet potato has been attributed mainly to its thin delicate skin which easily gets bruised during post-harvest handling and harvesting. Also, unfavourable environmental conditions coupled with weevil attack during storage also facilitate the deterioration of sweet potato. Despite the fact that, sweet potato cultivation is being encouraged in Ghana (Teye *et al.*, 2011), the perishable nature of the crop necessitates the need for its use in food development. In Sub-Saharan Africa, there is only one major growing season for Sweet potato, the crop is available from 4 to 8 months of the year and is regarded as a secondary staple eaten two to four times weekly (Low *et al.*, 2009).

2.4.1 Production of sweet potato in Sub-Saharan African Region

Sweet potato is one of the most widely cultivated root crops in Sub-Saharan Africa and its production is estimated to be 12.6 million tons of roots during the 2007 farming season (FAOSTAT, 2008 and national surveys). The popular term “poor man’s food” used for sweet potato is attributed to the fact that it is cultivated on small scale by farmers. Eastern and Central Africa countries including Malawi, Angola, Mozambique which live around the Great Lakes and Southern Africa country like Madagascar as well as Nigeria, a West African country have all reported that sweet potato is one of the important crops (Woolfe, 1992). The crop is rapidly expanding than other major field crop in Sub-Saharan Africa. Sweet potato is a good income generating food crop (Ewell, 1990).

2.4.2 Orange-fleshed sweet potato variety

In Sub-Saharan Africa, highly cultivated sweet potato varieties include the white, creamed and orange-fleshed varieties (Low *et al.*, 2009). In Ghana, nine varieties of sweet potatoes have been released. These include *Okumkom, Sauti, Faara, Santom Pona, Hi-Starch, Ogyefo, Otoo, Apomuden* and *Tek Santom* (Amankwaah, 2012). The *Apomuden* variety of sweet potato is an orange-fleshed sweet potato. Orange-fleshed sweet potato is energy dense (293 to 460 kJ/100 g) (Hagenimana, 2001), easy means to propagate vegetatively, and with the ability to withstand drought once the farmer gets it established. These properties make OFSP a viable food crop with the potential to address food security (Kurabachew, 2015). Orange-fleshed sweet potato (OFSP) is reported to be rich in beta-carotene, a compound from which vitamin A can be synthesized, promoting the alleviation of deficiency of vitamin A (Haile *et al.*, 2015). β -carotene, present in orange-fleshed sweet potato has received a widely known as a cheap source of antioxidant with several health benefits including boosting the immune system, anti-cancer activity, and preventing liver injury. The orange-fleshed sweet potato and is a biofortified crop with the potential to address malnutrition in developing countries. Vitamin A deficiency, which has been a public health concern can be addressed effectively addresses when orange-fleshed sweet potato is incorporated into food products (Mitra, 2012). Frequent consumption (100 grams per day or half-cup) of orange-fleshed sweet potato roots is reported to meet the recommended daily amount of vitamin A for children below the age of five years (400 μ g Retinol Equivalents [RE]) (Tsou and Hong 1992).

2.5 RICE FORTIFICATION

Rice, in its unmilled form is a good source of macro and micronutrients predominantly vitamins B1, B6, E, and niacin (USDA, 2012). However, during rice milling, the fat

and micronutrient-rich bran layers are removed resulting in the popularly consumed starch-rich white rice (Steiger *et al.*, 2014). Rice is an ideal fortification vehicle and fills a gap in the current fortification landscape (www.sightandlife.org). Several gluten-free products are low in terms of nutritional value mainly poor in minerals and bioactive compounds. There is therefore the need to pay attention to the nutritional quality of gluten-free products (Marti and Pagani, 2013). Utilization of OFSP into flour for use in food product such as pasta will help enhance its nutritional value since sweet potato is reported to be rich in carbohydrates, vitamin B6, beta-carotene, vitamin C, minerals including potassium, calcium, phosphorus, iron, zinc and magnesium. It also imparts flavour, dietary fibre and colour to food product (Ulm, 1988; Agbor-Egbe, and Richard, 1990; Purseglove, 1991). Complementing of soybean with grains and cereals food products help improve the amino acid profile in the sense that, soybean has sufficient lysine content, which is deficient in several cereal proteins (Hammond, 2014).

2.6 PASTA PRODUCTION

2.6.1 Mixing

During mixing, flour, water together with different ingredients which are previously weighed are first brought together at a fast speed and then at a minimum speed as well. In the industries, this takes about a total of about fifteen to twenty five minutes whereas most laboratories level scales take about 4 to 5 minutes in mixing (Wu *et al.*, 2006). Allocated time for mixing tends to also depend on the type of mixer used. The reason for mixing in noodles and pasta contrary to bread processing is to ensure a uniform distribution of ingredients as well as to promote hydration of flour particles. Mixers used commonly in noodle processing are the vertical mixer, horizontal mixer, low speed super mixer, the vacuum mixer and continuous high-speed mixer. Flour quality, quantity of water added, amount of certain ingredients, humidity and temperature of

environment where the processing takes place have all been reported to influence mixing flour. High damaged starch and protein content correlates with dough crumbs that are large during mixing, a factor found to militate against even hydration (Azudin, 1998).

2.6.2 Extrusion

Extrusion is a food processing technology, which operates by combining a lot of unit operations such as mixing, kneading, shaping, cooking, shearing, and forming. In extrusion processing, food ingredients mixed together are pushed out of a perforated die which has a unique shape it imparts to the food with blade that cuts the food into required sizes (Bordoloi and Ganguly, 2014). During extrusion, there is simultaneous mixing, kneading and cooking which causes the food material to undergo changes such as homogenisation, hydration of starches and proteins, melting of fats, plastification, gelation, shearing, denaturation or re-orientation of proteins and expansion of the structure of the food. Classification of extruders is done based on the method of operation (cold extruders or extruder-cookers) and the way it is constructed (single-or twin-screw extruders). In cold extrusion, the temperature of the food is maintained under ambient condition and is usually used for food mixing and shaping.

Examples of such foods include meat products and pasta. For extrusion cooking, food is cooked at a high-temperature and for a short period of time. This process has the tendency of reducing microbial load as well as enzyme inactivation in food (Fellows, 2000).

2.6.3 Drying of Pasta

Drying or dehydration involves the simultaneous application of heat under controlled conditions to remove a high of water normally present in food by though the process of evaporation or sublimation in the case of freeze-drying. The relevance of dehydration

is to ensure a reduction in water activity level of the food product. Factors found to affect drying are air temperature, air velocity and humidity. The mechanism of action behind drying is that, by blowing hot air over a wet food, there is the diffusion of water vapour through a boundary film of air that surrounds the food which gets removed away through the motion of air. There is the generation of driving force by the gradient created for the removal of water in the food (Fellows, 2000). Examples of dryers include bin dryer, cabinet dryer, conveyor/band dryer, drum dryer, spray dryer, trough dryer and tunnel dryer (Barr and Baker, 1997). In pasta drying, it is important to dry for longer hours to promote starch gelatinization which provides a strong starch network for acting as a binding agent to the pasta.

2.7 LYOPHILIZATION OF PASTA SAMPLE

Freeze drying or lyophilisation is a food processing operation which involves the preservation of food through the minimisation in water activity without heating the food and usually results in retention of the nutritional value as well as the sensory properties of food (Fellows, 2000). Freeze drying usually takes place at pressure and temperature conditions below the triple point, to facilitate the ice to sublime. Because low temperature and pressure are the conditions under which freeze drying takes place, it tends to be a suitable drying process for compounds that are thermolabile. Processes involved in lyophilization include the preparation of the sample with freezing, primary as well as secondary drying following. This results in obtaining a finally dried product with a desirable moisture level (Jeff, 2009). Between the drying front and condenser, there is the creation of water vapour concentration gradient, which then becomes the driving force for removal of water during lyophilization. During drying, an increase in vapour pressure of water corresponds with an increase in temperature. To prevent damaging the structure of the product, it is therefore important to keep the primary

drying temperature as high as possible, but beneath the critical process temperature (Adams and Irons, 1993).

2.8 BIOCHEMICAL CHARACTERISATION AND ATTRIBUTES OF PASTA

2.8.1 Protein quantification using Bradford test

The Bradford method is a colorimetric method for assaying total protein content in a sample. It is otherwise called the Coomassie Blue method and is based on the fact that, the amount of absorption or absorbance generated by species is proportional to the quantity of proteins present in a sample (Mikkelson and Corton, 2004). The principle behind the Bradford method involves the binding of the anionic form of the dye Coomassie blue G-250 noncovalently with proteins (Compton and Jones, 1985). Though the dye is found to react mainly with the positively charged side chain of arginine, slight interactions have also been found between the dye and basic amino acid residues (histidine and lysine) as well as aromatic residues (tyrosine, tryptophan and phenylalanine). The Bradford assay is very popular due to the fact that it is rapid in detecting proteins (5mins) and involves the single addition of a dye to the sample.

When there isn't any protein in a sample, the dye is pale red but upon binding to a protein (Mikkelson and Corton, 2004). Once the dye complexes a protein, there is a shift in its absorption maximum from 464 nm to 595 nm. The increase in absorbance at 595nm can be used as an indicator of the protein concentration in a sample (Holme and Peck, 1998).

2.8.2 SDS- PAGE for protein characterisation

Electrophoresis is a very useful biochemical method that separates proteins according to their charges movement. The relevance of electrophoresis as an analytical method is seen in its ability to get proteins visualised and separating them as well allowing scientists to characterise the several proteins present in a solution. Determination of the

approximate molecular weight of the protein is also done using electrophoresis (Lehninger *et al.*, 1993). SDS is a detergent that impairs protein folding (protein 3⁰ structure). Running of SDS-PAGE is found to take place in the midst of sulfhydrylreducing agents such as β -mercaptoethanol so as to disrupt any disulphide links between polypeptide chains are broken (Garrett, 1999). Proteins can be dissociated into their various polypeptide chains using (SDS) detergent following the reduction of disulphide bonds. There is the binding of the SDS to the polypeptide chain to produce a rod-shaped complex, the length of which is also dependent on the relative molecular mass of the protein. Electrophoretic mobility of all proteins complexed with SDS is almost approximately equal but the polyacrylamide gel acts as a molecular sieve resulting in a relative mobility inversely related to the size of the protein (Mikkelson and Corton, 2004).

Prior to the taking the sample through the electrophoresis process, the sample is diluted in a buffer containing SDS (10-25 g l^{-1}) and β -mercaptoethanol (10-50 ml l^{-1}), which ends up reducing the presence of disulphide bonds stabilising the protein. The sample is then heated at 100C for 2-5mins to ensure the protein is denatured and the total polypeptide chain length exposed to the detergent (Mikkelson and Corton, 2004).

After SDS has bound to most proteins, it imparts a large net negative, making of no effect the intrinsic charge on the protein. In SDS-PAGE electrophoresis protein separation is based on the basis of molecular weight, usually with smaller polypeptide molecules moving more rapidly exclusively. Visualization of proteins is made possible by the addition of Coomassie blue, which binds to proteins but not to the gel itself. Identification of the position of an unknown protein can help give an accurate measure of the proteins molecular weight once the position of the unknown protein is compared

to the position migrated by a protein with known molecular weight in the gel (Lehninger *et al.*, 1993).

2.8.3 Accessible Thiols

Thiols belong to a class of organic compounds characterized by having a sulfhydryl functional group (-SH). Biologically derived thiols commonly called Biothiols constitute an important antioxidants that have the ability to militate against oxidative stress (Sen and Packer, 2000; Wlodek, 2000). One of the widely studied biothiols, Glutathione, a very potent endogenous antioxidant is one of the most commonly studied biothiols (Meister *et al.*, 1983) and is reported to be synthesized from γ – glutamyl cysteine (GGC) by glutathione synthetase. The need to evaluate the accessible thiols in pasta is as a due to the essential role of thiols in foods acting as precursors for the synthesis of essential endogenous biothiols in the body. Accessible thiol content tends to provide information on the thiol-disulphide exchange events which provides insight into the “natural” network of protein interactions that covalently takes place. Also measurement of the thiol accessibility to suitable reagents under varied conditions (such as presence or absence of agents that have the ability to dissociate or enhance the stabilization of the protein structure or of interprotein contacts) tends to provide insight into the structural firmness of the protein network that is been observed (Bonomi *et al.*, 2013). According to Iametti *et al.* (2006), accessible thiol determination does not require any solubilisation step.

2.8.4 In-vitro protein digestibility

In-vitro protein digestibility approach is a quick, less expensive and simple method for assessing how the proteins in a food material are digested. This approach helps to allow close monitoring of the dynamics involved in protein breakdown by proteindigesting enzymes and involves the use of a relatively small amount of food product for the

evaluation (Dimes and Haard, 1994). The test is a technique that mimics how protein is digested by protein enzymes in humans. In humans, the role of the gastric juices which is acidic in nature (pH 1.0 to 2.5), is to also to serve as a denaturing agent promoting the denaturation of globular proteins ensuring the accessibility of their internal peptide bonds to hydrolytic enzymes present in the stomach. Trypsin and chymotrypsin play the role of further hydrolysing the peptides that are produced in polypeptide chains after proteins are denatured by pepsin. Protein digestion at this point is efficiently done since pepsin, trypsin and chymotrypsin have different amino acid specificities. Globular proteins from animal sources such as albumin are hydrolysed almost completely to amino acids in the GIT whereas fibrous proteins are only partly digested (Lehninger, 1993). The resulting mixture of free amino acids is then measured using a spectrophotometer.

