EFFECT OF EXTRUSION PROCESSING ON THE NUTRITIONAL VALUE AND TANNIN CONTENT OF SORGHUM – SOYBEAN COMPOSITE SUPPLEMENTARY PRODUCT

BY

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ABSTRACT

The study was designed to formulate sorghum-soybean composite supplementary foods and investigate the effect of extrusion on nutritional value and tannin content. The products were extruded and analysed for nutritional value, tannin content, urease activity, sensory and anti-oxidant activity. Results showed that, the extruded composite products had good nutritional value and conformed to the requirements for supplementary foods for older infants and young children. Nutritional composition of the composite supplementary foods were: protein 17.36 – 21.60 g/100 g DM, carbohydrates 67.35 – 77.46 g/100 g DM, fat 1.85 – 8.27 g/100 g DM, energy values 400.91 – 424.06 Kcal/100g and had appreciable quantities of Ca (77.57 – 272.37 mg/100 g DM), Zn (1.61 – 3.15 mg/100 g DM), Fe (2.72 – 11.21 mg/100 g DM), Mg (53.46 – 132.78 mg/100 g DM), Cu (0.51 – 0.83 mg/100 g DM) and K (74.14 – 255.61 mg/100 g DM). Extruded composite products had good physical and sensory attributes. A test of antioxidant activity using DPPH (1,1- Diphenyl-2-picryhydrazyl) demonstrated considerable radical scavenging activity for all the products (71.79 – 97.91 µg/ml). The highest radical scavenging effect was observed in plain red sorghum (RS-C) with EC_{50} value of 68.32 µg/ml. Extrusion resulted in loss of antioxidant activity by 1 – 20%. Extruded products had significantly lower concentrations of tannin and urease activity compared to the non-extruded products. The extruded products had higher WAI, WSI and were more acceptable than products that were not extruded. Based on the results, it may be concluded that, various blends of sorghum with sardines and soybeans can be used to produce high quality supplementary foods. Extrusion adds value to the products in that it transforms the products into instant, convenient products with better organoleptic properties. Therefore, extrusion technology should be adopted by small and medium scale food processors.

DECLARATION

I, DAVID MUGANYIZI NDIBALEMA, do hereby declare to the Senate of Sokoine University of Agriculture, that this dissertation is my original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

_____ _____

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The above declaration is confirmed

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Prof. MOSHA, T. C. E Date

(Supervisor)

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DEDICATION

This work is dedicated to my beloved parents Mr. and Mrs. John Ndibalema; for their love, encouragement and support.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	-	Analysis of Variance
AOAC	-	Association of Official Analytical Chemists
BSTID-NRC	-	Board on Science and Technology for International Development-National Research Council
CE	-	Catechin equivalent
DM	-	Dry matter
FAO	-	Food and Agriculture Organization of the United Nations
HTST	-	High Temperature Short Time
ICRISAT	-	International Crops Research Institute for the Semi-Arid Tropics
ORAC	-	Oxygen radical absorbance capacity
PEU	-	Protein-energy undernutrition
SD	-	Standard deviation
TBS	-	Tanzania Bureau of Standards
UNICEF	-	United Nations Children Fund
URT	-	United Republic of Tanzania
USAID	-	United States Agency for International Development
UNU	-	United Nations University
WAI	-	Water absorption index
WHO	-	World Health Organization
WSI	-	Water solubility index

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Childhood malnutrition remains a common problem in much of the developing world today. Many of the traditional supplementary foods used in developing countries are of low nutrient density. Traditional supplementary foods in Tanzania are based on starchy staples, usually cereals such as maize, rice, sorghum and finger millet and non-cereals such as cassava, sweet potatoes, yams, bananas and plantains (Mosha *et al*., 2000). Such foods are usually supplied without adequate supplementation with high quality protein sources (Kikafunda *et al*., 2006). Over dependence on such poor protein sources is the main cause for the widespread protein-energy malnutrition in these areas. Major food-related causes of malnutrition include inadequate feeding, foods with low energy and nutrient density, low bioavailability of nutrients, poor access to food, use of poor processing methods and microbial contamination (URT and UNICEF, 1990; Ijarotimi and Ashipa, 2005).

Traditional methods of preparing supplementary foods at household level involve cooking flours made from cereals or root/tubers in boiling water to porridge (*Uji*) or by mashing boiled roots or tubers such as cassava, yams or potatoes and diluting with some water to form paps. During cooking, the starch in the staple foods binds water, requiring considerable amounts of water to bring the consistency of porridges prepared from them to levels suitable for child feeding. This lowers the energy and nutrient density of the porridge considerably and makes it difficult for children fed on these gruels to obtain adequate amount of nutrients to support their optimal growth (Afoakwa *et al*., 2004).

The low cost, energy and nutrient rich infant foods is a constant challenge in developing countries. Effective use of readily available and inexpensive sources of protein e.g.

soybean protein has become a major focus of research in recent years. Low cost extrusion cooking to produce instant 'ready to feed' composite foods has been studied in a number of countries with encouraging success (Camire *et al.*, 1990; Bangoura and Zhou, 2007) and could offer the ever-elusive solution to the malnutrition problem. Extrusion cooking has become one of the most popular technologies in food processing. It is a low cost, high temperature short time (HTST) process, used worldwide for processing of a number of food products (Frame, 1994; Smith and Singh, 1996). Cereals have excellent expansion properties because of their high starch content and are well suited to thermal extrusion (Singh *et al*., 1998). Before a definitive conclusion can be reached on the use of extruded sorghum-soybean blends as supplementary foods, their nutritional composition and physical properties need to be evaluated. The study has been conducted to evaluate the nutritional composition, sensory and physical properties of extruded sorghum-soybean blends with a view to determine their suitability for use in supplementary feeding.

1.2 Problem Statement

Undernutrition characterized by underweight, stunting, wasting and infectious diseases are the most widespread problems affecting infants and young children in developing countries (Mamiro *et al*., 2005). Supplementary feeding can be a period of problems and vulnerability for the survival of a child. Nutritional status in children is most vulnerable during this period when both macro and micronutrients may be insufficient to maintain healthy growth and development. As infants transfer from nutritious and uncontaminated breast milk to the regular family diet, they become vulnerable to malnutrition and disease. A period of supplementary feeding is a time when a baby needs highly nutritious foods to supplement the mother's milk without affecting the growth and development of the baby. High cost and unavailability in rural areas of protein rich commercial foods have resulted in increased protein energy undernutrition among infants and young children in the

developing countries (Bell and Reich, 1988). Supplementary foods based on local starchy staples have been pointed out to contribute immensely to the prevalence of protein energy undernutrition among young children (Seenappa, 1987). Furthermore, the bulkiness of traditional supplementary foods and high concentrations of fiber and inhibitors are major factors reducing their nutritional benefits (Abebe *et al*., 2006). Sorghum, which is commonly used to prepare supplementary foods, is known to contain tannins that impair the digestion of proteins and absorption of minerals such as iron and zinc (Duodu *et al*., 2002).

Due to mothers' competing roles such as working in farms, collecting firewood, fetching water, washing clothes, cleaning house and caring for the other members of the family, they do not have time to prepare meals and feed the child in a frequency (4 – 6 times/day) that would provide adequate nutrients for optimal growth (Mosha, 2004). Most of the traditionally prepared supplementary foods require long time for preparations and are low in energy, macro- and micro-nutrients.

1.3 Justification

There is a need to apply other processing technologies other than traditional methods to prepare supplementary foods that are nutrient dense, relatively safe, ready-to-feed "instant" thereby reducing maternal workload in preparing meals several times in a day. Providing adequate nutrition during early childhood is of paramount importance for maintaining health. Inadequate intake of nutritionally balanced foods results in inhibition of growth. Therefore, development of nutritious supplementary foods has been suggested by FAO (1985) to combat malnutrition among children. Development of supplementary foods is guided by nutritional value, acceptability, availability and affordability of raw materials, and simplicity of food processing technologies and equipment (Dewey and Brown, 2003).

The nutritional quality of cereals and legumes can be improved and also exploited as human foods by processing techniques such as extrusion.

Sorghum is a potential low cost resource for combating energy undernutrition. On the other hand, soybean is a potential source of protein and it has the potential to supply the much needed protein in diet. Blending of soybean with sorghum can complement each other so that protein in the resulting product nearly resembles that of a complete or balanced protein.

Few reports have shown extrusion cooking of cereal-legumes blends as a tool to produce health foods. Likewise, little has been done to investigate the effect of extrusion cooking on the nutritive value and tannin content of sorghum-soybean composite foods. The present study thus focuses on the development of composite supplementary foods employing extrusion cooking using whole, dehulled and germinated sorghum flours blended with soybeans. In this study, the potential of extrusion processing to reduce tannin content and its effect on nutritional composition of sorghum-soybean composite supplementary foods was investigated.

1.4 Objectives

1.4.1 General objective

To investigate the effect of extrusion processing on the nutritive value and tannin content of sorghum-soybean composite supplementary foods.

1.4.2 Specific objectives

a) To formulate nutrient-dense supplementary food products using local sorghum and soybean.

b) To determine the proximate composition and mineral density of the extruded composite supplementary products and how they compare with TBS/Codex Alimentarius macronutrient specifications for cereal-based follow-on supplementary foods
c) To determine the residual levels of tannins under various extrusion profiles
d) To assess the sensory and acceptability of the extruded sorghum-soybean supplementary foods.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Supplementary Feeding

Adequate nutrition is essential for adequate growth and cognitive development of infants and young children and to resist and fight against infections (Silvia *et al*., 2007). Exclusive breast feeding for the first 6 months is the World Health Organization's recommended method of feeding full-term infants by healthy, well nourished mothers (WHO, 2003). Breast milk contains all the nutrients and immunological factors infants require to maintain optimal health and growth. Breast milk is a sole and sufficient source of nutrition during the first six months of infant life. However, towards the middle of the first year, breast milk becomes insufficient to support optimal growth of infants. Therefore, nutritious supplementary foods need to be introduced (Silvia *et al*., 2007).

King and Burgess (1993) defined supplementary feeding as the process of introducing foods other than breast milk to a child and gradually increasing the amount, so that eventually the child gets enough energy and nutrients from ordinary family food. The introduction of supplementary foods is often accompanied by stress and ill health for infants in developing countries, mostly because the foods are not properly tailored to the infant needs (Kikafunda *et al*., 2006). Many traditional supplementary foods in Africa are only a slight modification of adult foods, involving only mashing and dilution without taking into consideration the special nutritional requirements of young children (Uwaegbute, 1991). Adult diets, especially in developing countries, consist of highly starchy staples which are bulky and unless properly modified, they are unsuitable for infants and young children with their small gastric capacities (Mosha and Svanberg, 1983).

2.2 Supplementary Foods

The first foods for the baby are referred to as supplementary foods (King and Burgess, 1993). These foods should be nutritionally well balanced with appropriate mixture rich in protein, energy and micronutrients. They should have a soft texture with very low fibre content and specially prepared for the baby to swallow. Supplementary foods should also be clean, free from pathogenic microorganisms and parasites and easy to prepare (Kshirsagar *et al*., 1994). Their purpose is to supplement breast milk to ensure the young child has enough energy, protein and other nutrients to allow normal growth of the child. Impaired growth, retarded physical and mental development, a high frequency of infections and a variety of nutritional deficiencies may result from lack of these foods during early stages of a child's development.

Most of the imported industrial-processed supplementary foods are too expensive and difficult to obtain for the majority of families (Reddy *et al*., 1990). Children most at risk of under-nutrition are from poor families most in rural areas who cannot afford expensive industrial processed foods. The solution of infant nutritional problem may be to put more emphasis on the use of low cost supplementary foods that should at least combine some of the desired characteristics of high nutrient density, low bulk property basing on locally available resources, which can be easy to prepare at home (Reddy *et al*., 1990; Kshrisagar *et al*., 1994).

2.3 Protein Energy Undernutrition

Protein-energy undernutrition (PEU) is regarded as one of the public health problems in developing countries as a result of poor feeding practices due to poverty. Protein energy undernutrition is a nutrition related disorder associated with inadequate intake and/or poor utilization of protein, energy and micronutrients (UNICEF, 1996). PEU results when the

body's needs for energy, protein or both cannot be satisfied by the diet. It has a wide spectrum of manifestations, ranging in severity from weight loss to growth retardation, to distinct clinical syndromes frequently associated with deficiencies of vitamins and minerals (e.g. vitamin A, iron and zinc). The most severe clinical manifestations of PEU are kwashiorkor and marasmus. Protein energy undernutrition affects mostly children between the ages of 6 – 60 months and accounts for the high rates of childhood morbidity and mortality (Kavishe, 1992). Causes of marasmus and kwashiorkor are many, ranging from the microeconomics of the household to inadequate national and international policies. The immediate causes related to household economics include age at which supplementary feeding is introduced into the child's diet, method of food preparation, choices of foods, frequency of feeding and energy density of supplementary foods. Mothers may have difficulty in feeding children frequently if they are working in the fields, and this may be an important constraint on children's food intake (URT and UNICEF, 1990).

2.4 Sorghum

Sorghum is a staple food grain in many semi-arid and tropic areas of the world, notably in Sub-Saharan Africa because of its good adaptation to hard environments and its good yield of production. Sorghum outperforms other cereals under various environmental stresses and is thus generally more economical to produce. Among important biochemical components for sorghum processing are levels of starch (amylose and amylopectin) and starch depolymerizing enzymes (Dicko *et al*., 2006). More than 35% of sorghum is grown directly for human consumption. The rest is used primarily for animal feed, alcohol production and industrial products (FAO, 1995; Awika and Rooney, 2004). Sorghum while playing a crucial role in food security in Africa, it is also a source of income for house-holds (Anglani, 1998). Starch is the main reserve polysaccharide of the plant kingdom, and

the principal source of carbohydrate from agricultural origin, being the end product of photosynthesis. Sorghum grains like all cereal grains are comprised primarily of starch.

2.4.1 Description and production

Sorghum is the fifth most important cereal crop after wheat, rice, maize, and barley in terms of production (FAO, 1996). Table 1 shows the annual production (metric tons) of cereal grains, in 1961, 2005, 2006, and 2007, ranked by 2007 production (FAO 2008). All but buckwheat and quinoa are true grasses (these two are pseudocereals). Total world annual sorghum production is about 60 million tons from cultivated area of 46 millions ha. In terms of tonnage, sorghum is Africa's second most important cereal after maize. The continent produces about 20 million tonnes of sorghum per annum, about one-third of the world crop. However, these figures do not do justice to the importance of sorghum in Africa.

Table 1: Annual world production (metric tons) of various cereal grains

GRAIN	2007 (t)	2006 (t)	2005 (t)	1961 (t)
Maize	784 646 525	699 285 327	715 813 543	205 004 683
Rice	650 192 516	644 115 984	631 868 371	215 646 637
Wheat	607 045 333	598 440 593	626 562 256	222 357 231
Barley	136 209 179	139 056 564	138 888 612	72 411 104
Sorghum	64 579 247	58 302 622	59 094 912	40 931 625
Millets	31 874 597	32 073 257	30 908 287	25 703 968
Oats	25 991 961	22 758 002	23 382 343	49 588 769
Rye	15 749 613	12 722 572	15 198 310	35 109 990
Triticale	12 599 992	10 814 167	13 978 609	0
Buckwheat	2 461 159	1 992 753	2 083 925	2 478 596
Fonio	394 811	381 176	366 389	178 483
Quinoa	61 490	57 962	58 443	32 435

Source: FAO (2008)

It is the only viable food grain for many of the world's most food insecure people (Taylor, 2004). The problem of food shortage in sub-Saharan Africa is to a large extent due to the fact that much of the region is characterised by semi-arid and sub-tropical climatic

conditions. Africa is the only continent that straddles both tropics. Sorghum is crucially important to food security in Africa as it is uniquely drought resistant among cereals and can withstand periods of high temperature (Dicko *et al*., 2006). Sorghum is particularly adapted to hot, semi-arid tropical environments with 400-600 mm rainfall, areas that are too dry for other cereals. Sorghum is also found in temperate regions and at altitudes of up to 2300 meters in the tropics. It is well suited to heavy soils commonly found in the tropics. Most of the countries in Africa where sorghum is a significant arable crop are arid, and areas at risk of desertification. Sorghum is also an important crop in East Africa where overall there is good rainfall. This is related to the fact that the rain in sub-tropical Africa is intermittent and characterised by brief periods of very high rainfall. In fact sorghum is not only drought-resistant, it can also withstand periods of water-logging (Doggett, 1988).

2.4.2 Distribution

It is believed that sorghum originated in Africa, more precisely in Ethiopia, between 5000 and 7000 years ago (ICRISAT, 2005). From there, it was distributed along the trade and shipping routes around the African continent, and through the Middle East to India at least 3000 years ago. It then journeyed along the silk route into China. Sorghum was first taken to North America in the 1700-1800s through the slave trade from West Africa. It was re-introduced in Africa in the late 19th century for commercial cultivation, and spread to South America and Australia. Sorghum is now widely found in the dry areas of Africa, Asia (India and China), the Americas and Australia.

2.4.3 Worldwide utilization

Sorghum is grown in the United States, Australia, and other developed nations essentially for animal feed. However, in Africa and Asia the grain is used both for human nutrition and animal feed. Sorghum is widely used as a staple food in many parts of Africa. It is

estimated that more than 300 million people from developing countries rely on sorghum as a source of energy (Godwin and Gray, 2000). Sorghum in Africa is processed into a very wide variety of attractive and nutritious traditional foods, such as semi-leavened bread, couscous, dumplings and fermented and non-fermented porridges. It is the grain of choice for brewing traditional African beers. New sorghum products such as instant soft porridge and malt extracts have been a great success in places such as South Africa and Nigeria, respectively (Taylor, 2004). The food industry in Southern Africa has been exploring the use of sorghum in the production of ready-to-eat products, using extrusion cooking technology and gun-puffing. In sorghum, pericarp color, secondary plant color, endosperm color, and the presence of a pigmented testa are factors affecting the color and acceptability of the sorghum-based products (Waniska and Rooney, 2000). Traditionally, sorghum flour is used as food in the form of thin and thick porridges. Often sorghum porridges are characterized by thick pastes that may form rather stiff gels depending on variety used. Porridges prepared with malted sorghums have several orders of magnitudes lower viscosities than those of non-malted sorghums (Dicko *et al*., 2006). These porridges are particularly useful for the formulation of supplementary foods for infants because of their high energy density (Traore *et al.*, 2004).

A large number of traditional food products (i.e., porridges, alcoholic and non-alcoholic beverages) are prepared using high tannin sorghums. They are readily accepted by local people because these sorghums are well adapted, produce consistent crops and are often preferred for food production. Sometimes special procedures are followed to enhance food quality. Tannins bind to proteins, carbohydrates, and minerals and thus reduce digestibility of these nutrients. To reduce these negative effects, decortication, fermentation, germination, and chemical treatments (HCl, formaldehyde, and alkali) are used (Beta *et al*., 2000; Bvochora *et al*., 2005; Osman, 2004). Special products are made from high

tannin sorghums. High tannin sorghums with red/brown pericarps are often used in the production of opaque beers as the dark color imparted to the beer by the pericarp pigments is desirable. Some reports exist on antioxidant activity of fully processed products like cookies and bread containing sorghum bran (Awika *et al*., 2003a).

2.4.4 Sorghum grain composition and nutritive value

Sorghum grain quality is affected by factors such as genotype, climate, soil type and fertilizer use, among others, which can affect the chemical composition and nutrient value (Ebadi *et al.*, 2005). Starch is the main component of sorghum grain, followed by proteins, non-starch polysaccharides (NSP) and fat (Table 2). The average energy value of whole sorghum grain flour is 356 kcal/100g (BSTID-NRC, 1996). Sorghum has a macromolecular composition similar to that of maize and wheat (BSTID-NRC, 1996). The crude protein content of sorghum grains is highly variable. The protein content in whole sorghum grain is in the range of 7 to 15% (FAO, 1995). Using the solubility-based classification, sorghum proteins have been divided into albumins, globulins, kafirins (aqueous alcohol-soluble prolamins), cross-linked kafirins and glutelins (Jambunathan *et al*., 1975). The kafirins comprise about 50-70% of the proteins (Oria *et al*., 1995; Duodu *et al*., 2003). The storage proteins in sorghum grain are mostly kafirins, which are prolamins that are soluble in aqueous alcohol in the presence of a reducing agent. The nutritional quality of sorghum proteins is poor because these kafirins are protease resistant (Oria *et al*., 1995). The fat in sorghum grain (mainly present in the germ) is rich in polyunsaturated fatty acids. The fatty acid composition of sorghum fat (linoleic acid 49%, oleic 31%, palmitic 14%, linolenic 2.7%, stearic 2.1%) is similar in content to that of corn fat, but it is more unsaturated (Glew *et al*., 1997).

