THE EFFICACY OF HEAT, SPROUTING AND ACID TREATMENT ON THE REMOVAL OF PROTEASE INHIBITORS IN SOYA BEAN

(*Glycine max* (L.) Merrill)

BY

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DECLARATION

I, VICTORIA ANSAH, do hereby declare that except for the references cited which have been duly acknowledged; this thesis "***The efficacy of heat, sprouting and acid treatment in the removal of protease inhibitors in soya bean (Glycine max.* (L) Merrill*)"*** is the result of my own research. It has never been presented either in part or in whole for the award of any degree.

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ABSTRACT

Soya bean is an important legume used in fortifying cereal-based complementary foods for children. Despite its high protein content, it also contains anti-nutrients which makes the proteins unavailable. The objective of the study was to assess knowledge of consumers on anti-nutritional factors and their perception of how it could be processed out of soya bean as well as the efficacy of heat, sprouting and acid treatment in the removal of protease inhibitors in soya bean. The study was conducted in two phases; a survey and a laboratory experiment. Two hundred (200) open and close ended questionnaires were administered to weaning mothers (with babies from 6 to 24 months) in four selected health facilities within the Greater Accra region of Ghana. Three separate experiments were conducted for each of the above treatments to evaluate the effect of heat, sprouting and acid treatment on the removal of protease inhibitors in soya bean. The experimental design was a 2 x 3 full-factorial experiment laid out in a completely randomized design (CRD) for each of the experiments. The heat treatments were control, boiling and roasting. The sprouting levels were 0, 2 and 4 days while the acid soaking levels were 0, 12 and 24 hour The study revealed that the weaning mothers were not aware of anti-nutrients present in soya bean although they knew of its nutritional and health benefits. The most common method of processing used by consumers was roasting. It was also observed that most of the respondents (65.7%) did not process soya bean but rather purchased them from local processors within their respective communities. It was therefore recommended that education on anti-nutrients and how they could be processed out of soya bean should be intensified by all stakeholders. Future research into the levels of anti-nutrients in soya bean products sold on the Ghanaian market should be carried out.

Jenguma produced a higher protein content (48.55%) than Anidaso (45.79%). While Anidaso produced a highly significant protein content with the heat methods, Jenguma produced a highly significant protein content for sprouting and acid treatments. The in-vitro protein digestibility of soya bean treated with heat, sprouting and acid increased significantly ($p<0.05$) compared to the unprocessed one. Also, IVPD in Anidaso was better compared to Jenguma.

DEDICATION

I dedicate this thesis to my Husband, Jeremiah Obiwom Ansah, my Mum, Rosina Ansong, my Children Joyce, Ann and Joel and my Sister Priscilla Amarko Ntow of blessed memory.

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LIST OF ABBREVIATIONS

ANFs	Anti-nutritional factors
CWC	Child Welfare Clinics
FAO	Food and Agriculture Organisation
IVPD	In-vitro Protein Digestibility
MoFA	Ministry of Food and Agriculture
PEM	Protein Energy Malnutrition
PLWHA	People Living With HIV / AIDS
TIA	Trypsin Inhibitor Activity
W H O	World Health Organisation
WISHH	World Initiative for Soy in Human Health

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Soya bean (*Glycine max* (L.) Merrill) is a leguminous crop which originated from East Asia (USDA, 2011), belonging to the family leguminosae and sub family Papilionideae (IITA, 2009). In Ghana, soya bean was introduced to farmers in 1910 to be cultivated as food and also as an export commodity. As a foreign crop, a lot of promotion has been carried out by both governmental and non-governmental agencies in a quest to promote its production, marketing and utilization (Plahar, 2006 & Sarkodie -Addo *et al.*, 2006). It is referred to as the most essential legume and cultivated mainly in northern Ghana as a result of the favorable soil and climatic conditions. It is readily available on all markets within the country. Apart from its availability, soya bean is also known to be useful in the production of a wide variety of products (Hammond, 2014).

Soya bean is cultivated worldwide for its edible bean which is a rich source of proteins and fat, as well as a good source of energy, vitamins and minerals (Adu-Dapaah *et al*., 2004). According to Mondal *et al.* (2002), soya bean contains about 40% to 45% proteins and is also rich in essential amino acids which makes it equivalent to animal sources of proteins (Dixit *et al.*, 2011). This superior characteristic of soya bean makes it an excellent crop for combating Protein Energy Malnutrition (PEM) in especially infants and growing children, pregnant and lactating mothers, People Living with HIV/ AIDS (PLWHA) and convalescents. According to (Amagloh *et al.*, 2012), Sub Saharan Africa, which includes Ghana, is one of the areas in the world where the worst rates of malnutrition in growing children are recorded. They are also of the view that efforts have over the years been made by local and international bodies to combat PEM in these areas by fortifying complementary foods with legumes of which soya bean is the most commonly used.

As a plant, soya bean does not contain cholesterol, and therefore a diet consisting of soya bean reduces disease conditions such as high blood pressure, osteoporosis and diabetes. The presence of isoflavones in soya bean also helps to prevent obesity; a precursor to many diseases (Fabiyi, 2006).

In industry, oil extracted from soya bean can be used as raw material for processing several products such as vegetable cooking oil, margarine, soap and candle. The by-product, soya bean cake, is used as an essential feed constituent for livestock (Adu-Dapaah *et al.,* 2004).

Despite the high content of protein in soya bean, it also contains protease inhibitors which are anti-nutritional factors. These protease inhibitors prevent digestive enzymes from digesting proteins thereby making them unavailable for absorption and utilization by the body (Monteiro *et al.*, 2003). To facilitate the utilization of this important bean as an antidote to PEM, especially for weaning babies and older children, a study of food processing technologies capable of removing these protease inhibitors is key.

One of the most researched areas in postharvest is the minimization of post-production losses. In Ghana, and other parts of Sub- Saharan Africa, a lot of research on value added products of soya bean such as flours, soy sauce, weanimix, dawadawa (soya condiment) has been conducted in a bid to promote the utilization of the bean (Annan *et al.*, 2005; Dhore, 2011 and Dzogbefia *et al.*, 2007). Research by the World Initiative for Soy in Human Health (WISHH) revealed that consumers in Ghana have a relatively high knowledge about the nutritional and health benefits as well as a variety of products from soya bean (WISHH, 2006). However, data on their knowledge of anti-nutritional factors present in soya bean and how processing can be used to remove them is not known. It is therefore, essential that investigations are made to ascertain whether consumers know about anti-nutritional factors present in soya bean and also how they can be processed out.

Heat application, sprouting and acid treatment are technologies in food processing which have been found to remove these protease inhibitors. Mugendi *et al.,* (2010) reported that anti-nutritional factors can be removed by heat treatment. Sharma *et al.* (2013) observed that Trypsin Inhibitor Activity (TIA) in soya bean decreased significantly by 41% when treated with citric acid. Though these technologies have been identified to be useful in the processing of cereals and legumes into complementary foods; much work on the application of these technologies on soya bean varieties Jenguma and Anidaso have not been reported in Ghana. This research, is therefore, intended to investigate the efficacy of heat, sprouting and acid treatment in the removal of protease inhibitors in these local varieties.

The possibility of using these technologies in the removal of anti-nutritional factors will promote commercial and domestic production of soya foods which will in turn help to provide job opportunities, eliminate PEM and also improve the health conditions of Ghanaians.

Findings of this research will also inform policy makers and implementers to educate consumers and food processing industries on how to use these technologies so that the end users will obtain optimum availability and utilization of the proteins present in soya bean.

The main objectives of the research was to assess the efficacy of heat, sprouting and acid treatment in the removal of protease inhibitors in soya bean (var. Jenguma and Anidaso) as well as assess consumers' knowledge and perception of anti-nutritional factors in soya bean in selected localities within the Greater Accra Region. The specific objectives were to:

1. Assess consumers' knowledge and perception of anti-nutritional factors in soya bean within selected localities in the Greater Accra Region.
2. Evaluate the effect of heat, sprouting and acid treatments on the nutritional composition of soya bean.
3. Determine the In-vitro Protein Digestibility of soya bean processed from the three treatments above.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Distribution of Soya Bean

The initial domestication of Soya bean (Glycine max (L.) Merrill) can be traced as far back as the eleventh century B.C. or even a little earlier in the North eastern part of China. It has since then been one of the five major food crops cultivated in China. Soya bean was locally produced in China till the end of the Chinese-Japanese war in 1894-1895, when soya bean oil cake (used as fertilizer) was imported by the Japanese from Europe. By 1908, the shipment of soya bean to Europe had already caught worldwide attention (Gibson & Benson, 2005).

According to, Shurtleff and Aoyagi (2010), soya bean cultivation in Africa begun in 1896, in Algeria and by 1908, interest in cultivating the crop had overwhelmingly increased. Globally, the United States of America is the leading country in terms of production and export of soya bean, producing a total of 82,054,800 Mt, followed by Brazil (65,848,857 Mt) and Argentina (40,100,197 Mt) while in Africa, the leading producer is South Africa, producing (650,000 Mt), and followed by Nigeria producing (580,000 Mt) in 2012 (FAOSTAT, 2012). Ghana produces 1.5 Mt/Ha of soya bean annually which has a potential of rising up to 2.3 Mt/Ha (MoFA, 2011) and this is cultivated mainly in Northern Ghana with a few in Ashanti and Volta Region (Lawson *et al.,* 2008).

2.2 Botany of Soya bean

Soya bean, (*Glycine max* (L.) Merrill), is cultivated as an annual but some species are naturally perennial (Mullen, 2003). It is a bushy plant that grows up to 0.75 to 1.25 m high and may develop sparing or dense branches based on the cultivar or growing conditions.

Fine brown or gray hair cover the stem leaves and pods. Soya bean possess trifoliate leaves which bear three to four leaflets per leaf (Reinke & Joke, 2005).The inflorescence is either white or purple which is characteristic of Papilionideae (Chaturvedi *et al.*, 2011).

According to Mullen (2003), the beans are encased in pods of 3 to 5 cm which are clustered with each pod housing 2 or 3 beans. The colour of the seeds vary with some being yellow, green, brown or black with spots but most commercially cultivated types are yellow.

It possess a dicotyledonous seed which is devoid of endosperm and is enclosed in a testa. The two cotyledons stores food for use by the developing embryo. A seed scar of linear to oval shape marks the seed coat (hilium) which serves as a protective coat against infection of the embryo by fungi and bacteria.

The growth habit of soya bean may be determinate or indeterminate. The determinate types grow shorter, possess fewer leaves with more pods whereas the indeterminate types grow taller, with more leaves and pods which grow from the bottom of the stem to the tip (MoFA & CSIR, 2005).

The growth and development of soya bean is characterized by two main stages; the vegetative and reproductive stages. The emergence of seedlings, unfolding of uni-foliate leaves and their development into trifoliate leaves, formation of nodes on the main stem and branch development characterize the vegetative stage while flower bud formation and full bloom, pod formation and filling of the pod to full maturity constitute the reproductive stage (Reinke & Joke, 2005).

2.2.1 Varieties of Soya bean grown in Ghana

Soya bean varieties cultivated by Ghanaian farmers include Quarshie, Salintuya, Anidaso, Jenguma, Bengbie and Nankpanduri.

Anidaso is an improved variety with special nutritional attributes (Table 2.1). Jenguma is also an improved variety and it's widely cultivated in Ghana. It is resistant to pod shattering and therefore superior in preventing shattering losses (Kombiok & Buah, 2013).

Table 2. 1 Nutritional composition of Anidaso

Constituent	Percentage (%)
Moisture	12.7
Fat	17.6
Protein	45.8
Ash	5.4
carbohydrate	31.2

Source, (Plahar, 2006)

2.3 Constituents of Soya Bean

2.3.1 Proteins

Proteins are a major constituent of soya bean and are essential in promoting growth and repair of worn-out tissues. Proteins from plant sources unlike those from animal sources do not possess complete essential amino acids and therefore, do not provide all the eight essential amino acids needed for the production of human body proteins (Martínez Augustin & Martínez de Victoria, 2006). Soya bean contains 35% to 40% proteins which is almost the same as that of animal proteins (Barbalho & Flávia, 2011).

2.3.2 Fats

Soya bean has oil content of approximately 19.5% (William & Akiko, 2000). Oil from soya bean has triglycerides (99%) as the major constituent and other minor components such as phospholipids, also known as lecithin, as well as phytosterols and tocopherols and free fatty acids (Mateos-Aparicio *et al.*, 2008). Martins *et al.* (2003) reported that linoleic acids constitute approximately 54% of the total acid in soya bean. In addition, records depict that a large number of people's diets are deficient in this acid and therefore, the consumption of soya bean could be an essential source for improving linoleic acid intake (Martin *et al.*, 2008).

2.3.3 Carbohydrates

Soya bean contains about 35% carbohydrates of which non-starch polysaccharides are the most. Other types of carbohydrates present in soya bean are oligosaccharides which include, 4.4% of stachyose and 1.1% raffinose (Grieshop *et al.*, 2003). Polysaccharides present in soya bean are made up of insoluble dietary fibre and therefore useful as a supplement for fibre in diets. Also, soluble polysaccharides in soya bean makes it useful in modifying the physical properties of a variety of foods (Espinosa-Martos & Rupérez, 2006).

2.3.4 Vitamins and Minerals

Soya bean lacks vitamin B12 and vitamin C but in comparison to cereals it is a better provider of B complex vitamins. It also contains approximately 5% minerals and relatively has abundant potassium, phosphorus, calcium, magnesium and iron (Sugano, 2006).

2.3.5 Isoflavones

Soya bean is a rich natural source of isoflavones among plants (Cederroth & Nef, 2009). Isoflavones are phytochemicals and are also known as phytoestrogens as a result of the estrogenic effects they exhibit. Their chemical structure is similar to that of cholesterol except for a methyl or ethyl group present but they are much less absorbed than cholesterol in humans (Abumweis & Jones, 2008).

2.4 Nutritional and health benefits of Soya Bean

2.4.1 Nutritional Benefits

Soya bean, possesses a high amount of good quality protein, essential minerals and vitamins as well as essential fatty acids. According to Plahar (2006), it is a nutritional powerhouse and therefore excellent for combatting PEM in Ghana. Soya bean has a protein content of about (40 %) which is equivalent to proteins from animals. Also because of its high protein content, it serves as a good replacement for meat in vegetarian diets as well as processed foods (Fabiyi, 2006). Soya bean possess a balanced amino acid profile and is also rich in lysine, isoleucine, tryptophan, threonine and valine but limiting in methionine making it suitable for fortifying staples such as rice, millet and sorghum that are limiting in lysine but possess adequate methionine (Cromwell, 2012).

Soya bean serves as a major protein source used in the processing of soy meat (khebab), baby foods (weanimix) and dawadawa (local condiment for stews and soups).

2.4.2 Health Benefits

Several research work in the field of science and medicine have documented that most of the constituents of soya bean possess health benefits especially those relating to life style diseases such as high blood pressure and diabetes. Compared to carbohydrates and fats, proteins possess a high satiety power and this attribute makes soya bean (a high protein content food) able to minimize excessive food intake which could lead to obese conditions; precursor to heart diseases as well as type diabetes (Alfenas *et al.,* 2010 & Jequier, 2002). Studies in Japan and other Asian countries revealed that people who consume soya bean in large quantities had reduced rates of heart diseases (Hirayama *et al.*, 2010 & Reynolds *et al.,* 2006).

Phosphatidylserine derived from soya bean has proven to be essential in improving cognitive function which declines as a result of old age. In a study, Japanese men and women between the ages of 50 and 69 suffering from memory impairment, were found to have improved memory after six (6) months of receiving treatment form phosphatidylserine derived from soya bean (Kato-Kataoka *et al.,* 2010).

According to Cederroth & Nef (2009) soya bean is the only plant that contains the largest amount of isoflavone. They are phytochemicals known as phytoestrogens due to their estrogenic effects and studies have shown that they reduce different levels of cancer, osteoporosis, regulate blood pressure, obesity and diabetes when included in diets of people (Barbalho & Flávia, 2011; Dixit *et al.*, 2011). In addition, soy isoflavones are instrumental in the treatment of symptoms of menopause and studies have confirmed that women with prolonged consumption of soya bean have lesser symptoms during menopause (Cho *et al.*, 2010).

2.4.3 Other Benefits of soya bean

Soya beans are useful in industry and agriculture. Oil extracted from soya bean is used as raw materials in industries to such as food, soap and textiles to manufacture items such as printers' ink, cosmetics, glue, soap, margarine, shampoo and plastics. Soya bean is a legume and therefore possess the ability to fix nitrogen into the soil through its symbiotic association with nitrogen-fixing bacteria (Rhizobium sp.). This facilitates sustainable agriculture and improves the fertility of the soil particularly in crop production systems where soya bean is cultivated in rotation with cereals. Soya bean can also act as cover crops and thereby minimize erosion and suppress the growth of weeds. Another benefit of soya bean in agriculture is its ability to break pest and disease cycles when alternated with cereals Adu-Dapaah, *et al.,* (2004)

2.5 Anti-Nutritional Factors (ANFs) in Soya Bean

One of the major reasons why consumers show a low reception of soya bean products is due to the presence of anti-nutritional factors (ANFs) also known as anti-nutrients (Anjum, 2010). ANFs are chemical components of food produced as a result of metabolic activities in varying species of plants and other mechanisms that reduce or prevent the optimum utilization of specific nutrients (Soetan & Oyewole, 2009). Alternatively, it has been discovered that ANFs consumed in minute quantities help to prevent the occurrence of certain diseases such cancer and coronary diseases.

2.5.1 Classification of Anti-nutritional Factors

ANFs, according to Huisman & Tolman, (2001) can be classified based on their effects on the nutritional value foodstuffs and how the body's response to them biologically as:

1. Factors that depress protein digestion and their utilisation such as protease inhibitors, tannins saponins and lectins.
2. Factors that inhibit mineral availability such as phytates, gossypol pigments and oxalates.

3. Factors that cause the inactivation of vitamins or an increase in the body's requirement for vitamins.
4. Factors that negatively affect carbohydrate digestion such as amylase inhibitors, flatulence factors and phenolic compounds.
5. Factors that cause the stimulation of the immune system and hypersensitive reactions such as antigenic proteins.
6. Miscellaneous substances like mimosine, nitrates, alkaloids, phytoestrogen and mycotoxins.

Nahashon & Kilonzo-Nthenge, (2011) also classified ANFs based on their reaction to heat application as heat-stable and heat labile. The heat labile types are those that are sensitive to heat and can be reduced or removed by heat whereas the heat stable types are those that are not sensitive to heat application as shown in (Table 2.2).

Table 2. 2 Classification of Anti-nutritional factors in soya bean

Heat-labile	**Heat-stable**
Trypsin inhibitors	Saponins
Hemagglutinins	Estrogens
Goitrogens	Flatulence factors
Anti-vitamins	Lysinoalanine
Phytates	

Source: Nahashon & Kilonzo-Nthenge, (2011)

Soya bean in the raw state contains ANFs which include protease (trypsin) inhibitors, phytates, oligosaccharides, lectins (hemaglutinins) and goitrogens which reduce the nutritional value of legumes and pose health challenges to humans as well as animals who consume large amounts of the bean (Caprita *et al.,* 2010 & Mikic *et al.,* 2009) There is the need therefore, to inactivate or reduce ANFs in order to maximize the availability of nutrients to the human body especially weaning babies and growing children.