2.9 PASTING AND COOKING INDICES OF RICE BASED PASTA

2.9.1 Pasting properties

Initial viscosity refers to the viscosity of the flour suspension in Brabender Unit (BU) at the start of heating. The initial viscosity of the flour in water at 30 °C is called the cold viscosity and in the preparation of instant foods, this property relates the capacity of the flour to absorb water at room temperature to form a paste, gel or viscous liquid (Souza *et al.*, 2011). For the pasta samples, it indicates the capacity of the flour to absorb water at room temperature to form a paste, gel or viscous liquid. Physicochemical interactions between the amylose and amylopectin begin to take place once starch is heated in water resulting in a change of the starch granule structure. The series of phases that starch granule structure goes through include glass transition, gelatinization as well as the dissolution of the amylose-lipid complex (Schirmer *et al.*, 2015). In flour suspensions, the glass transition phase involves the change of the suspension from a

“glassy” state to a more rubbery progressive state (Roos and Karel, 1991). This tends to ensure the absorption of a high amount of water into the amorphous region prior to gelatinization setting in. Gelatinization refers to the phase transition that occurs during the heating of a starch suspension resulting in the change in the structure of a starch granule from an ordered to a disordered state (Babic *et al.*, 2009). It is reported that, to ensure complete starch gelatinization, at least fourteen molecules of water per one glucose unit is needed (Wang *et al.*, 1991). During the heating of a starch–water suspension, once the heating temperature exceeds the temperature at which the starch granule gelatinize, there is the initiation of the starch granule swelling. At a point in the starch granule swelling process, rupturing of the starch granules begin to take place which results in an increase in viscosity (Schirmer *et al.*, 2015). Peak viscosity refers to the rapid increase in viscosity that occurs when a significant number of starch granules become swollen (Mir and Bosco, 2013). According to Falade *et al.* (2014), breakdown viscosity is associated with the tendency of the cooked starch to disintegrate. Breakdown viscosity is the viscosity of the paste after been heated at the holding temperature of 95°C. A higher breakdown in viscosity has been found to correlate with a lower ability of the starch sample to resist shear stress and heating during cooking (Adebowale *et al.*, 2003). Setback viscosity is the measure of the tendency of starch granules to retrogradation (Abd Karim *et al.*, 2000). Once gelatinization is completed, the linear amylose chains that leach out begin to start reassociating with each other on cooling and this subsequently results in increased viscosity of flow pastes (Arif *et al.*, 2014).

2.9.2 Cooking Indices of Pasta

Cooking indices involves the cooking behaviour of the pasta in terms of its cooking time, water absorption and its firmness. Water absorption capacity involves the ability

of the matrix of a protein to absorb and retain bound, hydrodynamically, capillary physically entrapped water against gravity. It is a very important cooking index because it highlights the dynamics involved in protein water interaction in various food systems (Sangeetha and Devi, 2012). According to Brennan and Tundorica (2007), the quality and cooking properties of pasta tend to depend on the development of protein-starch matrix. Pasta firmness can be associated with the protein content of pasta and starch composition (Martin-Esparta *et al.*, 2013).

2.10 COLOUR ATTRIBUTES OF PASTA

Determination of colour attributes is a very important in food product development since colour informs consumers about their choice of a particular food. According to D'Egidio and Pagani (1997), factors such as processing conditions and formulations in terms of the characteristics of raw materials, and/or presence of specific ingredients tends to always affect luminosity and chromatic indices. Other factors that have also been found to impart the final colour of food samples include preparation conditions like the recipe, water temperature and its added amount (Nasehi *et al.*, 2009; Petitot *et al.*, 2010), extruder type used for the pasta production, the type of dryer used and the operating parameters (Jukic' *et al.*, 2007) besides the raw material profile. Miskelly (1984) also reported that, factors such as the milled kernel type and flour yield as well as flour composition such as ash, protein, pigments and damaged starch contents affects flour colour. Phytochemical pigments like the carotenoids and xanthophylls have been found to impart the colour of food samples (Humphries *et al.*, 2004; Fratianni *et al.*, 2005) likewise protein molecular composition (Ohm *et al.*, 2008). Botanical origin of flour as well as mixing ratio also tends to influence cereal colour composites for pasta colour. Crops that are most frequently used which imparts on colour formation as well as enhanced nutritional value include legumes such as

chickpea, soy, yellow or green pea, faba bean (Zhao *et al.*, 2005; Chillo *et al.*, 2008; Nasehi *et al.*, 2009; Petitot *et al.*, 2010).

2.11 NUTRITIONAL INTERVENTION

2.11.1 Celiac disease

Celiac disease is a chronic immune-mediated disorder of the small intestine that occurs when a person who is genetically exposed to the condition takes into the body dietary gluten (Ludvigsson *et al.*, 2013). The condition is usually aggravated when a genetically predisposed person ingest gluten, which is the protein component of wheat. The autoimmune inflammatory response results in villous atrophy, malabsorption, malnutrition and possibly malignancy (Mahan and Escott-stump, 2008). The prevalence of celiac disease is considered to be 1 in 133 persons in the US (Mahan and Escott-stump, 2008) and 1/200 in some European countries (Catassi and Fasano, 2008) necessitating the need for an appropriate nutritional intervention in the form of nutritionally enhanced rice-based pasta.

2.11.2 Burns conditions

Burns presents a major medical challenge accounting for a major portion of the workload for the Plastic Surgery and Burns Unit and it has been found that, 45% of burns patient are children (Thompson, 2011). Annually, 265,000 death cases attributed to Burns is reported in Ghana and children below 20 are the most affected (WHO, 2014). According to Prins (2009), there is an increase in protein requirements in burns patients due to the increased muscle breakdown, wound losses and tissue repair; necessitating the need for optimal protein administration. Development of ricebased pasta with egg albumen as the building body agent could be an effective intervention not only for people with celiac disease but also, burns patients mostly children in Ghana.

2.12 SENSORY EVALUATION OF PASTA USING ELECTRONIC NOSE AND TONGUE

2.12.1 Electronic nose

Commonly used sensors for e-nose are metal oxide semiconductor (MOS), conducting polymer, and surface acoustic wave (transducers) (Deisingh *et al.*, 2004, Ghasemi-Varnamkhasti *et al.*, 2009). Taste sensors for e-tongue exhibits qualities of low specificity and are of low selective chemical sensors with cross-sensitivity to different components in solution (Ghasemi-Varnamkhasti *et al.*, 2010). However, some e-tongue sensors are selective. Several e-nose and e-tongue instruments do not give information but instead through pattern recognition are able to give a digital fingerprint. However, in when electronic nose or tongue device is used in combination with a gas chromatography (GC) with solid phase microextraction (SPME) and/or mass detection by a mass spectrometer (MS) they do give information due to differences in chemical mass. E-nose that uses a short GC column which by giving a crude chromatogram that helps to give information on odour molecules in the sample has also been introduced on the market (Du *et al.*, 2010). Calibration of e-nose and tongue is done with chemicals and also by relating equipment responses to sensory data.

A clear difference observed between the operation of the e-nose and a biological system is that, the e-nose expose a limited number of sensors to volatiles whilst a biological system uses a large number of sensors with diverse binding proteins. The portable e-Mucosa System (PeM) is an example of an artificial system that mimic biology. The system operates through the utilization of three large sensor arrays, each with 200 chemoresistive sensors combined with two columns coated with different retentive layers (polar and non-polar compounds) giving pattern recognition that utilizes temporal information, improving the discrimination power of the instrument over

traditional e-noses (Che Harun, 2002). The Differential e-nose (Den-nose) is one of the latest inventions developed which uses two chemosensor arrays to discriminate odors (Brudzewski *et al.*, 2010). For the majority of e-nose systems, analysis of samples is usually carried out using an array of gas sensors and a pattern recognition algorithm requiring exposure to odors and flushing of the system for sensor recovery and this requires an environmentally-controlled (temperature and humidity) chamber. When the environment is dynamic, it causes the sensors to operate in a way that prevents them from reaching a steady state and the analysis tends to depend on the transient phase of the signal (Trincavelli *et al.*, 2009) for real time identification, achieved through the use of tin dioxide gas sensors for continuous monitoring application. The MS has been reported to have the potential to be used as e-nose and the head space (HS) coupled with MS. It involves injecting the headspace of a sample directly in the ionization chamber of the MS where there is fragmentation resulting in a global mass spectrum for each sample giving the fingerprint of a sample as with other e-nose systems.

2.12.2 Electronic tongue

The Electronic tongue (E-tongue) can be used to gather sensory attributes information such as bitterness, astringency and sourness of foodstuffs such as beers, wines and teas (Kaneda *et al.*, 2003, Polshin *et al.*, 2010, Rudnitskaya *et al.*, 2009 and Rudnitskaya *et al.*, 2010). The e-tongue works by identifying polyphenolic compounds and assessing attributes related to sensory qualities such as sweet, bitter, sour, caramel, fruity, body, intensity and burnt through the application of amperometric and or potentiometric chemical sensors. It does this by also applying the same pattern recognition techniques used by the e-nose. Scampiccio *et al.* (2008) have indicated that, taste sensation is the product of the physico-chemical interactions that occur between food molecules and the complex system of hundreds of cell buds located randomly all over the tongue. The e-

tongue works on the principle of signal combination from specific, non-specific and overlapping sensors using pattern recognition. There are four classes of the amperometric sensors and this includes metal, conducting polymer, phthalocyanine film and biosensors (Baldwin *et al.*, 2011). Metal sensors though are also used, tend to lack selectivity. They have been found to be very useful for classification applications than for evaluation of taste, like helping in the prediction of sensorial descriptors of Italian red wines of different origins (Burrati *et al.*, 2007). Polypyrrole and polyaniline are examples of conducting polymer sensors that show a variation of conductivity with adsorption of different analytes (Scampiccio *et al.*, 2008). Assessment of sweet, salty, astringent, acid and bitterness tastes of food samples has been carried out using e-tongues that bases its mode of action on conducting polymers (Apetrei *et al.*, 2004 and Arrieta *et al.*, 2004). Different chemical properties have been observed for Phthalocyanine. These exist as a group of compounds, which are complexed together whereby a transition metal is coordinated with a phthalocyanine ring. Cross-selectivity to antioxidant compounds like banillic acid, progallol, ascorbic acid and catechin has been shown by films of phthalocyanine, porphyrin and naphthalocyanine (Casilli *et al.*, 2005). Discrimination between model solutions of sweet, bitter, salty, acid and umami basic tastes and bitterness in olive oils have been carried out using e-tongues that operate using films of phthalocyanine sensors (Arrieta *et al.*, 2003, Apetrei *et al.*, 2004). E-tongues usually operate using biosensors that have an enzyme (biochemical transducer) with a solid electrode closely positioned. The enzymes are mostly oxidases that feed on oxygen as substrate and produce hydrogen peroxide or the reduced form of β nicotinamide adenine dinucleotide (phosphate) NAD(P)H as a dehydrogenase (Scampiccio *et al.*, 2008).

2.12.2.1 Principle of action of e-tongue sensors

The recognition element used by e-tongues mostly is a lipid membrane that changes taste relevant substances into electric potential charge across thin membranes. The membrane potential is not dependent on how thick the lipid membrane is and must be durable and reproducible even when repeatedly rinsed. Optimization of the concentration of the lipid in the membrane can be achieved and this affects the detection limit of the sensor adsorption taste substances (Liyama *et al.*, 2009).

2.13 SUMMARY OF LITERATURE REVIEW

The use of rice flour in pasta production possesses a technological challenge to rice processors. This has been attributed to the lack of gluten responsible for structure building in rice. The use of albumen as structure building agent has therefore been proposed. Rice is an ideal fortification vehicle and fills a gap in the current fortification landscape. Development of rice-based pasta fortified with orange fleshed sweet potato and soy flour enhances the nutritional value of the rice-based pasta.

Structural characterization of proteins evaluated through differential solubility indices and by measuring thiols reactivity gives an indication of the presence of protein aggregates stabilized by hydrophobic interactions and disulphide bonds in pasta samples. A higher network-forming ability in soybean-enriched pasta helps lower cooking losses of sample. Soybean and sweet potato enrichment imparts on the color of pasta samples. The E-nose and tongue sensing approaches is an objecting way of evaluating the sensory profile of the various pasta products and strongly depends on the perculiar enrichment. In terms of pasting properties, lower viscosity of enriched samples indicates that both soybean and sweet potato compete with water needed for starch granule hydration and subsequent gelatinization. This has a direct effect on the optimum cooking time of the various products. The application of a multidisciplinary

approach to the characterization of differently enriched rice-based pasta allowed to define the quality parameters of the samples.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SAMPLE PREPARATION

3.1.1 Soy flour preparation

The *Jenguma* soybean variety used for this work was sourced from a farmer in Wenchi, Brong Ahafo Region of Ghana and verified by an Agronomist. It was kept in an air-tight sack until ready to be used for flour preparation. The soybean was soaked in water for 2 days and manually dehulled. The dehulled soybean was dried in the sun to constant weight (which took 2 days with an average temperature of around 41°C) and milled to obtain the flour using a Rajex Grinding Mill (Serial Number 93, Type 2A).

3.1.2 Orange-fleshed sweet potato flour preparation

Apomuden, an orange-fleshed sweet potato variety was used for this work. The freshly harvested sweet potato samples were washed, cut into thin slices and oven-dried at a temperature of 45 °C for 8 hours. The dried sweet potato pieces were then milled into flour using a Rajex Grinding Mill (Serial Number 93, Type 2A). The flour was packaged into a dark polyethylene bag and kept in a cold room until ready to be used for the work.

3.1.3 Rice flour

A commercially produced pre-gelatinised low-grade milled rice flour from Italy was used for the study.

3.2 PASTA FORMULATION AND PREPARATION

Rice-based pasta was produced at the experimental pasta-making plant of the Department of Food, Environment and Nutritional Sciences (DeFENS), University of Milano (CRA, S. Angelo Lodigiano) by extruding a dough prepared with pregelatinized Italian rice flour and liquid egg albumen, and enriched with African soybean flour (cv *Jenguma*) and/or African sweet potato flour (cv *Apomunden*). The Pasta was formulated according to the formulation (Table 1) and was extruded and dried in the oven for 17 hours at a temperature of 65 °C. The addition of 20% albumen followed a report by Marti *et al.* (2014) who indicated that, addition of 15 % liquid albumen to parboiled rice results in significant improvement of the textural and structural features of rice-based gluten-free pasta.