Table 2: Proximate composition of sorghum grain[a]

Macro-components (g/100g f.m.)		**Essential amino acids (mg/100g)**		**Vitamins (mg/100g d.m.)**		**Minerals (mg/100g d.m.)**	
Carbohydrates	65 – 80	Leu	832	Vit. A	21 RE**	Ca	21
Starch	60 – 75	Ile	215	Thiamin	0.35	Cl	57
Amylose	12 – 22	Met/Cys	190	Riboflavin	0.14	Cu	1.8
Amylopectin	45 – 55	Lys	126	Niacin	2.8	I	0.029
Non starch	2 – 7	Phe/Tyr	567	Pyridoxine	0.5	Fe	5.7
Low Mw carbohydrates	2 – 4	Thr	189	Biotin	0.007	Mg	140
Proteins	7 – 15	Trp	63 - 187	Pantothenate	1	P	368
				Vitamin C	<0.001	K	220
α-Kafirins	4 – 8	Val	313			Na	19
β-Kafirins	0.2 – 0.5	Arg*	500			Zn	2.5
γ-Kafirins	0.7 – 1.6	His	200				
Other proteins	2 – 5						
Fat	1.5 – 6						
Ash	1 – 4						
Moisture	8 - 12						

[a]Sources: FAO (1995), BSTID-NRC (1996), Glew *et al*. (1997), Duodu *et al*. (2003), Dicko *et al*. (2006). *Not strictly essential amino-acids, **RE = retinol equivalent; f.m. = fresh matter, d. m. = dry matter; NSP = non starch polysaccharides.

The colors of sorghum grain and flour play an important role in its acceptance. In general, white sorghums produce the most acceptable colored food products. Food color is the result of factors such as grain color, pericarp color, pigmented testa, endosperm color, presence of tannins, degree of milling, and pH of the food system. Pigmentation in the pericarp and testa is primarily due to phenolic compounds. The color intensity greatly depends on pH. Anthocyanins are very unstable in acid medium and are readily converted to the corresponding anthocyanidin under slight acidic conditions (Hahn and Rooney, 1986).

2.4.5 Phenols and tannins in sorghum

Phenolic compounds in sorghum occur as phenolic acids, flavonoids and condensed tannins (proanthocyanidins). Phenolic compounds in sorghum grains are concentrated in the bran layer as a protective mechanism against insects and diseases (Awika *et al.*, 2005; Hahn and Rooney, 1986). Several factors such as plant type, age of the plant or plant parts, stage of development, and environmental conditions govern the polyphenol contents in plants. Sorghum is unique among major cereals because some cultivars produce polymeric polyphenols known as tannins (Butler, 1990). The sorghum tannins occur only in the pericarp and testa layers (Serna-Saldivar and Rooney, 1995). Sorghum tannins have been characterized as condensed tannins. Condensed tannins (proanthocyanidins) occur in sorghums with a pigmented testa which have dominant B_1B_2 genes (Waniska and Rooney, 2000). High tannin sorghums contain proanthocyanidins as part of their phenolic compounds but do not contain tannic acid nor hydrolyzable tannins. Tannin sorghums have a pigmented testa on the innermost layer of the pericarp. The pigmented testa is seen as a dark layer between the light endosperm and the pericarp when the caryopsis is scraped to remove the pericarp. Bleaching using the bleach test causes the constituents in the pericarp and testa to oxidize and yields black pigments on the surface of the caryopsis (Waniska, 2000). Sorghums with a pigmented testa and tannins remain black longer during bleaching than do non-tannin sorghums. Sorghums are classified as type I (without tannins), type II (tannins present in pigmented testa), or type III (tannins present in pigmented testa and pericarp).

Polyphenols, especially, the tannins have been reported to interact with proteins and form tannin-protein complexes leading to either inactivation of enzymes or making proteins insoluble. They are implicated in decreasing the activities of digestive enzymes, protein and amino acid availabilities, mineral uptake, vitamin metabolism, and depression of growth. They have therefore been regarded as antinutrients and considered nutritionally

undesirable (Hahn *et al.*, 1984; FAO, 1995). However, these compounds are also believed to have some favourable effects on human health, such effects as the lowering of human low-density lipoprotein, reduction of heart diseases and cancer (Awika and Rooney, 2004). The tannins in sorghums have the highest levels of antioxidants of any cereal analyzed (Gu *et al*., 2004). Despite their possible beneficial effects as antioxidants and possible protective effects on human health, tannins have been linked to reduced protein digestibility and they have also been erroneously reported to be toxic (Duodu *et al*., 2002). High tannin sorghums have condensed tannins, which are not toxic. High tannin sorghums are consumed as human food extensively in Africa and Asia without problems. High tannin sorghums do slow and reduce the digestibility of nutrients especially proteins. However, Elkin *et al*. (1996) demonstrated that, sorghums containing equivalent amounts of tannins have different digestibilities. This suggests that tannins are only partially responsible for lower protein digestibility.

Phenols (mainly condensed tannins) in sorghum kernel are considered a desirable agronomic trait since they can protect sorghum from being damaged by birds, insect pests and diseases (Waniska *et al*., 2001). In Southern Africa, small-scale farmers intercrop tannin and tannin-free sorghums in areas prone to high bird predation in order to reduce grain losses in the field. When harvested the grain is mixed and used in making porridge. Only brown, high tannin, bird resistant types contain condensed tannins; yellow sorghums contain low tannin and white sorghums contain no tannin. It is the phenolics which inhibit the growth of microorganisms. These beneficial effects ensure that brown sorghums will continue to be produced in certain pest-ridden areas of the world (Butler, 1990). In the view of nutritive value, tannins are considered undesirable due to their capacity of binding proteins and making them less digestible as well as producing astringent taste. Therefore, debates on the necessity and amount of tannins in sorghum kernels exist between

nutritionists, agronomists, growers and consumers. Sorghum breeders have been wandering between the two extremes, how to take the advantages of tannins in fields and to minimize their disadvantages of reducing nutritive value of sorghum grain (Ambula *et al*., 2003). The antinutritional activity of polyphenols can be reduced by removing polyphenols from the grains by chemical treatments or removing pericarp and testa. Food processing such as fermentation, germination and extrusion has been shown to have an effect on phenol and tannin content of sorghum-based foods (Dlamini *et al*., 2007). The reduction in tannins by processing occurs by the interaction of tannins with proteins and carbohydrates (Mehansho *et al*., 1987). These tannin complexes are less extractable, and give reduced tannin levels. Sorghum tannins have strong affinity for proteins high in proline content, like the prolamins (Emmambux and Taylor, 2003).

High tannin sorghums are preferred in some societies to make a variety of food products. In some African cultures, high tannin sorghums are actually preferred because porridges from such sorghums remains in the stomach longer and the farmer feels full for most of the day doing field work. Other pigmented sorghums are also preferred in some African cultures because of the characteristic color they produce in certain foods. High tannin sorghums have been used in the production of good-quality breads, malt, beer, and distilled beverages. Many acceptable products, such as porridges and alcoholic beverages, have been developed from high tannin sorghums in Africa (Awika and Rooney, 2004). Good quality breads containing high tannin sorghum bran have high antioxidant and dietary fiber levels with a natural dark brown color and excellent whole grain flavor (Gordon, 2001). In addition, healthy bread mixes containing high tannin sorghum bran, barley flour, and flaxseed have been developed (Rudiger, 2003). High tannin sorghums are often preferred for production of sorghum beers and alcoholic beverages because of their dark color (Awika and Rooney, 2004). Since high tannin in sorghum may impair the nutritional

quality of the final products, Dicko *et al.* (2005) suggested that, sorghum varieties which do not contain tannins or contain low levels of tannins after germination may be the best for the preparation of infant porridges from a nutritional stand-point. For infant porridges, the low tannin content is presumably more desired than high antioxidant activity (Dicko *et al.*, 2005).

2.4.6 Effect of processing on sorghum phenols, tannins and nutritional value

Traditionally, the various technologies used in the processing of sorghum into food have focused on improving its nutritional value, particularly in terms of improving protein digestibility (Mukuru, 1992). Technologies such as malting, fermentation and alkaline processing have been used to improve protein digestibility of high tannin sorghum (Monyo *et al.*, 1992; Mukuru, 1992). Processing high tannin sorghums into food products affects phenol levels. For example, Awika *et al*. (2003a) reported that, processing high tannin sorghum bran to produce cookies and breads decreased tannin content by 52 and 72 percent, respectively; the loss was mainly from the high-molecular weight tannins. Awika *et al.* (2003a) also reported that, extrusion of high tannin sorghum caused an 85% decrease in polymeric tannins while the lower molecular weight tannins increased by 29–47%. The issue of the fate of phenolic compounds during processing has been subject of investigation. The tannins form less extractable polymers either with other food components in particular proteins and carbohydrates or between themselves (Duodu *et al.*, 2002). Tannin interactions with food components are mostly non-covalent interactions, and may involve hydrogen bonding and hydrophobic interactions (Asquith and Butler, 1986; Mehansho *et al.*, 1987). The reduced extractability is observed as reduced measurable levels by methods such as the vanillin-HCl assay (Beta *et al.*, 2000).

2.4.6.1 Effect of sorghum dehulling

Generally, sorghum processing entails partial or complete decortication of sorghum grains before further processing and consumption, though whole grains may also be directly dry-milled to give a range of products such as fine flour or cracked grains and grits (Murty and Kumar, 1995). The objective of dehulling sorghum is to remove the outer layers (pericarp) of the seed, thereby reducing the fiber and tannin content and improve the appearance, cooking quality, palatability and digestibility of the grain (Reichert *et al*., 1986). The process has been found to reduce tannin by up to 98%. Despite the advantages, dehulling may remove protein up to 45% and is also associated with some loss of nutrients found in the outer layers of the grain (Chibber *et al*., 1978). In many parts of Africa, sorghum is milled as whole grain, or decorticated using traditional mortars and pestles or mechanical dehullers. Decortication removes the grain's outer layers where the polyphenols are concentrated, which reduces overall tannin content. This improves product color, reduces astringency and improves digestibility (Taylor and Dewar, 2001).

2.4.6.2 Effect of germination on sorghum composition

The physiological maturity of sorghum grain generally occurs 50 days after anthesis, and marks the end of nutrient delivery and the beginning of senescence, and caryopse desiccation (Waniska, 2000). The mature grain is then harvested and stored. In a dormant stage, it is characterized by dehydration and a dramatic decrease of metabolic activity. Germination is induced by rehydration of the seed, which increases both respiration and metabolic activity thus allowing the mobilization of primary and secondary metabolites (Limami *et al*., 2002). Therefore, the biochemical composition between ungerminated and germinated kernels is different. Germination induces the synthesis of hydrolytic enzymes, e.g. starch degrading enzymes and proteases.

The reduction of phytic acid, some flavonoids and proanthocyanidins has been observed during germination (FAO, 1995; Traore *et al*., 2004). The breakdown of protease resistant prolamins and the increase of the availability of minerals (e.g. iron and zinc) and essential amino acids (principally Lys, Tyr and Met) upon germination has also been reported (FAO, 1995; Anglani, 1998). Germination of sorghum is important for the preparation of supplementary foods with low paste viscosity and high energy density.

Germination and fermentation have been reported as ways of improving cereal-protein quality (Lorri, 1993). Another study has documented improved vitamin content in germinated soghum and maize (Asiedu *et al*., 1993). Despite the reported improvement in the nutrient status of germinated and fermented cereal based diets in sub-Saharan Africa, the nutrient needs of infants and sick adults are still not being met (Mbata *et al*., 2007). Earlier studies have documented the need for fortification of traditional fermented cereal porridges with legume (Gahlawat and Seghal, 1993).

2.4.7 Improving nutritional quality of sorghum

No one legume or cereal can provide adequate amounts of all nutrients to meet the nutritional requirements of a child (FAO, 1995). However, even before knowledge on protein content, protein quality, digestibility and the nutrient requirements of humans became available, it was recognized that mixing legumes with cereals in the diet could improve overall nutrition. The present and newly derived knowledge in these areas makes it possible to blend, mix or fortify one food material with others so that the resulting enriched or fortified mix has not only better nutritional quality but also the necessary attributes for consumer acceptance. The protein quality of sorghum is poor. Therefore attempts have been made to enrich these cereals with legumes or other cereals to make them nutritionally superior and acceptable. Sorghum has been successfully used in feeding

programmes after enrichment with legumes. Vimala *et al*. (1990) described various infant mixes based on sorghum and pearl millet enriched with soybean, green gram, red gram or Bengal gram flour.

2.5 Soybeans

Soybeans [*Glycine max* (L.)] have become an increasingly important agricultural commodity. Soybean is native to eastern Asia. It is widely believed that the soybean originated in China, probably 4000-5000 years ago and was considered as one of five sacred grains. From China soybean cultivation spread into Japan, Korea, and throughout Southeast Asia. By 19th century soybeans were planted in Europe. Currently, there are more than 150 varieties. The yellow soybeans are the dominant class used in the market. Other minor classes include green, brown and black varieties (Weingartner, 1987).

Soybean, an abundant and economical source of protein can be used to increase protein content and to improve the quality of cereal-based diets. Soybean protein is particularly valuable because it contains sufficient lysine and can serve as a valuable supplement to cereal foods where lysine is a limiting factor. Its addition to a mixed diet, greatly improves the quality of the diet's protein (Weingartner, 1987). The main attributes of soybeans as a source of protein include its abundance, low cost, high protein content and freedom from cholesterol. Generally, no other plant protein source can compete with soybean where good quality, low-cost protein is required (Aguilera and Lusas, 1981). Soybeans are the most abundantly produced crop among plant protein sources, and one of the world's leading cash crops because of its wide regional adaptability and ability to fix nitrogen. Although native to Eastern Asia, a bulk of soybean is produced in the United States followed by Brazil, Argentina, China and India (Keshun, 1997).

Soybeans were first introduced in Tanzania in 1907 and much effort to grow it in large scale began in 1947. Bossier (spherical and yellow) is the common variety in Tanzania and production is mainly in Mtwara, Lindi and Morogoro. Soybean is important in Tanzania as an alternative crop for correcting protein deficiency among the population (Myaka, 1990). Undernourished children in Zaire and Nigeria have used soybean for dietary management of protein-energy undernutrition. Children including those with kwashiorkor and marasmus consistently seem to thrive and gain weight when fed foods enriched with soybean (Weingartner *et al*., 1987). Martin *et al*. (2010) reported that soybean significantly improved the protein of soybean based supplementary foods. Soy protein is cheaper than animal protein sources (Myaka, 1990).

2.5.1 Nutritional composition of soybeans

The component present in the greatest amount in soybeans is protein averaging about 40% of total dry matter. Soybeans can be stored for up to one year if their moisture content is kept below 12% (Carrao and Gontijo, 1994). The majority of soy proteins are storage proteins. Storage proteins are localized in protein bodies, which are more or less spherical in shape. The majority of soy protein is a relatively heat-stable storage protein. This heat stability enables soy food products requiring high temperature cooking, such as tofu, soymilk and textured vegetable protein to be made. Whole soybean has a good balance of amino acids and is an excellent source of calories, minerals and vitamins (Mahan and Escott-stump, 1996). Protein from soybeans contains all the essential amino acids but it has minimum amounts of sulphur containing amino acids, methionine and cystine. Soybean protein has high levels of lysine which is the worldwide most limiting amino acid in most low cost diets (Weingartener, 1987: Carrao and Gontijo, 1994). It is also a good source of tryptophan and threonine, essential amino acids that are also limiting in most low cost diets (Carrao and Gontijo, 1994). Typical composition of Soybean is as follows: 42% protein,

33% carbohydrates, 20% oil and 5% ash, on a moisture-free basis (Pearson, 1983). The protein efficiency ratio (PER) of soy flour and grits generally falls in the range of 2.2 – 2.3 compared to 2.5 for casein which is normally taken as the control value in nutrition studies. The PER is computed by dividing the growth rate of the study animal by the amount of protein consumed (Torun *et al*., 1981).

2.5.2 Health promoting properties of soybean

Soybeans have been widely recognized for their health benefits. Soybean has received increasing attention in recent years from health care providers, biomedical researchers and the lay public alike because of its potential role in the prevention of a number of chronic diseases such as cancer, coronary heart diseases and osteoporosis. There is growing evidence that, consumption of soybean and soy products might protect against hormone-dependent cancers, like breast and prostate cancer, and have beneficial effects with regard to cardiovascular diseases, osteoporosis and menopausal symptoms (Devi ***et al.,* 2009).** Phytochemicals present in soybeans were once considered non-nutritive substances, but are now recognized for their positive implications on health (Craig, 1997). The phytochemicals in soybean are primarily isoflavones, although other phytochemicals such as saponins and phytates are also found. Soybean is a rich and relatively unique source of isoflavones, the most common being genistein, daidzein and glycitein, which occur as aglycone, glucoside, acetylglucoside and malonylglucoside (Wang and Murphy, 1994). Soy isoflavones have estrogenic and antioxidant activity. They may also have anticarcinogenic, anti-atherogenic, hypolipidemic and anti-osteoporotic activities (Fritz *et al*., 2003). The isoflavone concentrations in soybean seeds comprise about 72% of the total phenols and are affected by various genotypic and environmental factors (Heim *et al*., 2002). Epidemiological studies have shown that, populations with high intakes of soy foods, such as those of China, Japan and other Asian countries, usually have a reduced risk

of cancers of the breast, prostate, colon and uterus (Kwak *et al*., 2007). Consumption of soybean has been on the rise in recent years since a trend towards healthier living has begun. Consumption of foods containing significant amounts of antioxidants may help the human body reduce oxidative damage related to ageing and diseases, such as atherosclerosis, cancer and cirrhosis. Incorporation of soybean into food products may offer increased health benefits but there are also potential problems that may arise. The effect of processing on soybean may deplete some of their functional components, making them less effective or not effective at all (Davis, 2004). Processing of soybean to create different products must be closely monitored to prevent the loss of beneficial components.

2.5.3 Soybean processing

Soybeans are a versatile crop with many uses. But before they can be used in food, feed or industrial products, soybeans must be processed. Soybeans are associated with antinutritional factors such as trypsin inhibitors and haemagglutinins, among others (Ramamani *et al*., 1996). Industrial processes are designed to improve the food value of soybean by inactivating antinutritional factors and enhancing availability of nutrients (Carrao and Gontijo, 1994). Soybean is usually not consumed directly, but processed into a large number of varieties of popular products (Damardjati *et al*., 1996). Raw soybean does not promote growth due to the presence of antinutritional factors. However, soaking and cooking under pressure makes soybean a very nutritious source of protein (Liener and Tomlison, 1981).

2.6 Thermo - processing of Foods

The aims of food thermo-processing are to make the food edible by developing appealing texture, color, taste and flavor; pasteurizing/sterilizing the food and making it microbiologically safe; destroying natural toxins and anti-nutrients such as

phytohemagglutinins, enzyme inhibitors and cyanogenic glycosides; improving nutrient digestibility and bioavailability; improving the shelf-life and making foods more convenient for use (Friedman, 1992). Occurrence of antinutrients in plant foods is a notable disadvantage for their utilization if not processed (Taylor, 1982). There are a variety of thermo-processing methods that are commonly used in food preparation such as baking, roasting, frying, conventional cooking, drum processing and extrusion. Selection of any one of these processing methods is influenced by a number of factors including the nature and type of ingredients to be processed, the end product desired e.g. ready-to-eat products, availability of equipment and the cost of production. Interests of extrusion cooking are numerous (Harper and Jansen, 1985; Camire *et al.*, 1990). Extrusion results in inactivation of certain antinutritional factors such as trypsin inhibitors thus increasing protein digestibility. In addition, it induces a drastic reduction of microbiological load. Furthermore, it gives products which only need short time of preparation with possibility to produce either instant flour or ready-to-cook flour and improves organoleptic characteristics of the end products.