2.5.1 Protease Inhibitors (PIs)

According to (Soetan & Oyewole, 2009), PIs inhibit the action of protein digestive enzymes within the digestive system of animals. They are distributed widely within the kingdom Plantae as well as cultivated legumes. The main types of PIs are trypsin and chymotrypsin are the main types of ANFs mostly studied. Studies have proven that PIs account partially for retardation of growth which is a characteristic of raw legumes.

PIs contribute about 10 % of the overall protein content in plants and are generally found to be highly concentrated in the storage tissues of plants. Also, PIs are present in leaves and they function as a protection of plants against insects and pathogens. The possibility of PIs protecting plants was originally detected when observations on the larvae of some insects confirmed that those insects were not able to thrive on products of soya bean as a result of the trypsin inhibitor present (Habib & Khalid, 2007). Fan & Wu (2005), reviewed that PIs perform three major functions in plant protection which are to maintain seed dormancy, regulating protein synthesis and metabolism and inhibiting insect attack.

However, this function of PIs become no longer useful during the consumption stage as they hamper optimum digestibility, availability and utilization of the nutrients (proteins) present in soya bean. Processing is one of the major ways that has been used by researchers to reduce or inactivate PIs but the choice of processing method to improve protein quality of soya bean should function either by reducing the ANFs or enhancing optimum digestibility of the protein.

2.5.3 Phytates

Phytates (Inositol hexakiphosphate) are the salt forms of phytic acid and are present in plants, animals and soils. Phytates exist as salts of mono and divalent cations (K^{+}, Ca^{2+} and Mg^{2+}) and are concentrated in the seeds as ripening occurs. Phosphates and inositol are primarily stored in the form of phytates in seeds and grains Mueller, (2001).

Inadequate mineral in the diets of humans results in morbidity, growth retardation and poor pregnancy outcomes. It also lowers immunity against diseases and cognitive function.

2.5.4. Hemagglutinins (Lectins)

Soya beans contain hemaglutinins (also known as lectins) which are proteins that are mainly present as glycoproteins in oil seeds such as soya bean (Akande *et al.*, 2010). They possess a unique ability of binding sugar constituents (carbohydrates) in red blood cells. Those that agglutinate red blood cells with a particular sugar specificity are referred to as lectins whereas those that agglutinate red blood cells with an unknown sugar specificity are known as hemagglutinins. Lectins are capable of causing severe damage to the intestines, inefficient digestion and nutritional deficiency (Felix & Mello, 2000)

2.5.5 Oligosaccharides

These are hetropolysaccharides of monosaccharides that are made up of 3 to 6 molecules of simple sugars. Legume seeds are significantly dominated by raffinose, stachyose and verbacose. Oligosaccharide digestion require highly specific enzymes which are not secreted in animals but by some bacteria in the alimentary canal of animals. The oligosaccharides then become substrates for the bacteria and are then fermented anaerobically. This action leads to the release of gasses such as hydrogen, carbon dioxide and methane which in turn cause flatulence and stomach discomfort characterized by nausea, ulcers and dispersia which is one of the main setbacks in the promotion of soya bean and other legumes for consumption by humans (Chen *et al.*,2013).

2.6 Processing Methods used to remove/reduce PIs

2.6.1 Heat

Heat processing constitute one of the methods of removing protease inhibitors in soya bean. Heat applied may be either wet or dry. The most common and easier types of heat treatments used in most Ghanaian homes are boiling and roasting.

In boiling, pre-soaked beans are boiled in water until they become soft but this method, on the other hand can lead to a potential loss of some nutrients such as water soluble vitamins due to leaching (El-Adawy, 2002).

Roasting is a dry heat method where soy beans are roasted in heat generated by ovens, coal burners or direct flames. The most common methods of roasting used in most Ghanaian homes include heating of soya bean on solid carriers such as sand. Optimal results could be obtained when soya beans are roasted at temperatures between 90 ◦C and 200 ◦C (Caprita *et al.*, 2010). Roasting is influenced by particle size and in order to prevent overheating of small-sized beans, the beans must be separated into sizes.

Other methods of heat application include flaking whereby a low pressure steam generated at 300 ◦C is injected into a conditioner containing the seeds which makes the seeds boil and swell generating a characteristic "popping sound". The seeds are then forced through rollers which press and elongate the moist grains into flakes.

Expansion also constitute heat application methods and it involves the application of pressure and hot steam generated at temperatures ranging from 70 ◦C to170 ◦C between five to fifteen minutes

Extrusion involves the application of high temperatures to the beans over a short period of time. It also involves pushing the beans through a series of restriction rings which is aided by a system of coils resulting in high pressure and temperatures generated by friction and movement of the processed beans.

Anti-nutritional factors in soya bean compared to other legumes are sensitive to heat and this property of soya bean makes heat treatment a better method of processing which is one of the stress-free methods of processing soya bean (Akande & Fabiyi, 2010). Heat application improves upon the aroma and masks the characteristic beany of soya bean making it more appealing to eat (Gonzalo *et al.*, 2006).

The amount of heat applied is critical in obtaining a more nutritious produce in that mild heating of about 90 oC will denature the proteins and reveal fresh undigested portions for digestive enzymes to breakdown. Also, the heat labile ANFs including TIs are inactivated. On the other hand over heating will result in the Maillard reaction leading to a reduction of the available lysine and to a little extent, other amino acids (Caprita & Caprita, 2007). Soetan and Oyewole, (2009) reviewed that trypsin inhibitor activity (TIA) was almost removed when heat processing methods were used to treat soya beans. However, the quality of heat processing carried out can be assessed by conducting a urease activity test.

2.6.2 Sprouting

Sprouting involves the soaking of seeds and then allowing them to germinate in a controlled environment. The quality of sprouts is enhanced when germination is uniform and swift (Khattak *et al.*, 2007).

This method of processing requires relatively less energy and produces natural effects. Research has found sprouting to be an efficient means of removing anti-nutritional factors in legumes. Three major chemical processes are involved during sprouting which are the hydrolysis of some materials, transportation of materials and the production of new materials from the hydrolysed materials. In addition, sprouting initiates the breakdown of storage proteins into amino acids thereby enhancing the bioavailability of proteins (Akande & Fabiyi, 2010).

Amal, *et al.*, (2007) reported that the sugar content reduced when several plant species were germinated. The reduction of these sugars is attributed to the occurrence of the de-novo synthesis of α-galactosidase in order to mobilise stored food for the development of the embryo. The enzyme breaks down complex carbohydrates such as raffinose, stachyose and verbacose into simpler forms such as sucrose and galactose. In another study, Ramakrishna *et al.*, (2006) observed that soaking dry *Lablab purpureus* for 12 hours lowered TIA by 51 %. Sprouting the beans for 32 hours further reduced the TIA by 17%. Malomo *et al*. (2011) observed that the TIA in cowpea decreased as the period of germination increased in a trial where they sprouted cowpea for 0, 2, 3 and 5 days. The TIA reduced by 58% when sprouted for 2 days but by the 5th day, it was further reduced to 20% of the initial quantity in the raw cowpea.

2.6.3 Acid treatment (Citric acid)

Generally, soaking forms an important part in the processing of most traditional foods especially cereals and legumes. During soaking, the texture of the legumes are modified as the seeds approach an equilibrium and this makes it easier to extract proteins from them. The changes in texture is attributed to the absorption of water which influences the softening of the cotyledons and enhances grinding, reduces cooking time and improves product quality.

Soaking seeds in citric acid solution constitute one of the major ways of removing ANFs in soya bean. According to Prodanov *et al.* (2004), during soaking ANFs such as protease inhibitors, phytic acid, some minerals and α-galactosides fully or partly go into solution and they are processed out by discarding the solution after soaking.

However the amount of ANFs that goes into solution dependent on the species, pH and duration of soaking (Gibson *et al.,* 2006). It also serves as preliminary treatment before other treatments like sprouting, cooking and fermentation. Sharma *et al.* (2013) reported that TIA in soya beans soaked in 1 % citric acid solution was reduced by 41%.

2.6.4 Other methods of processing soya bean

Other processing methods that have been studied include addition of enzyme, fermentation with enzymes, irradiation with gamma rays and reduction of ANFs through breeding (Gonzalo *et al.*, 2006 & Foley *et al.*, 2013)

ANFs can be removed by the addition of enzymes such as β-glucosidase, α-galactosidase, phytases and proteases directly to the substrate (Bedford, 2000). These enzymes modify the chemical composition of the ANFs which results in their hydrolysis. Fermentation makes use of enzymes such as *Saccharomyces cereviseae,* and bacteria such as *Bacillus* *subtilis* and *Aspergillus oryzae* to hydrolyse the substrate into simpler forms that can be easily digested, absorbed and utilized by the body. In a study, Teng, *et al*., (2012) observed that fermenting soya bean meal with *Bacillus subtilis* and *Aspergillus oryzae* for 72 hours increased soluble protein by 63.11% and 19.4% respectively.

Fermentation improves protein quality by denaturing enzymes of protease inhibitors that inhibit the digestion of proteins. Foley *et al.*, (2013) reviewed that the protein digestibility in soya bean products is improved in fermentation due to increased solubilization of soya bean proteins. Fermentation has been found to breakdown macromolecules in soya bean resulting in an increase in water soluble constituents. Also, the biochemical changes that occur during fermentation lower the molecular weights of compounds in soya bean (Kiers *et al.*, 2000).

2.7 Soya Bean in Diets of Infants

The World Health Organisation (WHO), in her quest to promote positive health for both mothers and infants in the whole world, recommended that infants be first fed exclusively on breast milk for the first six months of life after which complementary foods could be introduced while breastfeeding continued till the child is 24 months old (WHO, 2002). Victora, *et al.*, (2010) are of the view that age 6 to 24 months is a period where infants stand the risk of nutritional deficiencies. To avert this risk, complementary foods of high nutritional value and digestiblity remains essential in order to prevent severe wasting and stunting which could lead to conditions such as "kwashiorkor" and marasmus among infants within this period of growth. Several nutritional interventions have been introduced by both local and international bodies to help prevent PEM. One of such in Ghana is the development of less expensive complementary foods, for example weanimix by the

Ministry of Health, from locally produced food crops that are readily available to replace those from animal sources that are much more expensive for most people in the developing countries (Amagloh *et al.*, 2010). A lot of research has been carried out in an attempt to develop high protein weaning foods from locally available food crops such as soya bean and cowpea (Amankwah *et al.*, 2009 & Amankwah *et al.*, 2010).

CHAPTER THREE

3.0 Materials and Methods

The study was carried out in two parts; a survey to assess consumers' knowledge and perception of anti-nutritional factors and laboratory experiments to assess the efficacy of heat, sprouting and acid treatment on the removal of protease inhibitors in soya bean.

3.1 Study Area

The Greater Accra Region occupies a total land area of 3,245 square kilometers. As the second largest populated region of Ghana, it has a population of 4,010,054 which represents 16.3% of the total population of Ghana and the largest population density of 1,236 per square kilometre (Ghana Statistical Service, 2012).

The study was conducted in four selected health facilities within four selected districts in the Greater Accra Region of Ghana. The health facilities were chosen purposively to represent the three types of service providers within the region which are government, private and mission. Table 3.1 shows the districts and their corresponding health facilities

Table 3. 1 List of District, Type of Service Provider and Health Facility Studied

DISTRICT	SERVICE PROVIDER	HEALTH FACILITY
Accra Metropolitan	Government	Princess Marie Louise
Tema Metropolitan	Private	Narh-Bita Hospital
La-Nkwatanang Madina Municipal	Mission	Alpha Hospital
Ledzokuku-Krowor Municipal	Government	LEKMA Hospital

3.1.2: Sample Size and Sampling Procedure

According to the method described by The Research Advisors, (2006), a sample size of 384 was obtained using a margin of error of 0.5% and was calculated based on the formula:

$$n = \frac{X^2 * N * P(1 - P)}{(ME^2 * (N - I) + (X^2 * P * (1 - P)}$$

Where:

n = sample size

$X^2 = chi\ square$

N = population size

P = population proportion

ME = desired margin of error

Finally, Two hundred (200) willing respondents were randomly sampled for the study due to time and financial constraints. Weaning mothers were defined as mothers who had children from ages six (6) months to two years and feeding their children with soya bean products.

3.1.3: Data Collection

A total of 200 pre-tested questionnaires (appendix I) comprising both open and closed ended questions were administered to weaning mothers who were willing to take part in the survey while they waited for their turns at the Child Welfare Clinics (CWC). The questionnaire was used to obtain data such as socio-demographic background of respondents, general knowledge of respondents on soya bean, their knowledge about anti-nutrients and their perception of how processing of soya bean could be carried out to reduce anti-nutrients.

3.1.4 Data Analysis

The data collected was analysed using Statistical Package for Social Sciences, SPSS (version 17) and Microsoft excel 2010 and tables, graphs and charts were used to present the results obtained.

3.2. Laboratory Experiment

3.2.1 Experimental Materials Used

Two varieties of soya bean namely Jenguma and Anidaso (TGX 1904) were obtained from Heritage seed growers in Tamale, Northern Region. The grains were sorted manually to remove extraneous materials and also seeds with holes and wrinkled ones. All the grains were washed with tap water prior to processing.

3.2.2 Experimental Design

The experimental design used was a 2 x 3 full factorial in a Completely Randomised Design (CRD).

3.2.3 Treatments

Two factors were used in this experiment. The first factor consisted of the 2 varieties of soya bean (Jenguma and Anidaso) while the second factor consisted of three processing methods; heat, sprouting and acid treatments. All treatments were replicated three times. The treatment combinations for the experiments are summarized in tables 3.2, 3.3 and 3.4 below:

Table 3. 2 Treatment combinations for heat treatment

Treatment	Treatment Combination	Description of Treatment
A	JB	Jenguma boiled
B	JR	Jenguma roasted
C	JC_O	Control for Jenguma
D	AB	Anidaso boiled
E	AR	Anidaso roasted
F	AC_O	Control for Anidaso

Table 3. 3 Treatment combinations for sprouting

Treatment	Treatment Combination	Description of Treatment
M	J_2	Jenguma sprouted for 2 days
N	J_4	Jenguma sprouted for 4 days
O	JC_1	Control for Jenguma
P	A_2	Anidaso sprouted for 2 days
Q	A_4	Anidaso sprouted for 4 days
R	AC_1	Control for Anidaso

Table 3. 4 Treatment combinations for acid treatment (1% citric acid)

Treatment	Treatment Combination	Description
G	J_{12}	Jenguma in citric acid for 12 hrs
H	J_{24}	Jenguma in citric acid for 24 hrs
I	JC_2	Control for Jenguma
J	A_{12}	Anidaso in citric acid for 12 hrs
K	A_{24}	Anidaso in citric for 24 days
L	AC_3	Control for Anidaso

3.3 Sample Preparation

3.3.1 Heat Treatment

Roasting and boiling methods were used since they are the easiest and most common methods used in most Ghanaian homes.

3.3.3 Boiling

250g of the grains were soaked for 24 hours at room temperature in distilled water with a grain to water ratio of 1: 10 (w/v). The grains were then rinsed with tap water and boiled in tap water at 100 ^{0}C on a hot plate for 30 minutes (Hefnawy, 2011). The grains were then cooled, dried in a wagtech air oven at 60 ^{0}C for 12 hours and milled into fine powder in a hammer mill with sieve size of 8 microns. The powder was then bagged into air-tight polythene bags and kept till the analysis was done.

3.3.4 Sprouting

250 g of soya beans were soaked in tap water for 24 hours and the water drained off and seeds washed again under running water. Heat sterilized pieces of jute sacks were spread in metal sheets and the seeds evenly spread unto it. Another sterilized piece of jute was used to cover the seeds while ensuring there was enough moisture. Water was sprayed unto the jute when they became dry. Sprouting was done for 0, 2 and 4 days after which the sprouted seeds were dehulled, dried in a wagtech air oven at 60 ^{o}C for 24 hours. The seeds were then milled in a hammer mill of sieve size 8 microns and then kept in sealed plastic bags until the analysis were done

3.3.5 Acid Treatment

250 g of soya bean was soaked in citric acid (1% W/V) for 0, 12 and 24 hours with a seed to solution ratio of 1:3 at room temperature after which the solution was drained off and the beans rinsed twice with tap water (Wanjekeche *et al.*, 2003 & Sharma *et al.*, 2013). The beans were then oven dried at 60 ^{0}C for 24 hours in a wagtech air oven and then milled in a hammer mill of sieve size 8 microns. The powder obtained was bagged in air-tight plastic bags and kept until the analysis were done.

3.4 Analytical procedure

Proximate analysis was conducted to determine the moisture content, ash, crude protein, fat and carbohydrate in accordance with (AOAC, 2003) standards at the Department of Nutrition and Food Science laboratory, University of Ghana..

3.4.1 Moisture Content Determination (AOAC, 2003)

The oven drying method was used. 2.0g of the sample was weighed into a dried crucible (W1). The Crucible together with the sample was weighed and put into an oven at 105°C for 4 hours after which it was removed and placed in a desiccator to cool and the weight taken again (W2). The procedure was repeated for each of the samples.

The percentage moisture content was calculated as:

$$\% \text{ moisture content} = \frac{W1 - W2}{W1} \times 100$$

Where, W1 = Initial weight of sample

W2= Final weight of sample.

3.4.2 Determination of ash (AOAC, 2003)

Clean empty crucible was placed in a muffled furnace at 600 ◦C for one hour. It was then cooled in a dessicator and the weight (W1) taken. 5 g of each sample was placed in the crucible and the weight recorded (W2). Ignition of the sample over a burner with a blowpipe, followed until the sample became charred. The crucible was placed in a muffle furnace at 550 ◦C for 3 hours until a grey white ash colour was observed. The crucibles were then cooled in a desiccator and weighed (W3).

$$\text{Percentage ash (\%ash)} = \frac{\text{Difference in Wt.of Ash}}{\text{Wt.of sample}} \times 100$$

Where Different in weight of ash is weight of crucible after incineration (W_3) and weight of empty crucible (W_1)

3.4.3 Determination of Crude Protein (AOAC, 2003)

The protein content was determined using the Khedjal method. One gram of each of the samples was put in a digestion flask. 15 ml of concentrated H_2SO_4 was added to the samples. K_2SO_4 and $CuSO_4$ (catalyst) were added to the samples in the ratio 8:1 and the mixture swirled in order to thoroughly mix the contents. The mixture was then heated for 2 hours until the mixture became clear (blue-green in colour). The digest was then cooled and transferred into a 100 ml volumetric flask and the volume topped up to 100 mL by adding distilled water. 10 ml of the digest was introduced into a distillation tube after which 10 ml of 0.5 N NaOH was added gradually through the same way. The process continued for about 10 minutes after the NH_3 produced was collected as NH_4OH in a conical flask which contained 4 % boric acid solution and a few drops of methyl red indicator. The distillate was then titrated against 0.1 N HCl solution till a pink coloration appeared. A blank was also run following the steps already mentioned.

Percentage of crude protein was estimated as:

% crude protein = correction factor (6.25) × % N

But

$$\% \text{ N} = \frac{(\text{S} - \text{B}) \times \text{N} \times 0.014 \times \text{D}}{\text{Weight of sample } \times \text{V}} \times 100$$

Where

S = Sample titration reading

B = Blank titration reading

N = Normality of HCl

D = Dilution of sample after digestion

V = Volume taken for distillation

0.014 = Milli equivalent weight of nitrogen

3.3.4 Determination of Crude fat (AOAC, 2003)

Five gram of each sample was placed in a Soxhlet apparatus and extracted using petroleum ether at a boiling point between 40 - 60 ^{o}C. The extract was oven-dried at 105 ^{o}C for 2 hours and then cooled in a dessicator. The percentage crude fat was calculated as:

$$\% \text{ Crude fat} = \frac{\text{Weight of ether extract}}{\text{Weight of sample}} \times 100$$

3.3.2 Roasting

250g of the grains were roasted in an oven at 120 ^{0}C for 15 minutes (Caprita *et al.*, 2010). All samples were roasted in triplicate with the exception of the control which was not roasted. The samples were then cooled and then milled with a hammer mill of sieve size 8 microns into fine powder and kept in air-tight polythene bags until analysis were executed.

