SOYBEAN CULTIVARS MILK YIELD AND RELATIVE ACCEPTABILITY OF THEIR FLAVOURED SOY-MILK

BY

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ABSTRACT

The objectives of this work were to study soybean cultivars milk yield and the acceptability of their flavoured soymilk. Whole soybeans varieties (TGX 1895-49F, TGX 1876-4E and TGX 1895-33F) were analysed for proximate composition, selected micronutrients and milk yield. The whole soybeans protein contents, with iron contents in brackets, for TGX 1895-33F, TGX 1876-4E and TGX 1895-49F were 43.998% (6.953 mg/100 g), 37.015% (19.670 mg/100 g) and 33.825% (10.580 mg/100 g) on dry matter basis respectively. Similarly the milk from the three varieties was analysed for proximate composition and selected micronutrients including β-carotene. The respective percentage milk yield for varieties TGX 1895-33F, TGX 1895-49F and TGX 1895-4E were 66.516, 66.063 and 56.122. These were found to be significantly different. The soymilk protein contents, with iron contents in brackets, for TGX 1895-33F, TGX 1876-4E and TGX 1895-49F were 4.499%w/v (9.569±1.868 g/100 g), 3.516%w/v (4.755±0.147 mg/100 g) and 4.271%w/v (4.072±0.057 mg/100 g) respectively. These together with moisture, oil, fibre, carbohydrates, ash and β-carotene were found to be significantly different. It was observed that the variety that contained the highest amount of protein had the highest iron and β-carotene. There was no significant difference among samples in calcium, zinc and copper content. Energy content for each of the three varieties was calculated. The milk yield, proximate and micronutrient data were ranked using Excel software to identify two suitable cultivars for efficient production of soymilk. The primary, secondary and tertiary selection criteria were milk yield, protein content and iron content respectively. After ranking, the milk from the two varieties (TGX 1895-33F and TGX 1895-49F) was fortified with three flavouring agents (vanilla, banana and pineapple) each at three levels i.e. 0, 0.015% and 0.030% with the 0 level serving as a control. Fortified products were assessed for colour, taste, aroma, mouth feel and overall acceptability on a 5 point hedonic scale.

Sensory evaluation results showed that of the three artificial flavours, pineapple flavour was the most effective compared with vanilla and banana flavour. The effectiveness was found significant ($p<0.05$) on the aroma and overall acceptability of soymilk with scores increasing with increasing level. Effectiveness was non significant ($p>0.05$) for colour, taste, and mouth feel. However, general comments were made by most panellists on the addition of sugar to improve taste. It was concluded in this study that TGX 1895-33F is the most effective cultivar for efficient production of soymilk and pineapple flavour being the most acceptable flavouring agent for soymilk.

DECLARATION

I, Lazaro Henry Msasalaga, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has not been submitted or concurrently being submitted for a higher degree award in any other University.

2008-11-22

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The above declaration confirmed

22nd Nov. 2008

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Date

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DEDICATION

This work is dedicated to my wife Purificator and our children Gloria and Savio.

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LIST OF ABBREVIATIONS AND SYMBOLS

ACC	-	Administrative committee on coordination
ARII	-	Agricultural Research Institute-Ilonga
ARVs	-	Anti-Retrovirals
CAST	-	Council of Agricultural Science and Technology
CNS	-	China National Standard
DASPL	-	Department of Animal Science and Production Laboratory
DFSTL	-	Department of Food Science and Technology Laboratory
DSSL	-	Department of Soil Science Laboratory
GMP	-	Good Manufacturing Practices
HACCP	-	Hazard Analysis Critical Control Point
HIV/AIDS	-	Human Immune-Virus/ Acquired Immune Deficiency Syndrome
ICB	-	International Congress on Biodiesel
IITA	-	International Institute of Tropical Agriculture
INTSOY	-	International Soybean Programme
NSRL	-	National Soybean Research Laboratory
PEM	-	Protein-Energy Malnutrition
SAA	-	Soyfoods Association of America
SPSS	-	Statistical Package for Social Sciences
SCN	-	Sub-committee on nutrition
TBS	-	Tanzania Bureau of Standards
USDA	-	United States Department of Agriculture
USFDA	-	United States Food and Drug Administration
VAD	-	Vitamin A Deficiency

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Protein energy malnutrition (PEM) is a major public health problem in most developing countries (Nti and Larweh, 2003). This is caused by poverty, which makes most families unable to get enough food to meet recommended dietary allowances. More than 2 billion people have been reported to be micronutrient deficient in the world (FAO, 1997). It is also estimated that 45% of children under age of five are affected by iron deficiency anaemia and 37% are estimated to have sub-clinical vitamin A deficiency (UNICEF, 2004). Moreover, about 80% of pregnant and lactating women are anaemic due to iron deficiency (Snow, 1998).

In Tanzania it is estimated that about 30% of the total population are malnourished (Laswai *et al.,* 2005). It has, therefore, become necessary to look for alternative sources of protein, especially those from leguminous plants. Of the legumes, soybean (*Glycine max,* L., Merrill) has the potential for alleviating PEM in the developing world. Soybean protein is cheap (Nelson *et al.*, 1978) and relatively high (40%w/w) with a good balance of all essential amino acids. The amino acid pattern compares well with that of animal protein (Nti and Larweh, 2003). Soy protein is higher in lysine and tryptophan than the common cereals (Singh *et al.*, 1987). Soybean is also rich in B-complex vitamins (except vitamin B_{12}), and vitamins A, E and K. Soybean is also rich in essential minerals notably iron, calcium, sodium, manganese, potassium and copper (Nelson *et al.*, 1971; Venter and Eyssen, 2001). Soy oil is made up of all essential oils namely oleic acid (30-35%), linoleic acid (45-55%) and linolenic acid (5-10%) with the respective percentages being that of the unsaturated fraction (Hinson and Hartwig, 1997). Laswai *et al.* (2005) also reported that soybean oil is of good quality and has highly digestible polyunsaturated oil with no cholesterol. Presence of essential oils in soybean oil is a property that excludes soybean from being associated with cancer, diabetes and other diseases/incidences (Wilcox, 1987; FAO, 1994; Heaney *et al.*, 2000; Bost, 2007).

As early as 1955, soybean was known in Tanzania as a useful crop to farmers because of price incentives and improvements in the country's marketing system (Sulzberger and Mclean, 1986), although its full potential has yet to be realized. Soybean, as human food, has several utilisation options that can vary from one place to another. These include porridges (uji, ugali, weaning food), drink (coffee-like), bread, buns, chapatti, cake, biscuits, fried snack (like groundnuts), oil and soymilk. Not all these utilisation options have been fully exploited and that most of it has been consumed as weaning food (Laswai *et al.*, 2005).

Traditional soymilk is an aqueous whole soybean extract which is generally a stable oil/protein emulsion in water. It sometimes goes by the names soybean milk, soy drink, soy beverage or "vegetable" milk. Soymilk, as other soybean products, is high in good quality protein (2.75-3.7 g/100 g) (Holden, 2004), which compares to cow's milk protein (3.4g/100 g) (Berk, 1992). This property, therefore, makes it important in fighting against PEM. It has so far been anticipated that promoting consumption of soymilk could lead to increased utilisation of soybeans, which consequently would bring about increased production and marketing of soybean crop. Moreover farmers are likely to gain economic power through sales of these legumes. Soymilk needs some promotion through flavour improvement as there is increased awareness of its benefits (Rweyemamu, 2004; Laswai *et al.*, 2005). Moreover, there are presently new demands in which utilisation as adult food especially those who have developed increased nutritional needs like the sick, pregnant/lactating women, the old and people living with HIV/AIDS. More utilisation of soybeans is also assumed to promote full pupil participation in class sessions hence promotion of education.

Soymilk is very low in vitamins A and E activity. Its water-soluble vitamin content is not outstanding except for thiamine and choline (Markley, 1951). Natural soymilk contains little digestible calcium (200 mg/l) but fortification to 1200 mg/l using calcium carbonate, tri-calcium carbonate or seaweed improves calcium bioavailability and absorption efficiency (Heaney *et al.*, 2000; Zhao, 2005). Soymilk is also suitable for people who are allergic to cow's milk (Markley, 1951).

Soymilk has grassy – beany and bitter flavours originating from soybean flavour compounds: ketones and aldehydes (Anon, 1987). These compounds impair development of novel beverages. Processing into soymilk must ensure reduction of these compounds. In Tanzania, utilisation of soybean as source of soymilk and subsequent utilisation of soymilk are still poor (Laswai *et al.*, 2005). Flavouring agents are likely to increase acceptability through masking of the bitter flavours.

1.2 General and Specific Objectives

In view of the above background information, the general objective of this work was to improve soymilk sensory quality by fortification with flavouring agents. The specific objectives were:

a) To identify a cultivar suitable for efficient production of soymilk.
b) To determine nutrient composition in soybean milk for the cultivars as given under (a) with a focus on proximate composition, minerals and vitamin composition.

c) To assess the effectiveness of artificial flavouring agents (banana, pineapple, and vanilla) on sensory quality.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Requirements for Quality Soybean

Soymilk quality is highly dependent on the quality of soybeans from which it is extracted (Wilson, 2003). The extracted soybeans should be sound and free from extraneous matter and harmful microorganisms. Variability in soybean composition is a function of cultivar, maturity, harvesting and storage conditions (Venter and Eyssen, 2001).

2.2 Soybean Nature, Grade and Nutrient Composition

It is increasingly recognised that the nutrient/phytochemical composition in foods can be optimised through agriculture and food technology and to put this strategy into practice, the compositional variation throughout the food chain has to be known (Azevedo-Meleiro and Rodriguez-Amaya, 2004). Soybean grains are spherical and the existing cultivars are mostly yellow in colour, with a few that are either black or greenish (Weingartner, 1987). Whole soybean is made up of 8% hull, 90% cotyledon and 2% hypocotyl (DM) (Wikipaedia, 2008).When ground, the soy flours are cream to light yellow in colour. Increased heat treatment causes darkening of the flour probably because of browning reactions (Wilcox, 1987).

According to US Standards, soybeans fall into four grades (Table 1). Soybeans not meeting standards in grades 1 to 4 and any that are musty or sour are categorised as sample grade. They are, therefore, not acceptable for human consumption. By percentage "coloured" means brown, black or discoloured soybeans other than either yellow or green.

Table 1: Soybean grades according to US Standards

Requirements	Grades			
	1	2	3	4
Maximum moisture (%)	13.0	14.0	16.0	18.0
Splits (%)	10.0	20.0	30.0	40.0
Maximum damaged (%)	2.0	3.0	5.0	8.0
Heat damaged (%)	0.2	0.5	1.0	3.0
Foreign matter (%)	1.0	2.0	3.0	5.0
Coloured (%)	1.0	2.0	5.0	10.0

Source: USDA (1988).