2.7 Extrusion

Extrusion cooking is a popular means of preparing snacks and ready to eat foods. In extrusion processes, foods are cooked at high temperature for a short time. Starch is gelatinized and protein is denatured, which improves their digestibility. Antinutritional factors that are present may be inactivated. Microorganisms are largely destroyed and the product's shelf-life is thereby extended. Extrudates become microbiologically safe and can be stored for long periods because of low moisture without need for refrigeration (Filli and Nkama, 2007). Extruded products are formulated from mixtures of cereals, legumes, and oil seeds and are completely precooked for easy reconstitution and use. They can be fortified with vitamin and minerals (Harper and Jansen, 1985). Food industries have been

applying extrusion technology to produce various products such as pasta, ready-to-eat cereals, meat analogs, flat bread and puffed snacks. Starchy materials from different kinds of cereals, legumes and tubers are commonly used in extrusion process (Nurtama and Lin, 2009).

The use of extruders for food cooking has been expanding rapidly in the food industry due to their versatility, high productivity, efficiency, hygiene conditions and low operation cost (Riaz, 2000). Extrusion alters the nature of many food constituents, including starches and proteins, by changing their physical, chemical and nutritional properties. High temperature short time (HTST) extrusion cooking technology has limitless applications in processing of cereal based products. Extrusion is a process whereby raw feed material is exposed to controlled conditions of high temperature, pressure and moisture. This is achieved by forcing the material through a tapering screw shaft and passing through a die plate under high pressure accompanied by an injection of steam or water.

During processing through the extruder, a dough-like mixture is forced through a stationary metal tube or barrel by a rotating screw shaft. As this occurs, heat can be added in the form of steam and is also generated by the mechanical energy of the turning screw and the friction of the barrel. As a result, very high temperatures (>150^{o}C) can be reached (Harper, 1992). Low-cost extruders, which process foods at moistures of less than 20 percent, have the lowest capital and operating costs.

2.7.1 Extrusion cooking technology

Extrusion has been practised for over 50 years in the production of breakfast cereals, snack foods and pet foods. Initially its role was limited to mixing and forming macaroni as well as ready-to-eat cereal pellets. Now the food extruder is considered a high-temperature

short-time reactor that transforms a variety of cereal grains into modified intermediate and finished products (Fast, 1991). The extruder basically consists of a feeder/hopper that feeds the ingredient; screws that rotates inside a cylindrical barrel; and a die that dictates the shape of the extruded products. The feed is mixed with water and compressed by the screws as they rotate and pushes the feed forward through the heated barrel. Due to the friction and the heat provided inside the barrel, the feed is quickly heated. As the mixture advances along the barrel, pressure and heat build up. This pressurized cooking transforms the mass into a thermoplastic “melt” (Berk, 1992). While the proteins undergo extensive heat denaturation, the directional shear force causes alignment of the high molecular components (Berk, 1992). At the end of the barrel the melt is forced through the die. The sudden release of pressure leads to instant evaporation of some of the water. This causes puffing of the extrudates, thereby resulting in a porous structure. The extrudate’s puffing or porous structure could be partially controlled by manipulating the melt temperature within the die.

Extrusion cooking is not a single-unit operation (Camire *et al*., 1990). While passing through the extruder, feed materials are subjected to a number of unit operations such as mixing, shearing, heating, cooking, texturizing, shaping and puffing. This is achieved by forcing the material through a tapering screw shaft and passing through a die plate. Cooking is accomplished by the combined effect of external heat and mechanical energy input to the material.

The general types of extruders are single screw and twin screw. Twin-screw extruders are commonly used for extruding different raw materials since their flexible design allows a fast change of product. They are also suitable for raw materials with a fat content of 18–22% whereas the fat content in single screw extruders cannot be higher than 12–17%

because the fat decreases the shear so that the energy cannot be transformed into heat for cooking (Harper, 1992). The single-screw extruders are relatively ineffective in transferring heat from the barrel jackets to the products. This is caused by the poor mixing within the extruder channel (Harper, 1989). Twin-screw extruders have considerably more heat exchange capability than single-screw extruders, which expand their application to heating and cooling of viscous pastes, solutions, and slurries. The twin-screw extruders, therefore, are more suitable for processing high moisture materials, due to better heat transfer. In addition, the direction of screw rotation, screw shape, screw configuration and relative position of screw sections minimize pressure and leakage flows (Harper, 1989; Noguchi, 1989). Twin-screw extruders have less interaction of process variables than single-screw extruders, making them easier to operate and control (Harper, 1989). Both types of extruders are widely used in the food industry. Due to their low cost, single-screw extruders remain to be an effective and economical choice to produce pet foods. The twin-screw extruders are mainly applied to products that require better control and operating flexibility.

The screws are the central components of an extruder and their configuration greatly influences the quality of the extruded product. Large extrusion screws (length/diameter > 10) tend to have greater operating flexibility and allow greater precision of control of the extrusion process. In some cases, screws are configured to reverse the flow of materials, which increases the shear, pressure, heat, and residence time of the food. Screws in the metering section of the barrel end have very shallow flights or flights with decreasing pitch. In this section, the shear rate is very high, internal mixing is increased dramatically and the dissipation of mechanical energy is at maximum. Correspondingly, the temperature rise in the metering section is very rapid and reaches maximum just before the product

emerges from the die. The puffed extrudates are usually chopped or molded to desired shapes (Harper, 1989).

2.8 Plant Antioxidants

Antioxidant compounds in food play an important role as a health protecting factor. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources (Miller *et al*., 2000). These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Scientific evidence suggests that, antioxidants reduce the risk for chronic diseases including cancer and heart disease. Several sources of natural antioxidants have been investigated, including plants and microorganisms (Arai *et al*., 2002). The best-known natural antioxidants that have proven important in the food industry and in human health are tocopherols, vitamin C and carotenoids (Shahidi, 1997). Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, cereal crops, spices and herbs as well as medicinal plants (Oke and Hamburger, 2002). Cereals and legumes containing a wide range of phenolics have been claimed to be a good source of natural antioxidants (Ragaee *et al.*, 2006). Specialty sorghum hybrids contain high levels of diverse phenolic compounds that may provide health benefits. High levels of polyflavanols (procyanidins), anthocyanins, phenolic acids, and other antioxidant compounds have been reported in sorghum (Hahn *et al*., 1984; Awika and Rooney, 2004).

Phenolic compounds are ubiquitous bioactive compounds and a diverse group of secondary metabolites universally present in higher plants. Flavonoids and other plant phenolics, such as phenolic acids, tannins, and lignin, are especially common in leaves, flowering tissues, seeds, and wood parts such as stem and bark (Kim *et al*., 1994). They are important in the plant for normal growth development and defense against infection and injury. Flavonoids also partly provide plant colours present in flowers, fruits and leaves. Bioactive polyphenols have attracted special attention because they can protect the human body from the oxidative stress which may cause many diseases. The antioxidant activity of phenolics is mainly as free radical scavengers, hydrogen donators and singlet oxygen quenchers. In addition, they have a chelating potential (Banias *et al*., 1992). It has been shown that, many plants are antioxidant sources, however, the wide varieties of methods used to measure antioxidant activity make it difficult to compare results from different studies (Sellapan, 2002).

2.9 DPPH (1, 1 diphenyl -2- picryl hydrazyl) Free Radical Scavenging Activity Assay

Numerous methods are used to evaluate antioxidant activities of natural compounds in foods or biological systems with varying results. The various methods used to measure antioxidant activity of food products can give varying results depending on the specific free radical being used as a reactant (Prakash, 2001). The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Lee *et al.,* 2003). DPPH, a paramagnetic compound with an odd electron, shows strong absorption band at 517 nm in methanol. The absorbance decreases as a result of color change from purple to yellow due to the scavenging of free radical by antioxidants through donation of hydrogen to form the stable DPPH–H molecule (Chandrasekar *et al*., 2006). The DPPH method is widely used to determine antiradical/antioxidant activity of purified phenolic compounds as well as natural plant extracts (Brand-Williams *et al*.,

1995). The method is based on the reduction of alcoholic DPPH˙ solutions at 517 nm in the presence of a hydrogen donating antioxidant (AH). The formation of the non-radical form DPPH-H by the reaction occurs as follows:

$$DPPH^{\cdot} + AH \rightarrow DPPH\text{-}H + A^{\cdot}$$

The remaining DPPH˙, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant. The sensitivity of the method is determined by the strength of absorption of DPPH˙ (Brand-Williams *et al.*, 1995). The method is rapid, a sample analysis takes 15 min in total and little manpower, no expensive reagents or sophisticated instrumentation are required. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. It is not discriminative with respect to the radical species but gives a general idea about the radical quenching ability (Koleva *et al*., 2002). A measure of total antioxidant capacity helps understand the functional properties of foods. Antioxidants in food may be water soluble, fat soluble, insoluble, or bound to cell walls and thus not necessarily freely available to react with DPPH, hence they react at different rates i.e. differing kinetics, and the reaction will often not go to completion in a reasonable assay time. Therefore, the sample size that can lower the initial absorbance of DPPH solution by 50% has been chosen as the endpoint for measuring the antioxidant activity. This change is compared to the change induced by a reference standard (Prakash, 2001). A good parameter for comparing the antioxidant activities of the various compounds could be expressing the results as EC_{50}, which represents the antioxidant concentration necessary to decrease the initial DPPH concentration by 50%. The activity is expressed as effective concentration EC_{50} (Sanchez-Moreno *et al*., 1999). Variations in the results obtained for antioxidant activity may occur due to variation in reagents quality,

environmental conditions, intrinsic errors, sample concentration, method of determination, and ways to present the results.

CHAPTER THREE

3.0 MATERIALS AND METHODS

This chapter presents the materials and methods employed in the formulation and extrusion of sorghum-soybean-sardine supplementary products. The chapter is divided into two major sections. The first section elucidates on the materials used and their sources, and the second section illustrates the methods and procedures used in material preparation, extrusion, chemical assays and sensory evaluation.

3.1 Materials

Red sorghum ‘Nkula’ was purchased from farmers in Shelui in Singida region. White sorghum ‘Lihonja’ was purchased from a farmer in Mikumi district in Morogoro Region. Dried sardines (*Rastrineobola argentea* from Lake Victoria) were purchased from Morogoro Municipal central market, Tanzania. Soybean (*Glycine max*) was purchased from a farmer in Morogoro region, Tanzania.

3.2 Methods

3.2.1 Sample processing

3.2.1.1 Sorting/cleaning/dehulling (sorghum)

Sorghum was sorted to remove extraneous matter and damaged grains. The sorghum was washed to remove mud and dust and then sun dried. The red sorghum was subdivided into 2 portions. One portion was germinated and the other portion was processed whole. The white sorghum was dehulled using a commercial dehuller machine (Intermech, Tanzania). Red sorghum was not dehulled to enable the study of effect of extrusion on tannin content; tannin compounds are concentrated in the outer layers (pericarp and testa).

3.2.1.2 Germination

Cleaned sorghum seeds were soaked in water overnight with water filled up to 15 cm above the level of the grains. Then the water was drained off. The soaked grains were spread in one cm thick layer between two wet white clothes and left to germinate in a dark cabinet for 48 hrs. In between, some water was sprayed on the wet cloth to ensure adequate moisture. The germinated seeds were then sun dried until a moisture content of 12% was attained.

3.2.1.3 Milling

Whole, dehulled and dried germinated sorghum was milled into fine flour (sieve size – 1mm) using a commercial hammer mill (Intermech, Tanzania). The flour was used for preparing sorghum-soybean blends.

3.2.1.4 Soybeans

The soybeans were sorted to remove extraneous matter and damaged (insect and moisture damaged, immature and broken) beans. The cleaned soybeans were weighed and blanched in boiling water for 30 min, allowed to cool to room temperature and then dehulled by hand machine to remove the outer hulls. Dehulled soybeans were washed thoroughly to separate the beans from the hulls. The dehulled soybeans were sun-dried to 10% moisture content thereafter milled into soybean flour. The soybean flour was used in the formulation of supplementary foods.

3.2.1.5 Sardines

The dry sardines (*Rastrineobola argentea* from lake Victoria) were sorted to remove pebbles and other extraneous materials. The sardines were then put in boiling water for 30 minutes. The water was then decanted. Cold water was added to the sardines and washed.

Then the sardines were put again in fresh boiling water for another 30 minutes and the washing process repeated. The sardines were then rinsed and sun dried. The sardines were thereafter ground into fine powder using a commercial hammer mill (Intermech, Tanzania).

3.2.2 Product formulation and composition

The ingredients were combined in proportions that provided the highest amino acid score possible or proportions that meet the FAO/WHO/UNU (1985) requirements for energy and essential amino acids (Table 3). Six supplementary food products namely, germinated red sorghum-soybean (GRSS), red sorghum-soybean (RSS), red sorghum -soybean-sardine (RSSSar), red sorghum plain (RS), dehulled white sorghum-soybean (WSS) and dehulled white sorghum -soybean-sardine (WSSSar) were formulated. Red sorghum plain was included and not white sorghum plain so as to serve as control in studying the effect of extrusion processing on tannin content of the various red sorghum-based food products.

Table 3: Composition (g/100 g) of the sorghum-soybean based supplementary food formulations

	Formulation[1]					
Ingredients	**WSS**	**WSSSar**	**RSS**	**RSSSar**	**GRSS**	**RS**
White sorghum	70	75	0	0	0	0
Red sorghum	0	0	N70	75	70	95
Soybean	25	15	N25	15	25	0
Sardines	0	5	0	5	0	0
Sugar	5	5	5	5	5	5
Total	100	100	100	100	100	100

[1] WSS =White sorghum-soybean; WSSSar=White sorghum-soybean-sardine; RSS =Red sorghum-soybean; GRSS =Germinated red sorghum-soybean; RSSSar =Red sorghum-soybean-sardine; RS =Plain Red sorghum

3.2.3 Preparation of extruded products

The formulations were processed into pre-cooked flour that could be reconstituted into porridge for child feeding. Each formulation was divided into three portions. First portion was packaged uncooked as control. Second and third portions were processed by extrusion at 100ºC and 140ºC respectively (Fig. 1).

Extrusion of the composite flours was carried out in a commercial twin-screw extruder (Model JS 60 D, Qitong Chemical Industry Equipment Co. Ltd, Yantai, China) (Fig. 2). Feed moisture content was adjusted to 24%. In this study, extrusion temperature was taken as the temperature at the exit end of the barrel (Zone 1). The foods were extruded at low temperature (100ºC) and at high temperature (140ºC). The following extrusion conditions were adopted: Temperatures 100º C or 140ºC (zone 1) and 90ºC or 130ºC (zone 2), main motor speed was set at 30.56 rpm and feeder speed at 13.79 rpm. The extruder consisted of two electrically heated zones. Desired barrel temperature was maintained by circulating tap water. Temperature was controlled by inbuilt thermostat and a temperature control unit. After extrusion, the extrudates were allowed to dry at room temperature and thereafter milled to obtain extruded flour. Extruded flour was packaged in polyethylene packets and kept at room temperature (22ºC) ready for chemical analysis. Samples for sensory evaluation were stored frozen until when sensory evaluation was conducted. Time of storage of extruded flour before chemical analysis and sensory evaluation ranged from one to three months.

3.2.4 Preparation of gruels for sensory evaluation

To make extruded gruels, boiling water was added to the extruded composite flour samples at a ratio of 7:1 (w/w) with mechanical stirring. For the samples that were not extruded (control), 150 g of flour was made into a slurry using 500 ml of cold water which was then

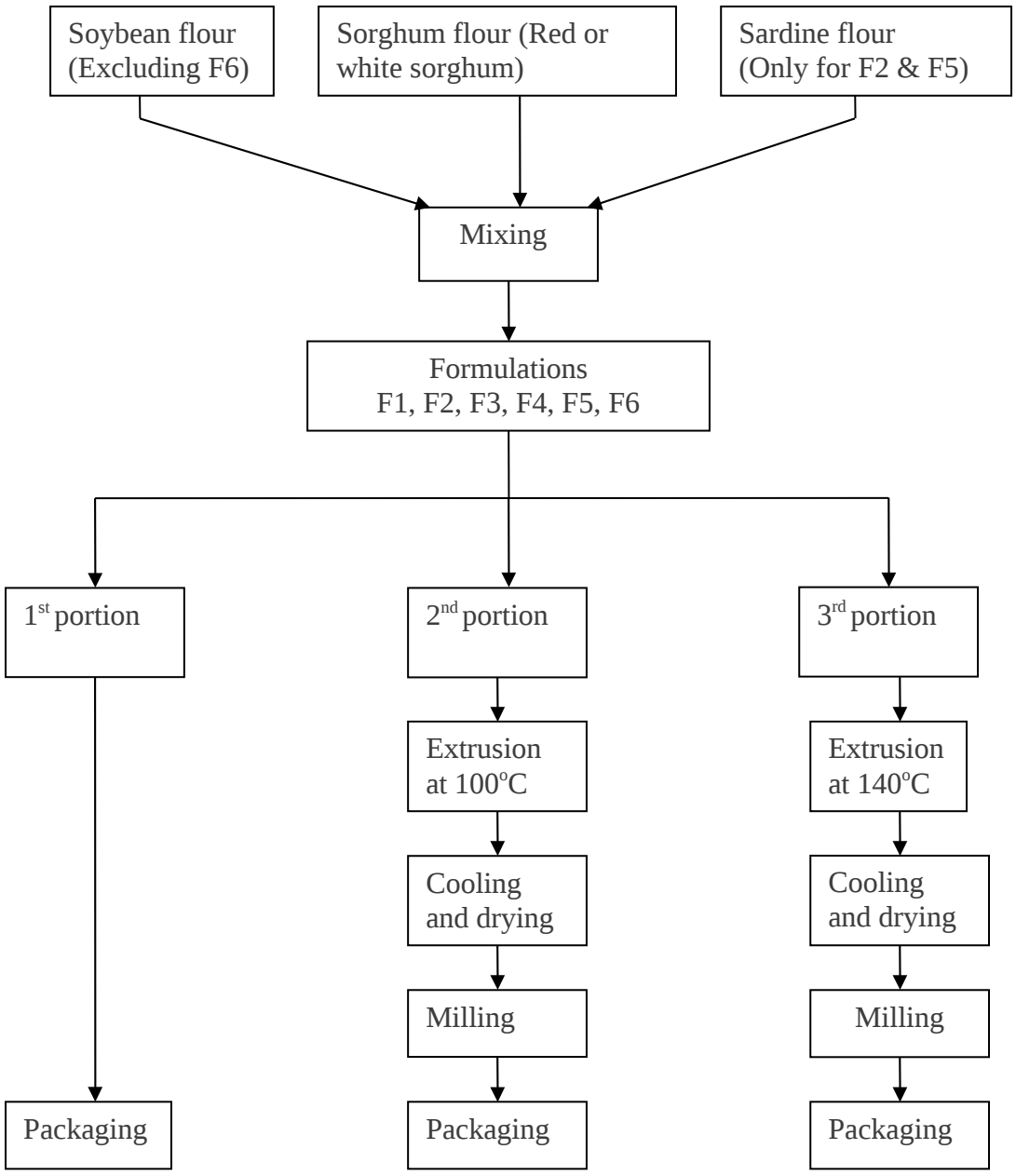

Figure 1: Flow diagram for the preparation of extruded foods

Key:

F1 – Formulation 1 (Dehulled white sorghum-soybean, WSS)

F2 – Formulation 2 (Dehulled white sorghum- soybean - sardine, WSSSar)

F3 – Formulation 3 (Germinated red sorghum-soybean, GRSS)

F4 – Formulation 4 (Whole red sorghum-soybean, RSS)

F5 – Formulation 5 (Whole red sorghum- soybean - sardine, RSSSar)

F6 – Formulation 6 (Red sorghum plain, RS)

poured in 1300 ml of boiling water and left to simmer for 15-20 minutes with constant stirring. The gruels were then kept in vacuum flasks ready for sensory evaluation. The gruels were prepared in the laboratories of the Department of Food Science and Technology, Sokoine University of Agriculture.