3.3.5Determination of Carbohydrate Content (AOAC, 2003)

The %CHO was calculated by difference. That is all the other parameters determined by proximate from 100.

%CHO = 100 – (% moisture + % crude protein + % crude fat + % crude fibre + % ash)

3.4.4 Urease Activity

The urease activity index was determined as a quality control measure for heat treated soya bean. The pH difference method as described by (AOCS Official Methods, 1997) was used. 200 mg of heat treated samples were incubated in 10 mL of phosphate buffered urea solution (0.07 M) at 30 ^{o}C for 30 minutes after which the difference in pH from (7.0) was recorded.

Table 3. 5 pH change indicating the level of heat processed soya bean

SOYA BEAN	UAI (p H change)
Under processed	> 0.20
Adequately Processed	0.05 – 0.20
Over – processed	< 0.05

Source: (AOCS official methods, 1997)

3.4.5 In-vitro Protein Digestibility (IVPD) Test

In-vitro protein digestibility was determined by modifying the method described by Laminu *et al.,* (2011). 1.0 mL of 11% trypsin was introduced into 3 test tubes. 4 mL of phosphate buffer with pH 7.5 was added to each test tube and 1 mL of 0.1 NHC1 was added and allowed to stand to equilibrate, after which 1 mL of 1 % soya bean flour was added to all the test tubes and allowed to digest for 3 h. The reaction was then stopped with 5 mL of 5% HCl (pH 2.46). The content of the test tubes were then filtered using filter paper. The filter papers were oven dried at 108 ^{o}C for 3 h. The nitrogen content of the undigested sample was determined by the Kjedahl method.

$$\text{IVPD (\%)} = \frac{\%\ \text{protein (total)} - \%\ \text{protein (undigested)}}{\%\ \text{protein (total)}} \times 100$$

3.6 Analysis of Data

Results obtained in this study were reported as means of three replicates. The averages determined for the parameters were subjected to Analysis of Variance using Gen Stat (12th edition). The Least Significant Difference (LSD) was used where differences were detected.

CHAPTER FOUR

4.0 Results

4.1 Knowledge and Perception of Weaning Mothers on Anti-Nutrients of Soya Bean

The results of this survey revealed the level of knowledge of weaning mothers on anti-nutritional factors and their perception of how it could be removed from soya bean. The survey showed that weaning mothers had little knowledge on anti-nutritional factors of soya bean.

4.1.1 Age of the Respondents

The age distribution of respondents who participated in the survey is summarized in Table 4.1. The ages are categorized into five groups. Weaning mothers within the range of 20 – 39 years formed the majority of respondents.

Table 4. 1 Age distribution of respondents

Age	Frequency	%
Less than 20	6	3.0
20 - 29	68	34.0
30 - 39	93	46.5
40 - 49	30	15.0
50 - 59	3	1.5
Total	**200**	**100**

Source: Field survey, 2015

4.1.2 Ethnic Background of respondents

In all the four hospitals, Akan (42.5%), Ga Adangbe (23.5%) and Ewe (21%) were the most common ethnic groups interviewed. Minority of the respondents (10.5%) and (2.5%) were of northern background and non-Ghanaians respectively (Figure 4.1).

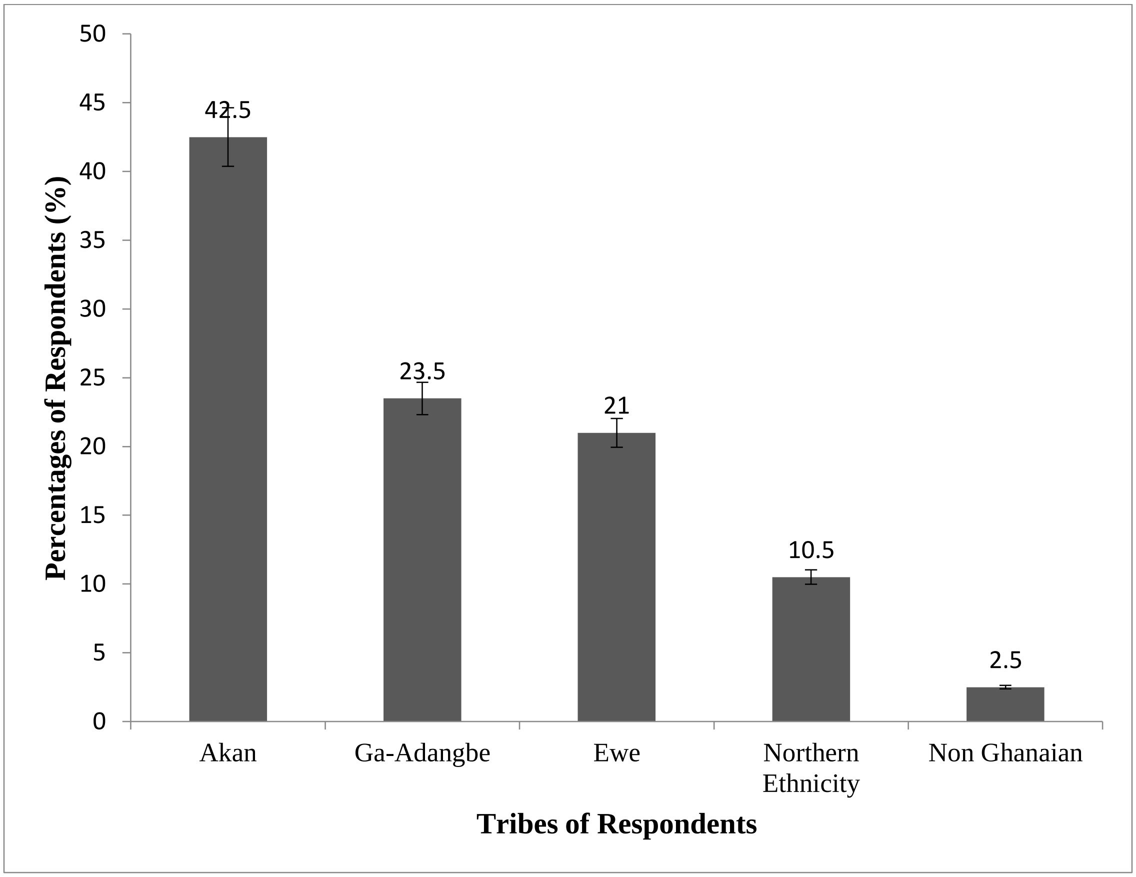

Fig 4. 1 Ethnic background of respondents

4.1.3 Educational background of respondents

The majority of respondents (92%) in the study had some form of formal education (Fig.4.2). Thirty-one per cent had at least Junior Secondary School or Middle school education while the remaining (8%) of the respondents had no formal education.

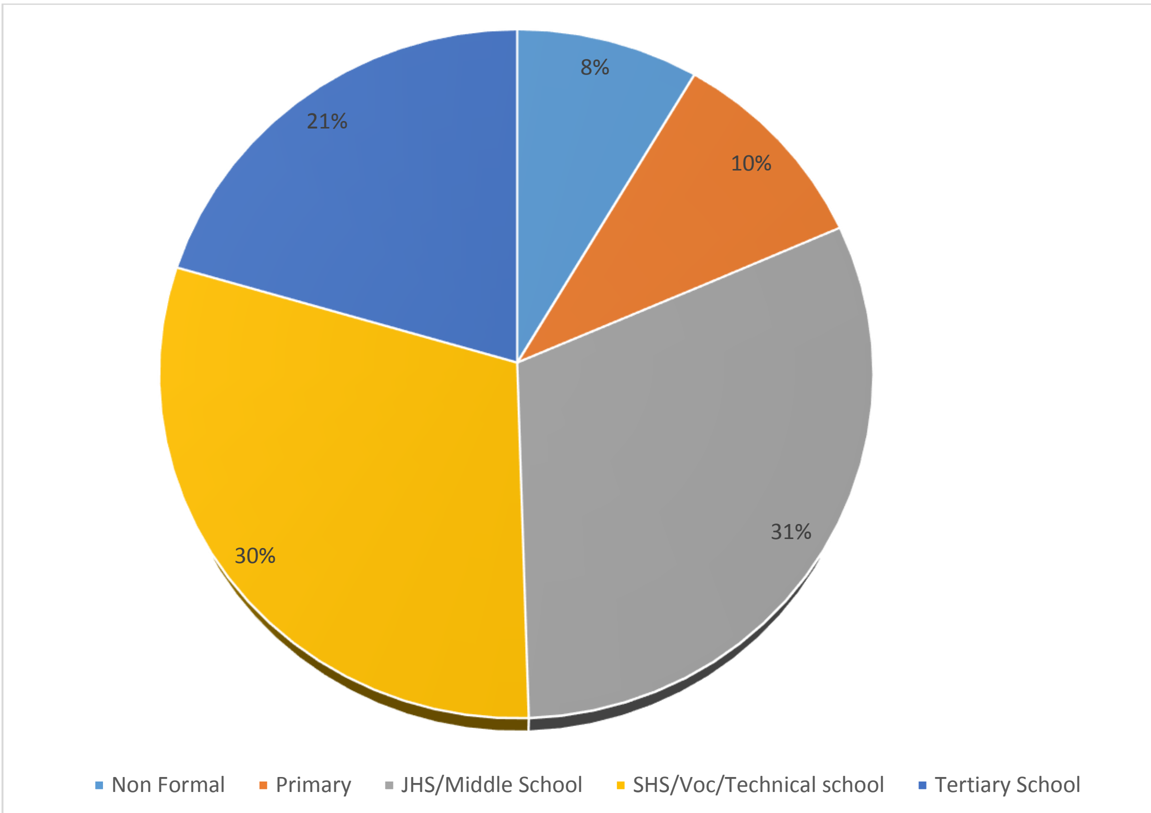

Fig 4. 2 Education background of respondents

4.1.4 Employment status of respondents

A greater proportion of the respondents were workers. Most of them were engaged in primary occupation such as petty trading 41.5% and vocational, technical or skilled work 25.5%. Professional workers were 21.5%, with l1.5% of respondents being unemployed

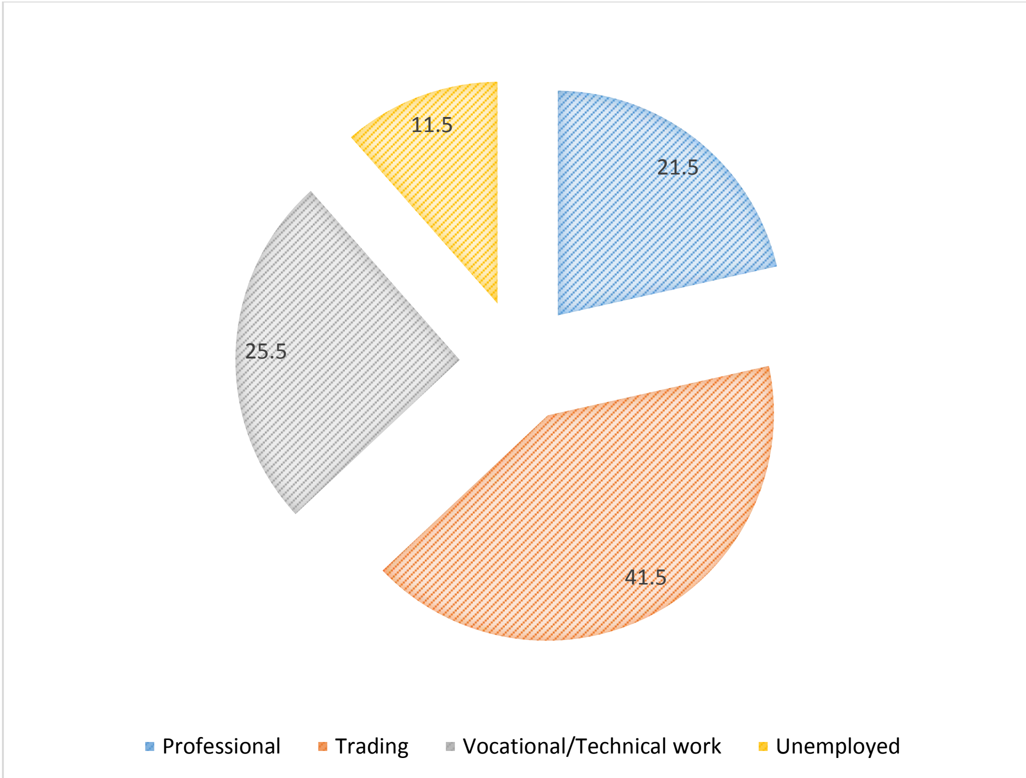

Fig 4. 3 Employment status of respondents

4.1.5 Knowledge of Respondents on Nutritional/ Health Benefits of soya bean

4.1.5.1 Soya bean consumption

While 34.5 % of respondents consumed soya bean at least once a week, others consumed it twice a week 30.5 % or even daily 26.5 %. Only a few respondents representing 8.5 % consumed soya bean once a month.

Table 4. 2 Percentage soya bean consumption

Period	Percentage (%)
Daily	34.5
Once a week	30.5
Twice a week	26.5
Once a month	8.5

Source: Survey Data, 2015

4.1.5.2 Perceptions of respondents on Major nutrient in soya bean

From the survey, 50.5% of the respondents know that the major nutrient in soya bean is protein while 8.5%, 8.0% and 1.5 % were of the view that the major nutrients were carbohydrates, minerals and vitamins respectively (Fig. 4.4). A good fraction (31.5%) of respondents had no idea of what the major nutrient in soya bean was.

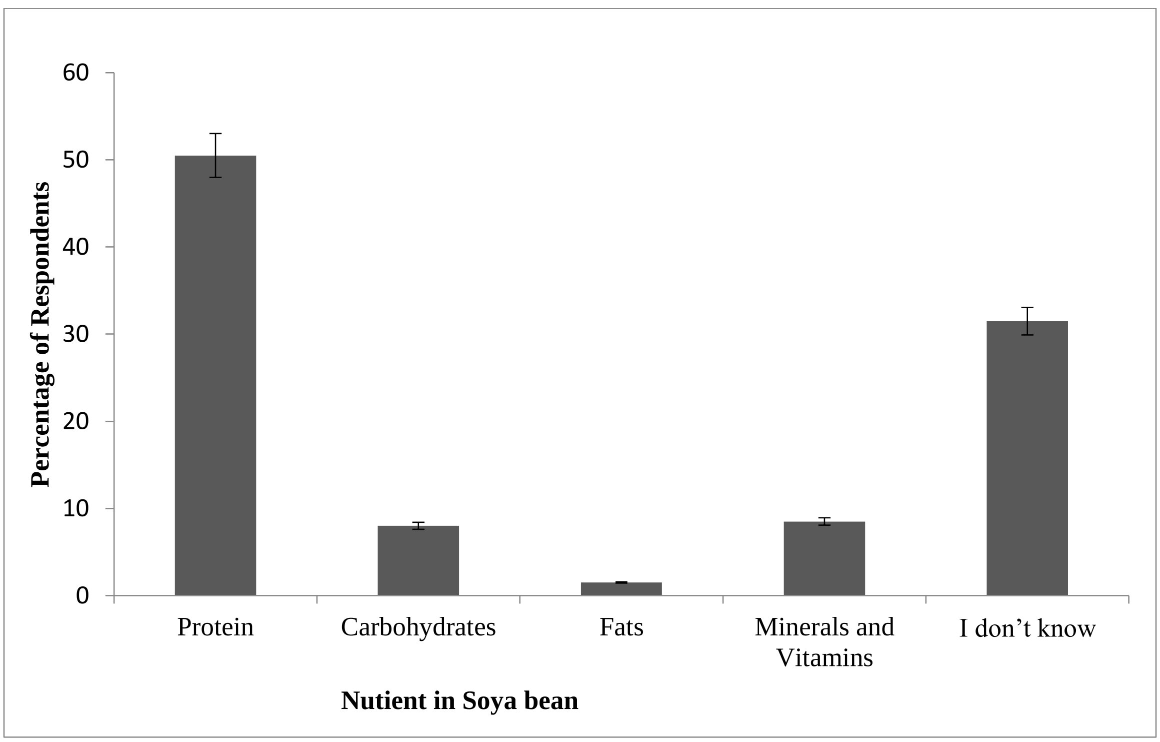

Fig 4. 4 Perceptions of major nutrients in soya bean

4.1.5.3 Reasons for consuming soya bean

The major reasons why the respondents consumed soya bean was based on the availability of the product, its rich nutrients, health benefits and low cost as compared to animal sources of protein (Fig.4.5). Fifty-one per cent of the respondents attributed their reasons for consumption to its health benefits while 44% attributed their consumption rate to nutrition. A few of the respondents, 3 % and 2 % attributed the reasons why they consumed soya bean to its availability and less expensive cost respectively.

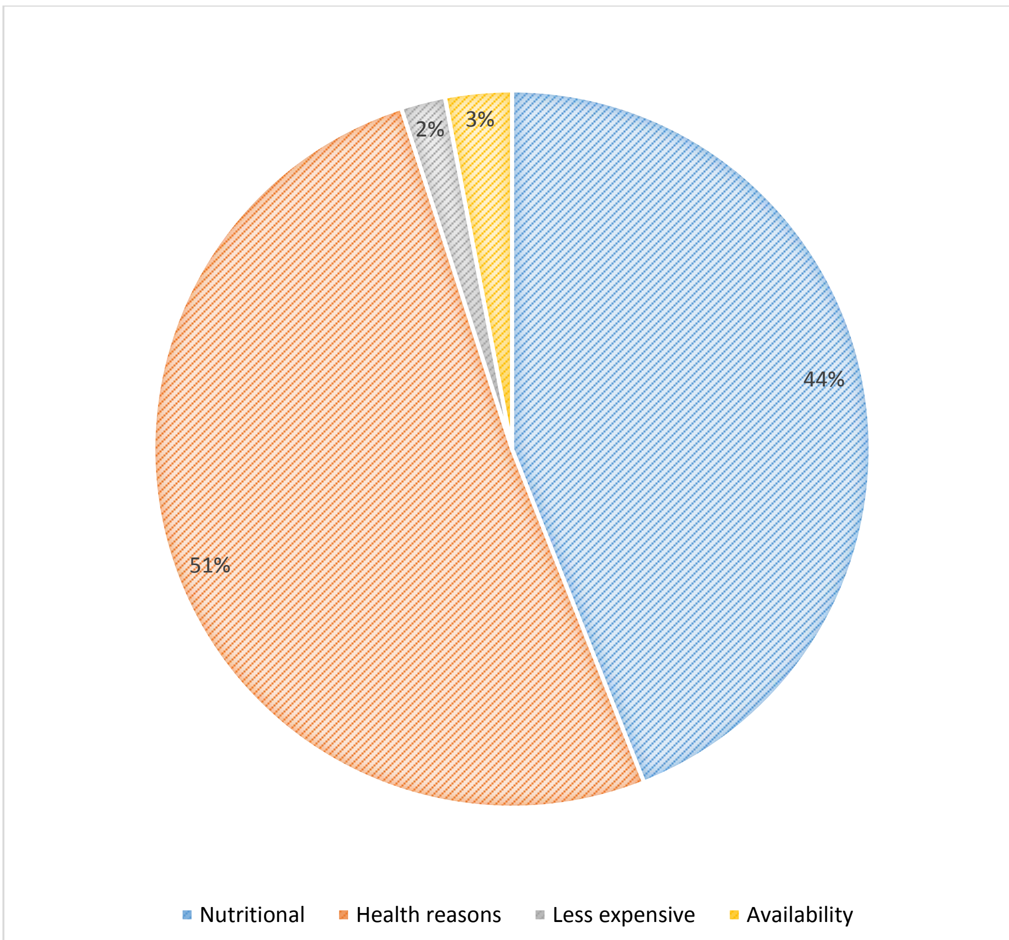

Fig 4. 5 Reasons for consuming soya bean

4.1.6 Knowledge of respondents on anti-nutritional factors

4.1.6.1 Education of respondents on anti- nutritional factors

The survey assessed the knowledge of respondents on anti-nutritional factors in soya bean (Table 4.3). Majority of weaning mothers 84% responded in affirmative to have received some form of education about soya bean. Alternatively, only 16% of the respondents have received no education on soya bean. Among those that received education on soya bean, 69.6% received educated from the Ghana Health Service (GHS), while 29.8% received education from other sources such as friends, relations, schools and the media. Only 0.6% received education from soya bean programmes organized by the Ministry of Food and Agriculture. Most of the respondents confirmed that the education received centered mainly on the nutritional health benefits of soya bean.