Table 1: Ingredients used for Pasta formulation

Sample Code	Egg albumen/ml	H ₂ O/ml	Rice/g	Sweet Potato/g	Soya flour/g
R	200	800	2000	0	0
RP	200	800	1800	200	0
RPS	200	800	1400	200	400
RS	200	800	1600	0	400

R= Rice only, RP= Rice and Potato, RPS= Rice, Potato and Soy, RS= Rice and

Soy



Plate 1. Single screw extruder



Plate 2. Extruder extruding rice-based pasta



Plate 3. Oven drying Pasta samples

3.3 BIOCHEMICAL CHARACTERISATION OF PASTA

3.3.1 Protein solubility

Protein solubility of pasta in native and denaturing conditions was determined using three different buffers. Thus buffer A: 0.05 M sodium phosphate monobasic dihydrate (NaH_2PO_4 , pH 7.0) containing 0.01 M NaCl ; buffer B: 0.05 M sodium phosphate monobasic dihydrate (NaH_2PO_4 , pH 7.0) containing 0.01 M and 6M urea; buffer C: 0.05 M sodium phosphate monobasic dihydrate (NaH_2PO_4 , pH 7.0) containing 0.1 M NaCl, 6M urea and 0.01 M Dithiothreitol (DTT). A 0.12g of finely ground sample was suspended in buffer A, B and C to assess the level of protein solubility. The suspensions were vortexed and agitated on a rotary shaker for 1 hour at 25°C. The samples were then centrifuged at 10,000 rpm, at a temperature of 20°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.

3.3.2 Electrophoretic pattern of the extracted proteins

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the produced pasta samples was performed according to the procedure 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°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.

3.3.3 Accessible Thiols

Accessible thiol groups were determined by suspending 0.25 g of finely ground samples in 5 mL of 50 mM sodium phosphate, 0.1 M NaCl, pH 7.0, in the presence/absence of 6 M urea, containing 0.2 mM 5,5-dithiobis-(2-nitrobenzoate) (DTNB) (Ellman, 1959). After 1 h stirring at room temperature, the suspension was centrifuged (13000*g, 30 min, 25 °C) and the absorbance of the supernatant was read at 412 nm. The blank was prepared by suspending 0.25 g of samples in 5 mL of 50 mM sodium phosphate, 0.1 M NaCl, pH 7.0, in the presence/absence of 6 M urea, without adding DTNB. Results are expressed as µmol thiols/g pasta.

3.3.4 In-vitro Protein Digestibility Test

This was carried out using the enzymes 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 13000rpm 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 13000rpm for 15 min. This was again repeated for 120 and 180min. The hydrolyzed peptide content in the supernatant was measured at 280 nm.

3.4 COLOUR ANALYSIS

A reflectance colour meter (CR 210, Minolta Co., Osaka, Japan) was used to measure the lightness and saturation of the colour intensity of the rice-based pasta by utilizing the CIE-LAB uniform colour space procedure. CIE-LAB system colour values L*, a*, and b* as measures of lightness, redness–greeness, and yellowness–blueness, respectively, were recorded for each sample. Each measurement was replicated at least four times, and the average value was used.

3.5 COOKING BEHAVIOUR

Determination of the cooking loss was carried out by evaluating the amount of solids that leached into the water used for cooking as described in AACC (2001). A 250 ml of distilled water is measured into a conical flask and heated over a burner until it starts to boil. A 25g of the rice-based pasta is weighed and transferred into the boiling water with no salt added. A timer was started to monitor the optimum cooking time. The boiling pasta is stirred intermittently to avoid sticking into the conical flask. The softness of the pasta is checked intermittently by smashing the cooked pasta over two glass plate. The time required for the central core to disappear when squeezed gently between two glass plates was taken to be the optimum cooking time according to AACC (2001). Recovered water from the cooked pasta was transferred into a 250 ml volumetric flask and topped up with distilled water to the 250 ml mark. A 40 ml of the diluted cooking water (stock) was collected into empty crucibles and dried in the oven

at 105 °C to obtain a constant weight. Residues obtained after oven drying of the collected water from the cooked pasta was weighed and the dry matter reported as percentage of the starting material. The result was expressed as grams of matter loss per 100 g of uncooked pasta. The water absorption capacity was determined according to the procedure by Marti *et al.*, (2014). Pasta samples were weighed before and after cooking to evaluate the weight increase. The result was expressed as the ratio of the weight increase to the weight of uncooked pasta. A texture analyzer TA.HD plus (Stable Micro System Ltd., Godalming, UK), calibrated for a load cell of 2.5 kN was used to determine the textural characteristics of the cooked pasta. Repetition of the analysis was done at least five times and for each replicate, 6 pieces of pasta were cooked at the optimal cooking time. The Kramer cell (test speed 0.67 mm/s) was used to analyse the results. Texture Exponent TEE32 software (v. 3.0.4.0) was used to evaluate the firmness (expressed in Newtons).

3.6 PASTING PROPERTIES

Pasting properties of formulations were determined according to the method of Marti *et al.* (2010). 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°C to 95 °C, held for 20 min at 95°C and cooled to 30°C and finally held for 1 min. Heating and cooling rates were 3°C/min and -3°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 °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 °C); setback (viscosity increase during cooling). The viscosity was measured in arbitrary Brabender units (BU).

3.7 MOISTURE CONTENT DETERMINATION

A 2g of the sample is transferred into a previously dried and weighed crucible and the weight of the wet sample recorded. The crucible with the sample is placed in a thermostatically controlled oven at 130 °C for 90 mins. The crucible with dried sample is then placed in a desiccator to allow for cooling to room temperature and the weight recorded. The percentage moisture (%) content of the sample is calculated as the ratio of the difference in weight between the wet and dried sample to the weight of the wet sample expressed in percentage.

3.8 SENSORY EVALUATION

3.8.1 Electronic Nose

A Portable Electronic Nose (PEN2) from Win Muster Airsense (WMA) Analytics Inc. (Schwerin, Germany) was used for the electronic nose evaluation of the sample. Its components include an array of chemical gas sensors producing an array of signals when confronted with a gas, vapor, or odor, a sampling apparatus, and an appropriate pattern-recognition software (Win Muster v.1.6) for data recording and elaboration.

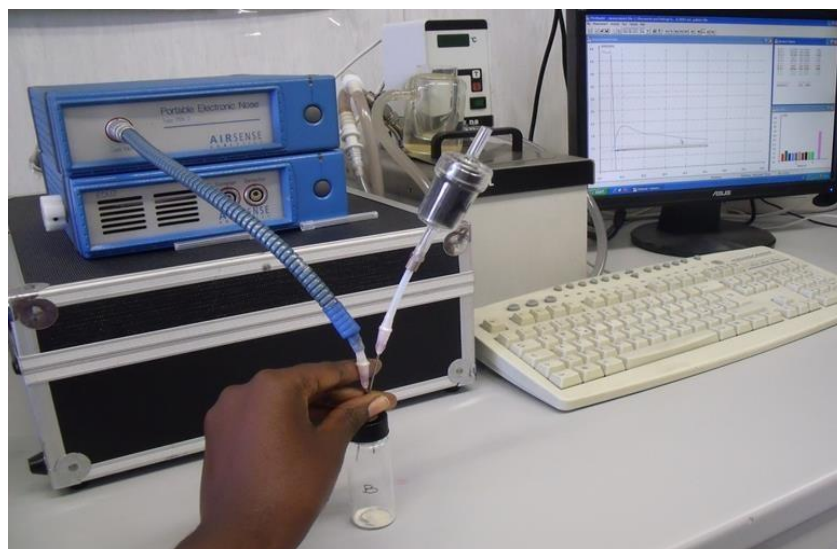


Plate 4. Electronic Nose (PEN2)

The electronic nose uses the principle of total headspace transfer of a sample to an array of sensors that have the ability to detect the presence volatile compounds. Based on the characteristics of the volatile compounds in the headspace, the sensors sensitivity and selectivity, a pattern of signals is produced. As a result of the interactions that occur between the gas sensors and the odour from the compounds, there is alteration of the state of the sensors resulting in the production of electrical signals which are captured on the instrument. The pattern of signals from the individual sensors is unique for the particular gas mixture measured. An interpretation is then given by multivariate pattern recognition techniques. Similar sensor response patterns are produced by samples that give similar odour whilst differences in patterns is observed for samples that produce different odour.

The composition of the sensor array in the electronic nose PENS involves ten Metal Oxide Semiconductor type of chemical sensors as seen in Table 2. Resistivity (Ω) is the unit of measurement for the sensor response.

Table 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 ₂ S 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)

3.8.1.1 Mode of operation

A 0.5 g of the lyophilized sample was weighed into a 500 µL of distilled water and the solution stirred until the sample is dissolved. The dissolved sample was then transferred into a 40mL airtight glass vial fitted with a pierceable Silicon/Teflon disk in the cap and allowed for headspace equilibration for 60 min at room temperature. The measurement sequence was then recorded. The conditions for operating include a flow rate of 300mL/min, injection time of 60 min, flush time 180 min. Air filtered through active carbon was used to clean the surface of the sensors. Analysis of the samples was done

in triplicate and the responses from the sensors used for subsequent statistical analysis.

The analyzed samples are reported in Table 3.

Table 3: Pasta and their coding for electronic nose analysis

Sample	Composition
1	R
2	RPS
3	RP
4	RS

3.8.2 Electronic Tongue

Taste-Sensing System SA 402B (Intelligent Sensor Technology Co. Ltd, Japan) namely Electronic Tongue (ET) was used for the analyses. The ET is a liquid analytical device that mimics the taste-sensing mechanism of the gustatory system; composed of two sensor arrays that are specific for liquid with the ability to evaluate tastes: sourness, saltiness, bitterness, umami and astringency as shown in Plate 5.



Plate 5. Taste sensing system

The part of the system for taste detection is made up of seven sensors with their surfaces attached to lipid membranes having different response properties to chemical substances on the basis of their taste as shown in Table 4.

KNUST

Table 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 the present 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 utilised by the electronic tongue is dependent on the ability of the taste substances to cause the potential of the detecting sensors to change as a result of its electrostatic or hydrophobic interaction with the hydrophilic and hydrophobic groups of the lipid membranes. The difference between the potential detected by the sensor and the potential of the reference electrode is the response of each sensor. Elaboration of the response of each sensor is aided by a computer and processed via a pattern recognition system.

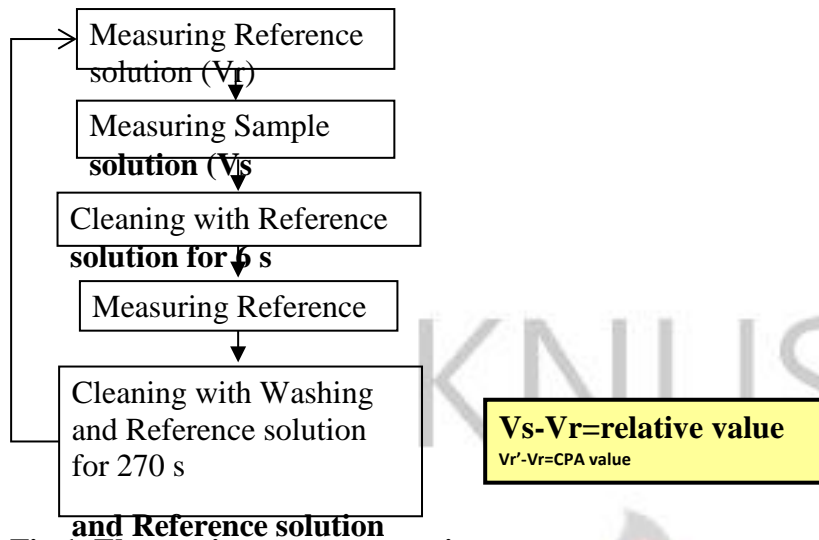


Fig 1. Electronic tongue measuring process

The detecting sensors and reference electrodes were calibrated by first dipping them in the reference solution (30 mM potassium chloride and 0.3mM tartaric acid) and the electric potential measured for each sensor was defined as V_r . This was then followed by immersing the sensors for 30 s into the sample solution. The measured potential recorded for each sensor was defined as V_s . For each sensor the “relative value” (R_v) was represented by the difference ($V_s - V_r$) between the potential of the sample and the reference solution as shown in Fig. 1. Fresh reference solution was used to rinse sensors for 6 s and then immersed into the reference solution again. The new potential of the reference solution was defined as V_r' . For each sensor, the difference ($V_r' - V_r$) between the potential of the reference solution before and after sample measurement is the CPA value (Change of Membrane Potential caused by Absorption) (CPA_v) and corresponds to the ET “aftertastes”. The electrodes were rinsed for 90 s with a washing solution and then for 180 s with the reference solution before starting with a new measurement cycle. 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:

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

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

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

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

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

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

$$\text{Umami} = -0.1575 \times R_v(\text{AAE})$$

$$\text{Richness} = -0.420 \times \text{CPAv}(\text{AAE})$$

3.8.2.1 Sample preparation

A 10 grams of each of the sample (R, RP, RS, and RPS) was weighed and added to 200 mL of distilled water. The solution was vortexed for about 5 min and centrifuged at 5000 rpm for 10 min at 20°C. The supernatant obtained after centrifugation was filtered and analyzed in triplicate.

3.8.3 Statistical Analysis

Apart from the electronic nose and tongue, data analysis was carried out on the biochemical and rheological parameters using SPSS (IBM version 20) One-way ANOVA with *P*-values (< 0.05) considered statistically significant.

3.8.3.1 Data Analysis for electronic nose and tongue

Processing of the multivariate output data generated by the sensor array signals represents a critical 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). A procedure that permits the extraction of 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 is the Principal Components Analysis (PCA). Its use in analyzing explorative data is as a result of the fact that, 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.