The nutritional content of soybean grain, according to Nelson *et al.* (1971), is: 3.34% sugar, 13.60% protein, 3.36% starch, 6.32% oil, 1.48% ash, 1.53% fibre and 10.63% nitrogen free extract. Laswai *et al.* (2005) reported soybeans to contain 335 kcal energy, 42% protein, 18% oil and 6.5mg iron. The International Congress on Biodiesel (ICB, 2007a) reported that soybeans are of high nutritional value and in comparison to many today's major food sources, they are truly a nutritional super power. The congress reported further that the protein content in soybeans is the highest of any grain or legume and that they contain substantial amounts of protein (35-40%DM), fat (15-20%DM), carbohydrates (30%DM), minerals and ash (5%DM), dietary fibre, vitamins and a virtual drugstore of phytochemicals useful for the prevention and treatment of many chronic diseases. The National Soybean Research Laboratory (NSRL) (2007) also stated that soybeans contain all three of the macronutrients namely: protein, carbohydrates and fat, as well as vitamins (folic acid) and minerals calcium and iron. According to ICB (2007c) soybeans contain 35-40% protein, 15-20% oil, 30% carbohydrates, 10-13% moisture content and 5% ash and minerals [The values show major differences due to cultivar type and the growing conditions of a given area]. Most recently reported values are obtained from highly improved varieties as Soyatech (2007) reports that genetically modified soybean varieties began to be commercially grown in 1996 and they quickly became dominant in the major soy producing countries.

2.2.1 Moisture content

The average moisture content of soybean at harvest is 14%. (Singh *et al.*, 1987) stated that suitable moisture content for storage for a period of 6-12 months is 13% while for a longer storage 10-11% moisture content is recommended. It is further explained that storage temperature below these may cause rupturing of the bean thus being susceptible to attack by spoilage microorganisms. The rupturing effect will depend on the climate of the given place since in areas where air is wet seeds constantly adsorb moisture.

2.2.2 Protein content

Soybean has relatively high protein content of high quality compared to that of other legumes. The content varies from 35% to 42% (ICB, 2007b). Yagasaki *et al.* (1997) reported that soybean seeds contain protein in a concentration of 40 – 45% (DM). It was further reported that glycinin, the major storage protein, accounts for about 35% of total soybean seed protein. Glycinin plays an important role in "tofu" gel formation and its functional properties have been compared to those of β-conglycinin, the other major storage protein in seeds (Poysa *et al.*, 2006).

Soy protein contains all of the essential amino acids and can sustain good health at all stages of human growth. Protein quality is determined by the balance of essential amino acids. Food legumes are known to be deficient in the sulphur containing amino acids, methionine and cystine, but they are relatively high in lysine which is low in cereal grains (FAO, 1977). Using the protein digestibility corrected amino acid score (PDCAAS) method, soy protein products generally receive scores of between 0.95 and 1.00, the highest value possible for plant protein (ICB, 2007b).

Due to its high protein quality in terms of essential amino acids composition, soybean flour is used as food ingredient to enhance the value of finished foods notably weaning foods and margarine. Soybean and soy fortified foods have been appraised for their protein content from a nutritional perspective and as such there is much interest among clinicians and researchers on their potential role in preventing and treating chronic diseases (Sawonola *et al.*, 2005). In a study by Anderson (1995), soy protein intake was associated with a 9.3% reduction in serum cholesterol, a 12.9% reduction in Low Density Lipoprotein (LDL) cholesterol and a 10.5% reduction in serum triglycerides. In the same study, High Density Lipoprotein (HDL), the "good" cholesterol concentrations increased by 2.4%. This serum cholesterol modification has the potential to reduce risk for coronary heart disease by 18-28%. It was concluded that soy protein was clearly potent in decreasing LDL-cholesterol levels. These health facts are supported by meta-analysis of 38 studies whose results suggested that soy protein may have antioxidant properties that help protect "bad" LDL cholesterol from oxidising (Anderson *et al.*, 1995). The formulation of soy-based foods is increasing as they are deemed suitable vehicles for fortification to meet known deficiencies of essential nutrients. Besides providing an alternative to traditional dairy products, soy protein is also being incorporated into many nutritious meat replacement beverages offering convenience to on-the-go consumers (Deshpande *et al.*, 2008).

2.2.3 Oil content

Soybeans, in comparison to other legumes and cereal grains, contain a high amount of oil (21%) with the saturated and unsaturated fatty acids being 13 and 87% respectively (ICB, 2007b). In a study by Nwar (1985), the saturated fatty acids consist of 9% palmitic ($C_{16:0}$)

and 4% stearic ($C_{18:0}$) while the unsaturated fatty acids consist of 24% oleic ($C_{18:1}$), 54% linoleic ($C_{18:2}$) and 8% linolenic ($C_{18:3}$) acids (Singh *et al.*, 1987).

Fortunately, the oil that is naturally found in soybean and that which ends up in whole soybean-based foods including most traditionally processed soy foods such as tofu, soymilk, tempeh, full fat soy flour, and liquid soybean oil can contribute to a reduction in cholesterol levels and decreased incidences of heart disease and cancer (Anderson, 1995). It is known that the amount of dietary fat and its composition are major determinants of serum cholesterol levels (ICB, 2007b). Approximately 50% of the fat is linoleic acid and 8% is alpha linolenic acid, which is an omega-3 fatty acid. This is the type of fat found in fish which is believed to be beneficial in lowering the risk of heart disease (ICB, 2007c). While saturated fatty acids and dietary cholesterol elevate serum cholesterol, polyunsaturated fatty acids have a modest cholesterol-lowering effect relative to carbohydrates (FAO, 1994).

Soybean oil is susceptible to flavour reversion because of its high linolenic acid content (8-9%) (Weingartner, 1987). Off-flavours occur when oxygen reacts with linolenic acid to form compounds such as 2-n-pentylfuran. Flavour reversion is a misnomer because the off-flavours are not present in fresh soybean oil. The off-flavour produced by these compounds has been described as beany and grassy (Singh *et al.*, 1987).

2.2.4 Carbohydrate content

Soybeans contain 35% soluble and insoluble carbohydrates. Of the carbohydrates (35%) contained in soybean, the insoluble portion consists of cellulose (4%) and hemi cellulose (15%) (Wagner *et al.*, 1977). The soluble carbohydrates consist of sucrose (4.5%) and the oligosaccharides stachyose (3.7%) and raffinose (1.1%). Stachyose is made up of galactose-galactose-glucose-fructose while raffinose is made up of galactose-glucose-fructose. The human body can not absorb intact oligosaccharides, as humans lack galactosidase for hydrolysis to their constituent simple sugars. The oligosaccharides, therefore, pass from the small intestine to the large intestine where they are fermented by intestinal microflora. These types of intestinal flora are considered important for human health, as it is believed that their presence can reduce incidence of many lower gastro intestinal tract, including colon cancer (ICB, 2007a). The microflorae, bifido bacteria, use these as nutrients to sustain and promote their growth through fermentation. This fermentation produces flatus (composed of carbon dioxide, hydrogen and a small amount of methane) – an unpleasant side effect of eating soybean foods. A linear relationship between the soy products oligosaccharide content and flatulence has been reported by Singh *et al.* (1987). Boiling whole soybeans for 20, 40, and 60 minutes in tap water removed, respectively 15%, 30% and 49% of the oligosaccharides, whereas boiling in 0.5% solution of $NaHCO_3$ removed 21%, 48%and 60% (Nelson *et al.,* 1976). The insoluble carbohydrates are nevertheless important for they are the major component of dietary fibre. The reported levels of fibre in soybean depend on the fat status in the flour. According to Caldwell (1973) full fat flour and defatted flour contain 2.8% and 3.2% fibre respectively. Lower levels of fibre (1.53%) have been reported (Nelson *et al.*, 1971). It is probable that variability in the fibre level is varietal dependent and that may include other factors like maturity status and growth conditions. The fibre level has nutritional implications in as far as being associated of oligosaccharides that cause flatulence.

2.2.5 Ash and mineral content

Soybeans are not a major source of minerals but, when included in a mixed diet, contribute to overall requirements. The major mineral components of soybeans are potassium, sodium, calcium, magnesium, sulphur and phosphorus. The ICB (2007a) reports ash content of 5% in soybeans. Mineral content can vary widely due to soil type and growing conditions. In general, the amount of minerals absorbed by the human body from consumed/ingested legumes and cereals is less than that from animal sources (CAST, 2003). This is due to presence of some chelating agents notably phytic acid that is present in whole soybeans. It chelates divalent cations, such as Ca, Fe and Zn, and reduces their bioavailability. Whole soybean consists of 0.28% Ca, 97ppm Fe, 0.22% Mg, 28ppm Mn, 0.66% P, 1.7% K and 22ppm Zn (Singh *et al.*, 1987). Presence of Fe in soybeans is of special interest since its utilisation may reduce incidence of anaemia. Iron deficiency anaemia is associated with substantial reduction in physical work capacity, poor attention span, inadequate fine motor skills, and reduced memory retention in adolescents (SCN, 1993). Moreover, maternal iron deficiency during pregnancy has been associated with low baby birth weight, altered placental foetal ratio and increased risk of cardiovascular diseases in adulthood (Gambling, 2006). Iron is important in blood formation and the above mentioned disorders are imminent on the ground that iron functions as an oxygen carrier, a medium for electrons transport within cells and as an integrated part of enzyme systems in various tissues since it is important in blood formation (FAO/WHO, 1988; FAO, 1999). It is a limiting element due to its susceptibility to chelation hence reduced bioavailability.

2.2.6 Vitamin content

Soybeans are not unique in being rich sources of any vitamin. Nevertheless, their inherent dietary levels definitely contribute to the overall nutritional well being. The water-soluble vitamins in soybeans include thiamine, riboflavin, niacin, panthothenic acid, biotin, folic acid, inositol and choline and fat-soluble vitamins notably vitamins A and E (ICB, 2007a). The fat soluble provitamin A (β-carotene) decreases as the soybean matures and dries (Kimura and Rodriguez-Amaya, 2003). The carotenoid composition varies markedly as influenced by such factors as variety, part of plant utilised, degree of maturity at harvest, climatic or geographical effects, cultivation and postharvest handling practices. Apart from nutritional point of view, tocopherol (vitamin E) is an important constituent of soy oil due to its antioxidant properties.

Deficiency of vitamins in human body has variable and far reaching impacts. Vitamin A Deficiency (VAD), for instance, causes progressive damage to the eye, eventually leading to blindness (ACC/SCN, 1992). VAD impairs children's resistance to infection and 37% of under-five children in developing countries have sub-clinical vitamin A deficiency (UNICEF, 2004). As earlier described, the β-carotene level in soybeans decreases with the maturity (Kimura and Rodriguez-Amaya, 2003).