Table 4. 3 Education of respondents on soya bean

Characteristics	Response
Have you ever received any public education on soya bean?	**N = 200**
Yes	168 (84.0%)
No	32 (16.0%)
Who educated you?	**N =168**
GHS Personnel	117 (69.6%)
MoFA personnel	1 (0.6%)
Others	50 (29.8%)

Source: Survey data, 2015

4.1.6.2 Consumers' knowledge of anti-nutrients

Majority of the respondents (94.5%) had no idea of what the term anti-nutrients were (Fig 4.6). The minority 5.5% of the respondents affirmed that they had an idea what anti-nutrients are but could not identify any food item that contains anti-nutrients.

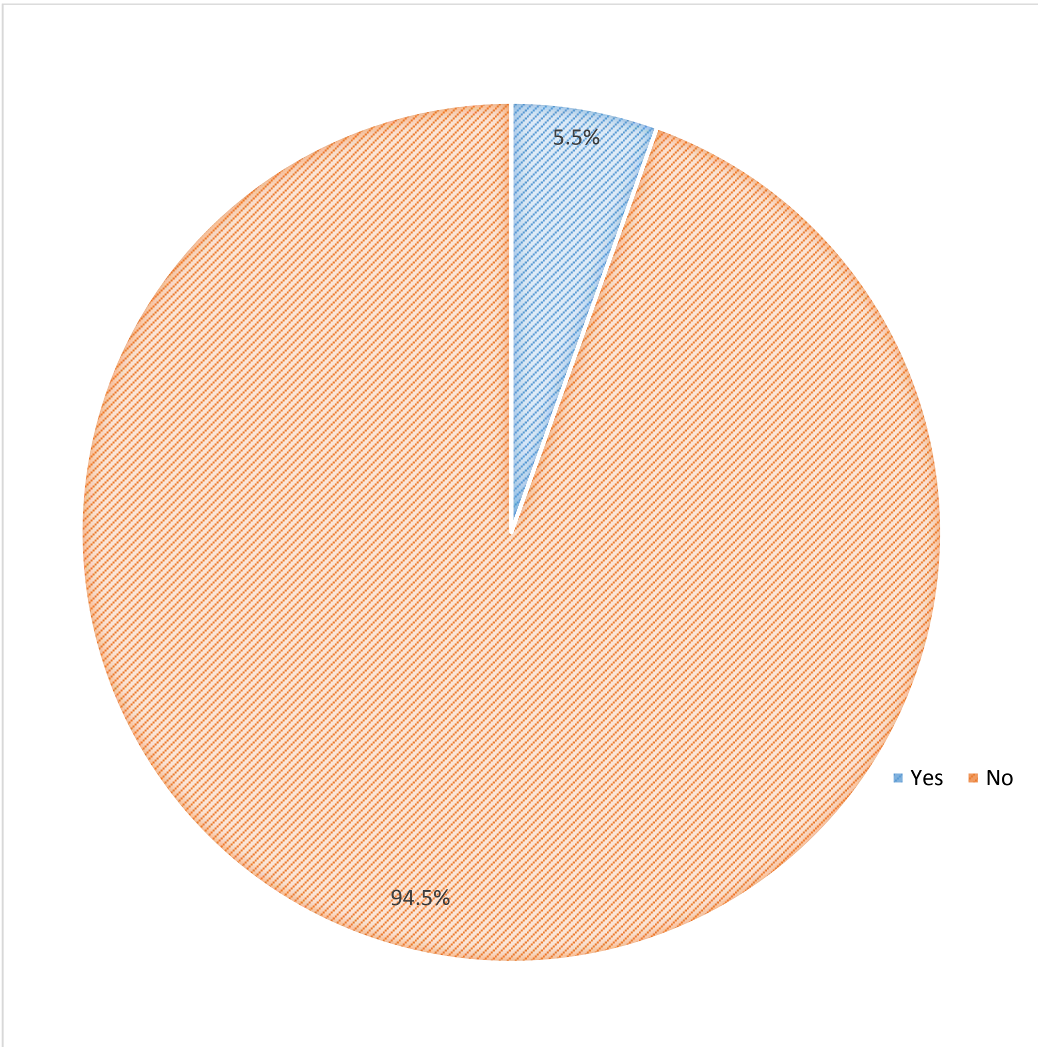

Fig 4. 6 Knowledge of consumers on anti-nutritional factors

4.1.6.3 Consumer Perception of How Anti-Nutritional Factors could be processed Out of Soya bean

4.1.6.4 Form in Which Weaning Mothers Use Soya bean

Soya bean is either used in raw or processed state (Fig. 4.7). A majority of mothers interviewed 65.7% use pre-processed soya bean to prepare meals for their children. On the other hand, 34.3% of respondents preferred to purchase the raw and process it themselves.

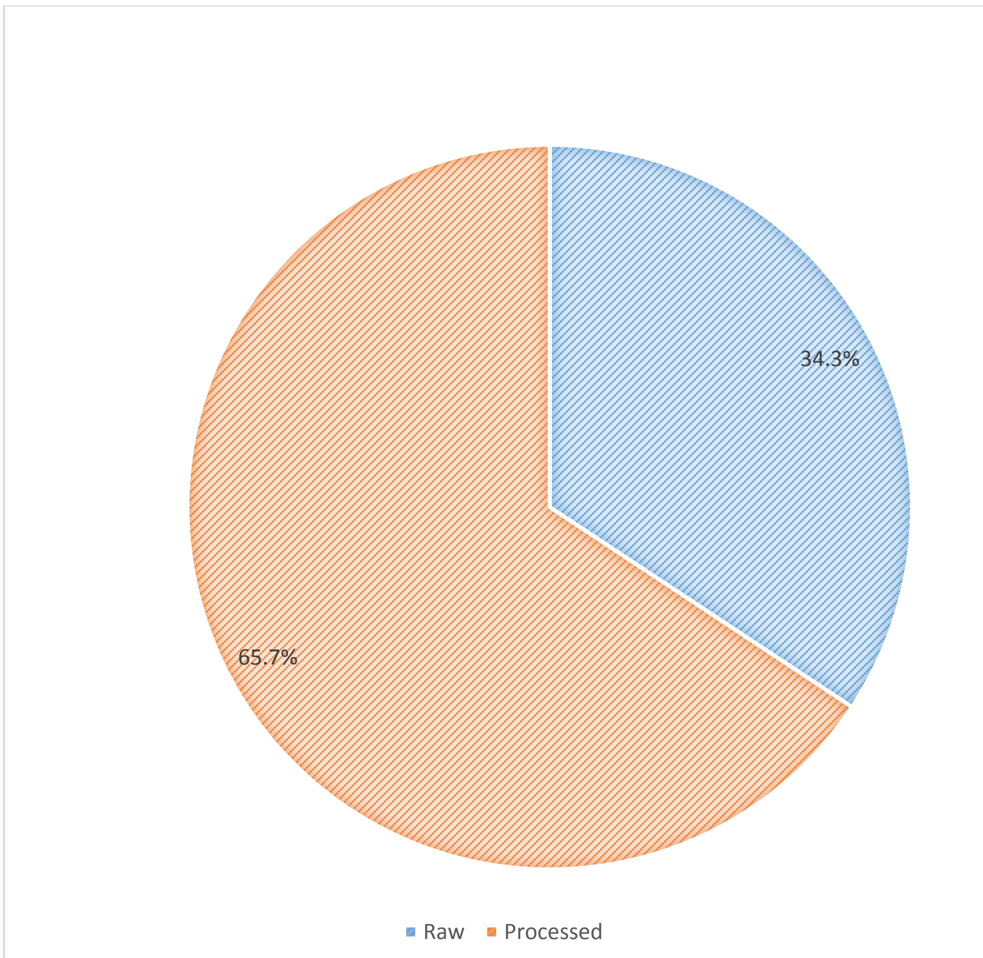

Fig 4. 7 Forms in which soya bean is used

4.1.6.5 Methods of Processing Soya beans by Weaning Mothers

The weaning mothers employed some processing methods in processing soya bean for their children (Fig 4.8). The study showed that the most common processing method employed by these women was roasting 60.6 %. This was followed by boiling 38.5%. Sprouting (0.9%) is an unpopular method used by weaning mothers as the survey indicated.

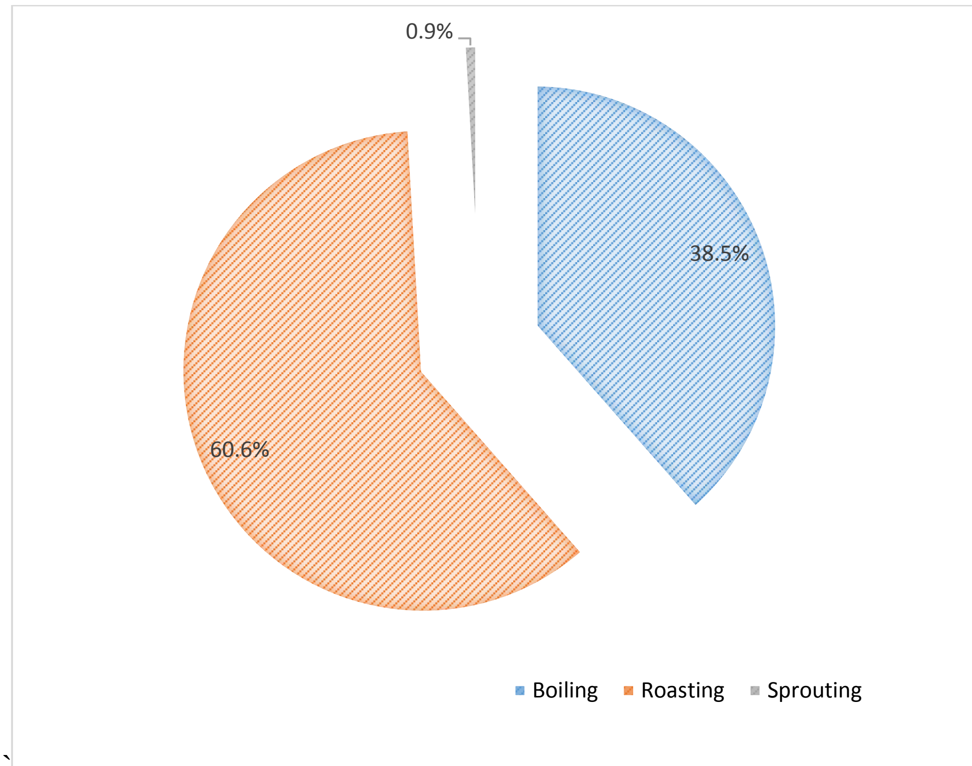

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Fig 4. 8 Methods of Processing of Soya Bean

4.1.6.6 Source of Processed Soya bean Used by Weaning Mothers

The weaning mothers purchase processed soya beans from the open market, family and friends, health facilities and some market outlets (Fig.4.9). A little more than half 53.3% of the weaning mothers buy processed soya bean products from the open market while

28.7% obtain the products from relations; and 18% get access to the processed soya beans from other sources such as health facilities, vendors and supermarkets.

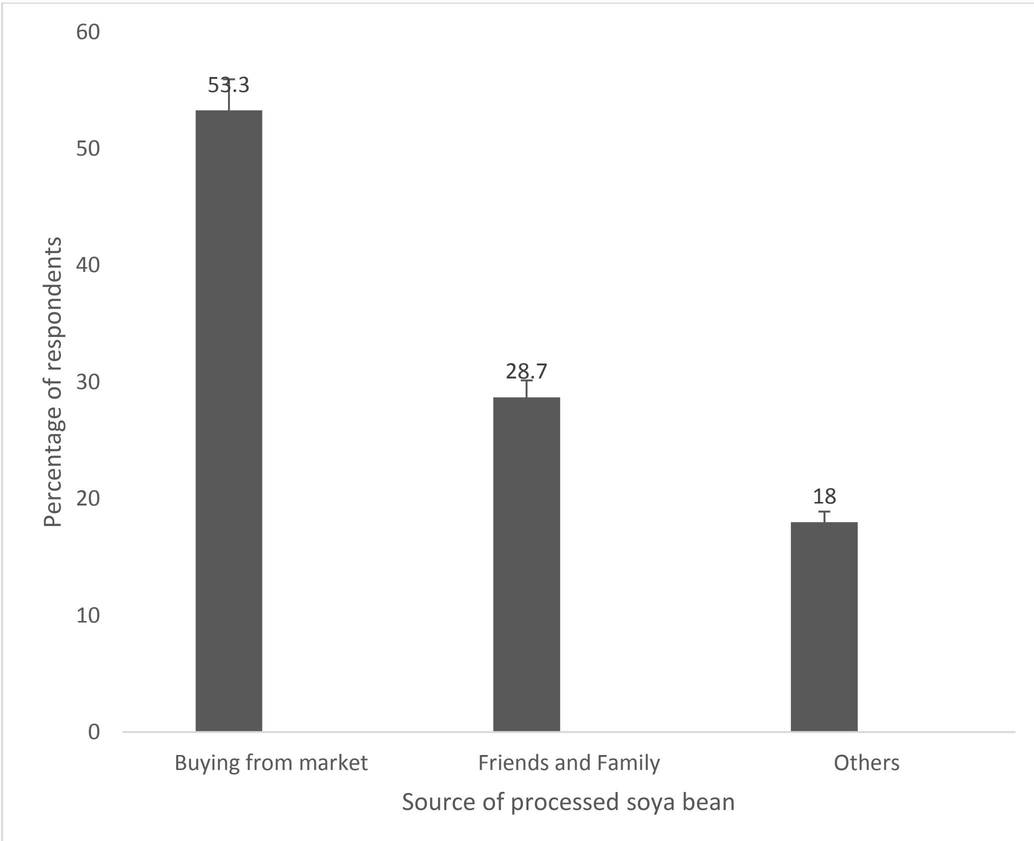


Fig 4. 9 Source of Processed Soya bean

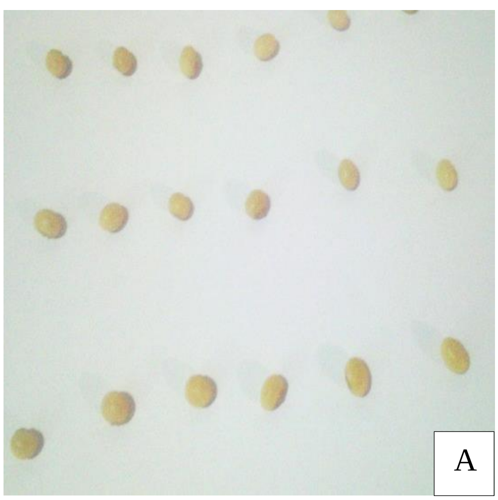
4.2 Nutritional composition of soya bean following heat application, sprouting and acid treatment

4.2.1 Physico-chemical characteristics of Anidaso and Jenguma

The physico-chemical characteristics of the two varieties of soya bean used for the experiment have been listed in Table 4.4 below. Jenguma has larger and pinkish seeds while Anidaso has generally smaller and yellowish seeds as shown in plate 1.

Table 4. 4 Physico-chemical characteristics of soya bean

Characteristic	**Variety**	
	Anidaso (%)	**Jenguma (%)**
Moisture	9.85	9.38
Ash	4.72	4.73
Fat	22.48	16.00
Crude protein	37.53	34.88
Carbohydrate	25.42	35.00



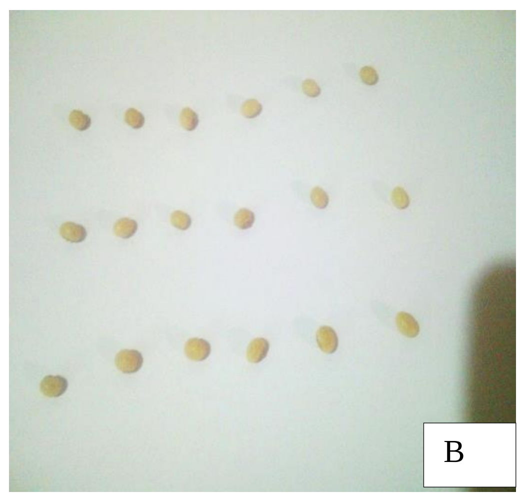


Plate 1 Soya bean varieties (A. Jenguma; B Anidaso)

4.2.1.1 Percent Moisture Content (%MC) following heat application.

Differences in MC existed among the soya bean flour produced after the application of the different levels of heat as shown in (Table 4.5). There was a significant reduction ($p<0.05$) of MC in the flour produced from boiling and roasting of both varieties as shown in (Table 4.5).The flour produced from raw Anidaso had a significantly ($p<0.05$) higher (9.85%) MC than that of Jenguma (9.38%). On the other hand, boiled Jenguma (5.51%) had a significantly higher MC than Anidaso (4.71%). In addition, roasted Jenguma (6.09%) had a significantly ($p<0.05$) higher MC than Anidaso (5.37%).

Table 4. 5 Nutritional composition of soya bean flour following heat application

Treatment	**Nutritional composition (%)**				
	MC	**ASH**	**CF**	**CP**	**CHO**
JC	9.38	4.73	16.00	34.88	35.00
JB	5.51	2.88	12.36	41.20	38.04
JR	6.09	5.00	15.33	36.30	37.27
AC	9.85	4.72	22.48	37.53	25.42
AB	4.79	3.32	25.59	44.31	21.98
AR	5.37	5.14	31.13	41.20	18.20
LSD≤0.05	0.04	0.12	0.71	2.35	2.34

Source: Generated from laboratory experiment, 2015

4.2.1.2 Percentage Ash content of soya bean following heat application

Generally, heat application affected % ash content in soya bean as shown in (Table 4.5). Ash content in raw Jenguma (4.73%) was not significantly different ($p>0.05$) from that of raw Anidaso (4.72%). However, ash content in boiled Jenguma (2.88%) was reduced significantly ($p<0.05$) than that of Anidaso (3.32%) as shown in (Table 4.5). Ash content in Anidaso (5.14%) was significantly ($p<0.05$) higher than that of Jenguma (5.00%).