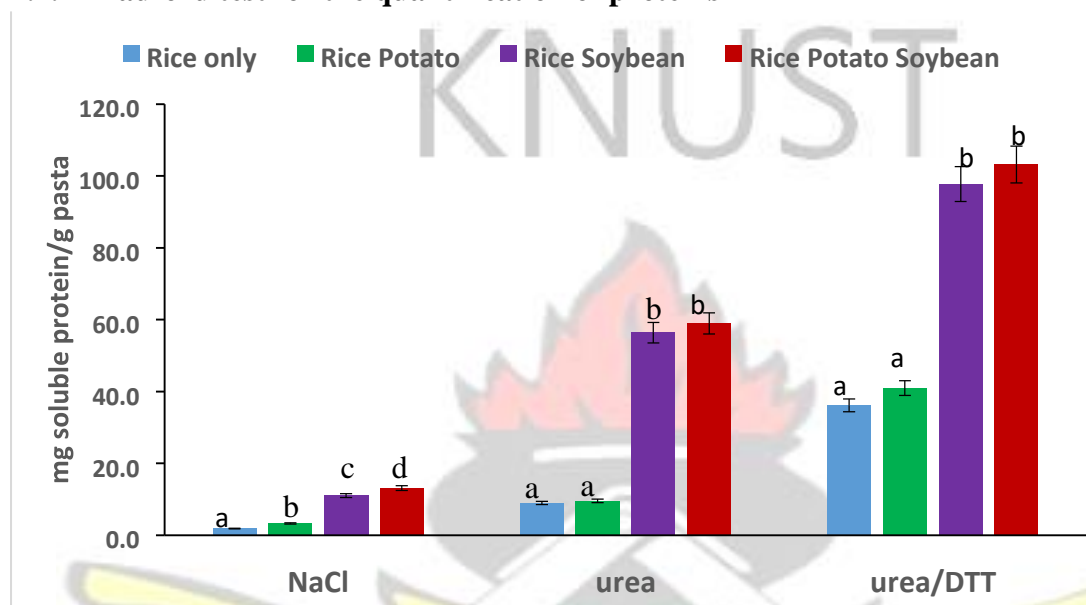


CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 BIOCHEMICAL CHARACTERISATION OF PASTA SAMPLES

4.1.1 Bradford test for the quantification of proteins



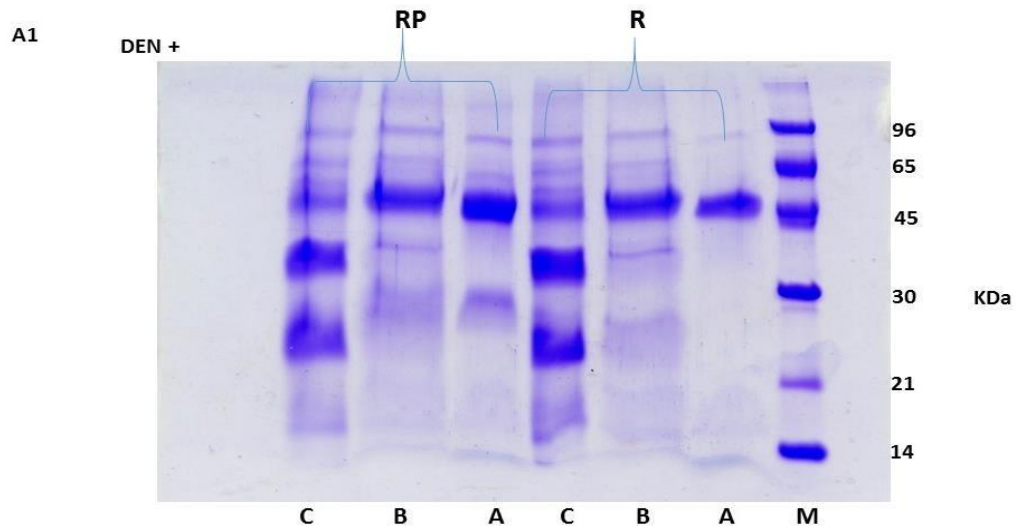
Mean values in a row with different letters for each buffer treatment are significantly different ($p < 0.05$)

Fig. 2. Effect of different buffer treatments on pasta samples

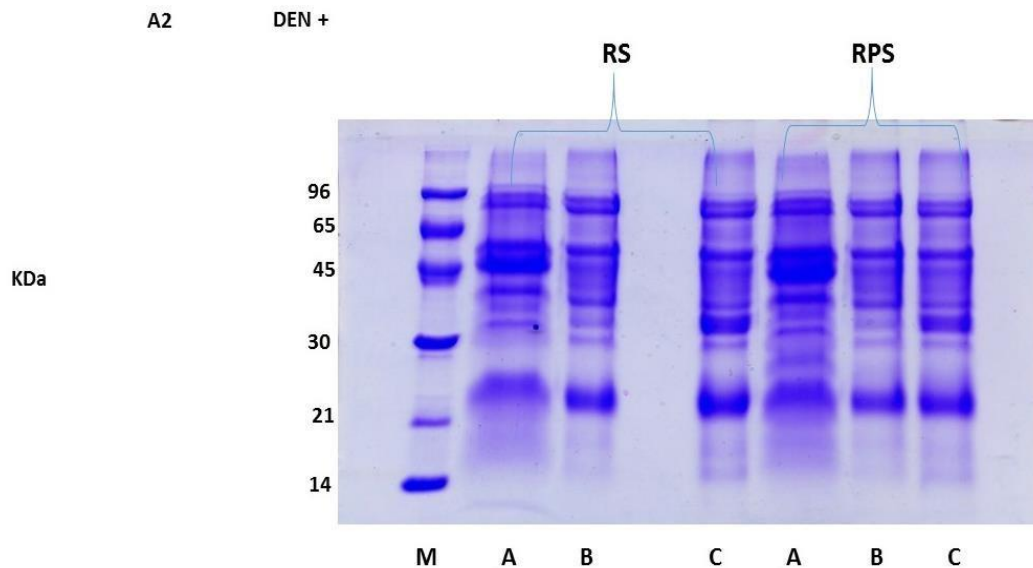
The assessment of the structural role of the covalent and non-covalent interactions among proteins on the stability of the pasta was done by detecting the quantity of protein solubilized in a media which has the potential to dissociate protein-protein complexes differently (Marti *et al.*, 2014). According to Lehninger *et al.* (1993), the covalent bonds in proteins are mainly peptide and disulphide bonds which help in the linking of amino acid residues in a polypeptide chain. The globular proteins, albumins and globulins, have been found to be soluble in a saline buffer whereas aggregate proteins stabilized by non-covalent and/or covalent interactions (interprotein disulphide bonds) are soluble in buffer with urea or urea/DTT respectively (Barbiroli *et al.*, 2013). In this work, it was observed that the amount of proteins in the pasta sample solubilized in the saline buffer

was very low but increased when urea and DTT were added (fig.2). Generally, it was observed that protein solubilization in buffer A < buffer B < buffer C for all the samples. This is consistent with the findings of Bhattacharya (2004) who reported that buffer A which is a saline buffer solubilized 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 thereby increasing the quantity of solubilized protein. On addition of dithiothreitol (DTT) to the saline + urea buffer, there was an increase in the solubilization of proteins due to dissociation of aggregates stabilized by disulphide linkages (Bonomi *et al.*, 2012) as shown in (fig. 2). This result is consistent with what was reported by Marti *et al.* (2014) that the amount of proteins in rice pasta with egg albumen and rice pasta with whey protein dissolved in saline buffer was very low but increased significantly when urea was added and increased further when urea and DTT were added. The increase in soluble proteins when urea and urea/DTT were added to the saline buffer could be attributed to the fact that, the proteins in the rice-based pasta were involved in a network stabilised by disulphide bonds and so were disrupted by the urea/DTT. The results also indicate higher levels of soluble proteins in the soy-containing pasta due to the rich protein content of soy flour.

4.1.2 SDS- PAGE Electrophoresis



M+ ; positive marker, A; Buffer A (50mM Sodium phosphate monobasic dehydrate + 100mM NaCl)
 B; Buffer B (Buffer A + Urea)
 C; Buffer C (Buffer B +DTT)
 RP; Rice + Potato
 R; Rice



M+ ; positive marker, A; Buffer A (50mM Sodium phosphate monobasic dehydrate + 100mM NaCl)
 B; Buffer B (Buffer A + Urea)
 C; Buffer C (Buffer B +DTT)
 RS; Rice + Soy flour
 RPS; Rice+ Potato + Soy flour

Fig. 3. Electrophoretic patterns of proteins in pasta using DEN (+)

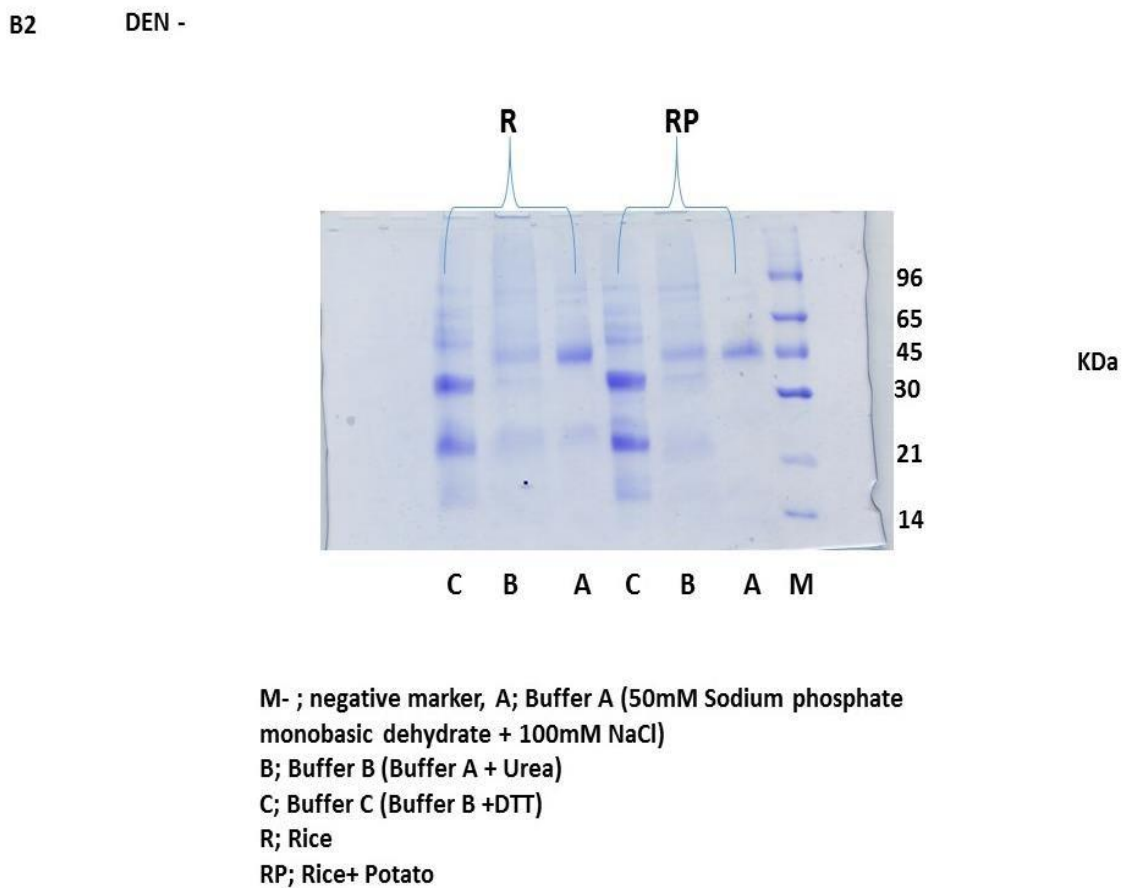
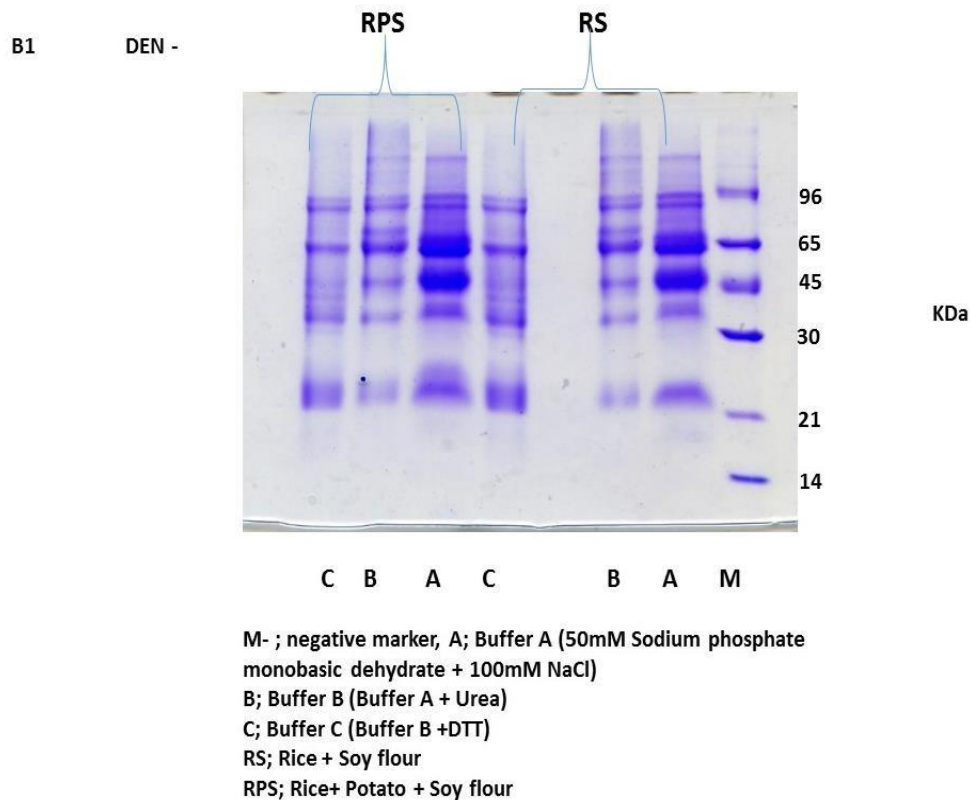


Fig. 4. Electrophoretic patterns of proteins in pasta using DEN (-)

From fig. 3 and 4, it could be seen that, the SDS PAGE showed a common protein band running through the pasta samples produced from RP, R, RS and RPS at the position around 45 kDa even when DEN (+) (containing β -mercaptoethanol) and DEN (-) (without β -mercaptoethanol) were used as denaturing buffers. This protein was identified as egg albumen. According to Nisbet *et al.* (1981), the amino acid sequence of chicken egg albumen -a phosphorylated glycoprotein- reveals the peptide portion of the molecule consists of 385 residues and has a molecular weight of 42.7kDa. The carbohydrate and phosphate portions account for an extra 1428 and 160 grams per mole respectively, making the total molecular weight of albumen add up to 44.3kDa (Tai, *et al.*,1977). Besides, egg albumen was used as a binding agent in the pasta used for this present study and so one would expect it band visualised in the SDS-PAGE profile. Albumen are water soluble proteins considered to have a nutritionally better amino acid compositions because of the higher lysine and methionine contents (Lasztity, 1984). They have been found to be composed of nonglutein proteins and are soluble in water and dilute buffers (Goesaert *et al.*, 2005). Bands of varying intensity were seen when buffers A, B and C were used for the extraction of proteins in the pasta. This could be explained that, the proteins in the pasta had a varied degree of solubilization in the various buffer media used for the protein extraction. The lightly observed total band intensity of lane A and B when compared with lane C could be attributed to the lower extractability of proteins by the saline and urea buffer prior to protein denaturation with DEN (+) and (-). The low protein solubility could be as a result of aggregation and/or cross-linking; with the aggregation of proteins attributed to polymerisation resulting from cross-linking of the proteins (Purnima *et al.*, 2012). Rice is known to contain a major disulphide-rich containing protein called oryzenin (Chrastil, 1990). The presence of the several disulphide bonds in rice accounted for the

intense band observed when buffer C was used as observed in lane C. This is attributed to the use of urea/DTT in buffer C for the protein extraction. DTT is known for its ability to dissociate protein aggregates stabilized by disulphide bonds. β -mercaptoethanol is a very potent reducing agent and in the presence of urea, has its activity enhanced. Urea impairs and disrupts hydrogen bonds that exist within protein aggregates resulting in the unfolding of proteins to enable the disulphide bonds in the proteins to be exposed and subsequently reduced (Nsiah, 2003). This could account for the intense coloured bands seen in the SDS-PAGE gels produced when DEN (+) was used. The β -mercaptoethanol reduced the several disulphide bonds in the pasta samples resulting in disaggregation into gels and with intense visualised bands.