Hence mature bean and soybean products like soymilk contain very little if not none of vitamin A precursor. Table 2 gives the estimated mean requirements and safe level of vitamin A intake for different age groups (FAO/WHO, 1988). The low levels of vitamin A precursor in soy products implies that there is a need for fortification of soymilk using other vitamin A sources notably green vegetables like *Moringa oleifera* and *Vigna unguiculata* (L) should soymilk be used as breast milk substitute. Vegetables play a highly significant role in food security to the underprivileged people in urban and rural settings (Schippers, 1997). They can serve either as primary foods or as condiments to dishes prepared from domesticated soybean varieties (Grivetti and Ogle, 2000).

Table 2: Estimated mean requirements and safe level of intake for vitamin A, by group

Group	Mean requirement (µgRE/day)	Recommended safe intake(µgRE/day)
Infants and children		
0-6 months	180	375
7-12 months	190	400
1-3 years	200	400
4-6 years	200	450
7-9 years	250	500
Adolescents		
10-18 years	330-400	600
Adults		
Females		
19-65 years	270	500
65+ years	300	600

Source: FAO/WHO (1988)

2.3 Isoflavones Content

Soy isoflavones are not only special because of the effect they are believed to have on health, but also because for all practical purposes, no other food contains as significant an amount of these chemicals as does the soybean (ICB, 2007b). The major isoflavones in soybeans are genistein, daidzein and glycitein. Of these, it is genistein, which is believed to have the outstanding potential of preventing or treatment of certain cancers. Isoflavones are also sometimes referred to as phytoestrogens, meaning plant estrogens, because they have structural similarity with estrogen (although the estrogenic effect of isoflavones is approximately one thousand times weaker than the natural hormone) (Yildiz, 2005). Soy phytoestrogens adsorbed onto the soy protein are the agents that reduce serum cholesterol levels (Nelson *et al.*, 1978). Lack of these in the diet does not lead to any characteristic deficiency syndrome nor do they participate in any essential biological function (Johnston, 2003). Hence, phytoestrogens can not be considered as nutrients.

2.4 Nutritional Comparison of Soybeans with Grains and Pulses

According to Singh *et al.* (1987), soybeans are among the most promising foods available to improve the diets of millions of people and research is underway to minimise the constraints to its production throughout the tropics, particularly in Africa. At present more than 1.45 x 10^8 ha of Sub-Saharan Africa would support cultivation of this crop, including areas in Ghana, Nigeria, Cameroon, Zaire, Tanzania, Kenya, Zambia and Zimbabwe (Singh *et al.*, 1987). Soybeans nutritional composition can be compared to grains such as rice, corn, and wheat. Their energy content is 400 – 430 cal. /100 g compared to 350 cal. /100 g of rice and other grains. Protein content is 38% for soybeans compared to rice (6.2%), corn (8.2%) and wheat (10.5%). Such protein-rich and high calorie soybeans, if directly used as food, can alleviate the shortage of protein food in the world, and for this purpose various recipes utilising soybeans have been investigated (Nelson *et al.*, 1976). From the economic, nutritional and health point of view it is worthwhile to explore means for enhancing soybean protein utilisation.

2.5 Soybean Antinutritional Factors

Soybean has several antinutritional factors. These include trypsin inhibitors, lectins, goitrogens, anti-vitamins, allergens and chelating agents. These have variable adverse nutritional effects including inherent differences in heat sensitivity. It is important to inactivate these factors in order to prevent adverse physiological conditions (Zhao, 2005).

Trypsin inhibitors are probably the best known, and certainly the most studied of all the anti-nutritional factors present in soy-beans. These inhibitors account for 6-10% of soybean protein (Kakade *et al.*, 1973). Thermal inactivation of these inhibitors is accompanied by a marked enhancement in protein nutritive quality (Nelson *et al.*, 1978). The extent by which the trypsin inhibitor in legumes is destroyed by heat is a function of the temperature, duration of heating, particle size, and moisture conditions- variables that are closely controlled in the commercial processing of soybean-oil meal in order to obtain a product with maximum nutritive value (Milner, 1975). The loss of the amino acids contained in trypsin is responsible for inhibiting growth (Liener, 1981). Trypsin inhibitors irreversibly bind trypsin, making the enzyme unavailable for its role in proteins digestion. This causes the intestines to release cholecytokinin to stimulate the pancrease to produce more trypsin. The increased secretory activity causes the pancrease to enlarge. The amino acids present in trypsin (sulphur containing methionine and cystine) can not be reabsorbed and thus are lost. Cooking of soybeans helps in inactivating trypsin inhibitors and makes the protein easier to digest (Kakade *et al.*, 1973). Lectins (Haemagglutinins) are readily inactivated by moist heat.

Goitrogens are present in soybeans as phenolic glycosides and they are water soluble (FAO, 1977). The phenolic metabolites formed from the glycoside are iodinated preferentially and thereby deprive the thyroid gland of available iodine. Thus the goitrogenic effect is counteracted by iodine supplementation but not by heat treatment. A dietary iodine supplementation at a level of 1.6 mg/kg of diet is usually recommended. Anti-vitamins are inactivated by heat treatment and their effects are counteracted by supplementation with vitamins and minerals. Chelating agents are heat stable anti-nutritional factors which interfere with bioavailability of minerals, e.g. Ca, Mg, Zn, Mn, Molybdenum, and iron (Chergan, 1980) by affecting their absorption. Whole soybeans contain 1-2% phytic acid which is found in plant but not in animal tissues and may be one of the plant's methods of storing phosphorus and carbon (Erdaman, 1979). They decrease availability of Ca, Zn and Fe by the formation of amino acid insoluble protein-phytic acid-mineral complex. It has been cited as causing reduced availability of zinc in soybean foods (Forbes and Parker, 1977) and calcium in whole wheat bread. However, phytic acid does not interfere with the bioavailability of added minerals to products, hence effective supplementation. Allergens responses are rare in humans but are common in soy-based infant formulae (Halpen *et al.*, 1973). Cases of allergy have been observed in workers within soybean processing plants or food plants using soy flour (Bush and Cohen, 1977). They cause gastrointestinal disturbances due to β-conglycinin and glycinin (Kilshaw and Sissons, 1979), the major storage soy proteins. They are quaternary in structure and sensitive to heat, pH, ionic strength and to organic solvent e.g. alcohol. Although heat treatment does not inactivate allergens, they are vulnerable to inactivation by alcohols (Sissons *et al.*, 1982).

Heat stable anti nutritional factors are removed from soybean through solubilisation in acid or water (Linden and Lorient, 1999). Moist heat treatment is used to remove or modify flavour compounds, though bound flavours persist and may be removed when soybean is incorporated into a food and during mastication (Berk, 1992). Fresh soybean may have residual antinutritional factors and agents that survive the heat treatment that is meant to rid soybeans of the antinutritional factors. Residual compounds that impart milk with off flavour may in a way decrease acceptability of soymilk based products. Fresh soymilk for human use, therefore, needs sensory quality improvement for it is made directly from the soybean (Berk, 1992).

2.6 Causes of Soybean Deterioration During Storage

Prior to storage, soybeans have to be dried to moisture content of 10-11% (Anon, 1987). Improperly dried and damaged soybean is susceptible to insect and mould infestation and especially if the grains are stored at higher moisture and temperature conditions (Anon, 1987). Deterioration by mould growth is compounded by liberation of aflatoxins in the grains (Singh *et al.*, 1987).

2.7 Soybean Utilisation in Tanzania

Tanzania has the potential of producing more than two million tons of soybeans per year. The under utilisation of soybean is due to lack of reliable market and knowledge on processing and utilisation at household level (Laswai *et al.*, 2005). Some varieties, through ARII, that have been demonstrated to be high yielding are TGX 1895-33F, TGX 1895 – 4F, TGX 1876 – 4E, and TGX 1895 - 49F (Myaka, 2005). Yield is expressed in terms of grain yield (kilogram per hectare) and seed size. Test genotypes were fifteen TGX series from the International Institute for Tropical Agriculture (IITA) with two check varieties namely Bossier and Uyole soya-1 (Myaka, 2005).

2.8 Conventional Scheme for Processing Soymilk

Soymilk is made by soaking soybeans, grinding them with water. The fluid which results after straining is called soymilk. Ogundipe *et al.* (1989) examined different processing methods for the production of soymilk. It was observed that the method with the highest yield was that in which soybeans were soaked overnight before blanching or grinding. Both the quality and yield of soymilk are important parameters since it is the production of quality soymilk and not diluted soymilk that will cause soymilk to achieve its aim as a protein supplement. The steps involved in the processing of soymilk are cleaning, soaking, de-hulling, blanching, grinding, filtration/decantation, pasteurisation, packaging and cooling (Berk, 1992). The quality and acceptability of soymilk is related to factors such as blanching time, added water, heating temperature, wholeness of beans, use of $NaHCO_3$ and incorporation of blanch water (Ogundipe *et al.*, 1989). The following account is a descriptive summary of the major soymilk processing steps (scheme). The inherent advantages for each step are also highlighted.

2.8.1 Cleaning

Cleaning of soybeans is done manually to remove extraneous materials like insects, stones, and damaged kernels/seeds. Presence of damaged seeds causes off-flavours and off-odours in the product (Berk, 1992). Hydrated sound beans do not develop beany or painty odours and flavours but addition of water to damaged/broken tissues results in instant off-odour and off-flavour development (Nelson *et al.*, 1978). Wholesomeness of soybeans is therefore, important at this very initial stage. It is a critical control point, according to HACCP, to ensure quality soymilk.

2.8.2 Soaking

Soaking is a means by which heat stable antinutritional factors are removed. It is also argued that soaking easies grinding and improves milk yield (Ogundipe *et al.*, 1989). It is recommended that the soaking duration be controlled to prevent fermentation, development of beany and bitter taste especially within the tropics. Soybeans are soaked in three times their weight of water (Gupta, 1997) and there are variable soaking durations that have been recommended. Although Plahar and Annan (1994) suggested 30 minutes as standard soaking time for beans in a 1:5 ratio, Gupta (1997) reported that 4-6 hours are generally required for soaking of soybeans in water at room temperature while soaking at 50-60^{0}C reduces soaking time to two hours. In temperate countries it is also recommended that soaking times of 4-6 and 8-12 hours are suitable during summer and winter respectively.

2.8.3 Dehulling

Dehulling, in a micro scale, is done by rubbing the beans between the hands to remove hulls. A separator may be used in large scale production (Berk, 1992; Debruyne and Koseoglu, 2006). The advantages of dehulling are to produce soymilk with attractive white colour (Anon, 1987), reduction in blanching time (Berk, 1992; Plahar and Annan, 1995) and increasing the rate of heat transfer during blanching. Furthermore, dehulling improves emulsion stability (Berk, 1992; Debruyne and Koseoglu, 2006). Anon (1987) reported that dehulling helps to remove bacteria and oligosaccharides present in hulls to avoid off-flavours and processing problems caused by foaming, hence better flavour and shelf life. These advantages not withstanding, the dehulling process may lead to removal of the germ which is rich in isoflavones; the important phytochemicals for treatment and prevention of cancer (Berk, 1992; Debruyne and Koseoglu, 2006; ICB, 2007a).