4.2.1.3 Effect of heat application on % crude fat (CF) of soya bean

Heat application had a significant ($p < 0.05$) effect on % crude fat in soya bean (Table 4.5). The fat content in the raw Anidaso (22.48%) was significantly higher than that of Jenguma (16.00%). The fat content in boiled Jenguma (12.36%) was reduced significantly ($p<0.05$) compared boiled Anidaso increased (25.59%). A similar trend was observed for flour produced by roasting for both varieties as shown in (Table 4.5).

4.2.1.4 Effect of heat application on % crude protein (CP) of soya bean

The crude protein content in boiled soya bean was significantly ($p <0.05$) higher than the control and roasted soya bean for both varieties as shown in (Table 4.5).

However, there was no significant ($p > 0.05$) difference in the % CP between the control and roasted soya bean as shown in (Table 4.5). Crude protein content in raw Anidaso (40.67%) was significantly ($p < 0.05$) higher than that of Jenguma (37.46%) (Table 4.5).

4.2.1.5 Effect of heat application on % carbohydrates (CHO) in soya bean

The CHO content in raw soya bean was higher in Jenguma (35.00) than in Anidaso (25.42). Generally, the CHO content in boiled soya bean was higher than that of roasted soya bean for both varieties but the difference was not significant ($p > 0.05$) in Jenguma as shown in (Table 4.5).

4.2.2.0 Effect of sprouting days on % MC of soya bean

There was a significant difference between the MC in the control and soya bean that had been sprouted days as shown in (Table 4.6). The % MC after 4 days of sprouting was lower than % MC after 2 days of sprouting for both varieties as shown in (Table 4.6). This trend was only significant in Jenguma but not in Anidaso.

Table 4. 6 Nutritional composition of soya bean flour following sprouting

Treatment	Nutritional composition (%)				
	MC	ASH	CF	CP	CHO
Jc	9.38	4.73	16.01	34.88	35.00
J2	4.16	3.20	22.38	44.38	25.14
J4	2.83	3.45	24.57	48.55	20.59
Ac	9.85	4.72	22.48	37.53	25.42
A2	2.99	3.22	21.67	40.15	31.96
A4	3.04	3.22	20.34	42.90	30.50
LSD≤0.05	0.09	0.13	0.60	0.60	0.85

Source: Generated from Laboratory experiment, 2015

4.2.2.1 Effect of sprouting days on % Ash in soya bean

Differences existed in the ash content in soya bean sprouted for 0, 2 and 4 days. The ash content in raw Jenguma (4.73%) was not significantly different ($p>0.05$) from ash content in raw Anidaso (4.72%) as shown in (Table 4.6). The ash content in Jenguma sprouts for 2 days (3.20%) was significantly ($p<0.05$) lower than those sprouted for 4 days (3.45%).

In contrast, there was no difference in ash retained by Anidaso (3.22%) for both 2 and 4 days of sprouting as shown in (Table 4.6).

4.2.2.3 Effect of sprouting days on %CF of soya bean

The %CF in soya bean between the control and the other sprouting days were different. The %CF in Jenguma increased significantly ($p<0.05$) along the sprouting days (0, 2 and 4 days) as shown in table (Table 4.6). However, %CF in Anidaso rather decreased along the sprouting days (0, 2 and 4 days) as shown in (Table.4.6).

4.2.2.4 Effect of sprouting days on %CP in soya bean

Significant differences ($p<0.05$) existed between the control and the other sprouting levels as shown in (Table 4.6). In addition, there was a significant ($p<0.05$) increase in CP along the sprouting levels (0, 2 and 4 days) for both varieties as shown in (Table 4.6). The CP content in raw Anidaso (37.53%) was significantly higher than the CP in raw Jenguma (34.88%). On the other hand, Jenguma had a significantly ($p<0.05$) higher protein content when sprouted for 2 and 4 days compared to the CP in Anidaso sprouted for the same period as shown in (Table 4.6).

4.2.2.5 Effect of sprouting on the % Carbohydrate (%CHO) of soya bean

The percentage carbohydrate of soy bean between the control and other sprouting levels were significantly ($p<0.05$) different. The %CHO in raw Jenguma (35.00%) was significantly ($p<0.05$) higher than that of raw Anidaso (25.42%). Additionally, %CHO reduced significantly ($p<0.05$) along the sprouting levels (0, 2 and 4 days) in Jenguma but that for Anidaso did not follow any particular trend as shown in (Table 4.6). There was a significant difference between Jenguma sprouted for 2 days and Anidaso sprouted for 2 days. Anidaso sprouted for 4 days had a significantly ($p<0.05$) higher CHO than Jenguma sprouted for 4 days.

4.2.3.1 Effect of soaking time on % MC of soya bean

The % MC of the flour produced from non-soaked soya bean was significantly ($p < 0.05$) higher than flour produced from soaked soya beans (Table 4.7). There was a significant ($p<0.05$) reduction in the MC of flour produced from soya bean soaked in citric acid along the soaking times (0, 12 and 24 hours) as shown in Table 4.7.The % MC was significantly ($p < 0.05$) reduced in Jenguma (4.13%) than in Anidaso (5.51%) when soaked for 12 hours. A similar trend followed when the beans were soaked for 24 hours as shown in Table 4.7.

Table 4. 7 Nutritional Composition of soya bean flour following acid treatment

Treatment	**Nutritional composition (%)**				
	MC	**ASH**	**CF**	**CP**	**CHO**
Jc	9.38	4.73	16.00	34.88	35.00
J12	4.13	3.28	19.52	46.25	26.82
J24	2.83	3.47	24.53	48.55	20.63
Ac	9.85	4.72	22.48	37.53	25.42
A12	5.51	3.72	18.65	37.26	34.86
A24	5.63	3.00	22.67	45.79	23.31
LSD≤0.05	0.08	0.14	0.28	0.47	0.45

Source: Generated from Laboratory experiment, 2015

4.2.3.2 Effect of soaking time on %Ash in soya bean

There was a significant ($p < 0.05$) difference between % ash content of the raw soya bean and soaked soya bean for both varieties as shown in Table. 4.7. There was no significant ($p<0.05$) difference in the ash content in raw Jenguma (4.73%) and Anidaso (4.72%).The % ash content in Anidaso decreased significantly ($p < 0.05$) along the period for soaking but ash content in flour produced from Jenguma did not follow any particular trend as shown in Table 4.7.

4.2.3.3 Effect of soaking time on % CF in soya bean

The % CF was significantly ($p < 0.05$) different for the three soaking times. The % CF content in Jenguma was significantly ($p<0.05$) increased along the soaking levels (0, 12 and 24 hours) while no trend was observed for Anidaso as shown in (Table 4.7).

4.2.3.4 Effect of soaking time on % Crude protein CP

Soaking time had some effect on % CP in soya bean Table. 4.7. The results revealed that there was a significant ($p<0.05$) increase in % CP from 0 to 24 hours in Jenguma but in Anidaso there was a drop (37.53% to 37.26%) after 12 hours of soaking. There was also a significant increase in % CP (45.79%) after 24 hours of soaking.

4.2.3.5 Effect of soaking time on % carbohydrate (%CHO) in soya bean

Differences occurred in the %CHO between the control and the other levels of citric acid soaking as shown in Table 4.7. Jeguma had a higher %CHO (35.00) while Anidaso had a lower %CHO (25.42%).The study showed that there was a significant decrease in % CHO from 0 to 24 hours in Jenguma. However, %CHO in Anidaso was high (34.86%) after 12 hours of soaking and then low (23.31%) after 24 hours of soaking.

4.3. Urease Activity Index (UAI)

The urease activity index (UAI) was determined to ensure that the heat treated samples were adequately processed (Table 4.8). The results showed that all the samples that were heat treated were adequately processed compared to the control that did not receive any heat treatment.

Table 4. 8 Urease Activity index (UAI) of heat treated samples

Sample	UAI
Control for Jenguma	2.96
Control for Anidaso	2.92
Roasting (Jenguma)	0.14
Roasting (Anidaso)	0.18
Boiling (Jenguma)	0.16
Boiling (Anidaso)	0.17

pH <0.2 (adequately processed); >0.2 (under processed); <0.05(over processed)

4.4 The Effect of heat, sprouting and acid treatment on in-vitro Protein Digestibility (%IVPD) of soya bean

4.4.1 Effect of heat application on %IVPD of soya bean

There were differences in the in-vitro protein digestibility between the two soy varieties as shown in Fig.4.10. The uncooked Anidaso had a significantly ($p<0.05$) lower (32.98%) in-vitro digestibility as compared with the Jenguma variety (56.12%). On the other hand when the varieties were boiled, the Anidaso variety showed a significantly $p<0.05$) higher (55.77%) in vitro digestibility than the boiled Jenguma (53.41%).

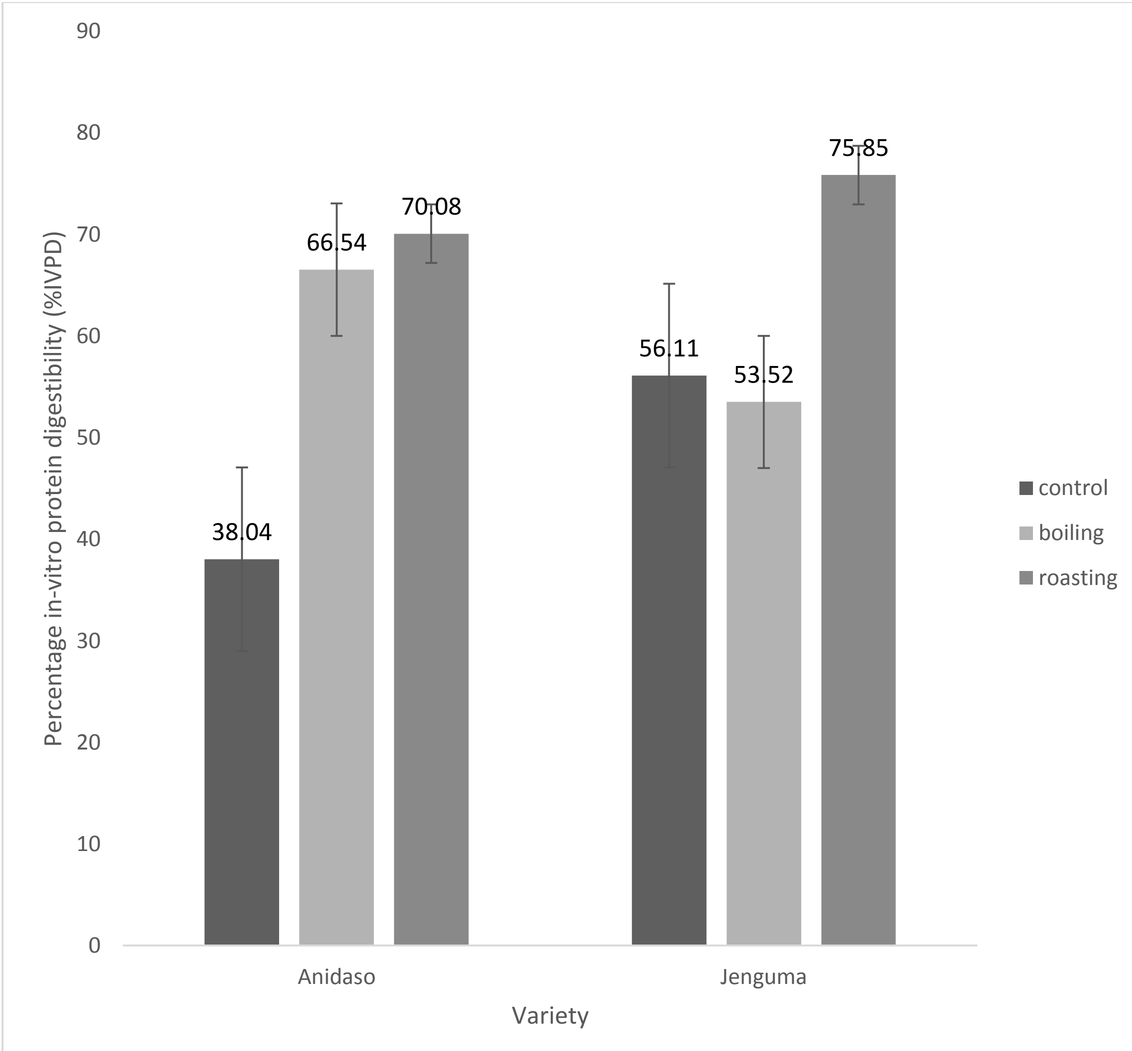


Fig 4. 10 %IVPD of soya bean flour following heat application

4.4.2 Effect of sprouting on %IVPD of soya bean

Sprouting significantly improved the IVPD in soya bean as shown in Fig.4.11. The IVPD in raw soya bean was significantly ($p<0.05$) higher in Jenguma (56.12%) than in Anidaso (32.98%). The IVPD in Anidaso increased as the number of days for sprouting increased whereas that of Jenguma did not follow any particular trend. The IVPD for Anidaso was higher (55.77%) after sprouting for 2 days than that of Jenguma (53.41%). Anidaso showed a significantly ($p<0.05$) higher IVPD (71.83%) when sprouted for 4 days as compared to that of Jenguma (62.81%).

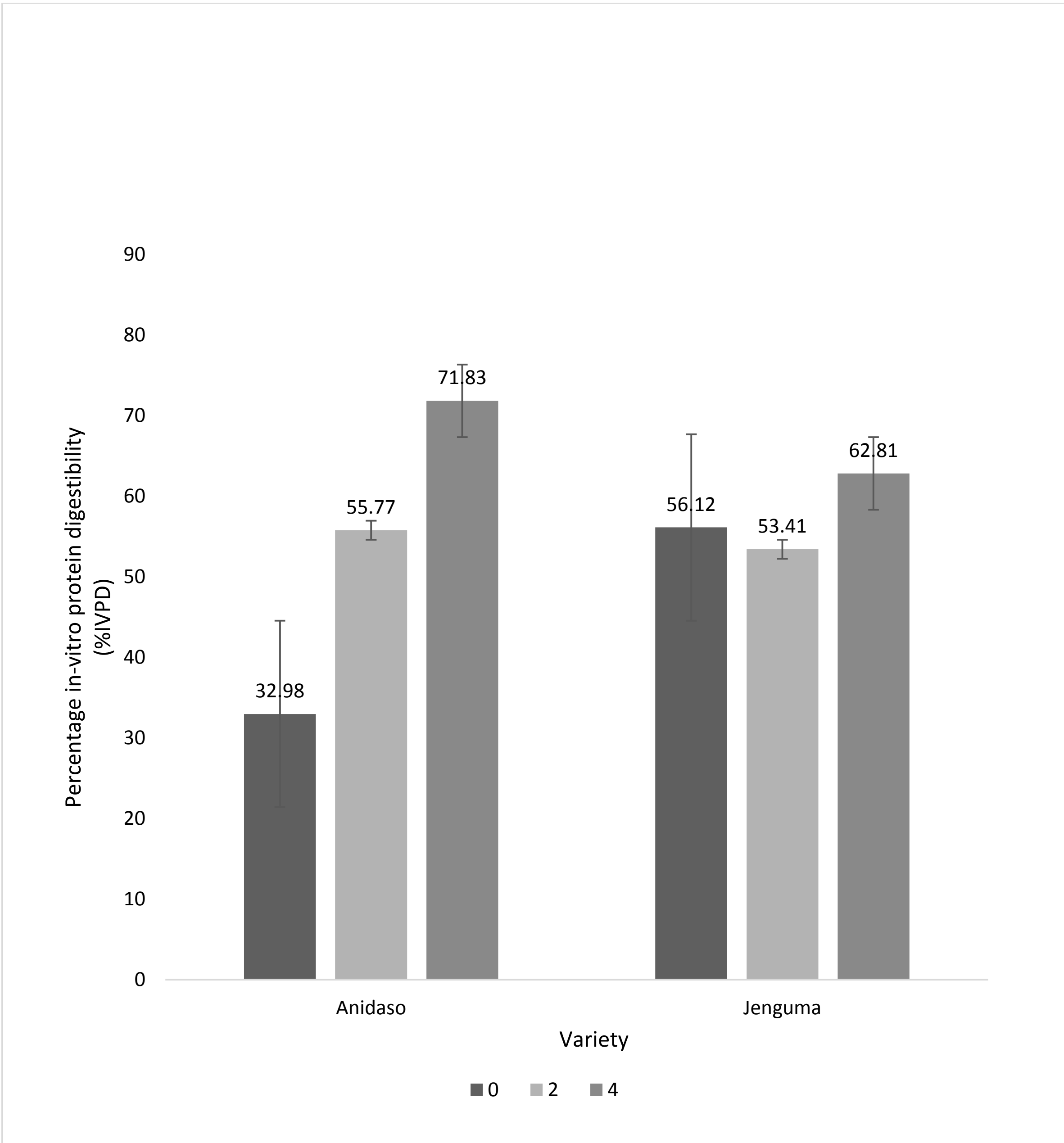


Fig 4. 11 %IVPD of flour obtained from sprouted soya beans

4.4.3 Effect of soaking time on the %IVPD of soya bean

Differences occurred between the two varieties as shown in (Fig.4.12). The IVPD increased in both varieties as the soaking time increased as shown in (Fig.4.12). The IVPD in raw Anidaso (39.27%) was lower than that of Jenguma (56.15%). On the other hand IVPD in Anidaso (56.9%) increased when soaked for 12 hours compared to Jenguma (58.15%). soaked for the same period. Although 24 hours of soaking time improved the IVPD for both varieties, the IVPD for Anidaso progressed faster than that of Jenguma as shown in (Fig.4.12).