4.1.3 In-vitro protein digestibility

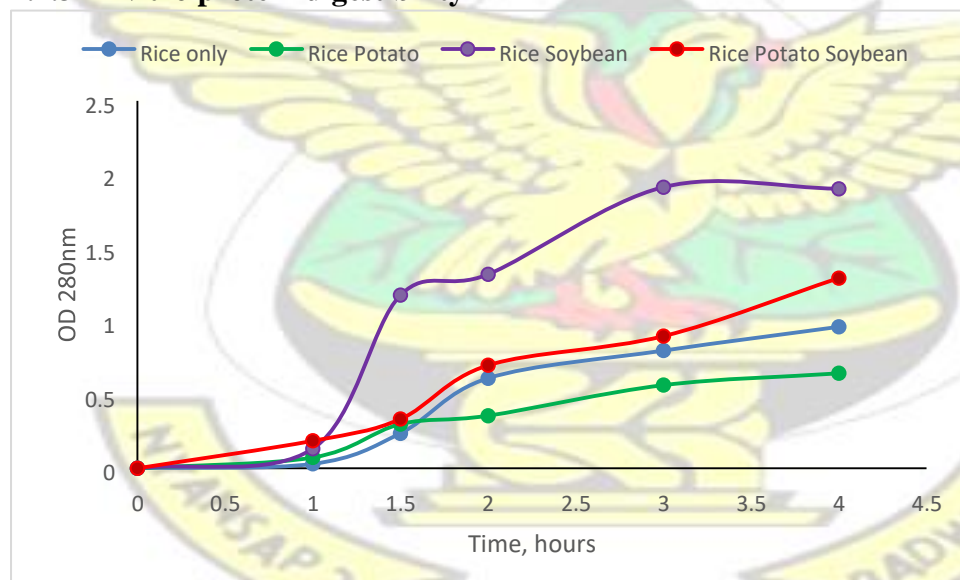


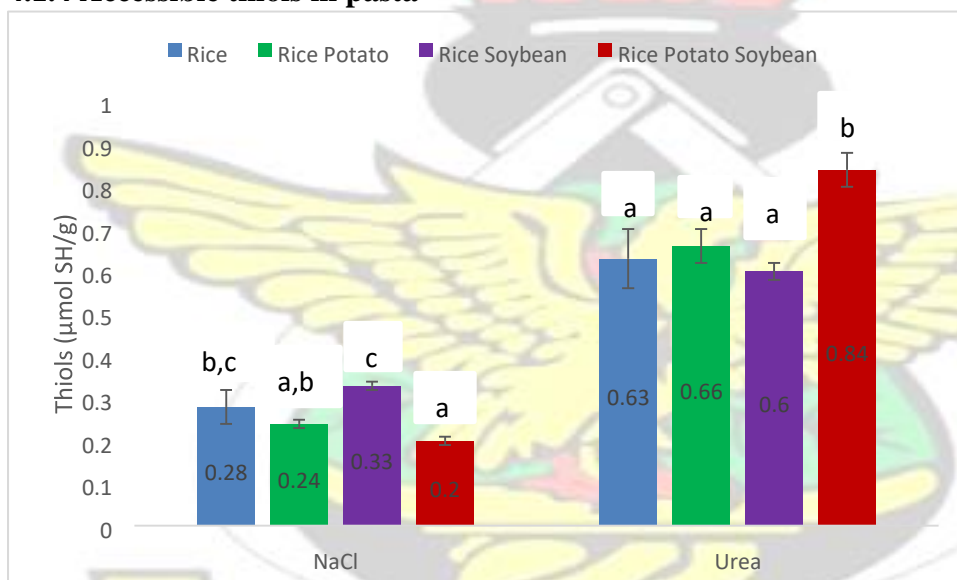
Fig. 5. In-vitro protein digestibility of cooked pasta samples

The in-vitro protein digestibility test is a technique that mimics how protein is digested by protein-digesting enzymes in humans. In humans, the role of the acidic gastric juices (pH 1.0 to 2.5) is to serve as a denaturing agent unfolding globular proteins and rendering their internal peptide bonds more accessible to enzymatic hydrolysis in the

stomach. Trypsin and chymotrypsin play the role of further hydrolysing the peptides that are produced in polypeptide chains after proteins are denatured by pepsin. Protein digestion at this point is efficiently done since pepsin, trypsin and chymotrypsin have different amino acid specificities. Globular proteins from animal sources such as albumin are almost completely hydrolysed to amino acids in the GIT whereas fibrous proteins are only partly digested (Lehninger, 1993). In this present work, it could be seen from the graph below (fig. 5) that, pasta with formulation of rice and soy flour had the highest amount of proteins digested by the trypsin and pancreatin enzymes followed by pasta with rice, sweet potato and soy flour, with pasta having rice only following and pasta with sweet potato having the least amount of proteins digested. It could then be inferred that, pasta developed from RS (rice and soy flour) had a higher composition of albumin and globulin as seen from the SDS-PAGE band profile (fig.3). Albumen and globulin have been found to be soluble in solution and therefore attacked easily by the enzyme trypsin (Mohammed *et al.*, 2009) resulting in the increased release of amino acids into solution after protein digestion by the trypsin and chymotrypsin enzymes. A higher protein digestibility value has been found to correlate positively with a higher nutritional value of the particular food protein digested by protein-digesting enzymes (Duodu *et al.*, 2003). It was also realised that, the formulation of rice and sweet potato flour had the lowest amount of proteins digested. This could be attributed to the presence of protease inhibitor, sporamin, present in sweet potato (Matsuoka *et al.*, 1990). Also, non-protein compounds such as polyphenols, non-starch polysaccharides, dietary fibre, tannins, starch, lipids and phytates which are exogenous as well as endogenous factors including changes that occurs within the proteins themselves have been found to interact with food proteins resulting in poor protein digestibility (Duodu *et al.*, 2003). It could be said that, pasta formulated from rice and sweet potato had a

higher amount of dietary fibre, phytates, polyphenols nonstarch polysaccharides which interacted with the available proteins resulting the poor digestibility of the proteins. Despite the fact that it is widely reported that, both sweet potato and soy have trypsin inhibitors, it could be said that, the drying of the pasta at 65 °C for 17 hours was able to reduce trypsin inhibitors in the soy than in the sweet potato flour. It could also be realised that, the longer the time allowed for protein digestion by protein-digesting enzymes, the higher the amount of proteins digested and the more amino acids released into solution. Soy flour contains about 40% proteins (Banaszkiewicz, 2011) and was found to enhance the protein content of pasta formulated with soy flour.

4.1.4 Accessible thiols in pasta



Mean values in a row with different letters for each buffer treatment are significantly different ($p < 0.05$)

Fig. 6. Effects of different treatments on pasta accessible thiols

Evaluation of the accessible thiols in the rice based pasta is very crucial since it tends to provide information on the thiol-disulphide exchange events that provide the “natural” network of covalent interactions. Measurement of the thiol accessibility to suitable reagents under varied conditions (such as presence or absence of dissociating agents or of agents that may contribute to stabilization of the protein structure or of

inter-protein contacts, such as divalent cations) tends to provide insight into the compactness of the protein network in the system under scrutiny (Bonomi *et al.*, 2013). Biologically derived thiols, commonly referred to as biothiols, constitute important antioxidants that have the ability to militate against oxidative stress (Sen and Packer, 2000; Wlodek, 2002). One of such biothiols is glutathione, a very potent endogenous antioxidant (Meister *et al.*, 1983) which is reported to be synthesized from γ -glutamyl cysteine (GGC) by glutathione synthetase. The need to evaluate the accessible thiols in pasta is as a due to the essential role of thiols in foods acting as precursors for the synthesis of essential endogenous biothiols such as glutathione in the body. It could be observed from this work that, there was a marked ureadependent increase in thiol observed for the pasta samples that were treated with urea. This is an indication of chaotropic destabilisation of a hydrophobically stabilised structure that causes a large number of thiols accessible to the specific reagent (Bonomi *et al.*, 2012). Protein aggregates in all pasta samples are stabilized by hydrophobic interactions and disulphide bonds with a higher network-forming ability is highlighted in soybean-enriched pasta. It was also observed that the amount of proteins in the pasta samples solubilized in the saline buffer was very low but increased when urea was added (fig.6). This is consistent with the findings of Bhattacharya (2004) who reported saline buffer solubilized water and salt soluble proteins such as albumins and globulins making accessible the thiols. The addition of a denaturing agent (urea) to the saline buffer enabled the dissociation of protein aggregates stabilized by hydrophobic interactions thereby increasing the quantity of solubilized protein and making available more accessible thiols.

4.2 PASTING AND COOKING INDICES OF PASTA SAMPLES

4.2.1 Pasting properties of raw pasta ingredients and extruded pasta

Table 5. Pasting properties of formulated raw materials for pasta preparation

Samples	Initial viscosity (BU)	Pasting temperature (°C)	Peak time (min)	Sample properties		
				Peak viscosity (BU)	Breakdown viscosity (BU)	Setback viscosity (BU)
R	33	88.5	19.57	372	258	191
RP	30	88.9	19.6	135	89	98
RPS	22	86.7	19	52	15	49
RS	30	89.5	19.9	100	47	98

R= Rice only, RP= Rice and Potato, RPS= Rice, Potato and Soy, RS= Rice and Soy

Table 6. Pasting properties of extruded and dried Pasta

Samples	Initial viscosity (BU)	Pasting temperature (°C)	Peak time (min)	Sample properties		
				Peak viscosity (BU)	Breakdown viscosity (BU)	Setback viscosity (BU)
R	18	88	19.37	172	102	150
RP	17	90.4	20.17	78	44	96
RPS	18	87.6	19.3	45	20	46
RS	21	89	19.73	93	46	102