Figure 2: Photo of extruder machine used in extrusion

3.2.5 Nutritional composition

The proximate composition (dry matter, crude protein, crude fibre, crude fat and ash content) of the uncooked and extruded products was determined according to standard AOAC (1995) methods. The results were presented as an average of triplicate determinations.

3.2.5.1 Dry matter

Dry matter was determined by oven drying method 925.10 (AOAC, 1995). Five grams of each sample was oven dried at 105°C for 24 h to a constant weight. The sample was dried in pre-dried and pre-weighed crucibles. Dry matter was obtained as the difference between moist sample before drying and dry sample after drying in the oven for 24 h. The difference obtained was expressed as percentage dry matter with respect to original amount of the sample taken.

$$\% \text{ Dry matter} = \frac{(C-B)\times 100}{A} \quad \text{.....} \quad (1)$$

Where: A = Weight of the sample taken (g)

B = Weight of crucible (g)

C = Weight of crucible and dry sample (g)

(C-B) = Weight of dry sample (g)

3.2.5.2 Ash

Ash content was determined according to AOAC (1995), method 923.03. One gram of dry sample from dry matter determination was taken for ash content determination. The sample was placed into a pre-heated and pre-weighed crucible and incinerated in a muffle furnace set at 550°C for 24 h until grey ash was obtained. Ash content was calculated as the difference between the weight of sample before and after incineration.

Percentage ash was calculated from the relationship:

$$\text{Ash (\%DM)} = \frac{\text{Weight of ash (g)}}{\text{Weight of dry sample (g)}} \times 100$$

……..... (2)

3.2.5.3 Crude protein

Crude protein content of the samples was determined using the micro-Kjeldahl method 920.87 (AOAC, 1995). The method consisted of three basic steps: i) digestion of the sample in sulfuric acid with a catalyst, which resulted in conversion of nitrogen to ammonia; ii) distillation of the ammonia into a trapping solution; and iii) quantification of the ammonia by titration with a standard solution. According to the method, % crude protein content of a sample = % nitrogen x 6.25. According to Pomeranz and Meloan (1994), it is generally assumed that a mixture of pure proteins will contain 16% nitrogen. Thus, the protein content of a sample was obtained by multiplying the determined nitrogen by the conversion factor: 6.25 = 100/16. This factor is commonly used for plant materials.

About 0.25 g of dried samples were weighed onto tared filter papers and quantitatively transferred into digestion tubes. About 10 g of catalyst (mixture of 10 g potassium sulphate, 0.5 g copper sulphate and 1.0 g titanium) were added into each tube with samples. Five ml of concentrated sulphuric acid were added to each tube. Samples were digested using Tecator digestion system 40 (Model 1016 digester, Sweden) for 3 hours to obtain a clear greenish solution. The digest was cooled and one tube at a time was mounted in the distillation unit (Foss Tecator, Model 2200 Kjeltec auto distilling unit, Sweden). Thirty ml of water were added to the digest followed by 30 ml of 40% sodium hydroxide and steam distilled for 3 minutes. About 50 ml of the distillate was collected in conical Erlenmeyer flask containing 20 ml of 2% Boric Acid. The distillate was thereafter titrated with 0.1N hydrochloric acid. Blank determination was carried out in the same manner using reagents without a sample.

Nitrogen content was calculated from the relationship:

$$\% \text{ Nitrogen } = \frac{1.4007 \times [titre(ml) - blank(ml)] \times Conc.of\ acid}{Weight\ of\ sample(g)} \quad \text{.....} \quad (3)$$

Percentage crude protein was calculated from the percentage nitrogen using the factor 6.25:

$$\% \text{ CP } = \% Nitrogen \times 6.25 \quad \text{.....} \quad (4)$$

3.2.5.3.1 Amino acid content

The essential amino acid content of the formulations was computed by using the USDA National Nutrient Database for Standard Reference (http://www.ars.sda.gov/ba/bhnrc/ndl). The amino acid scores were obtained by comparing the products' essential amino acids with the FAO/WHO/UNU (1985) reference patterns for pre-school age children. The essential amino acids reference pattern for pre-school age children was His 19, Ile 28, Leu 66, Lys 58, SAA (Meth + Cys) 25, AAA (Tyr + Phe) 63, Thr 34, Trp 11 and Val 35 (FAO/WHO/UNU, 1985).

3.2.5.4 Crude fibre content

Crude fibre was determined by using AOAC (1995) official method 920.86. Ankom fibre analyzer (Model ANKOM 220, USA) was used for the determination of crude fibre. Exactly 1.0 g of sample was first digested by dilute sulphuric acid (0.125M H_2SO_4) for 30 minutes and washed three times with hot water. The residues were then digested by dilute

alkali (0.125M KOH) for another 30 minutes and washed by hot water three times. Digested residues were dried in the oven for 12 h, cooled and weighed. The residues were then placed in muffle furnace and incinerated at 550°C for 2 h, cooled and weighed again. Total fibre content was taken as the difference between the residues before and after incineration.

$$\% \text{ Crude fibre} = \frac{W_1(g) - W_2(g)}{W(g)} \times 100 \quad \text{.....} \quad (5)$$

Where:

W_1 = Weight of sample residues before incineration (g)

W_2 = Weight of sample residues after incineration (g)

W = Weight of dry sample taken for determination (g)

3.2.5.5 Crude fat

Total fat was determined by Soxhlet ether extraction official method 920.85 (AOAC, 1995). The procedure involved continuous extraction of fat from the sample by light petroleum ether (40-60°C boiling point range) for eight hours. Petroleum ether was then evaporated and the weight of the crude fat determined.

Five grams of dry sample were placed into the extraction thimble, plugged with cotton wool and assembled to the soxhlet apparatus. One hundred ml of petroleum ether were used for continuous reflux for eight hours. Petroleum ether was then recovered by evaporation. Pre-weighed cups containing fat were dried in an oven at 80°C for 3 h to evaporate any remaining petroleum ether, cooled in a desiccator for 1 h and weighed. Percentage fat was calculated by using the formula:

% Crude fat = $\frac{Weight\ of\ crude\ fat(g)}{Weight\ of\ dry\ sample(g)} \times 100$ …….....

(6)

3.2.5.6 Carbohydrate

The carbohydrate content in this study was calculated as a percentage difference using the formula:

% Carbohydrate = 100 – (% protein + % crude fibre + % crude fat + % Ash)

3.2.5.7 Energy

Energy was calculated using the Atwater's conversion factors. Thus energy values were obtained by multiplying % fat by factor 9 and % protein and % carbohydrate by factor 4 each (AOAC, 1990).

Energy content = [(%Carbohydrate x 4) + (%Fat x 9) + (%protein x 4)] ……..…... (7)

3.2.5.8 Minerals

The samples of extruded and unprocessed products were incinerated and the ash was used for mineral analysis. The ash was dissolved in 6 N HCl and left for 12 h to allow extraction of minerals. The solution was filtered quantitatively into 100 ml volumetric flask. Mineral analysis for Calcium, Iron, Zinc, Magnesium, Potassium and copper was determined by using UNICAM Atomic Absorption Spectrophotometer (Model 919, Cambridge, U.K). Standards were set at highest sensitivity. On reading each corresponding lamp and wavelength were set. Zeroing was done after setting on flame and setting the wavelengths (Ca: Ca lamp, λ = 422 nm; Fe: Fe lamp, λ = 248 nm; Cu: Cu lamp, λ = 324 nm and Zn: Zn lamp, λ = 213 nm).

Mineral contents were calculated and expressed in mg/100 g on dry weight basis using the relationship:

Mineral content (mg/100g) = $\frac{GR}{1000\,ml} \times \frac{100\,ml}{SW} \times DF \times 100\,g$ ……..... (8)

Where;

GR = Absorbance

SW = weight of dry sample

DF = Dilution factor

3.2.6 The Bleach (Chlorox) test

The presence or absence of a pigmented testa in the sorghum grain was determined using the Bleach or Chlorox test (Waniska *et al.*, 1992). Chlorox reagent [5% NaOH in domestic bleach (3.5% sodium hypochlorite)] was added to 100 whole sorghum kernels just to cover the kernels. The beakers with sorghum kernels were then covered with aluminium foil. The containers were left for 20 min at room temperature with swirling every 5 min. The bleach solution was carefully poured off and the kernels were then tipped into small strainers, rinsed with tap water, dried by paper towel and the number of completely black kernels counted.

3.2.7 Tannin

Quantitative estimation of tannins was done using the modified vanillin-HCl method as described by Price *et al.* (1978). Blank determinations were used to counteract the effect of anthocyanins and other pigments in the samples. Tannin content was expressed as mg catechin equivalents per g (mg CE/g).

Tannin content of each blend was measured by the modified vanillin-hydrochloric acid method (Price *et al*., 1978). Exactly 0.25 g of dried sample was extracted with 10 ml of 4% HCL in methanol for 20 min at 22°C and the product centrifuged using a refrigerated centrifuge (Baird and Tatlock, Nottingham, UK). The supernatant was transferred into a volumetric flask and the residues were further extracted by 5 ml of 1% HCL in methanol on an electric shaker for 20 minutes. It was then centrifuged for 10 minutes at 4500 rpm. Supernatant aliquot was combined with the first one. To 1 ml of the extract, 5 ml of vanillin-HCL reagent was added. The reaction mixture was left for 20 min at room temperature (22ºC) after which the absorbance was read at 500 nm against a blank using a spectrophotometer (Wagtech, CE 2021, UK). The blank was prepared using 5 ml of 4% HCL in methanol. Tannin concentration in mg/g was extrapolated from a standard curve. Values were converted to mg/g using the relationship:

$$\text{Tannin content (mg/g)} = \frac{\text{OD500 x vol of extract}}{\text{Slope x Weight of sample}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(9)}$$

Whereby;

OD500 = Absorbance at 500 nm

Catechin standard solution was prepared by dissolving 1 mg of catechin into 60 ml of methanol in 100 ml volumetric flask and made to volume with methanol. Working standard solutions were prepared from the above stock solution. The pipetted standard solution was made to 1 ml with methanol before addition of Vanillin-HCl reagent.

3.2.8 Determination of urease actitivity

Residual urease activity was measured by the change in hydrogen ion concentration as described in the Tanzania national standard for processed cereal-based supplementary foods (TBS, 1983). About 0.2 g of the sample was weighed into a test tube containing 100

ml buffered urea solution, mixed and placed in a water bath at 30° C. Then, 1.0 g of sample was weighed into a small glass beaker and heated at 135°C for 30 minutes to inactivate urease enzyme. About 0.2 g of the inactivated sample was weighed into a test tube containing 100 ml buffered urea solution, mixed and placed in a water bath at 30° C. The test and blank test tubes were placed in the water bath at exactly 5 minutes intervals. The contents of both the sample and the blank test tubes were mixed every 5 minutes during the digestion period. At the end of 30 minutes, each test tube was removed from the water bath. Supernatant liquid was transferred to a 5 ml beaker and after exactly 5 minutes pH was determined. The difference between the pH of the test sample and that of the blank was taken as the measure of the urease activity present in the sample.

3.2.9 Water absorption index (WAI) and water solubility index (WSI) determination

WAI and WSI were determined in triplicate following the method described by Anderson (1982) with some modifications. About 0.5 g of each sample was suspended in 3 ml of distilled water and stirred intermittently over a 30 minute period in a water bath set at 30°C. Subsequently, the dispersions were centrifuged at 4000 g for 20 minutes. The supernatants were then poured into dry test tubes and stored overnight in an oven set at 100°C for the process of evaporation. The WAI is the weight of gel obtained after removal of the supernatant per unit weight of original dry solids. The amount of dried solids recovered from evaporating the supernantant was expressed as percentage of dry solids in the original dry sample.

WAI and WSI were calculated using the following equations:

$$\text{WAI} = \frac{Weight\ of\ gel}{Weight\ of\ dry\ sample} \quad \text{.....} \quad (10)$$

$$WSI = \frac{Weight\ of\ dissolved\ solids\ in\ supernatant}{Weight\ of\ dry\ sample} \times 100 \quad \text{.....} \quad (11)$$

3.2.10 Antioxidant capacity: DPPH radical-scavenging activity

3.2.10.1 Sample extraction

Methanol (80%) was used as a solvent. The extraction procedure for DPPH assay involved addition of 10 ml of solvent to a 1 g sample in 25 ml volumetric flask and the flask was shaken for 2 hours on a shaker at 30 rpm at room temperature (22ºC). Samples were then stored at -20 °C in the dark overnight to allow for maximum diffusion of phenolics from the cellular matrix. Samples were then removed from freezer and left to attain room temperature (22ºC). The supernatant was decanted. Each sample residue was rinsed with two additional 10 ml volumes of solvent with shaking by a laboratory shaker at 30 rpm for 5 min. The supernatant was decanted. The three aliquots were mixed and filtered using Whatman filter papers No. 1. The combined supernatants were used for analysis of free radical scavenging activity after being diluted to proper concentration with 80% methanol.

3.2.10.2 Determination of free radical scavenging activity

DPPH is a free radical, stable at room temperature, and produces a violet solution in methanol/ethanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncolored methanol/ethanol solution. The DPPH free radical-scavenging activity of extruded and non-extruded samples was determined with a stable radical, DPPH$^{\bullet}$, as described by Brand-Williams *et al.* (1995). A 0.1 mM solution of methanolic DPPH (1,1-Diphenyl-2-picryhydrazyl) solution was freshly prepared daily, stored in a flask covered with aluminium foil, and kept in the dark between the measurements. About 1.0 ml of this solution was added to 4.0 ml of each sample solution of different concencentration (four

serial dilutions: 0.125, 0.250, 0.375 and 0.5) and allowed to react at room temperature in the dark for thirty minutes. The free radical scavenging activity of ascorbic acid (Vitamin C) was also measured under the same conditions to serve as antioxidant agent (positive control). This was used to compare the biopotency of the sample extracts. The initial absorbance of the DPPH in ethanol was measured at 517 nm.

Absorbance of the DPPH/samples was measured at 517 nm at 0 min and after 30 min incubation at room temperature (22°C) in darkness. The EC_{50} values were calculated as the concentration of the compound which inhibited 50% of DPPH radical. The inhibition percentage of the absorbance of DPPH solution was calcultated using the relationship:

$$\% \text{ Inhibition} = [(Abs\ t0\,\text{min} - Abs\ t30\,\text{min}) / Abs\ t0\,\text{min}] \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (12)$$

Whereby, Abs t0 min represents the absorbance at the beginning of the measurement and Abs t30 min the absorbance after 30 min of incubation.

A calibration curve was constructed using Ascorbic acid, as an external standard with concentrations 0, 20, 40, 60, 80, 100 µg/ml (Appendix 2). Antioxidant activities were presented as EC_{50} i.e., the concentration of antioxidant required to cause 50% reduction in the original concentration of DPPH. Results were expressed as ascorbic acid equivalent. The EC_{50} values were used to compare the DPPH radical scavenging capacity of each sample extract.

3.2.11 Sensory evaluation

The extruded products and their controls were made into gruels and subjected to sensory evaluation which was done by a semi trained panel of 40 people. The panel consisted of 20

males and 20 females aged 21 to 31years. The panelists were students taking Bsc. and Masters degree programs at Sokoine University of Agriculture. Sensory evaluation was conducted to determine product acceptability. A 5-point Hedonic scale was used (Larmond, 1977). Rating scores were as follow: 5-like extremely, 4-like moderately, 3-neither like nor dislike, 2-dislike moderately and 1-dislike extremely (Appendix 1).

The gruels were placed in identical plastic cups coded in three digit random numbers. Samples were presented to the panelists in a cool environment. Sensory evaluation was conducted in the Food Science Laboratory of the Department of Food Science of Sokoine University of Agriculture. Panelists were asked to test each product at a time and express their degree of preference in relation to the following sensory attributes: colour, aroma, taste, mouth feel and overall acceptability. The degree of preference was converted into numerical scores ranging from 1 to 5, with 1 as dislike extremely and 5 as like extremely. After testing a product, the panelists were requested to rinse their mouth before testing the next product. To avoid fatigue, the panelists were allowed to test only six products per day. The remaining samples were tested on a different day by the same panelists.

3.2.12 Statistical analysis

The data obtained from proximate composition analysis, mineral determination, tannin determination, urease activity, WAI, WSI and sensory evaluation were subjected to one-way and two-way analysis of variance (ANOVA) where applicable, using CoStat statistical programme for Windows, version 6.311. The Duncan's Multiple Range Test was used to separate the means. Difference was considered to be significant at $p<0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSION

4.1 Overview

This chapter presents the results of the study. It is divided into four major sections. Section one gives the nutritional value of the extruded and unextruded food products. The second section looks at the residual levels of tannin and urease activity after extrusion while the third section highlights on the physical and functional properties of the products with focus on water absorption index (WAI), water solubiltity index (WSI) and anti-oxidant activity. The fourth section describes the sensory quality and acceptability of the gruels made from the extruded and unextruded products.

4.2 Chemical Composition of the Formulated Products

Nutrient composition of the formulated extruded and unextruded sorghum-soybean supplementary products was determined. The chemical composition (g/100 g dry weight) of the various products are summarized in Table 4.

4.2.1 Dry matter

Dry matter ranged from 89.96 to 95.23 (Table 4). The lowest dry matter was observed in the plain unextruded white sorghum flour (WS). Among the composite products, RSSSar-L had the lowest content of dry matter (92.3%). The highest dry matter content was observed in unextruded germinated red sorghum-soybean (GRSS-C). Dry matter ranged from 92.26 to 94.58 in the extruded composite supplementary products. This corresponds to a moisture content of 7.7% to 5.4% which is below the maximum level (8.0%) set by TBS (1983) for processed cereal-based supplementary foods. These results are in line with moisture content of less than 7.0% for extrudates obtained in a study by Filli *et al*. (2010).

The moisture contents of all composite flour samples except for plain white sorghum flour (WS) which served as a control, were below 10.0%. Such low moisture content of flours prevents microbial activity and extends the shelf life of the products (Tizazu *et al*., 2010; Kikafunda *et al*., 2006). The low moisture values of the products indicate that they would have good keeping quality. Generally germinated red sorghum soybean (GRSS) formulations, regardless of extrusion temperature, had the highest dry matter content among the formulations (Table 5). This may partly be attributed to the sun drying of germinated sorghum grains after germination. For the extrusion temperatures, samples that were extruded at high temperature (140^{o}C) had the highest dry matter content (Table 5).