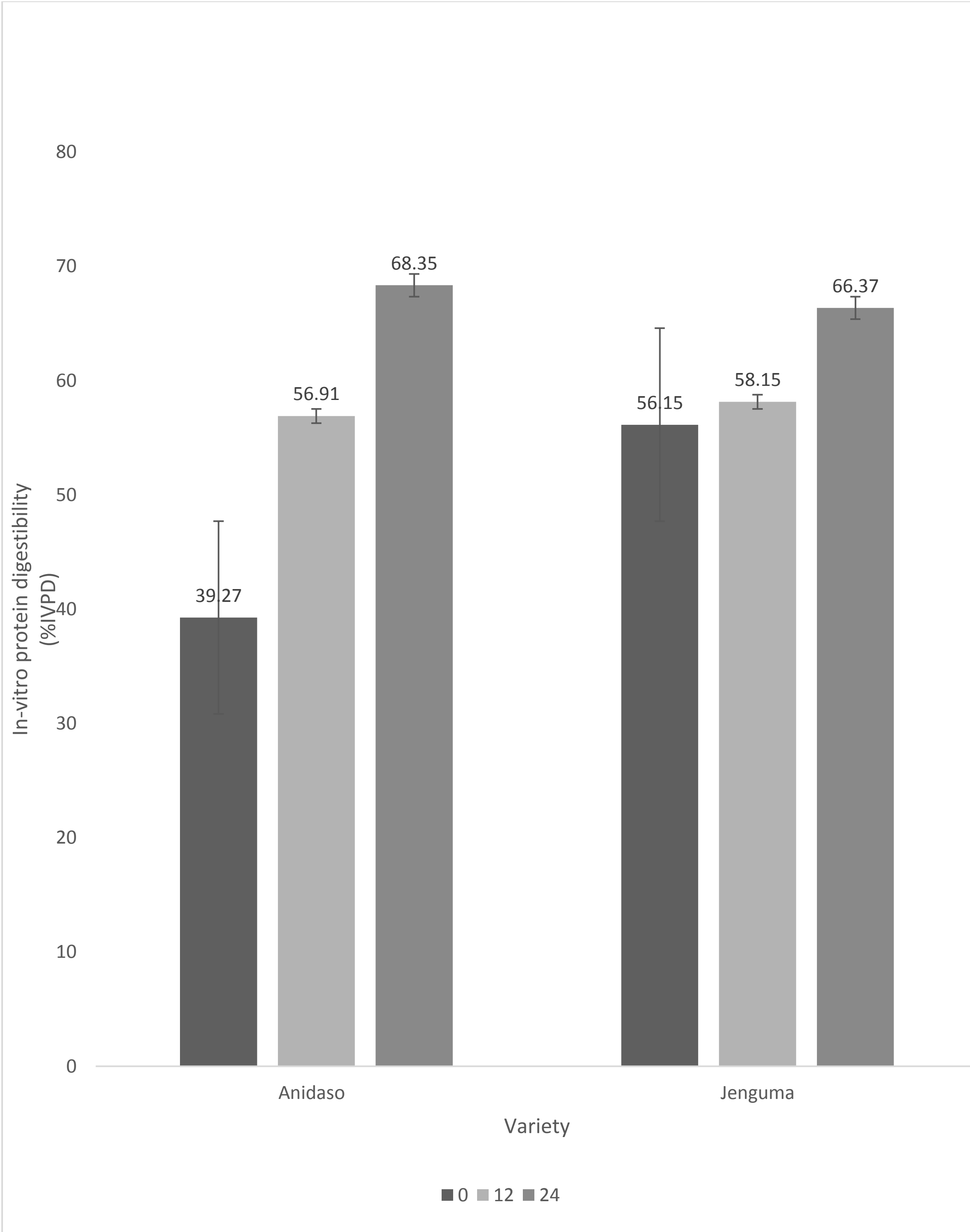


Fig 4. 12 %IVPD of soya bean flour following different soaking times

CHAPTER FIVE

5.0 DISCUSSION

5.1 Survey of the Respondents

5.1.1 Socio- Demographic Characteristics of Respondents

Most of the mothers interviewed were between the ages of 20 - 39 years which suggests that most of respondents were in the ages where they are economically active and therefore possess the purchasing power to provide meals for their children. This was more evident in the fact that only a small proportion (11%) were unemployed.

With the exception of 8% of the mothers interviewed who had no formal education, 92 % had at least basic education. This implied that the majority of these women might have been exposed to basic information about nutrition which might have influenced their choice of food and hence their preference for soya bean as a high protein constituent in the diets of their children. Furthermore, Burchi, (2012) reviewed that literacy is a powerful tool for assessing nutritional and health risk in children since it enables mothers to provide adequate nutrients for their families.

5.1.2 Knowledge of Nutritional and Health Benefits of Soya Bean

The soya bean consumption rate among the participants was high. This is in line with the findings of WISHH (2006) which stated that consumers have some knowledge of soya bean’s nutritional and health benefits. In addition, 50.5% of the consumers knew that protein is the major nutrient in soya bean. This explains why the respondents frequently fed their babies on soya bean. By observation, the most popular Ghanaian diet (Tom Brown; weanimix) is highly patronized by mothers for their growing children.

On the other hand, (31.5%) had no idea what the major nutrient in soya bean is. This suggest that formal education alone does not cater for nutritional education.

5.1.3 Knowledge of Respondents on Anti-nutritional Factors in Soya bean

Majority of the respondents (84.0 %) had received some form of education on soya bean. This could be attributed to programmes that emphasized on nutritional based education as documented by Plahar (2006). The study further revealed that most of the education was carried out by personnel of the Ghana Health Service and a little less (29.8%) of the mothers received education from other sources such as friends, relatives, schools and the media. This finding indicates that sources apart from the hospitals could be explored by implementers. Although the study took place in health facilities, it revealed that the activities of MoFA in the promotion of soya bean utilisation in the urban areas is rather low which may be attributed to the fact that most of their activities may be directed towards increase in production.

Almost all the mothers interviewed (94.5 %) had no idea what anti-nutritional factors were. This is a confirmation that most of the education on soya bean centered on the nutritional and health benefits as stated by respondents. The need for all stakeholders to create consumer awareness of the anti-nutrients present in soya bean and also how to effectively process them out is essential especially when it is used in feeding young children.

5.1.4 Perception of Respondents on How Anti-Nutrients Could be processed out of Soya bean

A third (34.3%) of the study population used primary processed soya bean to prepare food for their children while the remaining (65.7%) used secondary processed soya bean which is more acceptable and convenient for working mothers.

The study further found out that the mothers who used already processed soya bean products purchased them from the markets, health facilities, supermarkets and vendors. This finding has critical implications for the promotion of soya bean as food for growing children since there was an uncertainty as to whether the processors were adequately processing the soya bean products or not.

Roasting (60.6%) and boiling (38.5%) were common methods of processing used by the respondents. According to, (Caprita *et al.*, 2010) the amount of heat applied to soya bean plays an important role in improving its nutritional value. Mild temperature (below 90 ◦C) inactivates heat- sensitive protease inhibitors and expose new protein sites for hydrolysis while high temperatures (above 200 ◦C) results in the Maillard reaction thereby reducing the nutritional value. The amount of heat applied presents a challenge since by observation the average Ghanaian woman might not consider the temperature and time while roasting soya bean. Furthermore, soya has a high oil content and therefore retains a lot of heat even after cooking making it quite difficult maintaining a golden brown colour. Other methods of processing such as sprouting which is known to improve the nutritional value of soya bean can be explored.

5.2. The effect of heat, sprouting and acid treatment on the nutritional composition of soya bean

Food processing is one of the ways by which value can be added to soya bean in terms of improving its nutritive value. Each processing method possess unique characteristics that works to effect the desirable nutritional value expected by the processor. However, the choice of processing method must not cause a deleterious effect on the nutritional value of the commodity in question.The processing methods used in the present study resulted in changes in the nutritional composition of the two soya bean varieties studied.

5.2.1 Effect of heat on the nutritional composition of soya bean

Heat processing is an acceptable method of processing soya bean before its inclusion in human diet. This is due to its ability to improve the nutritive value of most legumes found in the tropics(Akande & Fabiyi, 2010). The results of this experiment showed a significant ($p<0.05$) reduction of % ash and fat in boiled soya bean than the control and roasted ones as shown in (Table 4.5). Similar losses of ash and fat was observed by Hefnawy (2011) when lentils were boiled for 90 minutes. Boiling in general is characterized by the leaching of nutrients into the boiling water which is discarded after the process. This could be a possible reason for the reduction in %ash that occurred in boiled soya beans. In addition, Anidaso retained a significantly higher ($p<0.05$) %ash than Jenguma (Table 4.5) which is likely due to differences in their physico-chemical characteristics. Jenguma possess larger grains which have broader surface areas compared to Anidaso which are smaller in size and smaller surface areas. This property of Jenguma accounted for the rapid loss of minerals from its grains during boiling. Again, %CP was significantly ($p<0.05$) higher in the boiled soya bean than roasted and raw soya bean (Table 4.5). Roasted soya bean, however, recorded a significantly lower ($p<0.05$) %CP compared to boiled soya bean which is an indication that moist heat is much more effective in processing soya bean than dry heat. In addition, the boiled soya bean was soaked prior to boiling which might have initiated the hydrolysis and subsequent solubilization of some nutrients thereby increasing the CP of soya bean. The CP increased in Anidaso for both boiling and roasting compared to Jenguma which is due to the differences in the physico-chemical characteristics between the two varieties. Anidaso possess smaller seeds than Jenguma and as a result was able to absorb heat faster unlike Jenguma with larger seeds.

5.2.2 Effect of sprouting on the nutritional composition of soya bean

Sprouting as a processing method is an easy and low cost method for improving the bioavailability of nutrients in diets from plants compared to heating. Gibson *et al.* (2006) reviewed that sprouting stimulates metabolic processes in cereals and legumes which leads to enzymatic hydrolysis of oligosaccharides, phytates and proteins. Thus, enhancing the availability of nutrients. This explains why changes in the nutritional composition occurred during the sprouting trials.

Ash content in soya bean was significantly higher ($p<0\ 05$) in the control than in the other sprouting treatments (Table. 4.6) This reduction of ash in the other treatments could possibly be due to the leaching of minerals into solution during the soaking of the beans as a pre-treatment to sprouting .

The %CHO reduced significantly ($p<0.05$) from 0 to 4 days of sprouting (Table.4.6). This is consistent with Mubarak (2005) who observed similar reduction in %CHO after sprouting mung bean for 3 days. This could be attributed to the hydrolysis of carbohydrates to release energy for the developing embryo by amylase produced by the embryo.

Percentage crude fat was increased significantly ($p<0.05$) as the sprouting days increased (Table 4.6) in Jenguma while the contrary was observed for Anidaso. This observation in Anidaso is consistent with Ghavidel & Prakash (2007) who reported a significant ($p<0.05$) reduction of fat content in some legumes following a germination trial. Osman (2007) observed a similar reduction of fat when mung beans were sprouted for three days. The decrease in fat content could be due to fat being used as energy source as sprouting progressed.

The crude protein content of soya bean increased significantly ($p<0.05$) along the three sprouting levels (0 to 4 days) (Table.4.6). Similarly, Syed *et al.* (2011), also found that mung bean yielded a significant increase in protein content when sprouted for 96 hours. This increase in protein content is due to hydrolysis of carbohydrates to release energy for the development of the sprouts as well as the synthesis of new proteins (Echendu *et al*,. 2009). Jenguma produced a significantly higher percentage of crude protein (48.55%) than Anidaso (45.79%) which suggests that Jenguma may be the best choice if the processor's interest is in increasing protein content.

5.2.3 Effect of acid treatment on the nutritional composition of soya bean

Soaking, generally, stimulates metabolic processes which results in changes in the constituents of soya bean. During soaking a concentration gradient is established which results in leaching of nutrients into solution. The solution is usually discarded with the leached materials. The present study resulted in some changes in the nutritional composition of soya bean.

The moisture content in soya bean on dry matter basis reduced significantly ($p<0.05$) from 0 to 24 hours of soaking (Table. 4.7) with Anidaso having a moisture content higher than Jenguma for both 12 and 24 hours of soaking. This was because Jenguma had larger cotyledons with larger surface area which enhanced a faster rate of drying unlike Anidaso. Moisture content also has implications on the shelf-life which the current study did not cover.

The fat content increased significantly as the period of soaking increased (Table .4.7) with Anidaso having a significantly ($p<0.05$) lower fat content than Jenguma. The ash content reduced significantly ($p<0.05$) between the control and the other hours of soaking (Table. 4.7) with 24 hours of soaking retaining the least percentage of ash. This is attributed to leaching and the solubilization of the mineral components which were removed as seeds were decanted and washed after soaking (Perlas & Gibson, 2002)

Protein and fat content of the two varieties increased significantly ($p<0.05$) after soaking in citric acid solution for 0, 12 and 24 hours (Table 4.7). The increase in protein content is consistent with observations made by Wanjekeche *et al.* (2003) who conducted a similar experiment on mature and immature mucuna beans. This observation was contrary to Sharma *et al.* (2013) who reported a decrease in protein and fat content after soaking soya bean in 1% citric acid overnight. Jenguma produced a significantly higher ($p<0.05$) protein content than Anidaso for both 12 and 24 hours of soaking which can be attributed to the differences in the physico-chemical characteristics between them. The increase in protein content might have been due to losses that occurred as CHO, anti - nutrients and minerals leached into solution resulting in a higher ratio of the proteins to the remaining nutrients.

The present study showed a significant ($p<0.05$) reduction in carbohydrate content between the control and soya bean soaked for 12 and 24 hours with the least amount of CHO being recorded after 24 hours of soaking for both varieties (Table 4.7). This could be due to hydrolysis of the complex carbohydrates into simpler forms which leached into the solution during soaking.

5.3. Effect of heat, sprouting and acid treatment on the in-vitro protein digestibility of soya bean

Digestion of food ensures the breakdown of complex food substances into forms that can be absorbed and utilized by the body. The bioavailability of these nutrients in plant food is dependent on factors relating to the chemical form of the nutrient present in the food, interactions existing between the nutrients and other organic components as well as methods used in the pre-treatment of the food while processing and/or preparing the food (Gibson *et al.,* 2006). In the case of soya bean, organic components such as trypsin inhibitors bind the protein and inhibit the hydrolysis of the complex proteins into amino acids making them unavailable for absorption and utilization by the body. A reduction or total removal of these inhibitors is known to improve protein digestibility of soya bean. The present study however, sought to improve the digestibility of proteins in soya bean by treating soya beans with heat, sprouting and acid.

5.3.1 Effect of heat on the %IVPD of soya bean

Trypsin inhibitors are heat –labile and are therefore better removed by heat. The urease activity index (UAI) is an indication of an adequately processed soya bean. The UAI for boiling and roasting processes employed in this study as shown in Table 4.8 indicates that the beans were adequately processed and also signifies a reduction in trypsin inhibitor activity (TIA) and a possible improvement of %IVPD. However, the extent to which the TIA was reduced was not studied.

The IVPD in the raw Anidaso was significantly ($p<0.05$) lower than the IVPD in raw Jenguma. This might be due to a possible high content of TIA in Anidaso. Additionally, the smaller seed sizes of the Anidaso variety enhanced the effective absorption of heat resulting in a faster reduction in the TIA resulting in the improved IVPD for the boiling and roasting treatments as shown in Fig: 4.10. In addition, these observations suggest that while the raw form of Anidaso may have higher anti-nutritive factors, they were more easily inactivated by boiling than those in Jenguma. More severe heat treatment such as roasting was able to inactivate the anti-nutrients in both of them, and so there were no significant differences in the in-vitro digestibility between the roasted flour of the two soybean varieties.

5.3.2 Effect of sprouting on %IVPD

By nature, seeds germinate when the necessary conditions for growth and development are met. This natural process often stimulates biochemical processes which changes the nutritional composition of the seed by hydrolyzing complex proteins into available amino acids which the embryo can absorb and utilize.

The in-vitro protein digestibility increased significantly ($p<0.05$) as the number of days of sprouting increased. Similar results were observed by Olu *et al.* (2013) in a study where the maize and cowpea were sprouted and found that the IVPD of maize and cowpea were highly correlated with the sprouting period.

Although raw Anidaso had a low IVPD than raw Jenguma as shown in Fig.4.11, the rate at which the IVPD increased along the sprouting levels was faster than Jenguma. This trend occurred due to the smaller seed size for Anidaso which resulted in a greater seed to volume ratio when the seeds were soaked prior to sprouting. Consequently, the absorption rate was faster than Jenguma and the hydrolysis of the complex nutrients into simpler forms was also faster resulting in the trend shown in Fig. 4.11.

5.3.2 Effect of acid treatment on %IVPD

Soaking soya beans in citric acid significantly increased ($p<0.05$) the IVPD as the period of soaking increased. This could be attributed to a reduction in TIA leading to a subsequent increase in IVPD. In soaking, a concentration gradient is built which causes the movement of constituents such as trypsin inhibitors into solution thereby reducing the trypsin inhibitor and increasing the IVPD (Osman, 2007). The IVPD increased as the soaking time increased for both varieties but the trend in Anidaso was faster than that of Jenguma due to its greater seed to volume ratio leading to a faster rate of absorption of the solution and a subsequent hydrolysis of complex nutrients into simpler forms. Similarly, the trypsin inhibitors were leached into solution faster in Anidaso than in Jenguma.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

Soya bean is a popular legume used to fortify infant food by most of the respondents. Weaning mothers were generally not aware of the presence of anti-nutrients in soya bean although they knew of its high protein content as well as its health benefits. The most common method of processing used by the respondents for soya bean was roasting.

Additionally, most of the respondents (65.7%) purchased their soya bean products from local processors who might not have any idea of anti-nutrients and how they could be processed out of soya bean.

The different processing methods used in the study caused some changes in the nutritional composition of the soya bean varieties. Generally, the processing methods increased the protein content in both Anidaso and Jenguma. However, heating produced a higher protein content in Anidaso than in Jenguma while Jenguma recorded a significantly ($p<0.05$) higher protein content (48.55%) for both soaking in citric acid and sprouting.

The study also showed that heat, sprouting and acid treatment had a significant ($P<0.05$) increase in IVPD following a possible reduction in TIA in soya bean but the extent to which they reduced TIA was not studied. The IVPD in Anidaso was generally higher than Jenguma.

6.2 Recommendations

1. It is recommended that education on anti-nutrients and how they could be processed out should be intensified by all stakeholders for both weaning mothers and processors on the media and households.
2. Further research into the knowledge of processors on anti-nutrients and how processing could help reduce or remove them is imperative.
3. Investigations into the levels of ANFs in soya bean products on the Ghanaian market must be carried out to ensure they are of acceptable levels especially for feeding infants.
4. It is recommended that Jenguma which is the most common variety on the Ghanaian market should be used by caterers of the Ghana School Feeding and weaning mothers since it yielded a higher protein content than Anidaso and therefore can play a key role in eliminating PEM among young children. However, breeders should further research into reducing the size of Jenguma so that IVPD could be improved.
5. Sprouting and citric acid soaking is recommended for processing of soy bean as they yielded high protein contents. Also, sprouting for 4 days and 24 hours citric acid soaking is recommended since they produced a high protein content and increased IVPD.
6. Further research into the extent by which trypsin inhibitor activity is reduced by these methods should be investigated.

REFERENCES

Abumweis, S. S., & Jones, P. J. (2008). Cholesterol-lowering effect of plant sterols. *Current Atherosclerosis Reports*, *10*(6), 467–72.

Adu-Dapaah, H. K., A.-A., Owusu-Akyaw, M. B., & Amoah, S. (2004). *Sustainable Soybean Production in Ghana. Paper presented at a Radio program on Soybean in Ghana 6 pages.*

Adu-Dapaah, H. K., Asafo-Adjei, B., M., Asiedu, E. A., M., O.-A., & Amoah, S. (2004). *Sustainable soybean production in Ghana.* (p. 8).

Akande, K. E., Doma, U. D., Agu, H. O. and Adamu, H. M. (2010). Major Antinutrients Found in Plant Protein Sources: Their Effect on Nutrition. *Pakistan Journal of Nutrition* 9 (8): 827-832.

Akande, K. E., & Fabiyi, E. F. (2010). Effect of processing methods on some antinutritional factors in legume seeds for poultry feeding. *International Journal of Poultry Science*, *9*(10), 996–1001. http://doi.org/10.3923/ijps.2010.996.1001

Alfenas, R. C. G., Bressan, J., & Paiva, A. C. (2010). Effects of protein quality on appetite and energy metabolism in normal weight subjects. *Arquivos Brasileiros de Endocrinologia E Metabologia*,, *54*(1), 45–51.

Amagloh, F. K., Weber, J. L., Brough, L., Hardcare, A., Mutukumira, A. N., & Coad, J. (2012). Complementary Food Blends and Malnutrition Among Infants In Ghana: Review Paper And A Proposed Solution. *Scientific Research and Essays*, *7*(9), 972–988.

Amal, B. K., Aurang, Z., & Nizakat, B. (2007). Influence of germination techniques on phytic acid and polyphenols content of chickpea (Cicer arietinum L.) sprouts. *Food Chemistry.*, *104*, 1074–1079.

Amankwah, E. A., Barimah, J., Nuamah, A. K. M., Oldham, J. H., & Nnaji, C. O. (2009). Formulation of weaning food from fermented maize, rice, soybean and fishmeal. *Pakistan Journal of Nutrition*, *8*(11), 1747–1752.