R= Rice only, RP= Rice and Potato, RPS= Rice, Potato and Soy, RS= Rice and Soy

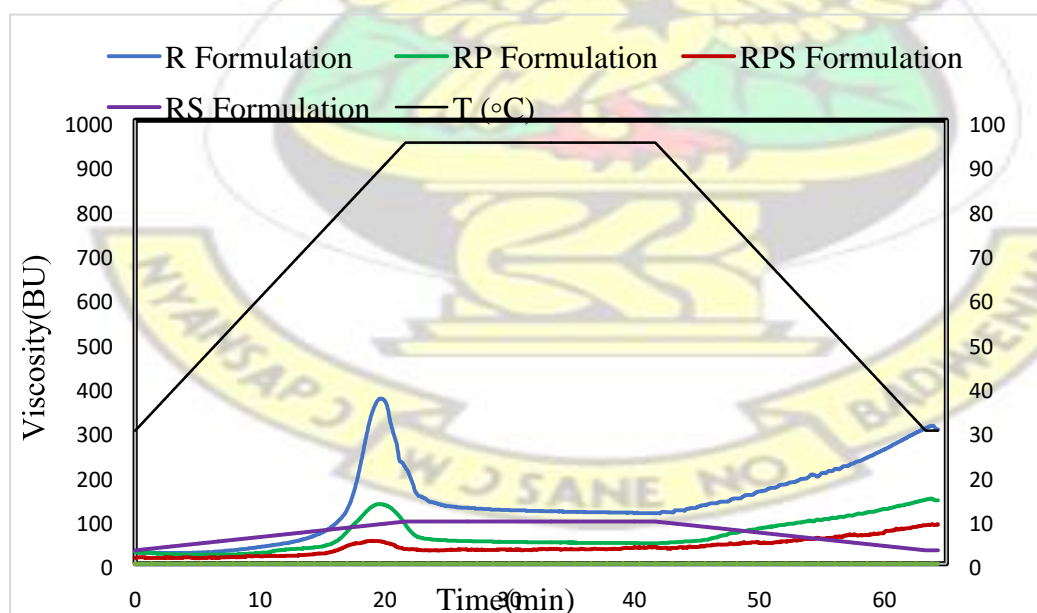


Fig. 7. Graph showing the pasting properties of formulated raw materials for

pasta preparation

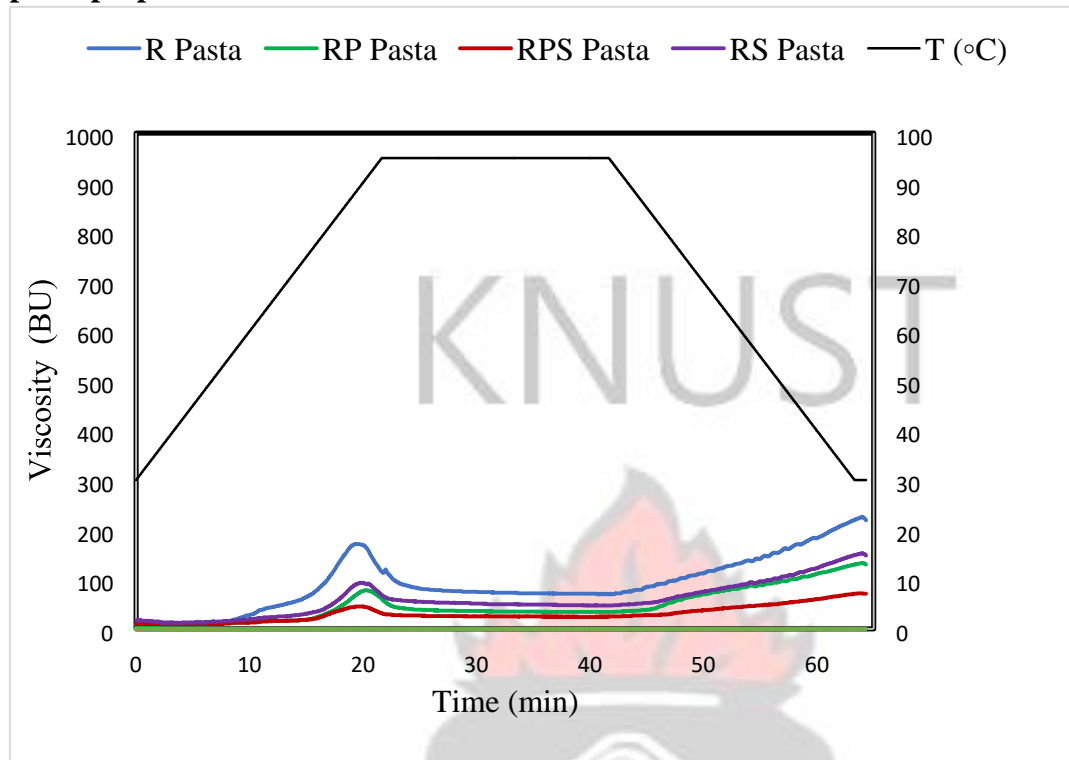


Fig. 8. Graph showing pasting properties of extruded and dried pasta

Table 5 and 6 as well as Figure 7 and 8 shows the result of the pasting properties of the pasta formulation and the extruded and oven-dried pasta. Initial viscosity refers to the viscosity of the flour suspension in Brabender Unit (BU) at the start of heating.

The initial viscosity of the flour in water at 30 °C is called the cold viscosity and in the preparation of instant foods, this property relates the capacity of the flour to absorb water at room temperature to form a paste, gel or viscous liquid (Souza *et al.*, 2011).

For the pasta samples, it indicates the capacity of the flour to absorb water at room temperature to form a paste, gel or viscous liquid. In this work, the formulations used for the pasta before extrusion and drying had an initial viscosity ranging from (22-33 BU) whereas that for the dried extruded pasta ranged from (17-21 BU). The higher values recorded for the formulations over the extruded dried pasta could be attributed to the fact that, the drying of the pasta resulted in the complete gelatinization of the

starches in the flour and so made the sample had a poor hydration ability to form a concentrated viscous paste.

Peak viscosity refers to the rapid increase in viscosity that occurs when a significant number of starch granules become swollen (Mir and Bosco, 2013). In this present work, the peak viscosity for the flour used for the pasta preparation before extrusion was higher than after the pasta had been extruded and oven-dried. This could be attributed to the fact that, during the extrusion and drying of the pasta, the shearing as well as the heat treatment destroyed the crystalline starch structure which resulted in a viscosity profile with no peaks and very low viscosity during the heating cycle. However, because the flour did not receive any severe heat treatment, a certain percentage of the starch granules preserved their structure, presenting relatively high values for the peak viscosity since most of the starch granules were in swollen condition (Souza *et al.*, 2011).

According to Falade *et al.* (2014), breakdown viscosity is associated with the tendency of the cooked starch to disintegrate. Breakdown viscosity is the viscosity of the paste after being heated at the holding temperature of 95 °C. Rice flour, R, had the highest breakdown viscosity in both the formulated and extruded form (258 and 102 BU) with RP (89 and 44 BU), RS (47 and 46 BU) and RPS (15 and 20 BU). The higher breakdown viscosities observed in rice and rice potato formulated and pasta samples could be attributed to the fact that, they had a higher total starch as well as low damaged starch as reported by ([www. air.unimi.it](http://www.air.unimi.it)).

Setback viscosity is the measure of the tendency of starch granules to retrograde (Abd Karim *et al.*, 2000). Once gelatinization is completed, the linear amylose chains that leach out begin to start re-associating with each other on cooling and this subsequently results in increased viscosity of flow pastes (Arif *et al.*, 2014). There was an increased

setback viscosity observed for the rice flour in both the formulated and extruded form (191 and 150 BU) with RP (98 and 96 BU), RPS (98 and 102 BU) and RS (49 and 46 BU) following in that order. The low setback viscosity recorded for the rice soy (49 and 46 BU) 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.

4.2.2 Cooking indices of Pasta

Table. 7: Cooking Indices of Pasta

	RPS	RP	RS	R
Cooking time (mins)	8	8	10	13
Cooking loss (g/100 g)	10.50 ^b	10.50 ^b	9.07 ^a	9.85 ^b
Water absorption (g/100 g)	70.50 ^b	68.30 ^a	72.10 ^c	67.20 ^a
Firmness (N)	233.61 ^a	224.23 ^a	341.34 ^b	535.87 ^c

R= Rice only, RP= Rice and Potato, RPS= Rice, Potato and Soy, RS= Rice and Soy

Mean values in a row with different alphabets are significantly different ($p < 0.05$)

Cooking indices of the rice-based pasta which involves cooking time, water absorption capacity, cooking loss and firmness were found to be affected significantly ($p < 0.05$) with the addition of the soy and orange-fleshed sweet potato flour to the pregelatinised rice flour used for this work. It was observed that, the cooking time for the pasta fortified with soy and sweet potato flour ranged between 8 and 10 mins. This compares well with the cooking times reported for noodles developed from broken rice and wheat flour which ranged from 6.20 to 11.23 mins, according to work done by Ahmed *et al.* (2015). Pasta developed from only the pre-gelatinised rice recorded a cooking time of 13 mins in this study. This is also consistent with cooking time reported by Rithurangdej *et al.* (2011) for wheat flour noodles which was within the range of 13.0-14.5 mins and that reported by Rosa *et al.* (2015) which was in the range of 11.33-17.66 mins for pastas prepared with buckwheat flour and different added proportions of amaranth and rice

flour. It could be seen from the cooking times recorded for this study that, the addition of the orange-fleshed sweet potato contributed to a decrease in the cooking time recorded for the pasta. This could be attributed to the fact that, the increase in fibre as a result of the milling of the skin (peels) and food material of the sweet potato reduced the strong starch network that was formed during the drying of the pasta promoting enhanced moisture penetration into the pasta. Marti *et al.* (2010) reported that, the higher fibre content in brown rice was responsible for a weakening of the starch network and consequently, for the increase in cooking loss when they prepared GF pasta from brown rice flour. They also reported that, the inclusion of fibre in the starch matrix partially reduced the extreme firmness and springiness found in pasta from milled rice flour.

Water Absorption Capacity (WAC) involves the ability of the matrix of a protein to absorb and retain bound, hydrodynamically, capillary physically entrapped water against gravity. It is a very important cooking index because it highlights the dynamics involved in protein water interaction in various food systems (Sangeetha and Devi, 2012). Marti *et al.* (2014) have reported WAC of 88.6, 88.9 and 90.1 g/100 g for rice-based pasta developed from parboiled rice only, parboiled rice with egg albumen and parboiled rice with whey protein. Rosa *et al.* (2015) worked the on technological properties and colour of pastas prepared with buckwheat flour and different added proportions of amaranth flour and rice flour and reported water absorption values ranging from (242-344%). These values are a bit higher than the WAC values observed in this study. In this present work, WAC of 70.5, 68.3, 72.1 and 67.2 g/100g were recorded for rice-based pasta developed from RPS, RP, RS and R only. The difference in WAC observed for the pasta developed in this study and that developed by Marti *et al.* (2014) could be due to the fact that, there was variation in the variety of the rice

used, differences in the gelatinization temperature as well as the variation in the various raw materials - soy, orange-fleshed sweet potato flour- used in this work as against the whey protein used they used. From Table 5, it could be seen that, RS had a WAC that was significantly higher ($p < 0.05$) than all the rest followed by RPS. There was no significant difference ($p > 0.05$) between the RP and R. This could be attributed to the fact that, the soy flour had a lot of soluble proteins with high solubility and hydration properties (Marti *et al.*, 2014). The presence of proteins with high soluble and hydration properties make them associate with water molecules easily make them have the ability to absorb water during cooking.

According to Brennan and Tundorica (2007), the quality and cooking properties of pasta tend to depend on the development of protein-starch matrix. Pasta firmness can be associated with the protein content of pasta and starch composition (MartinEsparta, 2013). Also, the use of egg albumen as a binding agent in GF pasta has been found to contribute to the formation of a more compact pasta protein network resulting in firmer product that has a tough texture (Alamprese *et al.*, 2011). Marti *et al.* (2014) reported pasta firmness of 275, 308 and 245 N for pasta produced from parboiled rice only, parboiled rice with egg albumen and parboiled rice with whey proteins. In the present study, firmness of 234, 224, 341 and 536 N has been reported for pasta made from RPS, RP, RS and R respectively. These values recorded for pasta firmness correlates well with the cooking times reported in this study. The higher firmness value recorded for R is an indication of a stronger texture which made it have the highest cooking time of 13 mins with RS and RP/RPS following. Pasta produced from RPS and RP compares well with the range of pasta firmness reported by Marti *et al.* (2014) which falls in the range of (245-308 N) with pasta from RS and

R having a higher firmness value of 341 and 536 N respectively. Resmini and Pagani (1983) explained that, anytime there is prevalence of protein coagulation during pasta cooking, trapping of the starch granules inside the protein network will occur with the consequent production of firm pasta. However, when starch swelling and gelatinization prevails, proteins assume a distinct or separate mass with no continuous structure forming and this result in pasta with a soft and sticky texture (Maningat *et al.*, 2009). Pasta produced from rice-potato and rice-potato-soy absorbed more water during cooking which has been attributed to the high fibre content contributed by the peels from the orange-fleshed sweet potato flour thereby reducing the firmness of the pasta from a textural point of view (Martin-Esparza, 2013). This result is also confirmed by Marti *et al.* (2010), who prepared gluten-free pasta from brown rice flour and reported that, the higher fibre content in brown rice was responsible for a weakening of the starch network and consequently, for the increase in cooking loss. They went further to report that, the inclusion of fibre in the starch matrix partially reduced the extreme firmness and springiness found in pasta from milled rice flour. It could be said that, the egg albumen was able to form a strong network with the proteins in the soy flour and pre-gelatinised rice flour whereas the addition of the OFSP flour reduced the strong protein-protein and protein-starch network created by the egg albumen which could be mainly attributed to the higher fibre content emerging from the peels of the OFSP.

4.3 COLOUR ATTRIBUTES OF PASTA

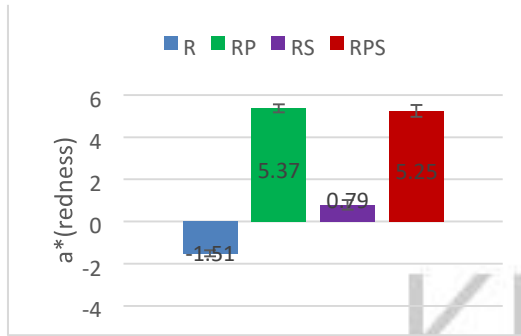


Fig. 9.1. Effect of different ingredients addition on pasta redness

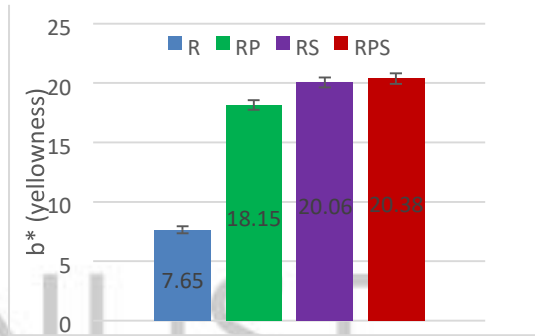


Fig. 9.2. Effect of different ingredients addition on the yellowness of pasta

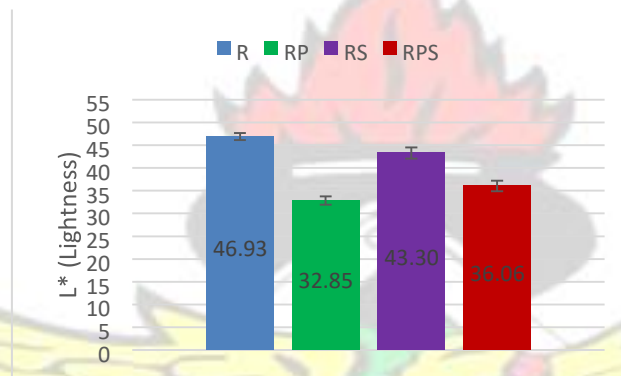


Fig. 9.3 Effect of different ingredients addition on the lightness of pasta

Determination of colour attributes is a very important in food product development since colour informs consumers about their choice of a particular food. In this present work, it could be seen from the figures that, at the L* coordinate which determines the whiteness-black scale, the pasta produced from only the pre-gelatinised rice flour recorded the highest measurement followed by pasta produced from RS, RPS and RP in that order. This could be attributed to the fact that, the fine pre-gelatinised rice flour which is whitish in colour had the highest absorbance in that range making the pasta formulated from pre-gelatinized rice only have the highest reflectance. Pasta formulated from RS had next higher absorbance since the soy flour in a way reduced pasta brightness in the whiteness region. The carotenoids in the pasta with sweet potato formulations were responsible for the reduction in pasta colour brightness at the

whiteness region. The reduction in the percentage of pre-gelatinized rice flour and the contribution of the orange colour from the carotenoids of the orange-fleshed made the pasta produced with the potato have a higher reflectance at the red-green scales.