4.2.2 Energy content

Low energy density in supplementary foods has been pointed out as a major cause of poor growth and undernutrition among children in developing countries (Dewey and Brown, 2003; Afoakwa *et al*., 2004). Formulation of composite food mixtures containing cereal, legumes, oilseeds and animal products has been proposed as the most practical and sustainable approach for improving the energy, protein, macronutrient and micronutrient status of supplementary foods in developing countries (WHO/UNICEF, 1998; Dewey and Brown, 2003). Enrichment of sorghum-based formulations with soybean and ground sardines significantly improved ($p < 0.05$) their energy densities (Table 4). Energy content ranged from 388.48 kcal/100 g to 424.06 kcal/100 g as shown in Table 4. The highest energy content was observed in unextruded red sorghum-soybean control (RSS-C) while the lowest energy content was observed in unextruded plain red sorghum (RS-C). Apart from the plain sorghum products, all the composite supplementary products had energy content above 400 kcal/100g which is the minimum value specified by Codex Alimentarius Standard CAC/GL 08-1991 (FAO/WHO, 1994a). Generally, the formulations with high proportions of soybean (sorghum-soybean formulations) had higher energy

Table 4: Proximate composition (g/100g), energy content (kcal/100 g) and protein-energy(%) of the various products [1,2]

Product[3,4]	Dry matter	Protein	Fat	Fibre	Ash	Carbohydrate	Energy	Protein-energy
RSS-H	93.80±0.39^{c}	19.44±0.26^{ef}	6.02±0.18^{e}	2.64±0.37^{bc}	1.80±0.02^{c}	70.09±0.30^{h}	412.33±0.70^{e}	18.85±0.23^{cde}
RSS-L	93.24±0.16^{def}	18.97±0.04^{fgh}	4.56±0.26^{g}	2.68±0.10^{bc}	1.93±0.08^{b}	71.86±0.47^{f}	404.37±0.59^{gh}	18.76±0.01^{def}
RSS-C	93.09±0.05^{f}	20.06±0.19^{bc}	8.27±0.12^{a}	2.53±0.31^{bcd}	1.79±0.01^{c}	67.35±0.38^{j}	424.06±1.80^{a}	18.92±0.26^{cde}
GRSS-H	93.31±0.01^{def}	20.02±0.09^{bcd}	6.16±0.17^{e}	2.83±0.25^{b}	1.73±0.01^{cd}	69.25±0.02^{i}	412.55±1.80^{e}	19.41±0.00^{bc}
GRSS-L	93.18±0.01^{ef}	18.83±0.21^{gh}	5.35±0.07^{f}	2.71±0.05^{b}	1.66±0.02^{d}	71.45±0.12^{fg}	409.28±0.22^{f}	18.41±0.22^{ef}
GRSS-C	95.23±0.11^{a}	19.25±0.05^{efg}	7.88±0.07^{b}	3.60±0.14^{a}	1.62±0.01^{d}	67.65±0.15^{j}	418.48±0.21^{c}	18.40±0.04^{ef}
RSSSar-H	93.26±0.03^{def}	18.11±0.70^{i}	4.43±0.27^{g}	1.61±0.11^{e}	2.34±0.09^{a}	73.52±0.77^{e}	406.36±2.16^{g}	17.82±0.59^{g}
RSSSar-L	92.26±0.01^{g}	17.38±0.18^{j}	3.91±0.09^{h}	1.58±0.05^{e}	2.28±0.05^{a}	74.85±0.37^{d}	404.11±0.06^{h}	17.20±0.18^{h}
RSSSar-C	93.33±0.38^{def}	17.36±0.24^{j}	6.47±0.07^{d}	2.86±0.12^{b}	2.28±0.02^{a}	71.03±0.21^{g}	411.76±0.75^{e}	16.87±0.20^{h}
WSS-H	92.62±0.02^{g}	19.66±0.22^{cde}	2.87±0.15^{i}	0.90±0.05^{f}	1.05±0.04^{f}	75.52±0.09^{d}	406.54±0.76^{g}	19.35±0.26^{bc}
WSS-L	93.11±0.13^{ef}	20.27±0.13^{b}	4.65±0.07^{g}	0.91±0.01^{f}	1.04±0.01^{f}	73.13±0.05^{e}	415.45±0.33^{d}	19.52±0.14^{b}
WSS-C	92.55±0.56^{g}	21.60±0.39^{a}	7.02±0.02^{c}	2.33±0.22^{cd}	1.09±0.00^{f}	67.96±0.59^{j}	421.46±0.97^{b}	20.50±0.42^{a}
WSSSar-H	93.55±0.07^{cde}	18.59±0.07^{hi}	1.85±0.05^{j}	0.61±0.02^{fg}	1.48±0.04^{e}	77.46±0.14^{c}	400.91±0.20^{i}	18.55±0.06^{ef}
WSSSar-L	93.64±0.00^{cd}	19.47±0.34^{def}	2.87±0.23^{i}	0.70±0.01^{fg}	1.44±0.15^{e}	75.52±0.27^{d}	405.81±1.78^{gh}	19.20±0.42^{bcd}
WSSSar-C	92.27±0.13^{g}	18.84±0.07^{gh}	4.70±0.08^{g}	1.32±0.25^{e}	1.46±0.04^{e}	73.68±0.21^{e}	412.35±0.44^{e}	18.27±0.04^{fg}
RS-H	94.58±0.04^{b}	8.52±0.30^{l}	0.59±0.05^{kl}	0.80±0.02^{fg}	1.63±0.05^{d}	88.46±0.42^{a}	393.21±0.03^{k}	8.67±0.30^{j}
RS-L	93.33±0.04^{def}	8.15±0.17^{l}	0.64±0.02^{kl}	0.71±0.01^{fg}	1.66±0.02^{d}	88.85±0.11^{a}	393.74±0.04^{k}	8.28±0.17^{j}
RS-C	90.22±0.01^{h}	8.47±0.05^{l}	0.87±0.05^{k}	2.18±0.08^{d}	1.78±0.02^{c}	86.69±0.09^{b}	388.48±0.65^{l}	8.73±0.07^{j}
WS	89.96±0.01^{h}	9.78±0.12^{k}	0.46±0.02^{l}	0.46±0.17^{g}	0.46±0.02^{g}	88.85±0.04^{a}	398.61±0.8^{j}	9.82±0.10^{i}

[1] Means ± SD based on triplicate determinations.
[2] Means within a column with different superscripts are significantly different at $p<0.05$
[3] RSS =Red sorghum-soybean; GRSS =Germinated red sorghum-soybean; RSSSar =Red sorghum-soybean-sardine; WSS =White sorghum-soybean; WSSSar=White sorghum-soybean-sardine; RS =Plain Red sorghum; WS = Plain white sorghum
[4] H =extruded at high temperature (140^{o}C); L =extruded at low temperature (100^{o}C); C =Unextruded (control

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density. This was because soybean is rich in fat and protein which contribute to energy density.

RSS, GRSS and WSS formulations had the highest energy density while plain red sorghum (RS) had the least energy density. Incorporation of soybeans and sardines in the formulations significantly increased the energy content ($p < 0.05$). For the extrusion temperatures, the products that were not extruded had higher energy density ($p < 0.05$) compared with products that were extruded at 140°C (H) and 100°C (L) (Table 5).

High dietary energy is essential for sparing protein from being used as a source of energy. It is recommended that, in order for the dietary protein to be used for the intended purpose of building and repairing body tissues, the proportion of protein to energy in the supplementary foods must not be less than 15% (Cameron and Hofvander 1976; Pellett and Mamabranchi, 1978). The percent of protein energy in sorghum-soybean composite foods ranged from 16.87% in RSSSar-C to 20.5% in WSS-C (Table 4). The percent of protein-energy in plain red sorghum: RS-L (8.28%), RS-H (8.67%) and RS-C (8.73%) were significantly lower ($p < 0.05$) than the level (15%) recommended by the FAO/WHO (1994a), Codex Alimentarius Commission and the FAO/WHO/UNU (1985). Among the formulations, WSS (white sorghum-soybean products) had the highest protein-energy percent (Table 5). There was no significant difference in protein energy values among RSS, GRSS and WSSSar formulations ($p > 0.05$). RS (plain red sorghum products) had the least protein energy (Table 5). Overall, the extrusion temperatures did not cause any significant difference ($p < 0.05$) in protein energy values among the formulations (Table 5).

Table 5: Multiple mean comparison of proximate composition (g/100g) between formulations and extrusion temperature[1,2]

Factors	Dry matter	Protein	Fat	Fibre	Ash	CHO	Energy	Protein-energy (%)
Formulation[3]								
WSS	92.76±0.38^{d}	20.51±0.91^{a}	4.85±1.87^{c}	1.38±0.74^{d}	1.06±0.03^{e}	72.20±3.47^{d}	414.48±6.74^{a}	19.79±0.60^{a}
WSSSar	93.15±0.69^{bc}	18.97±0.44^{c}	3.14±1.29^{d}	0.88±0.37^{e}	1.46±0.07^{d}	75.55±1.70^{b}	406.36±5.20^{b}	18.67±0.46^{b}
RSS	93.37±0.38^{b}	19.49±0.51^{b}	6.29±1.67^{b}	2.62±0.23^{b}	1.84±0.08^{b}	69.77±2.05^{e}	413.59±8.90^{a}	18.85±0.17^{b}
GRSS	93.90±1.03^{a}	19.37±0.55^{b}	6.46±1.16^{a}	3.05±0.45^{a}	1.67±0.05^{c}	69.45±1.71^{e}	413.44±4.25^{a}	18.74±0.53^{b}
RSSSar	92.95±0.56^{cd}	17.62±0.51^{d}	4.94±1.21^{c}	2.02±0.66^{c}	2.30±0.06^{a}	73.13±1.78^{c}	407.41±3.66^{b}	17.30±0.52^{c}
RS	92.71±2.01^{d}	8.38±0.24^{e}	0.70±0.14^{e}	1.23±0.74^{d}	1.69±0.08^{c}	88.00±1.05^{a}	391.81±2.61^{c}	8.56±0.27^{d}
Extrusion temperature[4]								
H	93.52±0.63^{a}	17.39±4.20^{ab}	3.65±2.17^{b}	1.57±0.94^{b}	1.67±0.40^{a}	75.72±6.66^{a}	405.32±7.07^{b}	17.11±3.99^{a}
L	93.13±0.45^{b}	17.18±4.31^{b}	3.67±1.63^{b}	1.55±0.90^{b}	1.67±0.41^{a}	75.94±6.22^{a}	405.46±6.83^{b}	16.89±4.10^{a}
C	92.78±1.57^{c}	17.60±4.47^{a}	5.87±2.62^{a}	2.47±0.74^{a}	1.67±0.38^{a}	72.39±7.08^{b}	412.76±12.28^{a}	16.95±4.00^{a}

[1] Means ± Standard deviation
[2] Means within a column with different superscripts are significantly different at $p<0.05$
[3] RSS =Red sorghum-soybean; GRSS = Germinated red sorghum-soybean; RSSSar = Red sorghum-soybean-sardine; WSS = White sorghum-soybean; WSSSar = White sorghum-soybean-sardine; RS = Plain Red sorghum.
[4] H =High extrusion temperature (140ºC); L =Low extrusion temperature (100ºC); C =Unextruded (control)

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4.2.3 Carbohydrate content

Carbohydrate content of the composite formulations ranged from 67.35 g/100 g DM in RSS-C to 77.46 g/100 g DM in WSSSar-H. Carbohydrate content of the plain sorghum product ranged from 86.69 g/100 g DM (RS-C) to 88.85 g/100 g DM (RS-L) (Table 4). The plain sorghum products had higher carbohydrate content compared to the composite products. All products containing high proportions of sorghum (RS-H, RS-L, RS-C and WS) had higher carbohydrate content compared to those that had lower proportions of sorghum. This agrees with the observation that, addition of legumes decreases the carbohydrate content of cereal-based traditional foods (Sefa-Dedeh *et al*., 2001). WSSSar and RSSSar (sorghum-soybean-sardine) composite products had significantly higher carbohydrate content ($p < 0.05$) compared to WSS, RSS and GRSS (sorghum-soybean products) (Table 5). This is because WSSSar and RSSSar had a higher proportion of sorghum in their formulations (Table 3). Extrusion temperatures had a significant effect on the carbohydrate content of the supplementary products (Table 5). The formulations that were extruded at 140^{o}C (H) and 100^{o}C (L) had significantly higher carbohydrate content ($p < 0.05$) than the formulations that were not extruded. The carbohydrate content in all composite supplementary products was above the minimum requirement specified in the Tanzania standard (TBS, 1983) for processed cereal-based supplementary foods (48.91 g/100 g DM).

4.2.4 Crude fat content

Fat concentration in the products ranged from 0.46 g/100 g DM in WS (plain white sorghum) to 8.27 g/100 g DM in RSS-C (unextruded red sorghum-soybean) (Table 4). Extruded and unextruded plain red sorghum and white sorghum had significantly lower fat content ($p < 0.05$) than the composite products. Fat content increased with increase in soybean proportions in the formulations. This can be explained by the high fat content in

soybean. The increment in fat content ranged between 302 and 1426% in composite white sorghum-based products and 349–851% in red sorghum-based composite products. Fats enhance the taste and acceptability of foods. Dietary lipids provide essential fatty acids and facilitate the absorption of fat-soluble vitamins (FAO/WHO, 1994b; Uauy *et al*., 2000). Fats also increase the energy density of the gruels. However, fat content that is too high is not recommended because it dilutes the density of protein and micronutrients per 100 kcal (Innis, 1991; Uauy *et al*., 2000). Red sorghum-based composite products had higher fat content compared to the white sorghum-based composite products. This was mainly because the plain red sorghum had higher fat content ($p < 0.05$) than plain white sorghum. Extrusion had an effect on fat content of the formulations (Table 5). Formulations that were not extruded (C) had significantly higher fat content ($p < 0.05$) than the formulations that were extruded at 140ºC and 100ºC. Therefore extrusion resulted in reduction of fat content. This is in agreement with a study by Davis (2004) whereby soybean flour was extruded to reduce fat content. With the exception of unextruded composite product (RSS-C), all the other unextruded and extruded composite products did not exceed the maximum limit for total fat (8.15 g/100 g DM) recommended by TBS (1983) hence meeting the requirement.

4.2.5 Ash content

Ash content ranged from 0.46 g/100 g DM in plain white sorghum unextruded flour (WS) to 2.34 g/100 g DM in red sorghum-soybean-sardine composite flour extruded at 140ºC (RSSSar-H) (Table 4). Ash content was higher in the sardine and soybean enriched composites than in the plain red and white sorghum products. This implies that soybeans and sardines are rich in inorganic nutrients. Plain red sorghum flour and red sorghum-based composite products had higher ash content ($p < 0.05$) than plain white sorghum and white sorghum-based products. Since white sorghum was dehulled, this could have led to

loss of some minerals and nutrients that are concentrated in the testa and outer pericarp. The red sorghum was not dehulled because it had soft floury endosperm which could have resulted in low decorticated yield. Red sorghum is known to have soft kernels that fragment easily resulting in higher endosperm loss to the bran when dehulled (Beta *et al*., 1999). Germinated red sorghum-based composite products (GRSS-H, GRSS-L and GRSS-C) had lower ash content compared to ungerminated red sorghum-based products (RSS-H, RSS-L and RSS-C).

This implied that, the germination process could have led to loss of some inorganic materials especially during steeping. Composite products that were enriched with milled sardines for both white sorghum and red sorghum-based products had higher ash content than the composite products that incorporated only soybean. The level of ash in food is an important nutritional indicator for mineral density and also a quality parameter for contamination, especially with foreign matter (Fennema, 1996). Among the formulations, red sorghum-soybean-sardine (RSSSar) formulations had the highest ash content ($p < 0.05$) (Table 5). Overall, extrusion at 140ºC (H) and 100ºC (L) did not result in significant difference ($p > 0.05$) in ash content among the formulations (Table 5). All the plain and composite products did not exceed the maximum level (5.43 g/100 g DM) for crude fiber recommended by TBS (1983).

4.2.6 Crude fibre

Results in Table 4 show significant variations ($p < 0.05$) in crude fibre content among the various products. The fiber content ranged from 0.61 g/100 g DM in white sorghum-soybean-sardines product extruded at 140ºC (WSSSar-H) to 3.60 g/100 g DM in germinated red sorghum-soybean that was not extruded (GRSS-C). Higher fibre content was observed in products containing red sorghum, e.g. red sorghum-soybean (RSS-L)

product extruded at 100ºC (L) had 2.68 g/100 g DM while the white sorghum-soybean (WSS-L) product extruded at 100ºC (L) had a fibre content of 0.91 g/100 g DM. Likewise, RSSSar-H had a fibre content of 1.61 g/100 g DM whereas WSSSar-H had a fibre content of 0.61 g/100 g DM. This could be because red sorghum was not dehulled whereas the white sorghum was dehulled. The seed hulls might have contributed to high fibre content in red sorghum-based composite formulations. Dehulling removes the hulls, which contain much fibre (Kikafunda *et al.*, 2006). Among the formulations, germinated red sorghum-soybean (GRSS) had the highest fibre content ($p < 0.05$) followed by red sorghum-soybean (RSS) (Table 5). Products made up of white sorghum-soybean-sardine blend (WSSSar) had the lowest fibre content. The results also show that, the formulations with high proportions of soybean had high fibre content. The products that were not extruded had higher fibre content than products that were extruded. Overall, there was no significant difference ($p > 0.05$) in fibre content between products that were extruded at 140ºC (H) and 100ºC (L) (Table 5). This implied that, apart from temperature, other factors such as high shear and mechanical transformations that the food materials goes through during extrusion could have caused reduction in the fibre content. Fibre is an important dietary component in the diet for adults as it is known to play a role in prevention of constipation, overweight, cardiovascular diseases, diabetes and colon cancer (Whitney *et al.*, 1990). However, too much fibre in the diet is not good for older infants and young children. Fiber has been reported to increase dietary bulkiness, hence limiting adequate food intake by infants and young children (Hofvander and Underwood, 1987). FAO (1985) Codex Committee recommended that, supplementary foods should not contain more than 5 g of crude fiber per 100 g of dry edible matter. The crude fiber contents in the products were in the range of 0.61–2.33 g/100 g DM in the white sorghum-based products and 0.71–3.60 g/100 g DM in the red sorghum-based products, lower than the maximum level (5 g/100 g) recommended by the FAO (1985) Codex Committee for supplementary foods.

4.2.7 Protein content

There was a significant difference in crude protein content among the different food products ($p < 0.05$). The protein content ranged from 8.15 g/100 g DM in RS-L to 21.60 g/100 g DM in WSS-C (Table 4). Soybeans contain 40 – 45 g/100 g crude protein on a dry-weight basis (Yagasaki *et al*., 1997). Thus formulations with higher proportions of soybean flour had higher protein content. Similar results using different types of legumes was reported in a study by Mosha and Vicent (2005). Although the protein content in plain red and white sorghum was low, both in extruded and unextruded flour, complementation of the sorghum flour with soybean and sardine significantly increased the protein content in the composite mixtures. This increase in protein content of the composite products was not unexpected since sardine and soybean are rich in protein. The increase in the protein content and energy values of foods that have been enriched with soybeans has been reported by many investigators (Plahar *et al*., 2003; Bangoura and Zhou, 2007; Martin *et al*., 2010). In the unextruded red sorghum-based formulations, protein content increased by 137, 127 and 105% in RSS-C (red sorghum-soybean), GRSS-C (germinated red sorghum-soybean) and RSSSar-C (red sorghum-soybean-sardine), respectively.

With the same proportions of soybean and sardines incorporated in their formulations, the white sorghum-based foods had higher protein content than the red sorghum-based foods; RSS-C (20.06 g/100 g DM) versus WSS-C (21.60 g/100 g DM), RSS-L (18.97 g/100 g DM) versus WSS-L (20.27 g/100 g DM), RSS-H (19.44 g/100 g DM) versus WSS-H (19.66 g/100 g DM), RSSSar-C (17.36 g/100 g DM) versus WSSSar-C (18.84 g/100 g DM), RSSSar-L (17.38 g/100 g DM) versus WSSSar-L (19.47 g/100 g DM). The high protein content in white sorghum compared to red sorghum may partly explain why the protein content in white sorghum-based foods was higher than the red sorghum-based products. The foods that incorporated sardines had lower protein content compared to the

foods that incorporated only soybean. This was because the proportion of sardines in their formulations was fairly small (5%) (Table 3), and it was purposely done to avoid problems in storage of the final products since sardines are known to have poor keeping quality. However, the inclusion of milled sardines in the formulations was nutritionally beneficial because fish have a high-quality protein which is more digestible compared with the plant protein in soybean. Addition of sardines also enhanced mineral density of the foods. Among the formulations, White sorghum-soybean (WSS) had the highest protein content and the products that were not extruded, that is kept as control, had slightly higher protein content compared to the extruded ones (Table 5). Protein is an essential macronutrient for the growth and maintenance of body tissues and its requirement is highest during the first year of life (FAO/WHO/UNU, 1985). All the composite foods had protein content that was above the minimum stipulated in the TBS Tanzania standard (TBS, 1983) for processed cereal-based supplementary foods (15.22 g/100 g DM) and 15 g per 100 g specified by Codex Alimentarius Standard CAC/GL 08-1991 (FAO/WHO, 1994a).

4.2.7.1 Protein quality

Table 6 summarizes the profile of both essential and non-essential amino acids in the formulations. Protein quality is influenced by the concentration of essential amino acids making up the protein. The greater the proportion of the essential amino acids, the greater is the biological quality. Proteins that are deficient in one or more of the essential amino acids are of poor quality and this is usually reflected in their amino acid scores. Proportionality pattern of amino acids in foods is the most important determinant of protein quality (FAO/WHO, 1991). Table 6 shows the proportion of the various essential amino acids in the formulations relative to FAO/WHO/UNU (1985) reference pattern for pre-school age children.