Amankwah, E. A., Firempong, C. K., Barimah, J., & Ahima, J. K. (2010). Some anthropometric, biochemical and haematogical studies of rats fed on different composite diets prepared from maize, groundnut and soybean. *Asia Pacific Journal of Clinical Nutrition*, *2*(4), 200–207.

Anjum, F. M. (2010). An overview of anti-nutritional factors in cereal grains with special reference to wheat-A review. *Pakistan Journal of Food Sciences*, *20*(1), 54–61.

Annan, N. T., Plahar, W. A., Poll, L., & Jakobsen, M. (2005). Effect of soya bean fortification on Ghanaian fermented maize dough aroma. *International Journal of Food Sciences and Nutrition*, *56*(5), 315–326.

AOAC. (2003). Official methods of analysis of the association of official's analytical chemists, 17th edn. Association of official analytical chemists, Arlington, Virginia.

AOCS Official Methods. (1997). Sampling and analysis of Oilseed by-products', Method BA 9-65 - Urease Activity, Champaign, IL.

Barbalho, S. M., & Flávia, M. V. F.-M. (2011). Soybean: Food or Remedy? In Prof. Hany El-Shemy (Ed.), *Soybean and Nutrition*. InTech.(pp. 412-432)

Bedford, M. R. (2000). Exogenous enzymes in monogastric nutrition - their current value and future benefits. *Animal Feed Science and Technology*, *1-2*(86), 1–13.

Burchi, F. (2012). Whose education affects a child's nutritional status? From parents' to household's education. *Demographic Research* Vol. 27 (23) pp 681-704. http://www.demographic-research.org/volumes/Vol27/23/DOI:10.4054/DemRES.2012.27.23

Caprita, R., and Caprita. (2007). Effect of heat treatment on soybean protein solubility. *Scientific Papers Animal Sciences and Biotechnologies*. 40(1) pp. 398-401

Caprita, R., Caprita, A., Ilia, G., Cretescu, I., & Simulescu, V. O. (2010). Laboratory Procedures for Assessing Quality of Soybean Meal, *II*, 20–23.

Cederroth, C. R., & Nef, S. (2009). Soy, phytoestrogens and metabolism: A review. *Molecular and Cellular Endocrinology*,, *304*(1-2), 30–42.

Chaturvedi, S. K., Debjyoti, S. G., & Rashmi, J. (2011). Biology of Food Legumes. In A. Pratap & J. Kumar (Eds.), *Biology and Breeding of Food Legumes*. CAB International.

Chen, L., Ronald, L. M., Praveen, V., V., Li, L., and Weiqun, W. (2013). Value - added Products from Soybean: Removal of Anti-Nutritional Factors via Bioprocessing, Intech, Available from: http://dx.doi.org/10.5772/52993

Cho, Y. A., Kim, J., Park, K. S., Lim, S. Y., Shin, A., Sung, M. K., & Ro, J. (2010). Effect of dietary soy intake on breast cancer risk according to menopause and hormone receptor status. *European Journal of Clinical Nutrition*, *64*(9), 924– 32.

Cromwell, G. L. (2012). Soybean Meal - An Exceptional Protein Source. Animal and Food Sciences Department - University of Kentucky.

Dhore, K. R. (n.d.) (2011). Value Addition in Sorghum cut- chips using different levels of Soyabean flour, *1*(2031), 1–3.

Dixit, A. K., Antony, J. I. X., Sharma, N. K., & Tiwari, R. K. (2011). 12 . Soybean constituents and their functional benefits, *661*(2), 367–383.

Dzogbefia, V. P., Arthur, P. L., & Zakpaa, H. D. (2007). Value addition to locally produced soybean in Ghana: production of soy sauce using starter culture fermentation. *Journal of Science and Technology (Ghana)*. http://doi.org/10.4314/just.v27i2.33036

Echendu, C. A., Obizoba, I. C., & Anyika, J. U. (2009). Effects of germination on chemical composition of grounbean (Kerstingiella geocarpa harm) seeds. *Pak. J. Nutr*, *8*(12), 1849–1854.

El-Adawy, T. A. (2002). Nutritional composition and antinutritional factors of chickpeas (Cicer arietinum L.) undergoing different cooking methods and germination. *Plant Foods for Human Nutrition*, *57*, 83–97.

Espinosa-Martos, I., & Rupérez, P. (2006). Soybean oligosaccharides. Potencial as new ingredients in functional food. *Nutricion Hospitalaria*, *21*(1), 92–96.

Fabiyi, E. F. (2006). Soya bean Processing, Utilisation and Health Benefits. *Pakistan Journal of Nutrition*, *5*(5), 453–477.

Fan, S. and Wu, G. (2005). Characteristics of plant proteinase inhibitors and their applications in combating phytophagus insects. Botanical Bulletin. Acad. Sin. 46:273-292

FAOSTAT. (2012). FAOSTAT: FAO statistical databases. FAO, UN, Rome.

Felix, J. P., & Mello, D. (2000). . Farm Animal Metabolism and Nutrition. United Kingdom: CABI.

Foley, J., Rosentrater, K. A., Budhi, L., & Poovia, N. (2013). *Processing Approaches to Improve Functionality and Value of Soybean Products* (pp. 1–28).

Ghana Statistical Service. (2012). *2010 Population and Housing Census.* (pp. 1–15).

Ghavidel, R. A., & Prakash, J. (2007). The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. *J. Food Sci. & Nutr.*, *40*, 1292–1299.

Gibson, L., & Benson, G. (2005). *Origin, History, and Uses of soybean (Glycine max) (ONLINE).*

Gibson, R. S., Perlas, L., & Hotz, C. (2006). Improving the bioavailability of nutrients in plant foods at the household level. *The Proceedings of the Nutrition Society*, *65*(2), 160–168. http://doi.org/10.1079/PNS2006489

Gonzalo, G. M., Maria, A. L., & Rosa, L. (2006). Processing soybeans, Department of animal production, UP Madrid.

Grieshop, C. M., Kadzere, C. T., Clapper, G. M., Flickinger, E. A., Bauer, L. L., Frazier, R. L., & Fahey, G. C. (2003). Chemical and nutritional characteristics of United States soybeans and soybean meals. *Journal of Agricultural and Food Chemistry*, *51*(26), 7684–7691.

Habib, H., & Khalid, Majid, F. (2007). Plant protease inhibitors : a defense strategy in plants. *Biotechnology and Molecular Biology*, *2*(3), 68–85.

Hammond, D. E. (2014). *Consumers' Perception of Attributes and Related Benefits of Soy and its Consumption in Ghana*.

Hefnawy, T. H. (2011). Effect of processing methods on nutritional composition and anti-nutritional factors in lentils (Lens culinaris). *Annals of Agricultural Sciences*, *56*(2), 57–61. http://doi.org/10.1016/j.aoas.2011.07.001

Hirayama, F., Lee, A. H., Binns, C. W., Hiramatsu, N., Mori, M., & Nishimura, K. (2010). Dietary intake of isoflavones and polyunsaturated fatty acids associated with lung function, breathlessness and the prevalence of chronic obstructive pulmonary disease: Possible protective effect of traditional Japanese diet (July 2010), pp. *Molecular Nutrition and Food Research*, *54*(7), 909–917.

Huisman, J., & Tolman, G. H. (2001). Anti-nutritional factors in plant proteins of diets for non-ruminants. Recent development in pig nutrition. Netherlands.

IITA. (2009). Soybean overview. Summary .IITA.

Jequier, E. (2002). Pathways to obesity. *International Journal of Obesity and Related Metabolic Disorders*, *26*(6), 12–17.

Kiers, J. L., Van Laeken. A. E., Rombouts, F. M., and Nout, M. J. (2000). In vitro digestibility of bacillus fermented soya bean. *International Jourmal of Food Microbiology* 60 (2-3): 163-169.

Kato-Kataoka, A., Sakai, M., Ebina, R., Nonaka, C., Asano, T., & T., M. (2010). Soybean-derived phosphatidylserine improves memory function of the elderly Japanese subjects with memory complaints. *Journal of Clinical Biochemistry and Nutrition*, *47*(3), 246–255.

Khattak, A. B., Zeb, A., Bibi, N., Khalil, S. A., & Khattak, M. S. (2007). Influence of germination techniques on phytic acid and polyphenols content of chickpea (Cicer arietinum L.) sprouts. *Food Chemistry*, *104*(3), 1074–1079.

Kombiok, J. M., & Buah, S. S. J. (2013). Tillage depth effects on nodulation , nitrogen fixation and yield of three soybean varieties in the Northern Savanna zone of Ghana, 1–6.

Laminu, H. H., Modu, S. and Numan, A. I. (2011) Production, In vitro Protein Digestibility, Phytate Content and Acceptability of Weaning Foods Prepared from Pearl Millet (Pennisetum typhoideum) and cowpea (Vigna unguiculata). *International Journal of Nutrition and Metabolism* Vol.3(9), pp.109-113.

Lawson, I. Y. D., Mensah, E. A., & Yeboah, E. N. (2008). Improving the Establishment and Yield of Soybeans through Planting Depth and Land Preparation Methods in Northern Ghana. *West African Journal of Applied Ecology*, *14*.

Malamo, O, Ogunmoyela O. A. B. and Oluwajoba S. O. (2011). Effect of Sprouting on trypsin inhibitor of cowpea (Vigna unguiculata).*Journal of Toxicology and Environmental Health Sciences vol.3(5)pp.*139-141.

Martínez Augustin, O., & Martínez de Victoria, E. (2006). Proteins and peptides in enteral nutrition. *Nutricion Hospitalaria : Organo Oficial de La Sociedad Espanola de Nutricion Parenteral Y Enteral*, *21*(2), 1–14.

Martin, C. A., Visentainer, J. V., Oliveira, A. N., Oliveira C.C., Matsushita, M. and Souza, N.E. (2008). Fatty acid contents in Brazilian soybean oils with emphasis on trans fatty acids. *Journal of the Brazilian Chemical Society*, Vol. 19, No. 1,(2008) pp 117-122. ISSN: 0103-5053

Martins, R. T., Cascabulho, A. R., Baião, N. C., & Afonso, R. J. C. F. (2003). Effect of different soybean oils on fatty acids composition of broiler carcass. *Arquivo Brasileiro de Medicina Veterinária E Zootecnia*, *55*(1), 92–98.

Mateos-Aparicio, I., Redondo Cuenca, A., Villanueva-Suarez, M. J., & Zapata-Revilla, M. A. (2008). Soybean a promising health source. *Nutricion Hospitalaria*, *23*(4), 305–312.

Mikic, A., Peric, V., Dordevic, V., Srebric, M., & Mihailovic, V. (2009). Anti-nutritional factors in some grain legumes. *Biotechnology in Animal Husbandry*, *25*(5-6), 1181–1188.

MoFA,(2011). Agriculture in Ghana, Facts and Figures (2010), Ministry of Food and Agriculture, Statistics, Research and Information Division (SRID) pp 12

MoFA and CSIR, (2005). Soyabean Production Guide. Food crops development project. Ghana's Ministry of Food and Agriculture. pp 38.

Mondal, M. R. I., Wahab, M. A. A., Ahmed, M. M. U. M. S., & Begum, F. (2002). *Leaflet of BARI soybean 5. ORC. BARI, Joydebpur, Gazipur.*

Monteiro, M. R. P., Moreira, M. A., Costa, N. M. B., Oliveira, M. G., & Pires, C. V. (2003). Avaliação da digestibilidade protéica de genótipos de soja com ausência e presença do inibidor de tripsina Kunitz e lipoxigenases. *Brazilian Journal of Food Technology*, *32*(4), 99–107.

Mubarak, A. E. (2005). Nutritional composition and antinutritional factors of mung bean seeds (Phaseolus aureus) as affected by some home traditional processes. *J. Food Chem.*, *89*, 489–495.

Mueller, I. (2001). Analysis of hydrolysable tannins. *Anim Feed Sci.Technol* 91: 3-20

Mugendi, J. B., Njagi, E. N. M., Kuria E. N., Mwasaru, M. A., Mureithi, J. A. and Apostolides, Z. (2010) Effects of Processing Techniques on the nutritional Composition and Anti-nutrient Content of Mucuna Bean (*Mucuna pruriens* L.). *African Journal of Food Science* Vol.4(4) pp.156-166

Mullen, R. (2003). *Soybean growth and development*. Iowa State University (ISU).

Nahashon, S. N. and Kilonzo-Nthenge, A. K. (2011). Advances in Soybean and Soybean By-Products in Monogastric Nutrition and Health, Soybean and Nutrition, Prof. Hany El-Shemy (Ed.), ISBN: 978-953-307-536-5, InTech, Available from: http://www.intechopen.com/books/soybean-and-nutrition/advances-in-soybean and-soybean-by-products-in-monogastric-nutrition-and-health

Olu, M., Alamu, A. E., & Oluwajoba, S. O. (2013). The effect of Sprouting on the In-Vitro Digestibility of Maize and Cowpea. *Journal of Advanced Laboratory Research in Biology*, *IV*(Ii).

Osman, M. A. (2007). Changes in Nutrient Composition, Trypsin Inhibitor, Phytate, Tannins and Potein Digestibility of Dolichos Lablab Seeds [Lablab purpureus (L) Sweet] Occuring During Germination. *Journal of Food Technology*,, *5*(4), 294–299.

Perlas, L., & Gibson, R. S. (2002). Use of soaking to enhance the bioavailability of iron and zinc from rice – based complementary foods used in the Philippines. *Journal of the Science of Food and Agriculture*, *66*, 614 –619.

Plahar, W. A. (2006). *Overview of the Soybean Industry in Ghana*. *Workshop on Soybean Protein for Human Nutrition and Health* (Vol. 233).

Prodanov, M., Vierra, I., & Vidal-Valverde. (2004). Influence of soaking and cooking on thiamin, riboflavin and niacin contents in legumes. *Food Chemistry*, *84*, 271–277.

Ramakrishna, V., Rani, P. J. and Rao, P.R. (2006). Anti-nutritional factors during germination in Indian bean (Dolichos lablab l.) seeds. *Journal of Dairy and Food Sciences.* 1:6-11.

Reinke, N. N., & Joke, N. (2005). Cultivation of soya and other legumes. *Agrodok-Series*, *10*, 69.

Reynolds, K., Chin, A., Lees, K. A., Nguyen, A., Bujnowski, D., & He, J. (2006). A Meta- Analysis of the Effect of Soybean Protein Supplementation on Serum Lipids. *The American Journal of Cardiology*, *98*(5), 633–640.

Sarkodie -Addo, J., Adu - Dapaah, H. K., Ewusi-Mensah, N., & Asare, E. (2006). Evaluation of Medium Maturing Soybean Lines for their Nitrogen Fixation Potentials. *Journal of Science and Technology*, *26*(2), 34 – 39.

Sharma, S., Goyal, R., & Barwal, S. (2013). Domestic processing effects on physicochemical, nutritional and anti-nutritional attributes in soybean (Glycine max L. Merrill). *International Food Research Journal*, *20*(6), :3203–3209.

Shurtleff, W., & Aoyagi, A. (2010). A Chapter from the Unpublished Manuscript, History of Soybeans and Soyfoods: Australia, New Zealand: 1100 B.C. to the 1980s.

Soetan, K. and Oyewole, O.E (2009). The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. *African Journal of Food Science*. Vol. 3(9).pp 223-232.

Sugano, M. (2006). Soy in Health and Disease Prevention, CRC Press, FL, USA.

Syed, A. S. Aurang, Z. Tariq, M. Nadia, N. Sayed, J. A. Muhammed, S. Md.Abdul, A. and Asim, M. (2011). Effects Of Sprouting Time On Biochemical And Nutritional Qualities Of Mungbean Varieties. *African Journal of Agricultural Research* vol:6(22), pp.5091-5098.

Teng, D., M., Gao, Y., Yang, B., Liu, Z., Tian, J., & Wang., . (2012). Bio-modification of soybean meal with *Bacillus subtilis* or *Aspergillus oryzae*. Biocatalysis and Agricultural Biotechnology 1(1):

The Research Advisors (2006). Sample Size Table. *http://research -advisors.com*. (accessed: 10/12/2014)

USDA. (2011). U.S. soybean inspection,.

Victora, C. G., M., O. de, Hallal, P. C., M., B., & R., S. (2010). "Worldwide timing of growth faltering: revisiting implications for interventions. *" Pediatrics,*.

Wanjekeche, E., Wakasa, V., & Mureithi, J. G. (2003). Effect of Germinatio,Alkaline and Acid Soaking and Boiling on the Nutritional value of Mature and Immature Mucuna (Mucuna Pruriens) Beans. *Tropical and Subtropical Agroecosystems,, 1*, 183–192.

WHO. (2002). "Infant and young child nutrition: Global strategy on infant and young child feeding." World Health Assembly Document A55/15. Geneva: World Health Organization.

William, S. and Akiko, A. (2000) Tofu and Soy milk production: *Book of Tofu*. 2(3). Soy foods centre.pp 23-28.

WISHH. (2006). World Initiative for Soy in human Health. Industrial application of soybean. St. Louis, Missouri, USA. Malnutrition International Development and Public Partnership review conference.

APPENDICES

APPENDIX I: QUESTIONNAIRE

SURVEY OF CONSUMERS' KNOWLEDGE AND PERCEPTION OF ANTI-NUTRITIONAL FACTORS IN SOYA BEAN WITHIN THE GREATER ACCRA REGION

I am a student of the University of Ghana, Legon undertaking a survey on the knowledge and perception of consumers on anti- nutritional factors in soya bean in the Greater Accra region. You are kindly requested to fill this questionnaire as honestly as possible to enable me achieve the objectives of this study. This information provided is strictly for academic purposes and will be treated with maximum confidentiality.

PART ONE (1): DEMOGRAPHIC DATA

(PLEASE TICK WHERE APPLICABLE)

1. Sex : Female ☐ Male ☐
2. Age : ☐ (20- 29) years ☐ (30 -39) years ☐ (40 – 49) years ☐ (50 – 59) years
3. Level of Education : ☐ Primary Education ☐ Junior High School / ☐ Middle School Senior High School Educ☐n ☐ Tertiary Education None

PART TWO (2): GENERAL QUESTIONS ABOUT SOYA BEANS

4. What products of soya bean do you produce?

   ………………………………………………..

5. What quantity of soya bean products do you process in a month?

………………………………………………

6. Who are your target consumers?

☐ Weaning babies ☐ Pregnant mothers ☐ Nursing mothers Others specify…………….

7. What is the major nutrient in soya bean?

☐ Proteins ☐ Carbohydrates ☐ Fats ☐ Minerals and Vitamins ☐ I don't know

8. What are your reasons for using soya bean in your product(s)?

……………………………………………………………………………….

PART THREE (3): KNOWLEDGE OF RESPONDENTS ON ANTI- NUTRIENTS IN SOYA BEAN

9. Have you heard of the term "anti-nutrients" in food?

Yes ☐ No ☐

10. If yes, what types of food contain anti-nutrients?

☐ Meat ☐ Soya bean ☐ Chicken ☐ Groundnuts

11. Can any nutritional problems occur when one consumes these types of food?

Yes ☐ No ☐

12. If yes, what problems do you know of?

…………………………………………………………

13. If no, why?

……………………………………………………………………………………

PART FOUR: PERCEPTION OF HOW ANTI-NUTRIENTS CAN BE PROCESSED OUT OF SOYA BEAN

14. What method of processing do you use in processing soya bean?

☐ Boiling ☐ Roasting ☐ Sprouting

Others specify ……………………………......