2.8.4 Blanching

The milk yield and indeed the extracted protein have been shown to vary with blanching time. According to Nelson *et al.* (1978), blanching of soybeans can be done by boiling the beans in sodium bicarbonate solution for 30 minutes. Blanching inactivates enzymes and heat labile anti nutritional factors, lipoxygenase and trypsin inhibitors (Anon, 1987; Laswai *et al.,* 2005). Although these anti-nutritional factors are highly resistant, they are easily removed if hydrated. Addition of sodium bicarbonate at 1% softens the soybean seed coat and thereby facilitating heat transfer to the inner parts of the bean which also renders easier grinding (Hango, 2002). Blanching enables unfolding of polypeptide chains that brings about the capacity to interact with each other and increase stability and viscosity of the final product. Otherwise blanching may cause leaching of minerals and water soluble vitamins into the blanching water (Linden and Lorient, 1999).

Singh *et al.* (1987) studied the influence of blanching time to soymilk quality. The percentage total solids in soymilk after blanching beans for 5,7,10 and 20 minutes were 6.6, 6.2, 6.4 and 4.76 respectively. The respective percentage proteins were 2.9, 2.9, 2.87 and 1.37. The resulting milk had objectionable off-odour. Milk boiled for 5 and 7 minutes had off-flavours. However the milk boiled for 10 minutes had no off-flavour and its protein level was reasonably high compared with lots boiled for 20 minutes. Hence boiling for 10 minutes was the optimum blanching time interval. Nelson *et al.* (1976) and Singh *et al.* (1987) reported the method used at the University of Illinois that whole or dehulled soybeans are blanched in 0.5% $NaHCO_3$ solution for 30 or 20 minutes respectively.

2.8.5 Grinding

Grinding is done to allow extraction of soy proteins, carbohydrates, minerals, vitamins and most of the oil from soybean. Apart from nutritional point of view, soy proteins are reported to stabilise the soymilk emulsion as they tend to accumulate at the oil-water interface and limit rupture of its emulsion (Linden and Lorient, 1999). It is further reported that soy proteins allow more water to be incorporated in the product and losses in cooking to be reduced. Blanched soybeans are ground while hot or cooled in blanching water. The use of cooled water in grinding reduces protein denaturation, solubility and extraction yield (Debruyne and Koseoglu, 2006). Processes selected for soymilk production, therefore, should be those that allow efficient extraction of soy proteins and other nutrients. In achieving this goal, soybean: hot water ratio of 1:8 and temperature 80-100^{0}C is preferable in grinding conditions that facilitate easier grinding, efficient soy protein extraction and improved sensory quality (Anon, 1987).

2.8.6 Filtration

Before filtration, the slurry is stirred well. Filtration improves taste, smoothness and emulsion stability. The process involves separation of solids from liquid portion (soymilk) using a fine muslin cloth filter in micro scale production or centrifugation and decanters in large scale processing (Berk, 1992; Fellows, 2001). Some proteins, vitamins, minerals, carbohydrates and oils are lost during filtration. The solids (residue) can be washed and be refiltered to improve milk yield (Debruyne and Koseoglu, 2006).

2.8.7 Pasteurisation

There are two methods: continuous and batch pasteurisation with the choice method depending on production scale. In large scale production, continuous method in which soymilk is brought to 72^{0}C for 15 seconds, flash pasteurisation which employs high temperature short time 130^{0}C for 5 seconds and ultra high temperature (UHT) heat treatments are employed (Fellows, 2001; Debruyne and Koseoglu, 2006). Batch pasteurisation is done to the entire volume of soymilk within the temperature range of 80-100^{0}C for 30 minutes (Fellow, 2001).

2.8.8 Packaging

Whereas at micro scale production level soymilk is packed in plastic bottles, Tetra-pack and glass bottles are used for packaging soymilk produced on a large scale (Jongen, 2002). The packaging containers should be sterile and free from cracks. They should not allow air and light penetration so as to protect micronutrients and enhance product shelf life (Jongen, 2002; Debruyne and Koseoglu, 2006).

2.9 Soymilk Composition, Classification and Comparison

Soymilk composition depends on some factors: variety used to extract soymilk, the manufacture and preference. Soyfoods manufacturers require different types of soybean varieties for each product for a given purpose (Soyatech, 2007). The following are typical soymilk compositions per 100 g: 93.27 g moisture, 2.75 g protein, 1.90 g fat, 0.27 g ash, 0.58 mg iron, 4 mg calcium and 3 micro-mg of provitamin A (Holden, 2004). The sedimentation rate is 5.8% while its specific gravity is 1.017 (Akintunde and Akintunde, 2005). Large proportions of these nutrients, when whole soybeans and soymilk are compared of their nutrient contents, are left in the residues after filtration to obtain soymilk. Unless these residues are used for feeding animals it is a great loss of nutrients. Soymilk composition is, apart from other factors, affected by the process used in its preparation. Singh *et al.* (1987) reported that the choice of a process is very important if quality soymilk was to be obtained.

USDA (1975) defined soybean milk as that soybean extract that contains 3.4%protein, 1.5% fat, 2.2% carbohydrates and 0.5% ash. This definition was revised by USDA (1986) as extract from soybeans that contains 2.75% protein, 1.91% fat, 1.81% carbohydrates and 0.27% ash. There are five subcategories of soymilk namely plain (or traditional) soymilk, dairy-type soymilk, soy beverages, cultured products and blends (SAA, 1996). Plain soymilk is made by water extraction of whole soybeans, using a bean to water ratio of 1:5. It has to contain approximately 4% protein. Dairy-type soymilk is formulated so as to have a composition roughly similar to that of dairy milk. Bean to water ratio should be 1:7. Protein content has to be 3.5%, slightly sweetened, and containing added oil and salt. It may also contain imitation milk flavour. Soy beverages are sweetened and flavoured drinks, containing about 1% protein. Bean to water ratio has to be 1:20. Cultured products are any of the above type after lactic fermentation or acidification with lactic acid. Blends are mixtures of soymilk and other vegetable or dairy milks. USDA (1986) classified soymilk basing on soy protein, soy oil and minimum total solids as shown in Table 3.

Table 3: Classification of soymilk according to soy protein, soy oil and minimum total solids content

Product	Soy protein	Soy oil	Total solids
Soymilk	≥ 3.0	≥ 1.0	≥ 7.0
Soymilk drink	1.5 – 2.9	≥ 0.5	≥ 3.9
Soymilk powder	> 38.0	≥ 13.0	≥ 90.0
Soymilk concentrate	≥ 6.0	≥ 2.0	≥ 14.0

Source: USDA (1986)

The International Soybean Programme (INTSOY) (1987), a programme supported by the US and other International Agencies and managed by University of Illinois, compared soymilk and cow milk as shown in Table 4.

Table 4: Comparison of soymilk with cow milk

Component	Soybean milk (at different moisture content)				cow milk
	1	2	3	4	
Water (%)	92.00	90.00	89.25	92.50	87.30
Protein (%)	3.70	4.95	3.15	3.02	3.42
Fat (%)	2.00	2.97	3.10	3.02	3.67
Carbohydrates (%)	1.80	1.34	3.02	0.03	4.78
Vitamin A, I.U.	Nil	Nil	Nil	Nil	Nil
Thiamine, mg/100g	Nil	Nil	Nil	Nil	Nil
Riboflavin, mg/100g	Nil	Nil	Nil	Nil	Nil
Vitamin D, I.U.	Nil	Nil	Nil	Nil	Nil
Ash (%)	0.50	0.44	0.45	0.41	0.73
Crude fibre, g/100g	0.14	0.20	-	-	-
Ash, g/100g	0.60	0.80	-	-	-
Ca, mg/100g	52.00	65.00	-	-	-
Fe, mg/100g	1.10	1.30	-	-	-

Source: Singh *et al.* (1987)

Calcium bioavailability is a problem for soymilk. While it was found that calcium from soymilk was absorbed at 75% the efficiency of calcium from cow milk, soymilk is still a significant source of calcium in the diet (Heaney *et al.*, 2000). In comparison with animal protein, soy protein decreases calcium excretion. This is, presumably, due to the lower sulphur amino acids content of soy protein. Consequently, a soy-based diet is able to maintain calcium balance with a lower calcium intake (Zemel, 1988).

Cow milk contains lactose while soymilk does not. Due to this, soymilk is suitable to persons who are lactose intolerant i.e. inactive lactase enzyme is unable to digest lactose (the sugar found in milk and other dairy products). However, there are 1-2% of all adult and 6-8% of infant Americans who are allergic to soymilk due to presence of Gly mBd 30K/P34 gene (USDA, 2002). It has been reported by Bost (2007) that studies are being conducted to ensure that the soybean is completely free of the allergy-causing protein.

2.9.1 Soymilk standards

The purpose of standards in any country is to promote honesty and fair dealing in the interest of consumers, to help to ensure that consumers receive quality products, to establish consistency and fairness in labelling and disseminate sound nutritional information. In countries where soymilk is commonly consumed, soymilk standards are already in place. In United States of America, the Soyfoods Association of America has standards that are approved by FDA (SAA, 1996). Some minimum requirements for some countries for different soy products are as shown in Appendix 3.

In achieving promotion of soymilk utilisation in countries like Tanzania, formulation and promulgation of standards for various soymilk products are of great importance.

2.9.2 Microbiological quality of soymilk

Soymilk has microbiological quality requirements given by SAA (1996). None of the following bacteria should be present- *Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, Vibrio parahemolyticus, Listeria monocytogenes* and *Campylobacter jejuni.* Also no *Yersinia enterocolitica* should be present and the final SPC should not exceed 20,000 CFU/g. Coliform bacteria should be absent in 1.0 ml samples.

2.9.3 Use of flavouring agents

In countries like USA, China and Japan, soymilk is most commonly flavoured and fortified with extra calcium (300-400 mg per cup) or vitamin A (400IU per litre) and is sold in aseptic cartons (Bost, 2007). The most popular flavours for soymilk are vanilla and chocolate (Wang *et al.*, 2000). Some producers add thickeners to their soymilk to give it a mouth feel of cow milk.

Flavour and colour of soymilk are influenced by presence of various compounds at low concentrations. These are alcohols, ketones, furans, aldehydes, phenols and phosphatides. They impart grassy, mouldy, mushroom, musty, potato-chip like, bitter and cooked off-flavours (Nelson *et al.*, 1978). It was in 1966 when scientists at Cornell University discovered that the enzyme lipoxygenase was responsible for creating the beany flavour in soymilk (SAA, 1996; Laswai *et al.*, 2007). This flavour is caused by interaction between enzyme lipoxygenase with free fatty acids present in raw damaged soybeans (Linden and Lorient, 1999). This is a common problem in traditional methods where grinding of cold water soaked soybeans is practised. These beany flavour compounds are not present in sound, dry soybeans but are produced as soon as the soybeans are wetted and ground. The dehulled soybeans should be subjected to another processing step within short time possible.