FAO/WHO/UNU (1985) recommended that, the dietary amino acid requirements for infants should be met by breast milk. The reference pattern for pre-school age children has been used for the formulations meant for supplementary feeding. The formulations were designed to maximize the amino acid scores as recommended by the FAO/WHO/UNU (1985). The amino acid score reflects the ability of the test protein to meet the protein needs of an individual and thus the ability to support optimal growth. The most limiting amino acid in the formulations was lysine whose amino acid scores are shown in Table 6. Lysine, SAA, tryptophan and threonine are the most common limiting amino acids in plant-based supplementary foods in developing countries (Kavishe, 1993; Millward, 1999). The combination of sorghum with sardines and soybeans (rich in lysine) helped to increase the protein quality of the products through nutrient complementation.

A study by Mosha (2004) showed that, thermal-processing methods including extrusion did not cause significant loss of the total amino acids in supplementary foods meant for rehabilitating undernourished populations in Tanzania. Likewise, a study by Bahnassey *et al*. (1986) showed that, thermo-processing of cereal- legume blends of pasta did not have any adverse effect on the amino acid composition. With the exception of plain sorghum (RS), all the composite formulations contained amino acid concentrations with amino acid scores that exceeded the FAO/WHO/UNU (1985) recommended score of ≥ 65%. The composite formulations therefore would be suitable for use as food supplements for older infants and young children.

Table 6: Amino acid composition (mg g^{-1} crude protein) of the various formulations

Amino Acids	Formulations[1]						
	WSS	**WSSSar**	**RSS**	**RSSSar**	**GRSS**	**RS**	**FAO[2]**
Asp	105	96	105	96	105	66	
Glu	216	205	216	205	216	216	
Ser	54	49	54	49	54	41	
Gly	42	40	42	40	42	31	
Hist	26	26	26	26	26	22	19
Arg	61	54	61	54	61	31	
Thr	40	39	40	39	40	31	34
Ala	71	74	71	74	71	91	
Pro	70	65	70	65	70	75	
Tyr	36	34	36	34	36	28	
Val	53	52	53	52	53	50	35
Ile	47	45	47	45	47	38	28
Leu	110	110	110	110	110	132	66
Trp	14	13	14	13	14	11	11
Lys	49	50	49	50	49	20	58
Phe	54	50	54	50	54	48	
SAA[3]	30	31	30	31	30	26	25
AAA[4]	89	84	89	84	89	77	63
AAS[5]	85	86	85	86	85	35	65
LAA[6]	Lys	Lys	Lys	Lys	Lys	Lys	

[1] WSS – White sorghum-soybean, WSSSar - White sorghum-soybean-sardine, RSS – Red sorghum-soybean, RSSSar - Red sorghum-soybean-sardine, GRSS - Germinated red sorghum-soybean, RS - Red sorghum-plain. [2] FAO/WHO/UNU (1985) essential amino acid reference pattern for pre-school age children. [3] SAA – sulfur containing amino acids – methionine + cysteine. [4] AAA – aromatic amino acids – phenylalanine + tyrosine. [5] AAS – amino acid scores (%) = (mg limiting amino acid per g of formulation protein/mg of amino acid per g of reference protein for pre-school age children) *100. [6] LAA – limiting amino acid based on the FAO/WHO/UNU (1985) reference pattern for preschool children.

4.3 Mineral Composition

Zinc and iron are two of the micronutrients that are most often deficient in developing countries, with children and women of reproductive age at a higher risk of such deficiencies (Gibson, 1994). The mineral contents (on dry weight basis) of the products (extruded and unextruded) are presented in Table 7. Potassium concentration was the highest in all products except in composite products containing sardines (RSSSar-H, RSSSar-L, RSSSar-C, WSSSar-H, WSSSar-L and WSSSar-C), in which Calcium was the highest. Copper values were the lowest in all products. Plain dehulled white sorghum flour (WS) was poor in all minerals. Consequently, white sorghum-based composite products were poor in mineral density compared to the red sorghum-based composite supplementary foods. This suggests that, there is a need to fortify the extruded supplementary products with vitamin-mineral premixes. Also, the high temperatures involved during extrusion are likely to destroy the heat labile essential vitamins in the products (Killeit, 1994).

4.3.1 Calcium

Results in Table 7 show that, calcium concentration was highest in red sorghum-soybean-sardine (RSSSar-H) extruded at 140ºC (272.37 mg/100 g DM) but lowest in plain unextruded white sorghum flour, WS (15.67 mg/100 g DM). Enrichment of the red and white sorghum with soybeans and/or ground sardines improved the Ca concentrations significantly ($p < 0.05$). Incorporation of both soybean and sardines in the formulations had the greatest effect in improving Ca concentration compared to incorporation of only soybean. Red sorghum-based composite products had higher concentration of Ca than white sorghum-based composite products (RSS versus WSS products; RSSSar versus WSSSar products). This was because plain red sorghum flour had higher concentration of Ca than plain white sorghum flour. Blending increased the concentration of Ca by 350–1495% (70.48–249.99 mg/100 g) in the white sorghum-based composite products and

275–1063% (87.98–272.37 mg/100 g) in the red sorghum-based composite products. Among the formulations, red sorghum-soybean-sardine (RSSSar) had the highest concentration of Ca followed by white sorghum-soybean-sardine (WSSSar) (Table 8). Overall, extrusion temperatures had an effect on Ca concentration of the formulations. The products that were extruded at 140ºC (H) had higher Ca concentration than the ones that were extruded at 100ºC (L) and those that were not extruded. Calcium is particularly essential for infants and young children for building up bones and teeth, muscles and nerves functioning, blood clotting and for immune defense (Whitney *et al*., 1990).

4.3.2 Magnesium

Magnesium is vital for the activity of more than 300 enzymes and plays an important role in neurochemical transmission and muscular excitability (Laires *et al*., 2004). Magnesium concentration differed significantly ($p < 0.05$) among the various composite products and plain sorghum (Table 7). Magnesium concentration was highest in the red sorghum-soybean (RSS-C) that was not extruded and lowest in plain unextruded white sorghum (WS) flour. Red sorghum-based composite products had higher concentration of magnesium than the white sorghum-based composite products. Magnesium concentrations were in the range of 123.22–132.78 mg/100 g in the red sorghum-based products and 13.16–55.54 mg/100 g in the white sorghum-based products. This could be attributed to the high concentration of magnesium in the plain red sorghum flour (RS-C) than in the white sorghum flour (WS). For red sorghum-based products, magnesium concentrations were not improved ($p > 0.05$) by the fortification of red sorghum with soybean and/or milled sardines. Red sorghum-soybean (RSS) formulations had the highest concentration of magnesium among the formulations (Table 8). Overall, the products that were extruded at high temperature (140ºC) seemed to have higher magnesium concentrations (Table 8).

PRODUCT[3,4]	Ca	Mg	K	Cu	Zn	Fe
RSS-H	101.36±0.08^{e}	126.60±0.61^{f}	222.70±1.49^{h}	0.72±0.01^{bc}	2.35±0.00^{de}	8.64±0.59^{b}
RSS-L	92.06±0.19^{g}	128.46±0.18^{cd}	242.30±0.71^{e}	0.68±0.00^{cd}	2.37±0.01^{cde}	8.28±0.11^{bc}
RSS-C	88.07±0.43^{h}	132.78±0.22^{a}	255.61±0.53^{c}	0.83±0.00^{a}	2.31±0.03^{e}	4.62±0.07^{def}
GRSS-H	94.40±0.23^{f}	131.60±0.69^{ab}	216.29±0.08^{i}	0.66±0.03^{d}	2.46±0.04^{c}	7.03±0.04^{c}
GRSS-L	87.98±0.37^{h}	127.07±0.17^{ef}	216.68±0.00^{i}	0.68±0.00^{cd}	2.30±0.01^{e}	7.20±0.07^{c}
GRSS-C	88.22±0.62^{h}	123.39±0.61^{g}	212.13±0.42^{j}	0.77±0.02^{b}	2.39±0.08^{cde}	3.69±0.04^{efg}
RSSSar-H	272.37±0.92^{a}	128.27±0.23^{cde}	216.65±0.19^{i}	0.55±0.00^{efg}	3.15±0.02^{a}	10.36±0.01^{a}
RSSSar-L	257.4±0.76^{b}	127.15±0.11^{def}	231.69±0.37^{f}	0.56±0.01^{efg}	3.10±0.01^{ab}	11.21±0.16^{a}
RSSSar-C	257.46±0.38^{b}	123.43±0.43^{g}	229.03±0.56^{g}	0.67±0.00^{cd}	3.02±0.09^{b}	5.15±0.06^{de}
WSS-H	70.48±0.21^{k}	54.44±0.71^{hi}	126.11±0.05^{l}	0.53±0.01^{fg}	1.61±0.01^{f}	5.36±0.03^{d}
WSS-L	72.36±0.17^{j}	53.46±0.41^{i}	138.61±0.09^{k}	0.58±0.01^{ef}	1.64±0.05^{f}	4.50±0.02^{def}
WSS-C	77.57±0.09^{i}	55.54±0.76^{h}	139.40±1.04^{k}	0.59±0.00^{e}	1.65±0.09^{f}	2.72±0.12^{gh}
WSSSar-H	242.5±0.80^{d}	42.37±1.72^{j}	74.14±1.80^{n}	0.38±0.05^{h}	2.37±0.05^{cde}	4.47±1.42^{def}
WSSSar-L	243.39±0.49^{d}	40.30±0.58^{k}	84.63±1.51^{m}	0.53±0.07^{fg}	2.40±0.04^{cde}	4.88±2.13^{de}
WSSSar-C	249.99±2.00^{c}	40.03±0.42^{k}	86.22±1.19^{m}	0.51±0.02^{g}	2.42±0.02^{cd}	3.23±0.93^{fg}
RS-H	24.97±0.03^{l}	131.11±0.05^{b}	277.60±0.02^{b}	0.35±0.02^{h}	1.43±0.08^{g}	4.31±0.11^{def}
RS-L	26.14±0.08^{l}	129.06±0.06^{c}	280.99±0.70^{a}	0.57±0.02^{ef}	1.41±0.01^{g}	4.32±0.05^{def}
RS-C	23.41±0.22^{m}	123.22±0.46^{g}	250.50±0.19^{d}	0.58±0.01^{ef}	1.34±0.00^{g}	3.33±0.19^{fg}
WS	15.67±0.22^{n}	13.16±0.54^{l}	48.69±0.09^{o}	0.20±0.00^{i}	0.49±0.01^{h}	1.71±0.04^{h}

Table 7: Mineral composition of supplementary products (mg/100g DM)[1,2]

[1] Means ± Standard deviation
[2] Means with n with different superscripts are significantly different at p<0.05
[3] RSS =Red s ybean; GRSS = Germinated red sorghum-soybean; RSSSar = Red sorghum-soybean-sardine;
WSS = Wh n- soybean; WSSSar = White sorghum-soybean-sardine; RS = Plain Red sorghum; WS = Plain white sorghum
[4] H =extrude mperature (140ºC); L =extruded at low temperature (100ºC); C =Unextruded (control)

4.3.3 Iron

Iron concentrations in the plain soghum flours and composite products ranged from 1.71 mg/100 g in plain white sorghum (WS) to 11.21 mg/100 g in red sorghum-soybean-sardine composite product extruded at 100°C (RSSSar-L) (Table 7). Addition of soybean and/or milled sardines increased significantly ($p < 0.05$) the concentration of Fe in the composite products. Overall, RSSSar (extruded and unextruded) had the highest concentration of iron ($p < 0.05$), followed by RSS (Table 8). The products that were extruded at 100°C (L) and 140°C (H) had higher concentration of iron ($p < 0.05$) than products that were not extruded. The higher concentration of iron in extruded products compared to the non-extruded products could not easily be explained but it is likely due to contamination during extrusion rather than to intrinsic iron. Guy (2001) reported that, high-fiber foods may abrade the interior of the extruder barrel and screws resulting in increased mineral content. Increased iron content and even increased iron availability in some extruded products has been reported elsewhere (Hazell and Johnson, 1989). The levels of Fe observed in this study were comparable with those reported in other findings (WHO/UNICEF, 1998; Mosha *et al.* 2000; Dewey and Brown, 2003). With the exception of plain sorghum products (WS and RS-C) and unextruded composite products (WSS-C, WSSSar-C and GRSS-C) (Table 7), all other composite products had iron levels that were within the recommended levels (4–8 mg/100 g) in supplementary foods (FAO/WHO 1994a). Iron deficiency is the most important cause of nutritional anaemia (Hallberg and Hulthen, 2000). Iron is vital for transporting oxygen in the bloodstream and for preventing anaemia. Iron also serves as an integral part of important enzyme systems in various tissues and as a transport medium for electrons within cells. For growing children, the need for iron increases with rapid growth and expansion of blood volume and muscle mass (Russell, 2001).

4.3.4 Copper

Copper is essential in the absorption and utilization of iron during haemoglobin and myoglobin biosynthesis and forms part of several enzyme systems (King and Burgess, 1993). For the composite products, copper concentration ranged from 0.38 mg/100 g DM in white sorghum-soybean-sardine (WSSSar-H) extruded at 140^{o}C to 0. 83 mg/100 g DM in red sorghum-soybean (RSS-C) that was not extruded (Table 7). In plain sorghum products, copper concentration was 0.2 mg/100 g in WS, 0.35 mg/100 g in RS-H, 0.57 mg/100 g in RS-L and 0.58 mg/100 g in RS-C. Red sorghum-based composite products had higher copper concentration than the white sorghum-based composite supplementary products. The composite products with higher proportions of soybean in their formulations (RSS, WSS, and GRSS) had higher concentration of copper ($p < 0.05$) than the ones that had lower proportions of soybean in their formulations (RSSSar and WSSSar). This implied that, soybean used was high in copper concentration and contributed to the improvement of copper concentration in the composite products. Among the formulations, red sorghum-soybean (RSS) and germinated red sorghum-soybean (GRSS) formulations had significantly ($p < 0.05$) higher concentration of copper (Table 8).

Extrusion affected the concentration of copper in the products. The products that were not extruded had significantly higher concentration of copper ($p < 0.05$) than the products that were extruded at both 100^{o}C (L) and 140^{o}C (H) (Table 8).

In light of the above results, all the composite products contained adequate amounts of copper that were above the minimum recommended amount of 0.16 mg/100 g for supplementary foods (WHO/UNICEF, 1998). Increased dietary intake of Cu along with Fe in the supplementary foods may have a beneficial effect of enhancing Fe uptake and utilization.

Table 8: Multiple mean comparison of mineral composition (mg/100 g) between formulations and extrusion temperature[1,2]

Factors	Ca	Mg	K	Cu	Zn	Fe
Formulation[3]						
RSSSar	262.41±7.73^{a}	126.28±2.27^{c}	225.79±7.19^{c}	0.596±0.06^{c}	3.09±0.07^{a}	8.91±2.93^{a}
WSSSar	245.29±3.79^{b}	40.90±1.41^{e}	81.66±5.99^{f}	0.473±0.09^{e}	2.4±0.04^{b}	4.19±1.44^{d}
RSS	93.83±6.16^{c}	129.28±2.85^{a}	240.2±14.83^{b}	0.742±0.07^{a}	2.34±0.03^{b}	7.18±2.01^{b}
GRSS	90.2±3.28^{d}	127.35±3.70^{b}	215.03±2.26^{d}	0.702±0.05^{b}	2.39±0.08^{b}	5.97±1.77^{c}
WSS	73.47±3.29^{e}	54.48±1.06^{d}	134.71±6.68^{e}	0.565±0.03^{d}	1.63±0.05^{c}	4.2±1.20^{d}
RS	23.84±1.23^{f}	127.80±3.67^{b}	269.70±14.95^{a}	0.497±0.12^{e}	1.39±0.05^{d}	3.99±0.52^{d}
Temperature[4]						
H	134.35±94.84^{a}	102.4±40.09^{a}	188.91±70.90^{c}	0.532±0.14 ^{c}	2.23±0.60^{a}	6.70±2.37^{a}
L	129.89±91.85^{c}	100.92±40.11^{b}	199.15±69.68^{a}	0.599±0.06 ^{b}	2.20±0.58^{a}	6.73±2.67^{a}
C	130.79±93.67^{b}	99.73±38.81^{c}	195.48±64.85^{b}	0.657±0.12 ^{a}	2.19±0.58^{a}	3.79±0.92^{b}

[1] Means ± Standard deviation
[2] Means within a column with different superscripts are significantly different at $p<0.05$
[3] RSS =Red sorghum-soybean; GRSS = Germinated red sorghum-soybean; RSSSar = Red sorghum-soybean-sardine; WSS = White sorghum-soybean; WSSSar = White sorghum-soybean-sardine; RS = Plain Red sorghum.
[4] H =High extrusion temperature (140^{o}C); L =Low extrusion temperature (100^{o}C); C =Unextruded (control)

4.3.5 Zinc

Results in Table 7 show the concentration of zinc in the composite products and plain soghum flour. The highest zinc concentration (3.15 mg/100 g) was observed in RSSSar-H while the lowest zinc concentration (0.49 mg/100 g) was observed in WS. In general, red sorghum-based composite products had higher concentration of zinc than white sorghum-based composite products. Zinc concentrations were significantly higher ($p < 0.05$) in the products that were enriched with milled sardines. Addition of ground sardines to the composite formulations was therefore helpful in improving the Zn status of the products. The analysis of variance showed that, among the formulations, red sorghum-soybean-sardine products (RSSSar) had the highest ($p < 0.05$) concentration of zinc. Extrusion temperatures did not cause any significant variation ($p > 0.05$) in zinc concentration in the products (Table 8). Zinc concentrations in all red sorghum-based composite food products and sardine-enriched white sorghum-based composite foods (WSSSar) were above the minimum recommended amount of 2 mg/100 g for supplementary foods (FAO/WHO 1994a).

The plain red and white sorghum flours (RS-H, RS-L, RS-C, WS) and white sorghum-based composite products (WSS-H, WSS-L, WSS-C), with the exception of those enriched with milled sardines, did not meet the recommended level of zinc concentration (2 mg/100 g DM). In children, zinc deficiency has been shown to lead to poor growth (Brown *et al*., 2002), impaired immunity, increased morbidity from common infectious diseases and increased mortality (Sazawal *et al*., 1998). Zinc is required as a component of more than 200 enzymes and as a structural component of many proteins, hormones, hormone receptors and neuropeptides. Zinc also plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity (Shanker and Prasad, 1998).

4.4 Residual Levels of Tannin Content and Urease Activity

4.4.1 Chlorox bleach test

The bleach test is a rapid method which qualitatively identifies sorghum with tannins (Waniska *et al.,* 1992). In the bleach test, the pericarp is dissolved by the bleach reagent, to expose the testa layer, which is black in tannin sorghum, and white to yellow in non-tannin sorghum. The chlorox bleach test was performed on red sorghum and white sorghum before processing to check for presence of pigmented testa. Red sorghum turned black hence indicating presence of tannin (pigmented testa) but white sorghum turned yellowish showing that it was a non-tannin sorghum variety. Absence of pigmented testa, may, however, not be taken as a confirmatory test for presence/absence of tannin (Hahn and Rooney, 1986). Thus a confirmatory test was carried out by the vanillin/HCl acid method whereby the plain red sorghum and red sorghum-based composite products had significant amounts of condensed tannins whereas white sorghum-based composite products did not show any significant quantities of condensed tannins. Not all red sorghums are high tannin and not all white sorghums are low tannin sorghum (Rooney and Miller, 1982; Taylor, 2001). Boren and Waniska (1992) investigated tannin content in a wide variety of sorghums varying in pericarp color. They showed that pericarp color and its intensity is not a good indicator of tannin content. Sorghums with white, yellow, red, or brown color pericarp may or may not have tannins depending upon the presence of a pigmented testa (Dykes and Rooney, 2006). A more definitive test must therefore be conducted to affirm presence or absence of tannins in food samples.

4.4.2 Tannin

Table 9 shows the tannin concentrations among the various composite food products and the plain sorghum flour. Tannin ranged from 0.09 mg CE/g in WSS-H to 33.63 mg CE/g in RS-C. Reduction in the amounts of tannin due to extrusion was comparable to findings

reported in other studies (Ngwenya, 2007; Dlamini *et al*., 2007). Tannins are antinutritional factors that bind with protein in foods to form insoluble complexes. They also chelate Fe, making it unavailable for absorption. Tannins therefore lower the overall quality of foods by reducing the bioavailability of protein and Fe. Tannin concentrations ranged from 33.63 mg CE/g in RS-C to trace levels in white sorghum-soybean-sardine (WSSSar-H) composite product that was extruded at 140^{o}C. Tannin concentrations were significantly lower ($p < 0.05$) in the extruded products than in unextruded products. Extrusion reduced tannin concentration of plain red sorghum (RS-C) by 94% when extruded at 100^{o}C (RS-L) and by 97% when extruded at 140^{o}C.