15. Which of these methods of processing do you think is best for processing soya bean into flour?

☐ Boiling ☐ Roasting ☐ Sprouting

Others specify …………………………………….

16. Please give reasons for your choice of method of processing.

…………………………………………………….

THANK YOU

APPENDIX II: STATISTICAL OUTPUTS

Analysis of variance for Heat treatment

Variate: Moisture_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.476939	0.476939	435.78	<.001
treatment	2	70.676044	35.338022	32288.55	<.001
Variety.treatment	2	1.424044	0.712022	650.58	<.001
Residual	12	0.013133	0.001094		
Total	17	72.590161			

Tables of means

Variate: Moisture_%

Grand mean 6.8328

Variety	Anidaso	Jeguma
	6.6700	6.9956

treatment	Boiling	Control	Roasting
	5.1517	9.6150	5.7317

Variety	treatment	Boiling	Control	Roasting
Anidaso		4.7900	9.8500	5.3700
Jeguma		5.5133	9.3800	6.0933

Standard errors of differences of means

Table	Variety	treatment	Variety treatment
rep.	9	6	3
d.f.	12	12	12
s.e.d.	0.01560	0.01910	0.02701

Least significant differences of means (5% level)

Table	Variety	treatment	Variety treatment
rep.	9	6	3

d.f.	12	12	12
l.s.d.	0.03398	0.04162	0.05885

Analysis of variance

Variate: Ash_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.158672	0.158672	16.49	0.002
treatment	2	13.233211	6.616606	687.64	<.001
Variety.treatment	2	0.161544	0.080772	8.39	0.005
Residual	12	0.115467	0.009622		
Total	17	13.668894			

Message: the following units have large residuals.

units 13	0.167	s.e.	0.080
units 14	-0.203	s.e.	0.080

Tables of means

Variate: Ash_%

Grand mean 4.299

Variety	Anidaso	Jeguma
	4.393	4.206

treatment	Boiling	Control	Roasting
	3.103	4.725	5.070

Variety	treatment	Boiling	Control	Roasting
Anidaso		3.323	4.717	5.140
Jeguma		2.883	4.733	5.000

Standard errors of differences of means

Table	Variety	treatment	Variety treatment
rep.	9	6	3
d.f.	12	12	12
s.e.d.	0.0462	0.0566	0.0801

Least significant differences of means (5% level)

Table	Variety	treatment	Variety treatment
rep.	9	6	3
d.f.	12	12	12
l.s.d.	0.1008	0.1234	0.1745

Analysis of variance

Variate: Crude_fat_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	630.4801	630.4801	1963.50	<.001
treatment	2	68.1907	34.0953	106.18	<.001
Variety.treatment	2	69.5149	34.7575	108.24	<.001
Residual	12	3.8532	0.3211		
Total	17	772.0389			

Message: the following units have large residuals.

units 17	1.37	s.e.	0.46
units 18	-1.23	s.e.	0.46

Tables of means

Variate: Crude_fat_%

Grand mean 20.48

Variety	Anidaso	Jeguma
	26.40	14.57

treatment	Boiling	Control	Roasting
	18.98	19.24	23.23

Variety	treatment	Boiling	Control	Roasting
Anidaso		25.59	22.48	31.13
Jeguma		12.36	16.00	15.33

Standard errors of differences of means

Table	Variety	treatment	Variety treatment
rep.	9	6	3
d.f.	12	12	12
s.e.d.	0.267	0.327	0.463

Least significant differences of means (5% level)

Table	Variety	treatment	Variety treatment
rep.	9	6	3
d.f.	12	12	12
l.s.d.	0.582	0.713	1.008

Analysis of variance

Variate: Crude_protein_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	46.272	46.272	13.24	0.003
treatment	2	134.843	67.422	19.29	<.001
Variety.treatment	2	1.119	0.560	0.16	0.854
Residual	12	41.949	3.496		
Total	17	224.183			

Message: the following units have large residuals.

units 16 5.15 s.e. 1.53

Tables of means

Variate: Crude_protein_%

Grand mean 39.06

Variety	Anidaso	Jeguma
	40.67	37.46

treatment	Boiling	Control	Roasting
	42.75	36.21	38.23

Variety	treatment	Boiling	Control	Roasting
Anidaso		44.31	37.53	40.16
Jeguma		41.20	34.88	36.30

Standard errors of differences of means

Table	Variety	treatment	Variety treatment

rep.	9	6	3
d.f.	12	12	12
s.e.d.	0.881	1.079	1.527

Least significant differences of means (5% level)

Table	Variety	treatment	Variety treatment
rep.	9	6	3
d.f.	12	12	12
l.s.d.	1.920	2.352	3.326

Analysis of variance

Variate: Carbohydrate_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	999.790	999.790	289.05	<.001
treatment	2	22.679	11.339	3.28	0.073
Variety.treatment	2	70.574	35.287	10.20	0.003
Residual	12	41.507	3.459		
Total	17	1134.549			

Message: the following units have large residuals.

units 16	-5.06	s.e.	1.52
units 18	3.44	s.e.	1.52

Tables of means

Variate: Carbohydrate_%

Grand mean 29.32

Variety	Anidaso	Jeguma
	21.87	36.77

treatment	Boiling	Control	Roasting
	30.01	30.21	27.73

Variety	treatment	Boiling	Control	Roasting
Anidaso		21.98	25.42	18.20
Jeguma		38.04	35.00	37.27

Standard errors of differences of means

Table	Variety	treatment	Variety treatment
rep.	9	6	3
d.f.	12	12	12
s.e.d.	0.877	1.074	1.519

Least significant differences of means (5% level)

Table	Variety	treatment	Variety treatment
rep.	9	6	3
d.f.	12	12	12
l.s.d.	1.910	2.340	3.309

Analysis of variance for sprouting

Variate: Ash_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sprouting_levels	2	8.46654	4.23327	379.48	<.001
Variety	1	0.02420	0.02420	2.17	0.167
Sprouting_levels.Variety					
	2	0.05410	0.02705	2.42	0.130
Residual	12	0.13387	0.01116		
Total	17	8.67871			

Message: the following units have large residuals.

units 8	0.200	s.e.	0.086

Tables of means

Variate: Ash_%

Grand mean 3.758

Sprouting_levels	2 days	4 days	Control
	3.212	3.337	4.725

Variety	Anidaso	Jeguma
	3.721	3.794

Sprouting_levels	Variety	Anidaso	Jeguma
2 days		3.223	3.200
4 days		3.223	3.450
Control		4.717	4.733

Standard errors of differences of means

Table	Sprouting_levels	Variety	Sprouting_levels Variety
rep.	6	9	3
d.f.	12	12	12
s.e.d.	0.0610	0.0498	0.0862

Least significant differences of means (5% level)

Table	Sprouting_levels	Variety	Sprouting_levels Variety
rep.	6	9	3
d.f.	12	12	12
l.s.d.	0.1329	0.1085	0.1879

Analysis of variance for sprouting

Variate: Crude_fat_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sprouting_levels	2	36.4105	18.2053	80.04	<.001
Variety	1	1.1961	1.1961	5.26	0.041
Sprouting_levels.Variety					
	2	89.2638	44.6319	196.23	<.001
Residual	12	2.7293	0.2274		
Total	17	129.5998			

Message: the following units have large residuals.

units 16	0.98	s.e.	0.39
units 18	-1.14	s.e.	0.39

Tables of means

Variate: Crude_fat_%

Grand mean 21.24

Sprouting_levels	2 days	4 days	Control
	22.03	22.45	19.25

Variety	Anidaso	Jeguma
	21.50	20.98

Sprouting_levels	Variety	Anidaso	Jeguma
2 days		21.67	22.38
4 days		20.34	24.57
Control		22.48	16.01

Standard errors of differences of means

Table	Sprouting_levels	Variety	Sprouting_levels Variety
rep.	6	9	3
d.f.	12	12	12
s.e.d.	0.275	0.225	0.389

Least significant differences of means (5% level)

Table	Sprouting_levels	Variety	Sprouting_levels Variety
rep.	6	9	3
d.f.	12	12	12
l.s.d.	0.600	0.490	0.848

Analysis of variance for sprouting

Variate: Crude_protein_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sprouting_levels	2	278.5473	139.2737	611.82	<.001
Variety	1	26.1847	26.1847	115.03	<.001
Sprouting_levels.Variety	2	59.1710	29.5855	129.97	<.001
Residual	12	2.7317	0.2276		
Total	17	366.6347			

Message: the following units have large residuals.

units 10	1.04	s.e.	0.39
units 16	-0.80	s.e.	0.39

Tables of means

Variate: Crude_protein_%

Grand mean 41.40

Sprouting_levels	2 days	4 days	Control
	42.27	45.73	36.21

Variety	Anidaso	Jeguma
	40.19	42.61

Sprouting_levels	Variety	Anidaso	Jeguma
2 days		40.15	44.38
4 days		42.90	48.55
Control		37.53	34.88

Standard errors of differences of means

Table	Sprouting_levels	Variety	Sprouting_levels Variety
rep.	6	9	3
d.f.	12	12	12
s.e.d.	0.275	0.225	0.390

Least significant differences of means (5% level)

Table	Sprouting_levels	Variety	Sprouting_levels Variety
rep.	6	9	3
d.f.	12	12	12
l.s.d.	0.600	0.490	0.849

Analysis of variance for sprouting

Variate: Carbohydrate_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sprouting_levels	2	67.1137	33.5568	73.69	<.001
Variety	1	25.5613	25.5613	56.13	<.001
Sprouting_levels.Variety					
	2	329.1841	164.5920	361.43	<.001
Residual	12	5.4647	0.4554		
Total	17	427.3237			

Message: the following units have large residuals.

units 6 -1.57 s.e. 0.55

Tables of means

Variate: Carbohydrate_%

Grand mean 28.10

Sprouting_levels	2 days	4 days	Control
	28.55	25.54	30.21

Variety	Anidaso	Jeguma
	29.29	26.91

Sprouting_levels	Variety	Anidaso	Jeguma
2 days		31.96	25.14
4 days		30.50	20.59
Control		25.42	35.00

Standard errors of differences of means

Table	Sprouting_levels	Variety	Sprouting_levels Variety
rep.	6	9	3
d.f.	12	12	12
s.e.d.	0.390	0.318	0.551

Least significant differences of means (5% level)\

Table	Sprouting_levels	Variety	Sprouting_levels Variety
rep.	6	9	3
d.f.	12	12	12
l.s.d.	0.849	0.693	1.201

Analysis of variance for acid treatment

Variate: Moisture_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Period_of_soaking	2	104.704744	52.352372	13598.02	<.001
Variety	1	10.857800	10.857800	2820.21	<.001
Period_of_soaking.Variety					
	2	4.160033	2.080017	540.26	<.001
Residual	12	0.046200	0.003850		
Total	17	119.768778			

Message: the following units have large residuals.

units 8	0.123	s.e.	0.051

Tables of means

Variate: Moisture_%

Grand mean 6.221

Period_of_soaking	12 hrs	24 hrs	control
	4.818	4.230	9.615

Variety	Anidaso	Jeguma
	6.998	5.444

Period_of_soaking	Variety	Anidaso	Jeguma
12 hrs		5.510	4.127
24 hrs		5.633	2.827
control		9.850	9.380

Standard errors of differences of means

Table	Period_of_soaking	Variety	Period_of_soaking Variety
rep.	6	9	3
d.f.	12	12	12
s.e.d.	0.0358	0.0292	0.0507

Least significant differences of means (5% level)

Table	Period_of_soaking	Variety	Period_of_soaking Variety
rep.	6	9	3
d.f.	12	12	12
l.s.d.	0.0781	0.0637	0.1104

Analysis of variance for acid treatment

Variate: Ash_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Period_of_soaking	2	7.60001	3.80001	289.59	<.001
Variety	1	0.00109	0.00109	0.08	0.778
Period_of_soaking.Variety	2	0.61201	0.30601	23.32	<.001
Residual	12	0.15747	0.01312		
Total	17	8.37058			

Tables of means

Variate: Ash_%

Grand mean 3.819

Period_of_soaking	12 hrs	24 hrs	control
	3.498	3.233	4.725

Variety	Anidaso	Jeguma
	3.811	3.827

Period_of_soaking	Variety	Anidaso	Jeguma
12 hrs		3.717	3.280
24 hrs		3.000	3.467
control		4.717	4.7

Standard errors of differences of means

Table	Period_of_soaking	Variety	Period_of_soaking Variety
rep.	6	9	3
d.f.	12	12	12
s.e.d.	0.0661	0.0540	0.0935

Least significant differences of means (5% level)

Table	Period_of_soaking	Variety	Period_of_soaking Variety
rep.	6	9	3
d.f.	12	12	12
l.s.d.	0.1441	0.1177	0.2038

Analysis of variance for acid treatment

Variate: Crude_fat_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Period_of_soaking	2	71.78474	35.89237	732.75	<.001
Variety	1	5.58894	5.58894	114.10	<.001
Period_of_soaking.Variety	2	66.23868	33.11934	676.13	<.001
Residual	12	0.58780	0.04898		
Total	17	144.20016			

Message: the following units have large residuals.

units 11 0.497 s.e. 0.181

Tables of means

Variate: Crude_fat_%

Grand mean 20.577

Period_of_soaking	12 hrs	24 hrs	control
	19.088	23.400	19.243

Variety	Anidaso	Jeguma
	21.134	20.020

Period_of_soaking	Variety	Anidaso	Jeguma
12 hrs		18.653	19.523
24 hrs		22.267	24.533
control		22.483	16.003

Standard errors of differences of means

Table	Period_of_soaking	Variety	Period_of_soaking Variety
rep.	6	9	3
d.f.	12	12	12
s.e.d.	0.1278	0.1043	0.1807

Least significant differences of means (5% level)

Table	Period_of_soaking	Variety	Period_of_soaking Variety
rep.	6	9	3
d.f.	12	12	12
l.s.d.	0.2784	0.2273	0.3937

Analysis of variance for acid treatment

Variate: Crude_protein_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Period_of_soaking	2	360.3817	180.1908	1280.47	<.001
Variety	1	41.3444	41.3444	293.80	<.001
Period_of_soaking.Variety	2	101.7285	50.8642	361.45	<.001
Residual	12	1.6887	0.1407		
Total	17	505.1432			

Message: the following units have large residuals.

units 10	1.037	s.e.	0.306
units 11	-0.623	s.e.	0.306

Tables of means

Variate: Crude_protein_%

Grand mean 41.710

Period_of_soaking	12 hrs	24 hrs	control
	41.753	47.168	36.208

Variety	Anidaso	Jeguma
	40.194	43.226

Period_of_soaking	Variety	Anidaso	Jeguma
12 hrs		37.260	46.247
24 hrs		45.790	48.547
control		37.533	34.883

Standard errors of differences of means

Table	Period_of_soaking	Variety	Period_of_soaking Variety
rep.	6	9	3
d.f.	12	12	12
s.e.d.	0.2166	0.1768	0.3063

Least significant differences of means (5% level)

Table	Period_of_soaking	Variety	Period_of_soaking Variety
rep.	6	9	3
d.f.	12	12	12
l.s.d.	0.4719	0.3853	0.6674

Analysis of variance for acid treatment

Variate: Carbohydrate_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Period_of_soaking	2	294.0695	147.0348	1166.79	<.001
Variety	1	0.6460	0.6460	5.13	0.043
Period_of_soaking.Variety					
	2	244.7968	122.3984	971.29	<.001
Residual	12	1.5122	0.1260		
Total	17	541.0246			

Message: the following units have large residuals.

units 10	-0.657	s.e.	0.290
units 12	0.613	s.e.	0.290

Tables of means

Variate: Carbohydrate_%

Grand mean 27.673

Period_of_soaking	12 hrs	24 hrs	control
	30.842	21.968	30.208

Variety	Anidaso	Jeguma
	27.862	27.483

Period_of_soaking	Variety	Anidaso	Jeguma
12 hrs		34.860	26.823
24 hrs		23.310	20.627
control		25.417	35.000

Standard errors of differences of means

Table	Period_of_soaking	Variety	Period_of_soaking Variety
rep.	6	9	3
d.f.	12	12	12
s.e.d.	0.2050	0.1673	0.2898

Least significant differences of means (5% level)

Table	Period_of_soaking	Variety	Period_of_soaking Variety
rep.	6	9	3
d.f.	12	12	12
l.s.d.	0.4466	0.3646	0.6315

Analysis of variance for heat (IVPD)

Variate: IVPD_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	98.561	98.561	84.72	<.001
treatment	2	3111.951	1555.975	1337.44	<.001
Variety.treatment	2	92.176	46.088	39.62	<.001
Residual	12	13.961	1.163		
Total	17	3316.648			

Message: the following units have large residuals.

units 14	-2.11	s.e.	0.88
units 15	2.12	s.e.	0.88

Tables of means

Variate: IVPD_%

Grand mean 70.39

Variety	Anidaso	Jeguma
	68.05	72.73

treatment	Boiling	Control	Roasting
	79.32	51.80	80.06

Variety	treatment	Boiling	Control	Roasting
Anidaso		78.61	51.03	74.52
Jeguma		80.02	52.58	85.60

Standard errors of differences of means

Table	Variety	treatment	Variety treatment
rep.	9	6	3
d.f.	12	12	12
s.e.d.	0.508	0.623	0.881

Least significant differences of means (5% level)

Table	Variety	treatment	Variety treatment
rep.	9	6	3
d.f.	12	12	12
l.s.d.	1.108	1.357	1.919

Analysis of variance for sprouting (IVPD)

Variate: IVPD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	46.06	46.06	4.29	0.084
Sprouting_levels	2	1041.79	520.89	48.57	<.001
Variety.Sprouting_levels					
	2	575.84	287.92	26.85	0.001
Residual	6	64.35	10.73		
Total	11	1728.04			

Tables of means

Variate: IVPD

Grand mean 55.49

Variety	Anidaso	Jeguma
	53.53	57.45

Sprouting_levels	2 days	4 days	Control
	54.59	67.32	44.55

Variety	Sprouting_levels	2 days	4 days	Control
Anidaso		55.77	71.83	32.98
Jeguma		53.41	62.81	56.12

Standard errors of differences of means

Table	Variety	Sprouting_levels	Variety Sprouting_levels
rep.	6	4	2
d.f.	6	6	6
s.e.d.	1.891	2.316	3.275

Least significant differences of means (5% level)

Table	Variety	Sprouting_levels	Variety Sprouting_levels
rep.	6	4	2
d.f.	6	6	6
l.s.d.	4.627	5.666	8.013

Analysis of variance for acid treatment (IVPD)

Variate: IVPD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	86.94	86.94	8.04	0.030
Treatment	2	772.44	386.22	35.71	<.001
Variety.Treatment	2	203.20	101.60	9.39	0.014
Residual	6	64.89	10.81		
Total	11	1127.47			

Tables of means

Variate: IVPD

Grand mean 57.54

Variety	Anidaso	Jeguma
	54.85	60.23

Treatment	12 hrs	24 hrs	control
	57.55	67.36	47.71

Variety	Treatment	12 hrs	24 hrs	control
Anidaso		56.91	68.35	39.27
Jeguma		58.18	66.37	56.15

Standard errors of differences of means

Table	Variety	Treatment	Variety Treatment
rep.	6	4	2
d.f.	6	6	6
s.e.d.	1.899	2.325	3.289

Least significant differences of means (5% level)

Table	Variety	Treatment	Variety Treatment
rep.	6	4	2
d.f.	6	6	6
l.s.d.	4.646	5.690	8.047