There are other modified technologies for producing less beany flavoured soymilk without flavour masking such as use of hot water instead of cold water during grinding. Soaking and blanching whole soybeans followed by bicarbonate treatment and pasteurisation of extracted soymilk have been reported to influence soymilk quality, composition and yield of the final product (Wilson, 2003).

The use of flavouring agents to foods and beverages must not exceed the maximum levels in mg/kg specified in the finished product (FAO/WHO, 1999). Vanilla, for instance, can be used to the maximum of 70 mg/kg of the ready-to-eat product. Flavouring agents must be used in the minimum quantity required to produce their intended effect and in accordance with all the principles of Good Manufacturing Practices (GMP) (FAO/WHO, 2007).

Vanilla flavour is naturally obtained via the shikimic acid pathway or as a lignin by-product during processing of wood pulp and paper while banana flavour (isomyl acetate) is from banana (Fennema, 1985). The pineapple flavour is due to 4-Hydroxy-5-methyl-3(2H)-furanone, a compound sometimes known as the "pineapple compound", which was first isolated from processed pineapple and that strongly contributes to the characteristic flavour (Fennema, 1985).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Three soy-bean varieties TGX 1895-33F, TGX 1876-4E and TGX 1895-49F were obtained from Agricultural Research Institute- Ilonga (ARII) in Kilosa District, Morogoro Region. The varieties were grown in two different seasons. Variety TGX 1895-33F was alone cultivated in year 2007 after going out of stock as a response to high demand by farmers due to its high yields per hectare. Three flavour improvers were used in this study namely vanilla (Langdale brand, made in England), banana and pineapple (Nandi brand, made in India). They were purchased from supermarkets in Dar es Salaam and Morogoro. These flavours were selected due to their availability and use as flavouring agents for various food products.

3.2 Methods

This study was conducted in three phases. Phase one was aimed at determining whole soybean nutrient composition. This was followed by phase two where determination of varieties` soymilk yield levels and nutrient composition were undertaken. These activities were followed by sorting using Excel software to select the best two soybean varieties using soymilk yield levels, protein and iron content as primary, secondary and tertiary criteria for sorting. The two selected varieties were then used in phase three where flavouring and sensory evaluation trials of soymilk were conducted.

3.2.1 Determination of nutrient composition in whole soybeans

3.2.1.1 Sample preparation and sampling

Each of the three varieties, i.e. TGX 1895-33F, TGX 1876-4E and TGX 1895-49F, was thoroughly mixed, sorted out and cleaned to enough working sample from each one's lot of 50 kg. Quarter sampling was used in order to obtain analytical samples for each. The soybean samples were milled into flour at the Department of Animal Science and Production Laboratory (DASPL), Sokoine University of Agriculture (SUA) using laboratory grinder machine type 8" LAB MILL, serial number 19911 manufactured by Christy Hunt Engineering Limited of England and were sieved through 0.1 mm mesh screen.

3.2.1.2 Percentage moisture determination

Moisture content of whole soybean flour was determined in quadruplicate by oven drying method according to the AOAC (1995) method. Exactly 2.000 g of sample were dried in an oven set at 105°C for 24 hours, and the moisture content was obtained by differences. The difference between wet and dried sample represented the dry matter (DM) and this was expressed as a percentage of the sample weight.

3.2.1.3 Percentage protein determination

Protein content of whole soybean flour was determined by Kjeldahl method (AOAC, 1995) official method 920.87. Exactly 0.5 g portions of dried sample, in quadruplicate, were weighed onto tared filter papers and quantitatively transferred into digestion tubes. Digestion was done at temperature 420°C followed by distillation using Tecator Kjeltec system. Titration was done and nitrogen content was calculated as follows:

$$\%N = \frac{14.01 \times (\text{titre (ml)} - \text{blank (ml)}) \times \text{Conc. of acid} \times 100}{\text{Weight of dry sample (g)} \times 1000}$$

Where, %N = percentage nitrogen

Percentage protein was calculated from the percentage nitrogen using the factor 6.25 for plant materials as follows:

$$\%\,CP = \%\,N \times \text{Factor}$$

Where; %CP = percentage crude protein

3.2.1.4 Percentage fat determination

Soybean flour oil was determined by Soxhlet ether extraction method (AOAC, 1995), Official method 920.85. A 5.000 g portion of sample was weighed onto a tared balance and the extraction unit was set at temperature of 40°C. Oil recovery was done using oven to evaporate any remaining n-hexane at 9-10°C for 1 hour. Percentage fat was calculated by using the formula:

$$\%\text{Fat (DM)} = \frac{((\text{Weight of flask} + \text{fat}) - \text{weight of empty dish})\,\text{g} \times 100}{\text{Weight of dry sample (g)}}$$

3.2.1.5 Percentage ash determination

Ash content was determined by using AOAC (1995), Official method 923.03. Four pre-cleaned crucibles for each sample were dried in an oven, cooled in desiccator and filled with samples. They were heated in the muffle furnace set at 550°C for 24 hours. Percentage ash was calculated as follows:

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

3.2.1.6 Percentage fibre determination

Percentage fibre content was determined by using AOAC (1995), Official method 920.86. Exactly 1.0 g of soybean flour was analysed using the 220 ANKOM Fibre-Tech set at 550°C for 2 hours. Fibre content was calculated and expressed as a percentage average of the quadruplicate samples on dry matter basis.

$$\text{Fibre content}\,(\%\,\text{DM}) = \frac{(C - B) - (E - D) \times 100}{A}$$

Where;

A = weight of dry sample (g)

B = Bag weight (g)

C = Bag + dry residue (g)

D = Crucible weight (g)

E = Crucible + ash (g)

3.2.1.7 Percentage carbohydrate determination

Carbohydrate content of the soybean flour was calculated as percentage difference (AOAC, 1995) using the formula:

Carbohydrate (%) = 100- (%mc + %CP + %CFat + %Ash + %CF)

Where;

mc	= moisture content
CP	= Crude protein
CFat	= Crude fat
CF	= Crude fibre

3.2.1.8 Energy content

Energy content for each of the three varieties was calculated according to AOAC (1990). This was done by multiplying percentage fat, percentage protein and percentage carbohydrate values by factors of 9, 4 and 3.57 respectively.

3.2.1.9 Mineral content determination

Mineral analysis for Ca, Fe, Cu and Zn was done 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 wavelength (Ca: Ca lamp, λ = 422nm; Fe: Fe lamp, λ = 248nm; Cu: Cu lamp, λ = 324nm and Zn: Zn lamp, λ = 213nm).

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

$$\text{Mineral content}\left(\text{mg/100g}\right) = \frac{\text{GR}(\text{mg})}{1000\text{ml}} \times \frac{100\text{ml}}{\text{SW}} \times \text{Dilution factor} \times 100\text{g}$$

Where;

GR = graph or machine reading

SW = Dry sample weight

3.2.1.10 β – Carotene content determination

Analysis of β-carotene was done in quadruplicate using the method by Rodriguez *et al.* (2004). This method involves the following steps: repeated extraction with cold acetone and filtration, separation into petroleum ether and lastly evaporation of excess petroleum ether. During extraction precautions were taken to avoid drying or exposure to strong sunlight since carotenoids are readily oxidised in the presence of light (Kimura and

Rodriguez-Amaya, 2003). According to this method, β-carotene is obtained after removal of chlorophylls and xanthophylls. Chlorophylls are removed through refluxing with anhydrous Barium hydroxide while xanthophylls are separated using aqueous methanol and β-carotene remains in the petroleum ether and is estimated at 470 nm.

Quadruplicate samples of 2.000 g each were ground and mixed with 4.000 g celite and 40ml cold acetone using mortar and pestle. After filtration washing was done and the filtrates were re-filtered into 50ml volumetric flasks through anhydrous Na_2SO_4 with the aid of glass wool. Petroleum ether was filled to the mark and absorbances were read at 470nm using UV- spectrophotometer. Percentage β-carotene was calculated as follows:

$$\beta\text{-carotene}(\%DM) = \frac{C(mg) \times \text{Acetone extract}(ml) \times 100}{1000 \times \text{acetone aliquot}(ml) \times \text{dry sample weight}(g)}$$

Where;

C = mg β-carotene obtained from the standard curve.

This analysis was repeated using the method by Allen (1989) and the results obtained were comparatively the same.

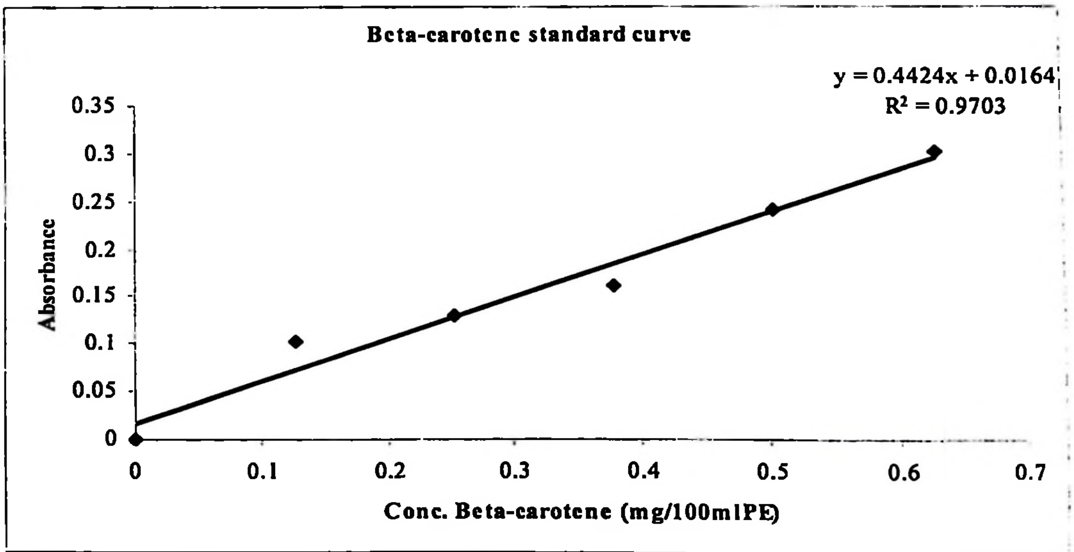

Figure 1: β-carotene standard curve

The β-carotene standard curve, shown in Figure 1, was prepared using $K_2Cr_2O_7$ standard instead of β-carotene standard. Other chemicals have been suggested as substitute standards instead of β-carotene. For example, 0.025% $K_2Cr_2O_7$ has been found to be equivalent to 0.158 mg β-carotene per 100 ml petroleum ether (Allen, 1989). Excess of $K_2Cr_2O_7$ was dried at 130°C for 2 hours. A stock solution of 1% $K_2Cr_2O_7$ was prepared. Working ranges of standards were prepared (recommended range is 0 – 0.20 mg β-carotene in 100 ml petroleum ether). Volumes to be drawn from the stock solution were calculated using the formula:

$$C_1V_1 = C_2V_2$$

Where,

C_1 = Concentration of the stock solution (1%).