According to D'Egidio and Pagani (1997), factors such as processing conditions and formulations in terms of the characteristics of raw materials, and/or presence of specific ingredients tends to always affect luminosity and chromatic indices. Other factors that have also been found to impart the final colour of food samples include preparation conditions like the recipe, water temperature and its added amount (Nasehi *et al.*, 2009; Petitot *et al.*, 2010), extruder type used for the pasta production, the type of dryer used and the operating parameters (Jukic' *et al.*, 2007) besides the raw material profile. Miskelly (1984) also reported that, factors such as the milled kernel type and flour yield as well as flour composition such as ash, protein, pigments and damaged starch contents affects final colour of a food sample. Phytochemical pigments like the carotenoids and xanthophylls have been found to impart the colour of food samples (Humphries *et al.*, 2004; Fratianni *et al.*, 2005) likewise protein molecular composition (Ohm *et al.*, 2008). Botanical origin of flour as well as mixing ratio also tends to influence cereal colour composites for pasta colour. Crops that are most frequently used which imparts on colour formation as well as enhancing nutritional value include legumes such as chickpea, soy, yellow or green pea, faba bean (Zhao *et al.*, 2005; Chillo *et al.*, 2008; Nasehi *et al.*, 2009, Wood 2009; Petitot *et al.*, 2010).

4.4 SENSORY EVALUATION OF PASTA SAMPLES

4.4.1 Electronic nose

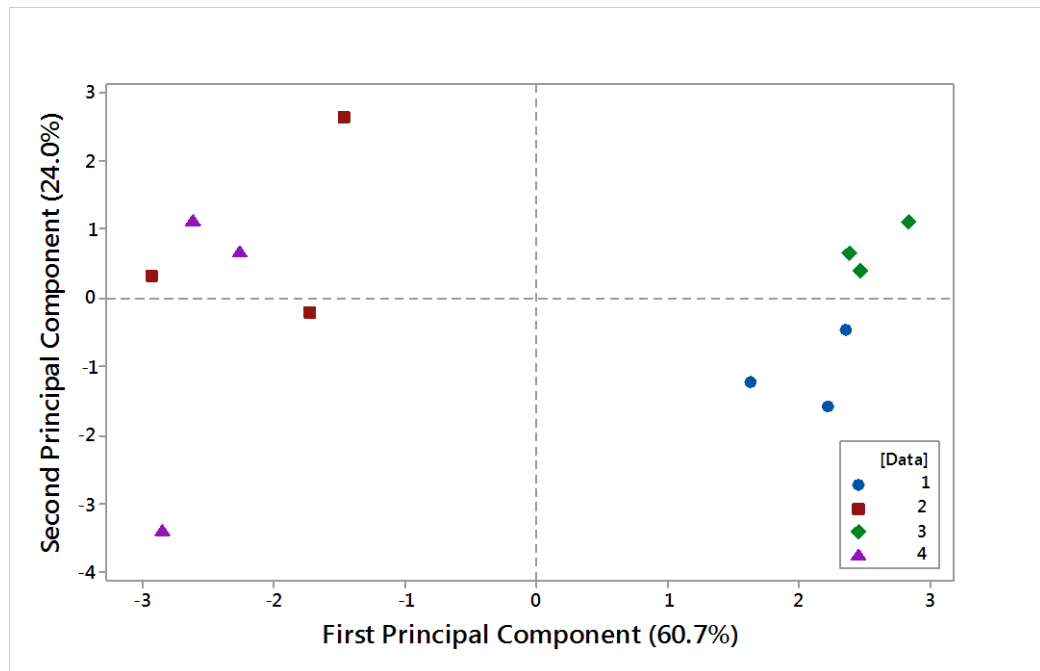


Fig 10A. PCA score plot of 1-4 samples

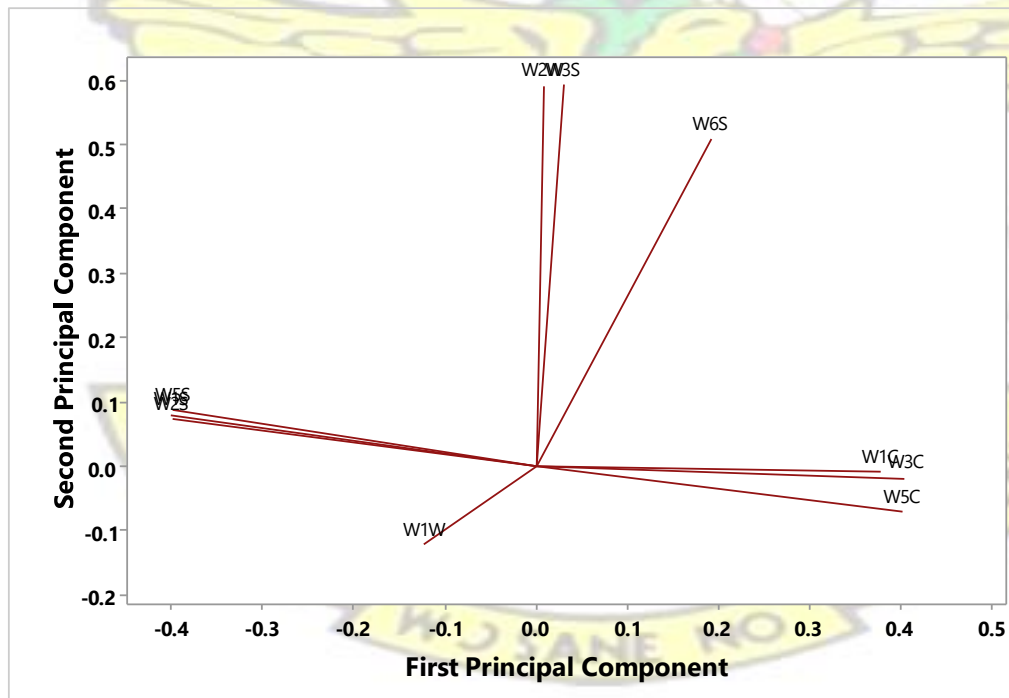


Fig 10B. PCA loading plot of 1-4 samples

The electronic nose was used to evaluate the aromatic profile of 1-4 samples. For each type of sample the electronic nose sensor responses were collected and elaborated by PCA performed in a correlation matrix to achieve a partial visualization of the data set in a reduced dimension. Figure 10A. reported the PCA score plot of the 1-4 samples distribution in the area defined by the first two PCs that explain the 84.7% of total variance. The score plot shows that samples are grouped along the first and second principal components into two clusters and there is a clear separation of samples 1 and 3 from 2 and 4 probably due to the presence of soya in the samples (2 and 4). E-nose responses are sample-specific and strongly depend on the presence/absence of soybean

Considering the loading plot (Fig 10B), it is possible to notice that WC sensors contribute to the aroma profile of 1 and 3 samples. On the left of the plot there were some WS sensors (W1S, W2S and W5S) that are relevant for the discrimination of 2 and 4 samples from 1 and 3 samples.

4.4.2 Electronic Tongue

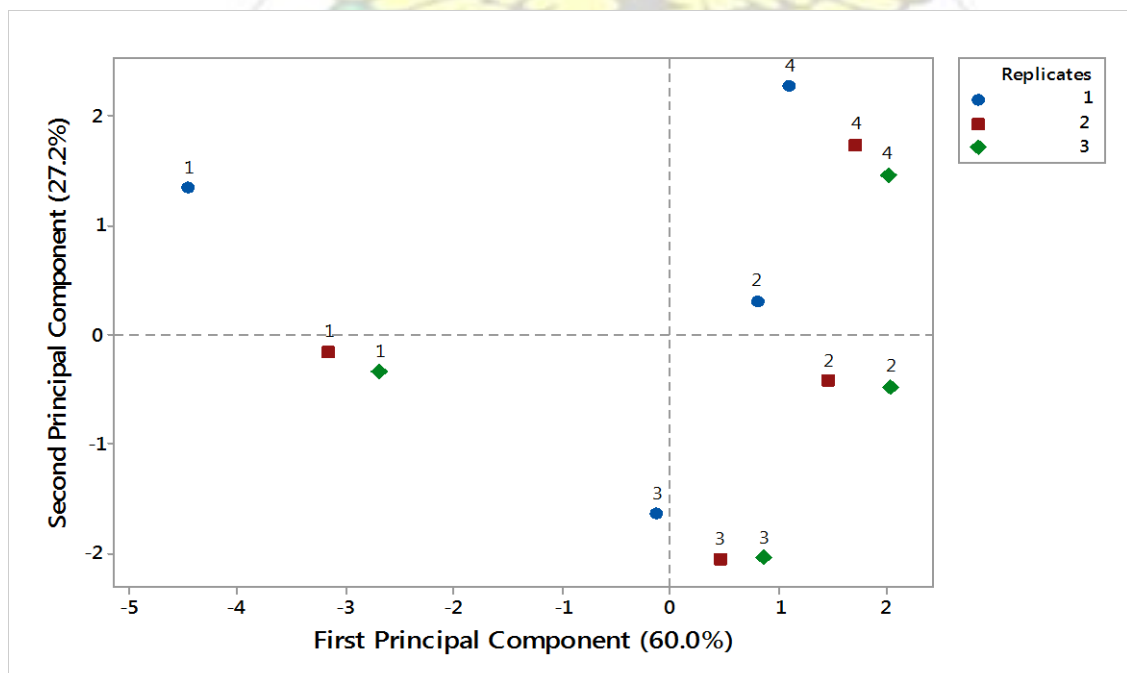


Fig.11 A: Score plot of samples1-4 in the plane defined by the first two principal component

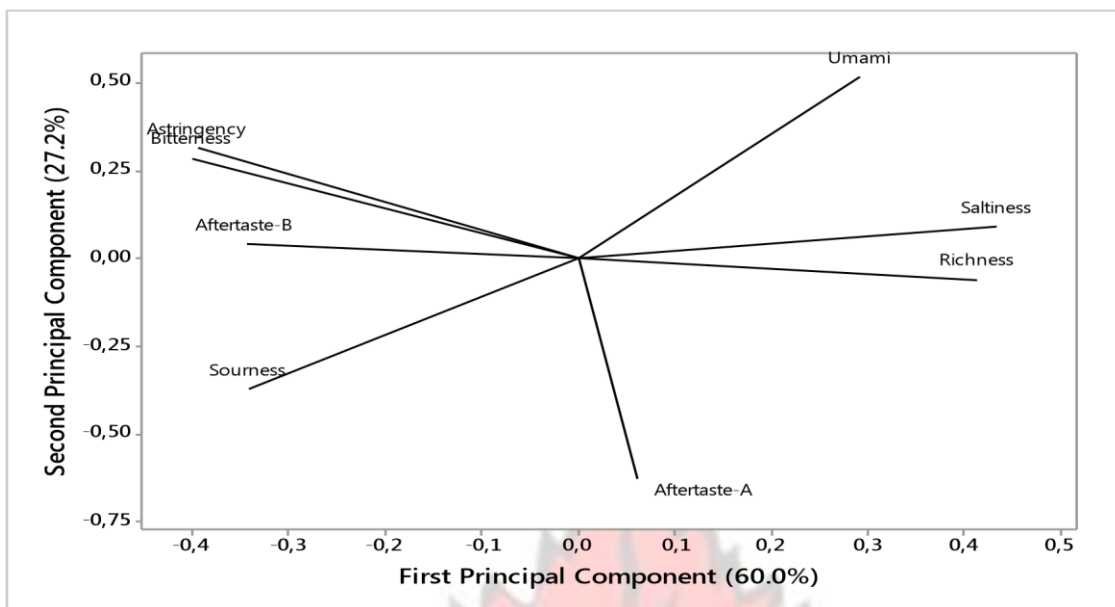


Fig. 11B: Loading Plot of electronic tongue variable in the plane defined by the first two principal components.

The taste values collected by ET were elaborated by Principal Component Analysis (PCA). PCA was used for explorative data analysis in order to achieve a partial visualization of the data set in a reduced dimension. PCA was performed in correlation (the variables were scaled). From the elaboration, two figures were collected: PCA-Score plot representing the relationship among the samples, and PCA-loading plot showing the relationship among the variables and how they influence the system.

Considering the score plot (fig. 11A) in the plane defined by the first two Principal Components (87.2% of the total variance) a clear separation of the samples is evident on the first (PC1) and second (PC2) Principal Component. In particular, sample 1, located in the negative part of the PC1, is discriminated by all the other samples and it is characterized by sourness, astringency and bitterness (fig. 11B); in the positive part of the PC1 sample 3 is characterised by the astringency aftertaste while samples 2 and 4 are perceived saltiest and are more characterized by the Umami taste (fig.11B). Umami is one of the basic tastes together with sour, sweet, salty and bitter imparted by a number

of substances mostly the amino acid *glutamate* and 5'-ribonucleotides such as *inosinate* and *guanylate* (Ninomiya, 2015). Umami-containing foods include vegetables (example, tomato, potato, cabbage, mushroom, carrot and soybean), seafood (example, fish, oyster, crab and prawn), meat (example, beef, pork and chicken) and cheese, which greatly imparts on the characteristic tastes of these foods (Kurihara and Kashiwayanagi, 2000).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Rice-based pasta fortified with soy and orange-fleshed sweetpotato was developed. Significant differences ($p < 0.05$) existed in the amount of proteins solubilised using the three different buffer treatments (saline, urea and urea/DTT buffer) for the Bradford test and thiol reactivity an indication of the presence of protein aggregates stabilized by hydrophobic interactions and disulphide bonds. Pasta with rice and soy had the highest protein digestibility whereas pasta with rice and sweet potato recorded the lowest. Soybean enrichment clearly affects pasta yellowness. Sweetpotato enrichment results in an increase of all indices, most likely as a result of browning Maillard-type reactions. Also, significant differences ($p < 0.05$) existed in the cooking loss (9.07-10.50)g/100, water absorption (67.20-72.10)g/100g and firmness (233.61535.87) N of the pasta samples produced. E-sensing approaches indicated that the sensory profile of the various pasta products strongly depends on the peculiar enrichment. Sweet potato increased the pasta astringency, whereas soybean enrichment resulted in a typical umami taste and a specific electronic nose response. This information can offer some

guidelines as for designing and producing snacks fit to the consumers' expectations that can also add value to local African raw materials.

5.2 RECOMMENDATION

Antioxidant micronutrients analysis can be carried out on the rice-based pasta to see the effect of fortification of the rice-based pasta with orange-fleshed sweet potato and soy flour. This is important because antioxidant micronutrients have been found to be very essential during the recovery phase of children who present with burns.