This observation was in agreement with the study by Dlamini *et al*. (2007) who reported that extrusion cooking reduced tannin content in Red Swazi sorghum by 97.3%. Percent reduction in tannin concentration in this study was higher than that observed in a study done by Awika *et al*. (2003a). This could be due to the high feed moisture content (24%) used in this study. The higher moisture content probably promoted phenolic and tannin polymerization which decreased extractability of tannins. Remy *et al*. (2000) reported that, high moisture may promote tannin polymerization during extrusion. Reduction of detectable tannins in thermal processing can be attributed to chemical rearrangement and formation of less extractable tannin complexes.

Techniques used in this study to reduce the tannin concentrations were germination and extrusion. As shown in Table 9, formulations that were blended with germinated red sorghum flour contained significantly lower levels ($p < 0.05$) of tannins compared to those blended with ungerminated red sorghum, although they were subjected to similar treatments (GRSS-C – 7.20 mg CE/g versus RSS-C – 9.67 mg CE/g, GRSS-L - 0.48 mg CE/g versus RSS-L – 0.64 mg CE/g and GRSS-H – 0.26 mg CE/g versus RSS-H – 0.34 mg CE/g).

Table 9: Tannin content and urease activity of various supplementary products[1,2]

Product[3,4]	Tannin (mg CE/g)	Urease activity (Units)
RSS-H	0.34±0.01^{gh}	0.00±0.00^{d}
RSS-L	0.64±0.00^{fg}	0.07±0.06^{d}
RSS-C	9.67±0.12^{c}	0.20±0.00^{c}
GRSS-H	0.26±0.00^{gh}	0.00±0.00^{d}
GRSS-L	0.48±0.01^{fgh}	0.03±0.06^{d}
GRSS-C	7.20±0.21^{d}	0.10±0.00^{d}
RSSSar-H	0.42±0.01^{fgh}	0.00±0.01^{d}
RSSSar-L	0.75±0.02^{fg}	0.06±0.00^{d}
RSSSar-C	11.18±0.32^{b}	0.10±0.00^{d}
WSS-H	0.09±0.01^{h}	0.00±0.00^{d}
WSS-L	0.10±0.01^{h}	0.07±0.05^{d}
WSS-C	0.44±0.04^{fgh}	0.20±0.10^{c}
WSSSar-H	0.09±0.01^{h}	0.01±0.00^{d}
WSSSar-L	0.10±0.01^{h}	0.03±0.05^{d}
WSSSar-C	0.49±0.01^{fgh}	0.10±0.10^{d}
RS-H	0.85±0.01^{f}	0.00±0.00^{d}
RS-L	1.92±0.55^{e}	0.01±0.00^{d}
RS-C	33.63±0.60^{a}	0.05±0.00^{d}
Dehulled soybean (control)		0.30±0.10^{b}
Raw whole soybean (control)		1.20±0.10^{a}

Means ± Standard deviation

[2] Means within a column with different superscripts are significantly different at $p<0.05$

[3] RSS =Red sorghum-soybean; GRSS = Germinated red sorghum-soybean; RSSSar = Red sorghum-soybean-sardine; WSS = White sorghum-soybean; WSSSar = White sorghum-soybean-sardine; RS =Plain Red sorghum

[4] H =extruded at high temperature (140^{o}C); L =extruded at low temperature (100^{o}C); C =Unextruded (control)

This decrease in tannin concentrations for formulations blended with germinated sorghum was in agreement with findings of other studies (Dicko *et al*., 2005; Chavan and Kadam, 1989). In a study of 50 sorghum varieties, Dicko *et al*. (2005) observed that germination decreased the content of condensed tannins. The decrease in extractable tannins after

germination could be due to leaching of water-soluble tannins which are located in the pericarp and testa (Beta *et al*., 1999) or due to formation of insoluble complexes with proteins (Riedl and Hagerman, 2001). Likewise, in a study of fava beans (*Vicia faba*), kidney beans (*P. vulgaris*) and red sorghum, Chavan and Kadam (1989) observed that germination reduced the concentration not only of tannins but also of other antinutritional factors (phytohemagglutinins and phytic acid). The decrease in tannin concentrations could be attributed to the interactions between tannins and proteins, enzymes and other organic compounds during germination to form complexes, which reduces the assayable tannins (Chavan and Kadam, 1989).

Extruded composite products contained lower ($p < 0.05$) concentrations of tannins than those that were not extruded. For example, the red sorghum-soybean product extruded at 140^{o}C (RSS-H, 0.34 mg/g) contained 96% less tannins than the same product that was not extruded (RSS-C, 9.67 mg/g) and red sorghum-soybean-sardine product that was extruded at 140^{o}C (RSSSar-H, 0.42 mg/g) contained 44% less tannins than the same product that was extruded at 100^{o}C (RSSSar-L, 0.75 mg/g). Overall, plain red sorghum (RS) products had the highest concentration of tannin ($p < 0.05$). Products that were not extruded had the highest concentration of tannin (Table 10).

Table 10: Multiple mean comparison of tannin content and urease activity between formulations and extrusion temperature[1,2]

Factors	Tannin(mg CE/g)	Urease activity(Units)
Formulation[3]		
RS	12.13±0.02^{a}	0.02±0.02 ^{b}
RSSSar	4.12±0.04^{b}	0.05±0.04^{ab}

RSS	3.55±0.09[c]	0.09±0.09[a]
GRSS	2.65±0.05[d]	0.04±0.05[ab]
WSSSar	0.17±0.07[e]	0.07±0.07[ab]
WSS	0.15±0.11[e]	0.09±0.11[a]
Extrusion temperature[4]		
C	10.44±0.08[a]	0.13±0.08[a]
L	0.64±0.05[b]	0.05±0.05[b]
H	0.31±0.00[c]	0.002±0.00[c]

[1] Means ± Standard deviation

[2] Means within a column with different superscripts are significantly different at $p<0.05$

[3] RSS =Red sorghum-soybean; GRSS = Germinated red sorghum-soybean; RSSSar = Red sorghum-soybean-sardine; WSS = White sorghum-soybean; WSSSar = White sorghum-soybean-sardine; RS = Plain Red sorghum.

[4] H =High extrusion temperature (140°C); L =Low extrusion temperature (100°C); C =Unextruded (control)

Extrusion cooking significantly reduced measurable tannins. The decrease in tannin concentrations could be attributed to the interaction of tannins with prolamins during extrusion (Emmambux and Taylor, 2003). Protein denatured by cooking, has open loose structures which promote tannin–protein interactions. The tannin levels for extruded products, observed in this study were comparable to those reported in other studies (Gu *et al*., 2004; Al-Kahtani, 1995; Mugula and Lyimo, 1999). Residual tannins in the extruded products were small and thus may not cause any adverse health effect to the consumers. Ngwenya (2007) reported that, tannin content of up to 11.4 mg CE/g was safe and tolerable in sorghum based foods.

4.4.3 Urease activity

Soybean and other legume products are able to liberate ammoniacal nitrogen from a urea solution when they have not been properly cooked. Loss of urease activity indicates improvement in the cooking of the soya products; and hence improvement in the protein quality of the products (TBS, 1983). Proper cooking of soybean-cereal mixtures improves

flavor, protein and starch digestibility and inactivates antinutritional factors such as trypsin and chymotrypsin inhibitors. Cooking doneness in soybean-cereal mixtures can be evaluated by the residual level of urease enzyme after cooking (TBS, 1983). According to the Tanzania Bureau of Standards (1983), supplementary foods containing legumes must not contain more than 0.8 units of urease activity. Table 9 shows the urease activity for the plain and composite foods that were extruded and those which were not extruded (control).

In the products that were not extruded, urease activity ranged between 0.05 and 0.2 units. In the products that were extruded at low temperature (100^{o}C), urease activity ranged between 0.01 and 0.07 units while in the products that were extruded at high temperature (140^{o}C), urease activity ranged between 0.00 and 0.01 units. Raw whole soybean flour and dehulled soybean flour were run as checks during urease activity determination. Fresh raw soybean is expected to give a pH change of over 1 unit during urease activity determination (TBS, 1983). Raw whole soybean flour had urease activity of 1.2 units, however dehulled soybean flour had lower urease activity of 0.3 units. This could be attributed to the fact that, during dehulling of soybean the beans were soaked in hot water to facilitate removal of hulls. This heat treatment probably inactivated the urease enzyme. Hence, milled dehulled soybean flour used in the composite formulations already had very low urease activity. Extrusion further reduced the urease activity by 65 – 100%. Table 10 shows multiple mean comparison of urease activity between the formulations and extrusion temperatures whereby products that were not extruded had significantly higher urease activity ($p < 0.05$) than those that were extruded at 100^{o}C and 140^{o}C. Urease activity in all the composite supplementary food products were much lower than the maximum level (0.8 units) recommended by TBS (1983), suggesting preliminary processing and extrusion were effective in inactivating the urease enzyme and the food products were thus suitable for children consumption.

4.5 Physical and Functional Properties of Sorghum-Soybean Products

Process treatment of raw materials is known to affect their hydration properties. Water absorption characteristics are attributed to the protein and starch granules present in the samples as well as their arrangements (Phillips *et al*., 1988). Generally extrusion increased the hydration power of products, which is a desirable effect for utilization of instant products. Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence (Miller *et al*., 2000) suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Highly reactive free radicals and singlet oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative diseases.

Table 11 shows the antioxidant activities of the various food products. Significant differences ($p < 0.05$) were observed in the antioxidant capacities of methanolic extracts of sorghum and sorghum-soybean composite products. WAI and WSI values of extruded composite supplementary foods and the control samples (unextruded) are also presented in Table 11.

4.5.1 Water Absorption Index (WAI)

WAI in products that were not extruded (control samples) ranged from 2.29 to 2.57 g/g. WAI in products that were extruded at 100^{o}C ranged from 3.01 to 4.11 g/g, while WAI ranged from 3.66 g/g to 4.73 g/g in the composite products extruded at 140^{o}C. The highest WAI was observed in WSSSar-H (4.73 g/g) while the lowest WAI was observed in RS-C (2.29 g/g) (Table 11). These results are comparable to WAI values of extruded products obtained in other studies (Filli *et al*., 2010; Dlamini *et al*., 2009). Dlamini *et al*. (2009)

obtained WAI value of 4.65 g/g for whole extruded plain sorghum (Framida). In a study of influence of extrusion variables on physical properties of extruded millet/soybean flour mixtures, Filli *et al*. (2010) obtained an optimal WAI of 4.6 g/g. Bressani *et al*. (1978) extruded blends of corn and soybean materials and recorded WAI values of 4.6-5.1 g/g, which compared favourably with the results obtained in this study for samples extruded at 140ºC. In the current study, the composite foods that were extruded at 140ºC had significantly higher WAI ($p < 0.05$) than those that were extruded at 100ºC and control samples (Table 12). These results showed that, extrusion cooking significantly increased the WAI. Typically, water absorption ability of an extrudate increases with increasing processing temperature, which results in a higher expansion (Rosentrater, 2005). General increase in water absorption of sorghum extrudates with increase in extrusion temperatures was reported by Gomez *et al*. (1988).

Gujska and Khan (1990) reported a threefold increase in WAI by extrusion cooking of the high starch fraction of beans compared to non-extruded samples. Arambula *et al*. (1998) reported increased water absorption capacity, water absorption index and water solubility index of instant corn flours as a result of extrusion. These changes responded positively with increase in temperature. They also associated the changes with the degree of starch gelatinization of the material.

Table 11: Water absorption index, Water solubility index and Antioxidant activity of sorghum-soybean supplementary products[1,2]

Products[3,4]	**WAI (g/g)**	**WSI (%)**	**EC_{50} (µg/ml)[5]**
RSS-H	3.66±0.14^{cde}	5.26±0.26^{h}	80.56±1.44^{f}
RSS-L	3.90±0.15^{bc}	6.51±1.19^{fg}	74.84±0.65^{hi}
RSS-C	2.57±0.00^{g}	6.09±0.22^{gh}	71.79±0.69^{j}
GRSS-H	3.82±0.09^{cd}	9.05±0.47^{d}	97.91±1.25^{a}
GRSS-L	3.58 ±0.19^{de}	10.95±0.40^{c}	93.60±1.42^{b}
GRSS-C	2.44±0.07^{g}	8.79±0.44^{de}	78.09±0.65^{g}
RSSSar-H	4.16±0.14^{b}	6.52±0.59^{fg}	73.11±1.17^{ij}

RSSSar-L	4.11±0.11[b]	6.95±0.42[fg]	73.81±1.12[ij]
RSSSar-C	2.48±0.02[g]	7.34±0.19[fg]	72.30±0.36[j]
WSS-H	4.70±0.26[a]	5.18±0.64[h]	86.63±1.23[c]
WSS-L	3.55±0.13[de]	7.07±0.16[fg]	85.98±1.01[cd]
WSS-C	2.30±0.07[g]	6.72±0.65[fg]	82.13±1.19[ef]
WSSSar-H	4.73±0.19[a]	6.66±0.24[fg]	93.13±1.10[b]
WSSSar-L	3.02±0.29[f]	9.56±0.51[d]	88.41±0.45[c]
WSSSar-C	2.29±0.05[g]	6.50±0.13[fg]	76.73±1.69[gh]
RS-H	4.70±0.29[a]	13.55±1.78[b]	83.00±1.35[ef]
RS-L	3.43±0.18[e]	35.18±0.90[a]	83.68±1.31[de]
RS-C	2.29±0.03[g]	7.71±0.44[ef]	68.32±1.30[k]
Ascorbic acid (control)			68.77±0.69[k]

[1] Means ± Standard deviation

[2] Means within a column with different superscripts are significantly different at $p<0.05$

[3] RSS =Red sorghum-soybean; GRSS = Germinated red sorghum-soybean; RSSSar = Red sorghum-soybean-sardine; WSS = White sorghum-soybean; WSSSar = White sorghum-soybean-sardine; RS =Plain Red sorghum, [4] H =extruded at high temperature (140ºC); L =extruded at low temperature (100ºC); C =Unextruded (control), [5] The DPPH free radical scavenging activity was evaluated as the concentration of the test sample required to decrease the absorbance at 517 nm by 50%.

Water absorption index (WAI) indicates the extent of starch gelatinization and is associated with dispersion of starch in excess water; the dispersion is increased by the degree of starch damage due to gelatinization. Extrusion induces starch fragmentation which reduces the molecular weight of amylose and amylopectin molecules (Yagci and Gogus, 2008). Protein denaturation, starch gelatinization and swelling of the crude fibre, which occur during extrusion, could all be responsible for the increased WAI of extruded flour.

Low water absorption values for extrudates extruded at low temperatures indicate restricted water availability for the starch granule due to a more compact structure. However, when temperatures are increased, amylose and amylopectin chains are separated, forming an expansible matrix which results in a higher water holding capacity (Filli and Nkama, 2007). The WAI is a measure of the ability of the flour to associate with water (Ijarotimi and Ashipa, 2005). Water absorption capacity is an important index, which gives

valuable information on the behaviour of composite food products during reconstitution in hot or cold water. A high WAI is a desirable property in ready to eat porridges (Pelembe *et al*., 2002).

The ability to absorb water is particularly important if the food will have to be reconstituted in water before consumption. The above results show that, products of reasonable quality were obtained when sorghum-soybean/sardine composite products were extruded, especially at 140ºC, and gruels can be made from the extruded flour of these composite products by reconstitution in hot water or cooking for a short time.

4.5.2 Water Solubility Index (WSI)

WSI ranged from 5.2% in WSS-H to 35.2% in RS-L (Table 11). These results are comparable to WSI values obtained from other extruded products (Kadan *et al*., 2003). In food samples that were not extruded, the WSI ranged from 6.1 to 8.8%. In food samples that were extruded at 100ºC, WSI ranged from 6.5 to 35.2% while in samples that were extruded at high temperature of 140ºC, WSI ranged from 5.2 to 13.6%. The trend shows WSI progressively increased with an increase in extrusion temperature up to a certain temperature and started to decrease as temperature increased. RS samples had the highest WSI followed by GRSS samples (Table 12).

Table 12: Multiple mean comparison of WAI, WSI and anti-oxidant activity between formulations and extrusion temperature[1,2]

Factors	WAI (g/g)	WSI (%)	EC_{50} (µg/ml)
Formulation[3]			
RSSSar	3.58±0.83[a]	6.94±0.52[cd]	73.07±1.00[e]

WSS	3.52±1.05[ab]	6.33±0.99[de]	84.91±2.35[b]
RS	3.47±1.06[abc]	18.81±12.57[a]	78.33±7.83[c]
RSS	3.38±0.62[bcd]	5.95±0.83[e]	75.73±4.06[d]
WSSSar	3.34±1.10[cd]	7.57±1.52[c]	86.09±7.61[b]
GRSS	3.28±0.65[d]	9.60±1.09[b]	89.87±9.37[a]
Extrusion temperature[4]			
H	4.30±0.48[a]	7.70±3.08[b]	85.72±8.55[a]
L	3.60±0.39[b]	12.70±10.49[a]	83.39±7.44[b]
C	2.40±0.12[c]	7.19±0.97[c]	74.89±4.84[c]

[1] Means ± Standard deviation, [2] Means within a column with different superscripts are significantly different at $p<0.05$, [3] RSS =Red sorghum-soybean; GRSS = Germinated red sorghum-soybean; RSSSar = Red sorghum- soybean-sardine; WSS = White sorghum-soybean; WSSSar = White sorghum-soybean-sardine; RS = Plain Red sorghum. [4] H= High extrusion temperature (140ºC); L =Low extrusion temperature (100ºC); C =Unextruded (control).

Cumming *et al*. (1973) stated that, as processing temperature increases during extrusion, proteins within a soy-based food become increasingly altered and redistributed and can become insoluble. Early works revealed that, heat treatment of soy protein leads to loss of solubility due to the formation of disulfide bonds, hydrogen bonds and hydrophobic bonds (Stanley, 1989). Balandran-Quintana (1998) reported that, high protein content decreased WSI in extruded pinto bean meal.

The effect of protein content on WSI was confirmed by Gujska and Khan (1991), when they added a protein fraction at 10.0% level to the high starch fraction of pinto bean flour and a decrease in WSI was observed. The WSI obtained in this study for extruded plain red sorghum flour extruded at 140ºC (13.6%) was much lower than WSI (31.0-34.2%) obtained by Dlamini *et al*. (2009) for plain sorghum extruded at the same temperature range. This could be attributed to the high feed moisture (24.0%) used in this study compared to a feed moisture content of 18.0% used by Dlamini *et al*. (2009). Gujska and Khan (1991) found that WSI decreased significantly with increasing moisture in the

extrusion of pinto bean flour from 35.3% at 20.0% feed moisture to 21.1% at 30.0% feed moisture. They indicated that, this was caused by greater shear degradation of starch during extrusion at low moisture. The water-solubility index reflects the extent of starch degradation.

Plain sorghum flour and composite formulations that had high proportions of sorghum had higher WSI. Therefore, addition of soybean reduced the WSI of extruded products. Changes in WSI with incorporation of soybean could be due to reduction in the amount of starch in the feed. Jones *et al*. (2000) reported that, fibre, starch and protein contents affect WSI. The formulations that incorporated germinated red sorghum had higher WSI than formulations that did not incorporate germinated red sorghum (GRSS-C – 8.8% versus RSS-C – 6.1%, GRSS-L - 11.0% versus RSS-L – 6.5% and GRSS-H – 9.1% versus RSS-H – 5.3%). This could be attributed to starch conversion that occurred during germination. The WSI often is used as an indicator of degradation of molecular components especially starch (Kirby *et al*., 1988). At high temperatures or shear, but within certain limits, the starch is degraded or dextrinized to smaller soluble molecules, thus increasing WSI (Ding *et al*., 2005).