V_1 = Volume to be drawn from the stock solution.

C_2 = Concentration of $K_2Cr_2O_7$ equivalent to the concentration of β-carotene.

V_2 = Total volume of the solution of $K_2Cr_2O_7$ equivalent β-carotene (i.e. 100 ml).

Absorbance for each sample was read at 470 nm.

3.2.2 Soymilk processing

Samples were prepared for each of the three varieties (TGX 1895-33F, TGX 1876-4E and TGX 1895-49F). Quartering was used to obtain a working and analytical sample. From the working sample, 3 replicas each 1 kg were weighed. Cleaning was done thrice to ensure complete removal of damaged kernels and extraneous matter. Soymilk was prepared in accordance with the scheme outlined in Appendix 4. Soymilk for proximate and selected micronutrients analysis was filled in plastic containers at 30°C and refrigerated. Flavours were added into fresh soymilk immediately after cooling to 35°C. Sensory evaluation was undertaken in the afternoon of the same day. Thermos flasks were used to prevent soymilk temperature variation.

3.2.2.1 Chemical analysis of soymilk

The methods that were used in soymilk chemical composition analyses were as described in 3.2.1.2 to 3.2.1.10 except for oil determination. It should be noted here that in determining fibre, ash and minerals, soymilk was oven-dried and the residues were pounded using mortar and pestle as described by Pomeranz and Meloan (1994). Determination of the oil percentage in soymilk was done using Tecator Soxtec System (HT 1043 Tecator AB, Sweden) according to Gerber's method (AOAC, 1995) official method 920.85 as described by Kurwijila (2000). The percentage fat was read with accuracy of 0.01%.

3.2.2.2 Flavour concentration determination

Fortification of soymilk samples from the two selected varieties was carried out after determining the concentration of three flavouring agents i.e. vanilla, banana and pineapple flavour essence as shown in Table 5. Each flavour essence was replicated thrice. A pipette was used to draw 2ml for each replica and its contents were filled into pre-weighed beakers of the same volume. The replicas were placed into the water bath and heated at 80°C for 60 minutes. Weights were measured and recorded. These included weight of empty beaker, weight of beaker filled with flavour essence before heating and weight of beaker plus essence after heating. Weight losses and average weights were calculated and recorded. Flavour concentrations were calculated and expressed as percentage of the initial weight.

Table 5: Amount of flavour added per each level

Flavour	Required level (%)	Weight (g)	%	Amount added (ml/l)
Vanilla	0.000	18.033	64.405	0.000
	0.015			0.233
	0.030			0.466
Pineapple	0.000	16.786	59.950	0.000
	0.015			0.250
	0.030			0.500
Banana	0.000	9.688	34.600	0.000
	0.015			0.434
	0.030			0.867

3.2.3 Fortification

Three types of flavours were added at three levels for each of the two soymilk variety sources. The levels were 0, 0.015% and 0.030% with the ensuing formulations/arrangement being 2x3x3 factorial design with varieties, flavours and flavour levels being primary, secondary and tertiary factors respectively.

3.2.3.1 Sensory evaluation

Flavoured samples of soymilk were evaluated by a semi trained panel of 30 people. Five attributes (i.e. colour, taste, aroma, mouth feel and overall acceptability) were evaluated using a 5 point hedonic scale (Larmond, 1977). Sensory evaluation for the soymilk from the two soybean varieties was done in two different days. Each panellist was presented with nine samples on a tray. The samples and plastic cups were labelled. The terms used to describe the attributes were such that 1 = dislike extremely, 2 = dislike moderately, 3 = neither like nor dislike, 4 = like moderately and 5 = like extremely (Appendix 2)

3.2.3.2 Data analysis

The proximate and selected micronutrients composition data for both whole soybean varieties and soymilk were analysed using SAS software. The sensory evaluation data were subjected to Analysis of Variance (ANOVA) based on 3 x 3 factorial model in a Completely Randomised Design (CRD). This was done using MSTAT-C computer programme where panellists (replication), flavours (factor A), levels (factor B), and second order interactions (AB) were the sources of variation. Tests for significance between means were done by Duncan's Multiple Range Test (DMRT) at $P<0.05$. Unpaired T-test was also carried out to test for significance between varieties with respect to sensory variables.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Overview

This chapter presents and describes the results and findings of the study and it consists of four parts: chemical composition of whole soybeans, chemical composition of soymilk, selection of two varieties by ranking and fortification and sensory evaluation.

Variety TGX 1895-49F was subjected to test for its grade. A sample of weight 1161.38 g was sorted out. The weight of extraneous matter plus damaged kernels was found to be 161.26 g, which was 13.8852% of the total sample. This figure compares well with US Standards value of 14.2% contamination given by USDA (1988) that categorises such soybeans to grade one. According to this standard, the soybeans were of grade 1. Referring to Table 1, grade 1 soybeans should contain 10% splits, 2% maximum damaged kernels, 0.2% heat damaged kernels, 1% foreign matter and 1% coloured soybeans.

Several observations were made during soymilk processing. Soaking was done for six hours and TGX 1985-33F was observed to contain a substantial amount of soybeans that were resistant to soaking while the rest had few of these. During dehulling, TGX 1876-4E variety was found hard to dehull while TGX 1895-49F and TGX 1895-33F varieties were easily dehulled. Grinding/blending was done using a blender. The blended soybeans were found to differ in particle size. The particles for TGX 1876-4E looked coarse while those for variety TGX 1895-49F were the finest. This had effect on filtration and variety TGX 1895-49F was difficult to filter compared to the other two. TGX 1895-33F was very easily filtered.

4.2 Chemical Composition for Whole Soybean Varieties

The chemical composition for each of the three varieties of soybean is summarised in Table 6. According to USDA (1988), grade one soybeans have to contain 13% maximum moisture content. Suitable moisture content for storage for a period of 6-12 months is reported to be 13% while for a longer storage 10-11% moisture content is recommended (Singh *et al.*, 1987).

Table 6: Proximate composition for whole TGX 1895-49F, TGX 1876-4E and TGX 1895-33F soybean varieties (on dry matter basis)

Parameter	Variety			S.E.
	TGX 1895-49F	TGX 1876-4E	TGX 1895-33F	(±)
Moisture (%)	5.609[a]	5.460[a]	5.037[a]	0.216
Protein (%)	31.935[c]	34.815[b]	41.448[a]	0.092
Oil (%)	20.500[a]	20.195[a]	16.217[b]	0.269
Ash (%)	5.679[a]	5.475[a]	5.873[a]	0.134
Fibre (%)	5.763[c]	13.323[a]	10.519[b]	0.176
Carbohydrates (%)	30.537[a]	20.733[b]	20.907[b]	0.218
β-carotene (μg/100g)	998.040[c]	1229.370[b]	1542.690[a]	20.201

Means within the same row superscripted by the same letter are not significantly different at $p<0.05$ following separation by Duncan's Multiple Range Test.

The moisture contents of the three varieties showed non-significant differences ($P>0.05$) and the values were far below the maximum requirement for safe storage. It has been observed that lower moisture content than recommended can cause bean physical damage though such moisture content is good versus susceptibility to microbial attack. Egan *et al.* (1988) stated that flour, with moisture content in excess of 13%, is susceptible to attack by micro-organisms, mites and insects. The storage of soybeans at 5-6% moisture content might have taken into consideration the damp climate and low temperatures of the area that can not cause physical damage of soybeans. With time soybeans can absorb the surrounding moisture thus increase in moisture content. When this happens, moisture may come up but yet cause no microbial growth. The insignificant difference on moisture content shows that soybeans were stored under similar storage conditions.

The protein content results were in agreement with that reported by several researchers. Nelson (1978) reported 40% protein content in soybeans. Phillips (1993) reported protein content in soybeans between 32.2% and 45.2% and its protein was of high quality with a good balance of all essential amino acids. Yagasaki *et al.* (1997) reported the percentage protein soybean seeds within 40 – 45 on a dry-weight basis. Furthermore, the ICB (2007a) reported that soybeans vary widely in nutrient content based on the specific variety and growing conditions, but typically they contain 35-40% protein. There were very high significant differences in protein among soybean varieties. Samples of TGX 1895-33F were rated significantly higher and the variation was due to variety type.

There were very high significant differences in oil among the three soybean varieties. The differences are 94.59% due to variety. According to Singh *et al.* (1987), whole soybeans contain 21% oil. An oil content of 18% was reported by Laswai *et al.* (2005), the amount that is lower than that reported by Singh *et al.* (1987). TGX 1895-49F samples showed small but insignificant difference from TGX 1876-4E samples.

Laswai *et al.* (2005) reported ash content in soybeans of 1.48%. The ICB (2007a) reported ash content of 5% in soybeans. The three soybean varieties in the present study were insignificantly different in ash content ($P>0.05$) but higher values than the reported ones. The value by Laswai *et al.* (2005) was the lowest of all while that by ICB (2007a) was closer to the values in the current study. The variation was 68.95% due to other factors.

The insignificant differences show that the varieties were grown in the same area and the small differences are due to variety type.

Soybeans are reported to contain 35% soluble and insoluble carbohydrates. The values obtained were lower than the reported ones. Of the carbohydrates (35%) contained in soybean, the insoluble portion consists of cellulose (4%) and hemi cellulose (15%) (Singh *et al.*, 1987). There were very high significant differences in carbohydrate content among the three varieties. This was mainly due to variety type whereby TGX 1895-49F was rated significantly higher.

The results for minerals are presented in Table 7. There were significant differences in Fe ($P<0.05$), Ca ($P<0.01$) and Cu ($P<0.05$) and insignificant differences in Zn ($P>0.05$) for the three varieties samples. The iron content was rated higher in TGX 1876-4E but lower in TGX 1895-49F and TGX 1895-33F. For the purpose of alleviating calcium and iron deficiency, TGX 1876-4E is rated the highest. Calcium content in TGX 1876-4E and TGX 1895-49F was however below recommended calcium allowances (FAO/WHO, 1988). The low mineral content in TGX 1895-33F might have been due to variation in growing periods and soil condition/fertility. This variety yields highly (in kg/h) in the field and farmers cultivate more of this variety.