Since the E-nose and tongue is objective, human subject can also be used to evaluate the sensory attributes to confirm the results of the E-nose and tongue.



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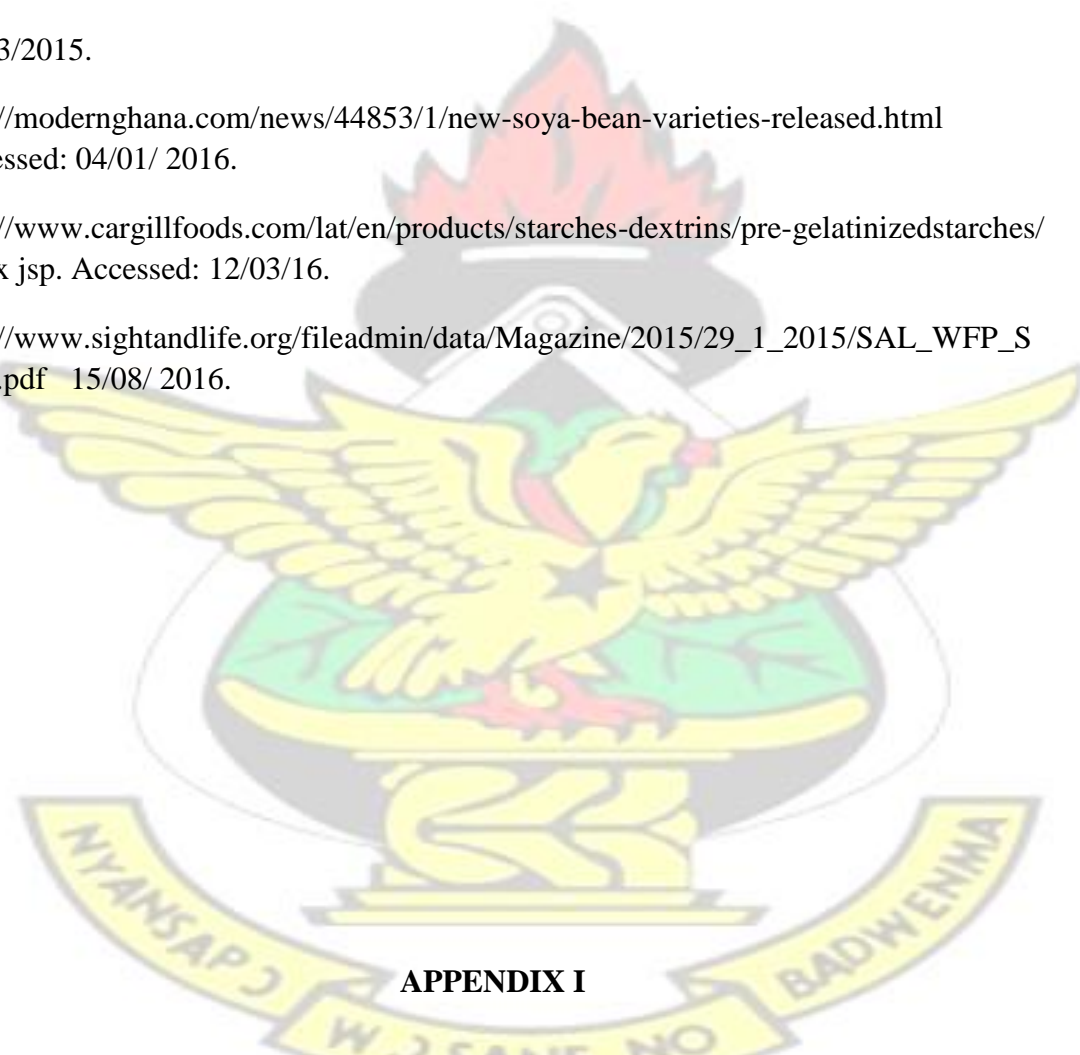
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APPENDIX I

RESULTS OF BRADFORD TEST ON PASTA SAMPLES

	1	2
	Dilution factor 1: 5	
RSA₁	0.306	0.319
RSA₂	0.326	0.317
RPSA₁	0.367	0.368
RPSA₂	0.377	0.385

Dilution factor 1:2		
RA₁	0.132	0.138
RA₂	0.155	0.154
RPA₁	0.240	0.241
RPA₂	0.239	0.230
Dilution factor 1:20		
RSB₁	0.384	0.391
RSB₂	0.414	0.410
RPSB₁	0.418	0.406
RPSB₂	0.418	0.428
Dilution factor 1:4		
RB₁	0.322	0.323
RB₂	0.318	0.319
RPB₁	0.343	0.340
RPB₂	0.342	0.338
Dilution factor 1:40		
RSC₁	0.353	0.335
RSC₂	0.337	0.370
RPSC₁	0.378	0.365
RPSC₂	0.368	0.358
Dilution factor 1:8		
RC₁	0.264	0.250
RC₂	0.267	0.268
RPC₁	0.285	0.291
RPC₂	0.302	0.303

APPENDIX II

Result of the in-vitro protein digestibility test

Sample	Abs.	Sample	Abs.	Sample	Abs.	Sample	Abs.
At time t ₀							
RSA	0.216	RPA	0.139	RPSA	0.343	RA	0.064
RSB	0.228	RPB	0.143	RPSB	0.372	RB	0.060
RSC	0.229	RPC	0.148	RPSC	0.301	RC	0.062
RS	0.211	RP	0.136	RPS	0.320	R Blank	0.059
Blank		Blank		Blank			

At time t ₁							
RSA	0.328	RPA	0.166	RPSA	0.382	RA	0.082
RSB	0.340	RPB	0.171	RPSB	0.373	RB	0.079
RSC	0.335	RPC	0.155	RPSC	0.378	RC	0.086
RS	0.209	RP	0.132	RPS	0.342	R Blank	0.057
Blank		Blank		Blank			
At time t ₂							
RSA	0.369	RPA	0.173	RPSA	0.404	RA	0.128
RSB	0.370	RPB	0.174	RPSB	0.406	RB	0.123
RSC	0.371	RPC	0.177	RPSC	0.431	RC	0.141
RS	0.220	RP	0.134	RPS	0.334	R Blank	0.061
Blank		Blank		Blank			
At time t ₃							
RSA	0.441	RPA	0.203	RPSA	0.465	RA	0.148
RSB	0.458	RPB	0.210	RPSB	0.426	RB	0.149
RSC	0.422	RPC	0.205	RPSC	0.446	RC	0.155
RS	0.207	RP	0.137	RP Blank	0.336	R Blank	0.053
Blank		Blank					
At time t ₄							
RSA	0.460	RPA	0.211	RPSA	0.484	RA	0.182
RSB	0.467	RPB	0.214	RPSB	0.461	RB	0.170
RSC	0.490	RPC	0.232	RPSC	0.502	RC	0.205
RS	0.222	RP	0.134	RPS	0.312	R Blank	0.059
Blank		Blank		Blank			

APPENDIX III

Results of moisture content- Pasta formulations Sample Crucible W wet W dry
 Dry Moisture Average Average ds

	Weight		matter			Dry matter	Moisture	
RPS	51.2286	53.2286	53.0398	90.5600	9.4400	90.62	9.38	0.09
RPS	44.7872	46.7871	46.6009	90.6895	9.3105			
R	51.9306	53.9304	53.7189	89.4239	10.5761	89.55	10.45	0.17
R	43.9736	45.9739	45.7672	89.6666	10.3334			
RP	45.3483	47.3483	47.1351	89.3400	10.6600	89.30	10.70	0.05
RP	38.4881	40.4881	40.2735	89.2700	10.7300			
RS	43.9739	45.9738	45.7928	90.9495	9.0505	90.98	9.02	0.04
RS	43.0464	45.0466	44.8666	91.0009	8.9991			

Results of moisture content- Extruded pasta

Sample	Crucible Weight	W wet	W dry	Dry matter	Moisture	Average Dry matter	Average moisture	ds
RPS	44.5900	46.5900	46.4113	91.0650	8.9350	91.01	8.99	0.08
RPS	41.4607	43.4610	43.2801	90.9564	9.0436			
R	45.6771	47.6773	47.4927	90.7709	9.2291	90.84	9.16	0.10
R	42.6589	44.6590	44.4772	90.9105	9.0895			
RP	47.1943	49.1944	49.0196	91.2604	8.7396	91.18	8.82	0.11
RP	43.9754	45.9754	45.7975	91.1050	8.8950			
RS	46.4748	48.4748	48.3007	91.2950	8.7050	91.47	8.54	0.24
RS	40.4345	42.4345	42.2672	91.6350	8.3650			

APPENDIX IV

Results of Accessible Thiols Absorbance

	umol/g	Sample	sd sample	Buffer	
0.25	0.30	R	NaCl	0.28	0.04
0.58	0.68	R	urea	0.63	0.07
0.34	0.32	RS	NaCl	0.33	0.01
0.63	0.57	RS	urea	0.60	0.04
0.25	0.24	RP	NaCl	0.24	0.01
0.67	0.64	RP	urea	0.66	0.02
0.21	0.20	RSP	NaCl	0.20	0.01
0.81	0.87	RSP	urea	0.84	0.04

APPENDIX V

Colour analysis

Sample RS		
L	a	b
44.35	0.6	19.88
42.18	0.73	20.42
44.35	1.13	19.54
42.31	0.7	20.38

Sample RP		
L	a	b
33.66	5.35	18.39
34.07	5.3	18.57
32.99	5.57	18.49
32.29	5.44	17.97
32.26	5.51	17.97
31.8	5.03	17.51

APPENDIX VI

Water Absorption Capacity

Sample RPS		
L	a	b
35.4	5.26	20.8
35.18	5.27	20.72
34.96	5.59	19.76
34.98	5.61	19.72
37.24	5.1	20.51
37.32	4.94	20.56
37.37	4.95	20.59

Sample R		
L	a	b
47.06	-1.48	7.37
47.50	-1.55	7.92
46.92	-1.45	7.78
47.63	-1.48	7.42
45.45	-1.75	8.04
47.01	-1.33	7.37

Sample	Raw (g)	Cooked (g)	Water Absorption (%)
RPS	25	42.8	71.2
RPS	25.02	42.72	70.7
RPS	24.96	42.29	69.4
RP	24.94	42	68.4

RP	25.07	42.3	68.7
RP	25.05	42.01	67.7
RS	25.02	43.11	72.3
RS	24.9	42.83	72.0
RS	24.98	42.93	71.9
R	25.01	41.84	67.3
R	25.07	41.79	66.7
R	24.97	41.83	67.5

KNUST



APPENDIX VII

Texture

Sample	Firmness (N)				
	R	565.33	558.269	524.351	547.384
RP	219.068	220.648	230.361	226.99	224.073
RPS	242.241	242.85	243.54	211.592	227.83
RS	315.632	316.656	324.894	372.266	377.26

APPENDIX VIII

Cooking loss

Sample	Crucible tare(g)	Crucible+dry solids (g)	Dry solids (g)	Pasta before cooking (g)	Water before cooking (ml)	Volume poured (mL)	Cooking loss/100g
RPS	41.9408	42.3669	0.4261	25	250	40	10.653
	54.3559	54.7773	0.4214	25	250	40	10.535
	42.0851	42.5051	0.4200	25	250	40	10.500
	51.9309	52.3428	0.4119	25	250	40	10.297
RP	41.1027	41.5028	0.4001	25	250	40	10.003
	45.6748	46.0951	0.4203	25	250	40	10.508
	42.8608	43.3088	0.4480	25	250	40	11.200
	46.4731	46.8859	0.4128	25	250	40	10.320
RS	51.9298	52.2935	0.3637	25	250	40	9.093
	47.2251	47.5795	0.3544	25	250	40	8.86
	43.4487	43.8068	0.3581	25	250	40	8.953
	47.3277	47.7031	0.3754	25	250	40	9.385
R	38.4874	38.8864	0.399	25	250	40	9.975
	44.9793	45.387	0.4077	25	250	40	10.193
	43.9715	44.3703	0.3988	25	250	40	9.97
	41.4587	41.8296	0.3709	25	250	40	9.272

APPENDIX IX

Statistics

Homogeneous Subsets

WAC

Tukey HSD

factor	N	Subset for alpha = 0.05		
		1	2	3
R	3	67.1667	70.4333	72.0667
RP	3	68.2667		
RPS	3			
RS	3			
Sig.		.171	1.000	1.000

Cooking loss Tukey HSD

factor1	N	Subset for alpha = 0.05	
		1	2
RS	4	9.0728	10.5078
R	4		
RPS	4		
RP	4		
Sig.		1.000	.087

Firmness

Tukey HSD

VAR00011	N	Subset for alpha = 0.05		
		1	2	3
2.00	5	224.2280		
1.00	5	233.6126		
3.00	5		341.3418	
4.00	5			535.8694
Sig.		.922	1.000	1.000

Bradford Analysis for Protein Quantification Homogeneous Subsets NaCl

Tukey HSD

factor	N	Subset for alpha = 0.05			
		1	2	3	4
r	4	1.8750			
rp	4		3.2509		
rs	4				
rps	4			11.0522	
Sig.					13.1586
		1.000	1.000	1.000	1.000

Urea

Tukey HSD

factor	N	Subset for alpha = 0.05	
		1	2
r	4	8.9500	
rp	4	9.5500	
rs	4		56.4000
rps	4		59.0000
Sig.		.904	.053

Urea_DTT

Tukey HSD

factor	N	Subset for alpha = 0.05	
		1	2
r	4	36.1500	
rp	4	41.0000	
rs	4		97.7500
rps	4		97.7500
Sig.		.257	1.000

Accessible Thiol Analysis Homogeneous Subsets NaCl

Tukey HSD

Factor	N	Subset for alpha = 0.05		
		1	2	3
rps	2	.2000		
rp	2	.2400	.2400	
r	2		.2800	.2800
rs	2			.3300
Sig.		.146	.146	.078

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Urea

Tukey HSD

Factor	N	Subset for alpha = 0.05	
		1	2
rs	2	.6000	
r	2	.6300	
rp	2	.6600	
rps	2		.8400
Sig.		.103	1.000

APPENDIX X



Preparing cooked pasta for water absorption determination



Drying off stock from cooked pasta to determine cooking loss