4.5.3 Anti-oxidant activity

Evaluation of the antioxidant activities of the plain and composite products are summarized in Table 11. All the samples exhibited appreciable ($p < 0.05$) anti-oxidant activity. The lower the EC_{50} value is, the greater the anti-oxidant activity. As shown in Table 11, the anti-oxidant activity of the plain and composite sorghum-soybean products varied considerably. EC_{50} values of the various products ranged from 68.32 to 97.91 µg/ml. EC_{50} of the standard compound, ascorbic acid, was 68.77 µg/ml. Of all the food samples, RS-C had the least EC_{50} value (68.32 µg/ml). A low EC_{50} value is an indication of strong

antioxidant activity. Therefore RS-C had the highest anti-oxidant activity with EC_{50} value of 68.32 µg/ml, followed by RSS-C (71.79 µg/ml) and RSSSar-C (72.30 µg/ml). The lowest anti-oxidant activity was shown by GRSS-H (97.91 µg/ml).

Products that were not extruded exhibited considerably higher anti-oxidant activities than products that were extruded at 100ºC and 140ºC (Table 12). The values of EC_{50} for all the extruded and unextruded products, with the exception of RS-C, were significantly higher than that of ascorbic acid ($p < 0.05$), a compound which is used as a standard for antioxidant activity. This shows that, the antioxidant activities of the tested food samples were significantly lower than that of the standard reference (ascorbic acid). Interestingly, RS-C showed an anti-oxidant activity almost equivalent to that of Ascorbic acid. Awika (2000) reported that, high tannin sorghums have the highest oxygen radical absorbance capacity (ORAC) and phenolic content among sorghums. It has been reported in several studies (Gomez *et al*., 1988; Awika *et al*., 2003; Dlamini *et al*., 2007) that, sorghum possess antioxidant activity due to its content of phenolic compounds. Tannins have been reported to be responsible for the high antioxidant activity in high tannin sorghums (Turner, 2004; Ngwenya, 2007; Awika and Rooney, 2004). Soybean is also known to possess antioxidant activity. It has been documented in several studies that isoflavones found abundantly in soybeans are the principle components responsible for antioxidant activity (Fritz *et al*., 2003; Tripathi and Misra, 2005; Devi *et al*., 2009). The composite products investigated in this study were blends of sorghum and soybean. Therefore, the antioxidant activity exhibited is likely to be contributed by different phenolic compounds from both sorghum and soybean.

The literature on effect of extrusion on antioxidant activity shows contrasting information. While in most cases thermal processing results in a decrease in antioxidant activity, some

authors have reported an increase in antioxidant activity (Dewanto *et al*., 2002; Patras *et al*., 2008). Gumul and Korus (2006) reported an increase in antioxidant activity in extruded rye bran. A study by Shih *et al*. (2009) showed that, extrusion process significantly increased the anti-oxidant ability of yellow and orange sweet potatoes. The reason given by the authors was that, heat treatment increased the release of potent radical-scavenging antioxidants from the cell matrix and enhanced availability of phytochemicals. Therefore, the effect of extrusion on antioxidant activity depends largely on the components in foods that are responsible for antioxidant activity and how they behave under extrusion conditions.

Limsangoun *et al*. (2010) reported that, extrusion process slightly decreased the antioxidant capacity (3.61-13.07%) in cereal and legume based extruded snack foods fortified with by-products from herbs and vegetables. In the current study, extrusion resulted in some loss of antioxidant activity. The loss in antioxidant activity due to extrusion ranged from 1% to 20% when the antioxidant capacity of the extruded products was compared to the unextruded products. This is in agreement with results by Awika *et al*. (2003b) who observed that extrusion of sorghum and sorghum products resulted in loss of antioxidant activity. When processed into foods, most of the antioxidant activities of the raw sorghums were retained, 57-78% for baked and 70-100% for the extruded products. Awika (2003) reported further that, high tannin sorghum extrudates retained 21% of their original assayable tannin content and 89% of their original antioxidant activity. This implies that, the sorghums can be processed into foods that are functional.

It has been reported that, the mechanical energy supplied by the extruder can influence the nature of some complexes formed between the flour components and influence the degradation of larger molecules, such as starch. Results of the current study showed some

loss of antioxidant activity for the extruded products. The decrease in antioxidant levels in extruded products may have been partly due to interaction of antioxidant components with proteins and other food components into non-extractable complexes (Turner, 2004).

4.6 Sensory Evaluation

Table 13 shows the results of the sensory evaluation of the gruels made from the extruded composite products and their controls (unextruded). Sensory evaluation of food products is important for determining consumer acceptability (Samuel *et al*., 2006). Panelists detected significant differences ($p < 0.05$) among the samples in colour, aroma, taste, mouth feel and overall acceptability. Colour of food strongly influences acceptability. GRSS-H had the highest mean score for colour. The extruded products, GRSS-H, RS-H, RSS-H, WSS-L, WSSSar-H, WSS-H and GRSS-L were mostly liked in terms of colour. GRSS-C, which was not extruded, was the least liked among the products in terms of colour. Therefore, extrusion improved the colour of the products. In terms of aroma, RS-H (control) and GRSS-H had the most appealing smell ($p < 0.05$), while RSSSar-H, RSSSar-L and RSSSar-C were the least appealing to the panelists in terms of smell. The panelists did not like the residual smell of sardines that remained after processing. The white sorghum-based products that incorporated sardines were better liked in terms of smell than the red sorghum-based products that incorporated sardines. For the red sorghum-based composite products, the products that were based on germinated red sorghum (GRSS-H, GRSS-L and GRSS-C) were more liked in terms of smell than the composite products that were not germinated (RSS-H, RSSSar-H, RSS-L, RSSSar-L, RSS-C, RSSSar-C). Therefore, germination of the red sorghum improved the aroma of the final products. A study by Kikafunda *et al*. (2006) revealed that, germination leads to development of desirable flavours.

Composite product WSSSar-H had the most superior taste ($p < 0.05$). The composite products WSSSar-L and GRSS-H were also ranked high in terms of taste preference. RSSSar-C was the least preferred in terms of taste. Again, extruded products were most preferred in terms of taste than the ones that were not extruded. For the composite products, the white sorghum-based composite products seemed to be preferred in terms of taste compared to the red sorghum-based composite products. The extruded white sorghum-based composite products that incorporated sardines (WSSSar-H and WSSSar-L) had higher mean scores for taste than the other composite products. This was interesting because the residual sardine smell adversely affected their mean scores for smell. For the red sorghum-based composite products, the products that were based on germinated red sorghum (GRSS-H, GRSS-L and GRSS-C) had taste that was more liked than the composite products that were based on ungerminated red sorghum (RSS-H, RSSSar-H, RSS-L, RSSSar-L, RSS-C, RSSSar-C). This implied that, germination contributed to the improvement of the taste of the final products. This could be attributed to conversion of starch to simple sugars and other biological products that induced favourable taste to the products.

Data in Table 13 indicate that, WSSSar-H and RS-H (control) gruels had the best texture (mouth feel) followed by WSSSar-L. RSSSar-C was the least liked in terms of texture. Texture is an important sensory attribute that influences customer selection and acceptability of a product. Texture is influenced by the physical characteristics of the flours such as particle size but also by fibre content. In this regard, composite products e.g. WSSSar-H and WSSSar-L which had low fibre content had smooth texture and were ranked high in terms of mouth feel. Most panelists preferred the smoothness of the extruded white sorghum-based gruels (WSSSar-H, WSSSar-L, WSS-H) compared to the red sorghum-based composite gruels which had a coarse texture. The soft mouth feel of

white sorghum-based gruels could be attributed to the fact that white sorghum was dehulled hence having low fibre whereas the red sorghum used in the formulations was not dehulled. Dehulling reduces fibre content in the flour which makes the flour develop a smooth texture when cooked. Lower sensory rating scores for texture were observed in the gruels that were not extruded. This means that, extrusion of the ingredients and subsequent grinding of the extrudates resulted in a more acceptable texture of the final products (gruels).

Table 13: Sensory attributes of the plain and composite sorghum-soybean gruels[1,2]

Products[3,4]	Colour	Aroma	Taste	Mouthfeel	Overall acceptability
RSS-H	3.80^{a}	3.33^{abcdef}	3.00^{efgh}	2.97^{defg}	3.27^{cdef}
RSS-L	3.23^{abcd}	3.07^{cdef}	2.77^{ghi}	2.90^{efg}	3.07^{defg}
RSS-C	3.30^{abcd}	2.80^{efg}	2.47^{hi}	2.77^{efg}	2.47^{gh}
GRSS-H	3.97^{a}	3.77^{ab}	3.77^{abcd}	3.60^{bcd}	3.73^{abc}
GRSS-L	3.70^{a}	3.63^{abc}	3.33^{cdefg}	3.43^{bcde}	3.37^{bcdef}
GRSS-C	2.60^{d}	2.90^{def}	2.93^{fgh}	2.60^{fg}	2.73^{fgh}
RSSSar-H	3.40^{abc}	2.73^{fg}	3.20^{defg}	2.97^{defg}	3.20^{cdef}
RSSSar-L	3.33^{abc}	2.70^{fg}	2.97^{fgh}	3.17^{cdef}	3.00^{efg}
RSSSar-C	2.77^{cd}	2.2^{g}	2.27^{i}	2.27^{g}	2.37^{h}
WSS-H	3.73^{a}	3.60^{abc}	3.63^{bcde}	3.47^{bcde}	3.70^{abcd}
WSS-L	3.80^{a}	3.70^{abc}	3.57^{bcdef}	3.10^{cdef}	3.57^{abcde}
WSS-C	3.30^{abcd}	3.47^{abcd}	3.07^{efgh}	2.7^{fg}	3.10^{cdef}
WSSSar-H	3.77^{a}	3.40^{abcde}	4.10^{ab}	4.27^{a}	3.93^{ab}
WSSSar-L	3.53^{ab}	3.20^{bcdef}	3.87^{abc}	3.77^{abc}	3.63^{abcde}
WSSSar-C	2.97^{bcd}	2.90^{def}	3.43^{cdef}	2.80^{efg}	3.03^{efg}
RS-H	3.87^{a}	3.90^{a}	4.30^{a}	4.07^{ab}	4.17^{a}
RS-L	2.83^{bcd}	3.60^{abc}	3.73^{abcd}	3.73^{abc}	3.60^{abcde}
RS-C	3.27^{abcd}	3.60^{abc}	3.33^{cdefg}	2.77^{efg}	3.30^{bcdef}

[1] Rating scores for sensory attributes based on a 5-point hedonic scale

[2] Mean scores within a column with different superscripts are significantly different at $p<0.05$

[3] RSS =Red sorghum-soybean; GRSS = Germinated red sorghum-soybean; RSSSar = Red sorghum-soybean-sardine; WSS = White sorghum-soybean; WSSSar = White sorghum-soybean-sardine; RS =Plain Red sorghum

[4] H =extruded at high temperature (140^{o}C); L =extruded at low temperature (100^{o}C); C =Unextruded (control)

It was observed that grinding the extrudates after extrusion resulted in fine textured flour that made the cooked products more appealing. Therefore dehulling and extrusion resulted in products with texture that was more liked by the panelists. Among the composite gruels, the white sorghum-soybean-sardine extruded at 140^{o}C (WSSSar-H) was liked very much in terms of mouth feel.

Overall, all the extruded composite gruels were acceptable to the consumers, with the exception of some red sorghum-based composite products that were not extruded (RSS-C, GRSS-C and RSSSar-C). These composite products (RSSSar-C, RSS-C and GRSS-C) had lower rating scores for overall acceptability hence slightly disliked by the panelists. Plain red sorghum extruded at high temperature (140^{o}C) (RS-H) had higher rating scores in overall acceptability followed by extruded composite products - WSSSar-H and GRSS-H.

Table 14 shows multiple comparison of means, with factors being formulations and extrusion temperatures. For the composite products, WSSSar and WSS had higher rating scores in overall acceptability followed by GRSS. WSSSar had the highest mean scores in taste, mouth feel and overall acceptability. WSS had the highest mean scores in colour and aroma. RSSSar had the least mean scores in almost all sensory attributes.

For all formulations, the products that were extruded, especially at 140^{o}C, had the highest mean scores in all sensory attributes (Table 14). Extrusion has been reported to have an organoleptic benefit of changing not only the flavor, aroma and texture but also the physical appearance (colour) of cooked products (Jones *et al.*, 2000).

Table 14: Multiple mean comparison of sensory attributes between formulations and extrusion temperature[1]

Factors	Colour	Aroma	Taste	Mouthfeel	Overall acceptability
Formulation[2]					
RSS	3.44^{ab}	3.07^{c}	2.74^{c}	2.88^{cd}	2.93^{c}
GRSS	3.42^{ab}	3.43^{ab}	3.34^{b}	3.21^{bc}	3.28^{b}
RSSSar	3.17^{b}	2.54^{d}	2.81^{c}	2.80^{d}	2.86^{c}
WSS	3.61^{a}	3.59^{a}	3.42^{b}	3.09^{cd}	3.46^{ab}
WSSSar	3.42^{ab}	3.17^{bc}	3.80^{a}	3.61^{a}	3.53^{ab}
RS	3.32^{ab}	3.70^{a}	3.79^{a}	3.52^{ab}	3.69^{a}
Extrusion Temperature[3]					
H	3.76^{a}	3.46^{a}	3.67^{a}	3.56^{a}	3.67^{a}
L	3.41^{b}	3.32^{a}	3.37^{b}	3.35^{a}	3.37^{b}
C	3.03^{c}	2.98^{b}	2.92^{c}	2.65^{b}	2.83^{c}

[1] Mean scores within a column with different superscripts are significantly different at $p<0.05$

[2] RSS =Red sorghum-soybean; GRSS = Germinated red sorghum-soybean; RSSSar = Red sorghum-soybean-sardine; WSS = White sorghum-soybean; WSSSar = White sorghum-soybean-sardine; RS =Plain Red sorghum

[3] H= High extrusion temperature (140^{o}C); L =Low extrusion temperature (100^{o}C); C =Unextruded (control)

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The study has shed light on the potential of using sorghum for developing supplementary foods for older infants and young children. Evident from this study is the fact that it is possible to formulate and manufacture by extrusion cooking ready-to-eat supplementary foods of high nutrient density using combinations of whole, dehulled, germinated sorghum flours and soybean/sardine which are locally available food commodities.

It is concluded from this study that, porridges made from extruded composites of sorghum and soybean could offer the best route to address the identified problem of child undernutrition. Such local supplementary products can fairly substitute the more expensive proprietary formulae in the market. The results from this study suggest that, proper formulation and fortification of local diets can provide nutritious foods that are suitable for supplementary feeding. It is hoped that use of extrusion would increase small scale industrial utilization of sorghum.

According to FAO/WHO (1994a), supplementary foods must contain adequate amount of high-quality protein, high energy density, must be free of antinutritional factors and toxins, should be formulated from locally produced, easily accessible inexpensive food ingredients and must be culturally and organoleptically acceptable to adults and children. The white sorghum-soybean-sardine product extruded at 140^{o}C (WSSSar-H) was ranked the highest in colour, mouthfeel, taste and overall acceptability. The product also had good nutritional value that met the recommended levels for macronutrient requirements for supplementary foods for older infants and young children as stipulated by TBS and Codex alimentarius.

WSSSar-H could be adopted as a supplementary food for young children as long as it is fortified with vitamin-mineral pre-mixes during processing. Extrusion at 140^{o}C resulted in more desirable products as revealed by sensory evaluation. Also, extrusion at 140^{o}C resulted in products with fairly high WAI and WSI values, and lower residual levels of tannin and urease activity. Extrusion at 140^{o}C did not have damaging effects on the nutritional components of the products. Therefore, extrusion temperature of 140^{o}C is recommended for extrusion of sorghum-soybean composite supplementary products.

5.2 Recommendations

The problem of undernutrition is still prominent in Tanzania. Therefore, it is important that manufacturers of infant foods should consider producing supplementary foods from locally available food materials. Such foods will be readily available and affordable by the low income families both in urban and rural areas.

Extrusion has many advantages such as improving the quality of the food by making it more digestible and making the products ready to feed. Supplementary foods that are ready to feed reduce the women's work load by reducing the time spent in their preparation. Also chances for contamination due to unhygienic handling are reduced. Therefore, small food processing industries should adopt the extrusion technology and use it for food production to cater for more clients in the country.

It is also recommended that, further research should be done to assess the efficacy of the extruded sorghum-soybean composite foods in promoting growth and well being by using animal model to evaluate true protein digestibility and protein digestibility corrected amino acid scores (PDCAAS).

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APPENDICES

Appendix 1: Sensory Evaluation Form

Name……..
Sex……..
Age…….
Time……
Date…….

Please look at and taste each of the SIX (3-digit) coded samples. Indicate how much you like or dislike each sample by checking the appropriate sample attribute and indicate your preference (5 to 1) in the column against each attribute by putting the appropriate number.

KEY: 5- Like extremely
4- Like moderately

3- Neither like nor dislike

2- Dislike moderately

1- Dislike extremely

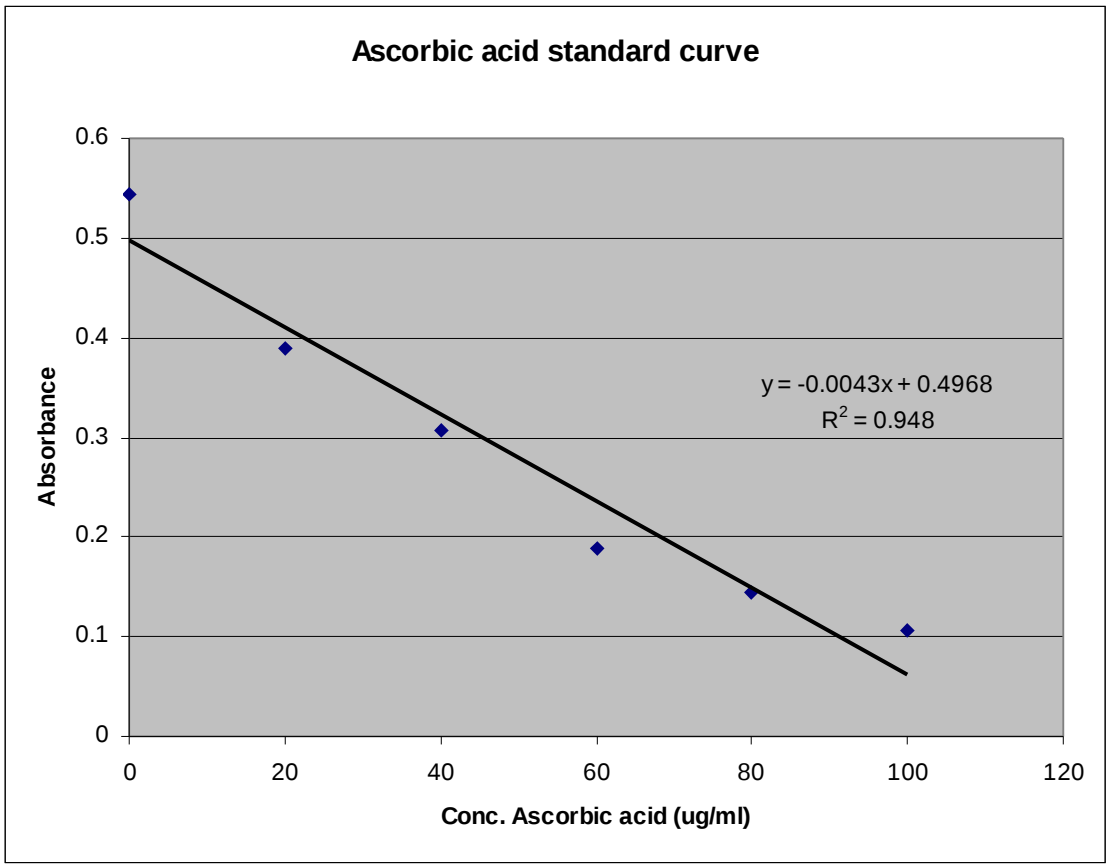
	SAMPLES (CODES)					
Colour						
Aroma/smell						
Taste						
Mouth feel						
Overall acceptability						

Comments……

……

……

Appendix 2: Ascorbic acid standard curve


Ascorbic acid standard curve
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
y = -0.0043x + 0.4968
R2 = 0.948
0
20
40
60
80
100
120
Conc. Ascorbic acid (ug/ml)