Table 7: Mineral content for whole soybeans

Mean mineral content (mg/100g)	Variety			S.E.
	TGX 1895-49F	TGX 1876-4E	TGX 1895-33F	(±)
Fe	10.580^{b}	18.600^{a}	4.366^{b}	2.040
Ca	211.750^{b}	280.860^{a}	172.580^{c}	11.099
Zn	2.323^{a}	2.144^{a}	2.248^{a}	0.102
Cu	1.845^{a}	0.720^{b}	0.720^{b}	0.203

Means within the same row superscripted by the same letter are not significantly different at $p<0.05$ following separation by Duncan's Multiple Range Test.

β- carotene, α- carotene and β- cryptoxanthin are provitamins A. Structurally, vitamin A (retinol) is essentially one-half of the β- carotene molecule (Rodriguez-Amaya *et al.*, 2004). In general, provitamin A loss increases from micro waving, steaming and boiling. Deep frying, prolonged cooking, combination of several preparation/processing methods, baking, and pickling result in substantial losses (Rodriguez-Amaya, 1997). However, soybeans contain trace amount of provitamin A at maturity. The varieties in this study (Table 6) were found to differ significantly in β- carotene content ($P<0.001$) and the variation was mainly due to variety type. This means that the processing method involved does not destroy all β- carotene.

4.3 Soymilk Yield and Chemical Composition

Tables 8 and 9 summarises results that were obtained after preparing soymilk and determining its yield, proximate, selected minerals and vitamin composition. There was significant difference in yield ($P<0.05$) of soymilk among the three varieties in this study. TGX 1895-33F and TGX 1895-49F yield levels were not significantly different while TGX 1876-4E produced the least quantity of soymilk. TGX 1895-33F was easily filtered and contained the highest amount of protein. The variety TGX 1876-4E was rated the lowest in yield although it was second in easiness to filtration. The variety TGX 1895-49F was last in easiness to filtration but second in soymilk yield.

Table 8: Yield and proximate composition for soymilk from TGX 1895-49F, TGX 1876-4E and TGX 1895-33F

Parameter	Variety			S.E.
	TGX 1895-49F	TGX 1876-4E	TGX 1895-33F	(±)
Yield (kg)	3.303^{a}	2.806^{b}	3.326^{a}	0.136
Mc (%)	90.229^{c}	92.490^{a}	91.080^{b}	0.197
Protein (%w/v)	4.271^{b}	3.509^{c}	4.499^{a}	0.026
Oil (%w/v)	2.325^{a}	1.500^{b}	2.375^{a}	0.150
Fibre (%w/v)	1.013^{a}	0.793^{b}	0.721^{b}	0.025
Ash (%w/v)	0.397^{a}	0.333^{b}	0.391^{a}	0.011
Carbohydrate (%w/v)	1.765^{a}	1.382^{a}	0.932^{b}	0.133
β-carotene (μg/100g)	0.002^{b}	0.003^{b}	0.008^{a}	0.001

Means within the same row superscripted by the same letter are not significantly different at $p<0.05$ following Duncan's Multiple Range Test (DMRT).

4.3.1 Moisture content and macronutrient composition

The three varieties were found to differ significantly in moisture content ($P<0.001$) and this difference was mainly due to variety type. During processing these varieties were found to influence grinding and filtration efficiency. However, the moisture content values for the three varieties were within the literature values for soymilk. Holden (2004) reported 93.27% while Singh *et al.* (1987) reported 89-92.5% moisture content in soymilk.

The varieties in this study were found to differ significantly in protein content ($P<0.001$). The soymilk from variety TGX 1895-33F was rated the highest in protein content followed by TGX 1895-49F and TGX 1876-4E respectively. Holden (2004) reported a protein content of 2.75% and moisture content of 93.27%. Singh *et al.* (1987) reported soymilk protein content of 8.9% in 237ml soymilk, which is equivalent to 3.755% in 100ml soymilk. The International Soybean Programme (INTSOY) (1987) reported the protein contents of 3.4%, 3.5% and 3.6% in whole cow milk, skimmed cow milk and soymilk respectively. Generally speaking, all the three varieties are suitable for soymilk preparation as they give soymilk whose protein content compares well with cow milk protein. The significant difference observed in protein content was due to variety type.

The differences in oil and ash contents were statistically significant ($P<0.05$). The variety TGX 1876-4E had the lowest oil and ash contents compared to the other two which differed insignificantly by 0.050%w/v and 0.006%w/v respectively. The significant differences were mainly due to variety. Holden (2004) reported oil and ash contents of 1.900% and 0.270% at moisture content of 93.27%. The carbohydrate contents were found by difference and the values are within the range as per available literature values. The fibre content for the three varieties were found to be significantly different ($P<0.001$) and the values were percentage-wise higher compared to 0.140% and 0.200% (at moisture content of 92% and 90% respectively) as reported by Singh *et al.* (1987).

4.3.2 Micronutrient composition

There was significant difference in iron content ($P<0.05$) among samples from the three varieties and this was due to variety type and other factors. Although TGX 1876-4E soymilk had the lowest ash content, it ranked second for iron content. There was no significant difference in calcium, copper and zinc content ($P>0.05$). Fortification can be done to soymilk with low mineral content. In US, for instance, calcium is added in the range 300-400 mg per cup (Bost, 2007).

The β-carotene values (in μg/100g) were relatively low. There is very little or no β-carotene in soymilk (Singh *et al.*, 1987). Holden (2004) reported β-carotene content of

3µmg in soymilk. There was significant difference in β-carotene ($P<0.001$) among samples of the three varieties in this study. Variety TGX 1895-33F had the highest amount of β-carotene. No significant difference was observed in the other two varieties. Maturation or ripening in fruits and vegetables is usually accompanied by enhanced carotenogenesis, which differs from soybeans whose carotene content decreases with maturity (Azevedo-Meleiro and Rodriguez-Amaya, 2004).

Table 9: Mineral content for soymilk from TGX 1895-49F, TGX 1876-4E and TGX 1895-33F

Minerals (mg/100g):	Variety			S.E
	TGX 1895-49F	TGX 1876-4E	TGX 1895-33F	(±)
Fe	4.072^{b}	4.755^{b}	9.569^{a}	1.083
Ca	142.796^{a}	203.577^{a}	162.460^{a}	20.980
Zn	1.818^{a}	1.927^{a}	2.282^{a}	0.142
Cu	0.788^{a}	0.792^{a}	0.662^{a}	0.060

Means within the same row superscripted by the same letter are not significantly different at $p<0.05$ following Duncan's Multiple Range Test (DMRT).

The results of this study agree with the finding even if improved varieties are used. This brings in the importance of fortifying soymilk with other sources of vitamin A. Bost (2007) reported that the majority of soymilks on the US market today are fortified with vitamin A to levels similar to cow milk fortification (400 IU/l) as well as being fortified with vitamin B_{12}. In developing Tanzania standards for soymilk, this should be considered as an important aspect.

4.3.3 Energy content

Energy content results for the three varieties soymilk are as shown in Table 10. The values were below 49 kcal as reported by INTSOY (1987). TGX 1895-49F contained the highest amount of energy followed by TGX 1895-33F. These two varieties can be recommended for PEM alleviation as they contain higher protein and energy content.

Table 10: Energy content in kilocalories per 100 g of soymilk

Parameter	Energy		
	TGX 1895-49F	TGX 1876-4E	TGX 1895-33F
Protein energy	17.083	14.062	17.997
Fat energy	20.925	13.500	21.375
Carbohydrate energy	6.210	4.911	3.326
Total energy	44.218	32.473	42.697

4.4 Sensory Evaluation

Statistical analysis of the data on the organoleptic assay showed that there was significant difference ($p<0.05$) among the flavour levels on aroma and overall acceptability for TGX 1895-33F. There was no significant difference on colour, taste and mouth feel. Samples without added flavour had lower mean scores than those with flavours. In an attempt to establish procedures to improve flavour, stability and acceptability of soymilk, Wang *et al.* (2000) reported that flavoured soymilk had 18% higher panel scores in aroma than plain soymilk. Vanilla flavoured soymilk from TGX 1895-33F soymilk showed no improvement in aroma from flavour level 0 to 0.015%, but from the latter to 0.030% (equivalent to 0.466ml/l) (Table 11). Mnkeni and Nyaruhucha (1994) reported that 0.5 ml/l of vanilla are enough to bring soymilk to acceptable sensory quality. Banana flavour showed an increasing trend on aroma acceptability from lower to higher concentration. The difference was statistically insignificant for the concentrations 0.015% and 0.030%. Pineapple had a clear increasing trend on aroma from lower concentration to the higher concentration. It also received a higher mean score than vanilla and banana as far as flavour types are concerned. It can, therefore, be concluded that to improve aroma in soymilk the pineapple flavour is more efficient than either vanilla or banana flavour.

Table 11: The effect of flavour levels on aroma scores of TGX 1895-33F soymilk

Flavour type	Aroma scores for percentage fortification at:-			Flavour means	S.E.
	0.000	0.015	0.030		
Vanilla	3.300^{b}	3.300^{b}	3.500^{a}	3.367^{A}	±0.105
Banana	3.333^{b}	3.700^{a}	3.733^{a}	3.589^{A}	±0.105
Pineapple	3.200^{b}	3.800^{a}	4.067^{a}	3.689^{A}	±0.105
Level means	3.278^{B}	3.600^{A}	3.767^{A}		
S.E.	±0.105	±0.105	±0.105		

Means (±0.182 S.E.) within rows of the main body superscripted by the letters of the same case are not significantly different at $p<0.05$ following separation by Duncan's Multiple Range Test (DMRT). Similarly marginal means superscripted by the same upper case letter are statistically similar following separation by Duncan's Multiple Range Test at $p<0.05$.

Results show that any increase in flavour concentration will lead to increased acceptability of soymilk aroma though the difference is statistically insignificant. The use of these flavours, however, should not exceed the recommended maximum levels for safety. FAO/WHO (1999) directed that the use of flavouring agents to foods and beverages must not exceed the maximum levels of 70 mg/kg of the final product ready for consumption.

Flavour levels had also a significant effect ($p < 0.05$) on overall acceptability of soymilk from TGX 1895-33F. A clear increasing trend was observed for pineapple flavour indicating that it is the most suitable flavour (Table 12).

Table 12: The effect of flavour levels on overall acceptability scores of TGX 1895-33F soymilk

Flavour type	Overall acceptability scores for percentage fortification at:-			Flavour mean	S.E.
	0	0.015	0.030		
Vanilla	3.467^{b}	3.367^{a}	3.467^{b}	3.433^{A}	±0.097
Banana	3.067^{b}	3.433^{a}	3.300^{a}	3.267^{A}	±0.097
Pineapple	2.800^{b}	3.367^{a}	3.600^{a}	3.256^{A}	±0.097
Level means	3.111^{B}	3.389^{A}	3.456^{A}		
S.E.	±0.097	±0.097	±0.097		

Means (±0.169 S.E.) within rows of the main body superscripted by the letters of the same case are not significantly different at $p<0.05$ following separation by Duncan's Multiple Range Test (DMRT). Similarly marginal means superscripted by the same upper case letter are statistically similar following separation by Duncan's Multiple Range Test at $p<0.05$.

Flavour type had a significant effect ($p < 0.05$) on aroma of soymilk from TGX 1895-49F (Table 13). Pineapple flavour had the highest mean score followed by banana and vanilla flavours respectively.

On interaction, pineapple had the highest scores followed by banana and vanilla respectively. Pineapple flavour excelled in both soymilk variety samples. It is, therefore, a recommendable flavour in improving the aroma of soymilk. Furthermore, the increase in levels of flavours improves the aroma acceptability of soymilk though a maximum level needs to be established.

Table 13: The effect of flavour type on aroma scores of TGX 1895-49F soymilk

Percentage fortification	Aroma scores for flavour type			S.E.
	Vanilla	Banana	Pineapple	
0	3.300^{b}	3.467^{ab}	3.367^{a}	±0.108
0.015	3.500^{b}	3.533^{ab}	3.967^{a}	±0.108
0.030	3.333^{b}	3.500^{ab}	3.967^{a}	±0.108
Flavour means	3.378^{B}	3.500^{AB}	3.767^{A}	
S.E.	±0.108	±0.108	±0.108	

Means within rows of the main body superscripted by the letters of the same case are not significantly different at $p<0.05$) following separation by Duncan's Multiple Range Test (DMRT). Similarly marginal means superscripted by the same upper case letter are statistically similar following separation by Duncan's Multiple Range Test at $p<0.05$.

The mean sensory scores for aroma and overall acceptability of the two varieties were subjected to unpaired T-test. Their mean and standard deviation values are as shown in Table 14. The results showed that there was no statistical significant difference ($p>0.05$) between varieties on sensory characteristics of soymilk samples assessed.

Table 14: T-test values

Variety	Mean	Standard deviation	F
TGX 1895-33F	3.3994	0.3386	1.2545
TGX 1895-49F	3.4408	0.3023	

Hence the two soybean varieties give soymilk of almost the same sensory properties if care is taken on the quality of soybeans and the process used.

4.5 General Comments on Sensory Evaluation Results

The analysis of variance at $p< 0.05$ showed that there was no significant difference in colour ($p>0.05$) for the two varieties (i.e. TGX 1895-33F and TGX 1895-49F), three flavours together with their respective levels. This may be due to the nature of colours of these flavours and the amount added per litre. Soymilk from these varieties had creamy colour that the small amount of flavours added at different levels could not affect the creamy colour of soymilk.

There was no statistical significant difference in taste ($P>0.05$) for the two varieties, three flavours and their respective three levels. There was also no panellist who reported presence of beany-grassy flavour of soybean in flavoured and non flavoured soymilk samples. Most panellists recommended addition of sugar to bring soymilk to taste. This is a common practice by most of consumers to add sugar before drinking cow milk. The process used and the quality of soybeans might have had effect in reducing the beany-grassy flavour of soymilk to undetectable level. Wilson (2003) reported that there are modified technologies for producing less beany flavoured soymilk without flavour masking such as the use of hot instead of cold water during grinding. It is further reported that soaking and blanching whole soybeans, followed by bicarbonate treatment and pasteurisation of extracted soymilk influence soymilk quality, composition and yield of the final product. Therefore, the use of flavours in small quantities may be useful in improving aroma and sugar addition could improve the taste. The flavour addition in soymilk should not affect other soymilk organoleptic and sensory properties. They should also be applied to levels acceptable to avoid health hazards. Mnkeni and Nyaruhucha (1994) recommended the use of vanilla at 0.5ml/l in order to improve soymilk taste. Most of the values used in this study were calculated following the concentration of the flavour in the particular flavour essence and were below or equal to 0.500ml/l except for banana at 0.030% (0.867ml/l). FAO/WHO (2007) stated that flavouring agents must be used in the minimum quantity required to produce their intended physical or technical effect and in accordance with all the principles of GMP. The choice of process for soymilk production is very important in ensuring that it is efficiently extracted, palatable and safe. This will help in ensuring that any additives added for sensory quality improvement, be it sugar or flavours, are used at their minimum levels.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Soymilk has the potential to substitute dairy milk in the fight against PEM. The results show that variety TGX 1895-33F was ranked first followed by TGX 1895-49F and TGX 1876-4E respectively. It can therefore be concluded that variety TGX 1895-33F could effectively be used in efficient production of soymilk compared to the rest.

All the three varieties had their nutrient contents in agreement with the available literature. Typical composition varies depending on the soybean cultivar, maturity, harvesting and storage conditions. These varieties were obtained from one research station. The variation in chemical composition was therefore due to cultivar type and probably some yet unknown factors. Under controlled processing conditions, cultivar TGX 1895-33F had the highest yield (3.326 kg) of soymilk with highest protein content (4.499%) followed by TGX 1895-49F (3.303 kg, 4.271%) and TGX 1876-4E (2.806 kg, 3.516%) respectively. The iron content in TGX 1895-33F soymilk was also the highest though there was variation in growth seasons and soil. In general, however, variation in yield, proximate and selected micronutrient composition may be due to variety type, methods of extraction and analysis.

In the assessment of the effectiveness of artificial flavouring agents (vanilla, banana and pineapple) on soymilk sensory quality, the pineapple flavour was the most effective over vanilla and banana. It had the highest mean (3.767 pineapple, 3.500 banana and 3.378 vanilla) score and the panellists were able to discern its aroma in an ascending manner according to level of aroma fortification. It can be concluded that pineapple flavour is the most suitable over banana and vanilla flavours.

The choice of the process for soymilk production has shown to be important if we were to minimise the amount of these artificial flavours. There was no significant difference (P>0.05) between samples with and those without flavours for colour, taste and mouth feel as observed in this study. Moreover, all the three varieties had soymilk meeting specifications for protein and solids content according to Japan, Taiwan, China and France Standards (Appendix 3). The process used was one of the improved/modified technologies for production of soymilk that may not require flavour masking. Sugar addition was recommended to improve taste, which is a common practice even for cow milk. The choice of the process for soymilk production is important since soymilk is intended for the eradication of PEM problem, which implies that the product would be required daily by children (especially those who are lactose intolerant), old people, pregnant and lactating women and sick people (especially those who are HIV/AIDS victims). Minimising the amount of artificial flavours consumed per day per consumer, through proper choice of processing methods, is of importance in order not to exceed the recommended use of these flavours.

In this study it has been demonstrated that fortification of soymilk with flavours and addition of sugar, enhances its acceptability and consumption. Comparison shows that of the three flavours, pineapple is more effective for this purpose.

5.2 Recommendations

It is recommended that variety TGX 1895-33F, which ranked first, be the first choice in soymilk production and pineapple flavour be used in soymilk fortification. There is a need to develop other products from the residues obtained after filtration. A lot of nutrients are lost in the residues. Whole TGX 1895-33F soybean cultivar, for instance, contains 43.000% (w/w) protein. Soymilk of the same cultivar contains 4.499% (w/v) protein. The substantial amount of protein lost in residues after filtration can be recovered by developing other products in order for more protein to be available for PEM eradication.

Complementary research studies can be undertaken in order to develop other products from soymilk, e.g. yoghurt, and determine their shelf life. It is also recommended that investigation into identification of more flavouring agents be carried out. Diversity in soymilk products will sensitise the community on the soymilk potential consumption with a view of supplementing cow milk.

Efficient machines should be fabricated focusing on minimisation of processing difficulties especially cleaning, dehulling and filtration. Simple and yet efficient machines can be a valuable asset in small scale soymilk production. These will, with time, help in expanding soymilk production from small to large scale as is the case to countries like US, Japan and China. As a consequence, a large part of the population will get access to packaged soymilk that will easily be available in the market; people will develop interest in drinking it and in the long run have an impact on reducing PEM in the country. Furthermore, the risk of soy plant workers to allergy due to dust will be minimised.

In order to promote soymilk utilisation in Tanzania, standards for soymilk products have to be formulated and promoted by Tanzania Bureau of Standards (TBS). This is very crucial in commercialising soymilk products for PEM alleviation. Standards will promote honesty and fair dealing in the consumer's interest; help in ensuring that consumers receive quality soymilk products and in establishing consistency and fairness in labelling and disseminating sound soymilk nutritional information.

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APPENDICES

Appendix 1: Soymilk Laboratory Chemical Analysis Form

Name...

Type of analysis requested...

Date...

Parameters	Samples																
Moisture content (%)																	
Protein (%)																	
Fat (%)																	
Carbohydrates (%)																	
Crude fibre (%)																	
Ash (%)																	
Iron (mg/100g)																	
Calcium (mg/100g)																	
Zinc (mg/100g)																	
Copper (mg/100g)																	
β-carotene (μg/100g)																	

Analysis done by...

Position...

Name of Laboratory...

Date...

Appendix 2: Sensory evaluation form

Name...

Sex...

Age...

Time...

Date..

Please look at and taste each of the EIGHT (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								
	947	432	684	936	571	195	432	749	486
Colour									
Taste									
Aroma									
Mouth feel									
Overall acceptability									

Comments...

....

....

Appendix 3 : Various categories and compositional characteristics for soymilk for the given countries (minimum requirements)

Country	Product	Protein (%)	Fat (%)	Solids (%)
USA	Soymilk fluid	2.75	1.91	
China	Soymilk	3.4	1.0-2.0	
	Low fat soymilk	2.6	0.5	
Japan	Soymilk	3.8	-	8.0
	Blended soymilk	3.0	-	6.0 – 8.0
	Soymilk beverage	1.8	-	4.0 – 6.0
	Soy protein beverage	1.8		
Taiwan	Soymilk	2.1	0.5	
	Formulated soymilk	2.0	0.5	
	Soy drink	1.4	0.5	
Singapore	Soymilk	2.0		
	Soy drink	>2.0		
France	Soymilk (tonyu)	>3.6	>1.5	>7.3
	Fortified soymilk	>3.8		
Thailand	Soymilk	2.0	1.0	

Source: USDA (1986) and SAA (1996)

Appendix 4: Flow chart for soymilk production

Cleaning, sorting and sampling

↓

Weighing

↓

Soaking

(1:3w/w soybeans/water, 6 hours)

↓→ (soaking water discarded)

Weighing of soaked soybeans

↓

Dehulling

(Manually)

↓→ (Hulls + loss of soybeans discarded)

Blanching

(1:6w/w soybeans/water; 1% $NaHCO_3$;98°C, 8min.)

↓

Blending

(1:4w/w soybeans/water; 3minutes)

↓

Filtration

(Sieve size 210 microns)

↓→ (Residue discarded)

Boiling

(98°C, 30 min.)

↓

Cooling

↓

Chemical analysis/flavouring