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Germination as a processing technique for soybeans in small-scale broiler farming

By

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Declaration

I, Ndonga Charles Kayembe, declare that this thesis, for the degree **Magister Institutionis Agrar** (Animal production management) at the University of Pretoria, has not been submitted by me for a degree at any other University.

.....

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ABSTRACT

The effect of germination on the levels of certain antinutritional factors, nutritional composition and *in vitro* protein digestibility (IVPD) of soybean seeds were determined. Raw soybeans contain antinutritional factors, such as trypsin inhibitors (83.05 mg/g), total polyphenols (10.83%) and condensed tannins (1.24%). These factors limit the use of soybeans as a source of protein, carbohydrates and minerals in the poultry industry. In general, soybeans are subjected to heat treatment to reduce the amount of antinutritional factors within the seeds, but it is costly and needs high technology equipment. In order to assist small-scale chicken farmers or people living in developing countries, traditional or domestic processing methods are implemented to address the problem of antinutritional factors (ANFs) in soybeans. This study focused on germination as a versatile and low cost practice. Different lengths of germination time were assessed and compared to each another. Soybeans were soaked for 24 hours and allowed to germinate from one day up to six days. Changes within seeds were noted for nutritional and antinutritional factors during germination. Condensed tannins and trypsin inhibitors decreased significantly ($P < 0.05$) while total polyphenols increased from 5 to 6 days. Protein and fat content increased significantly ($P < 0.05$), but starch content decreased with germination. There were no significant changes for IVPD during germination of soybeans. Germination was also compared to other traditional methods such as roasting, soaking and dehulling. Results showed that dehulled soybeans had the highest proximate composition which was significantly different from other treatments ($P < 0.05$), but the total polyphenols increased. It was concluded that germination for a period of three days effectively improved the nutritional value of soybeans and can be considered as an alternative treatment of soybeans in situations where heat treatment is impossible or impractical.



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INTRODUCTION

Hunger and malnutrition continue to be serious problems in the world, even in countries that report a surplus food grain production (Dolberg, 2003). The United Nations' Food and Agriculture Organisation (FAO) provides a startling depiction of the number of people in the world who are under-nourished. Poultry production is the most widely used animal production system in the world, and it can be an excellent tool for combating food insecurity (Mack *et al.*, 2005). Recently, there has been tremendous growth in poultry and egg production in North America, Europe, South America and Asia. However, the poultry production systems of Africa are based mainly on scavenging indigenous chickens which are reared in small villages and the majority of poultry products that are sold at markets come from indigenous smallholder flocks (Sonaiya, 1990; FAO, 1998). These rural poultry systems rely on a minimal input of resources. Although indigenous chickens are poor producers of eggs and meat, they play an important role in providing the rural population with a substantial income and with high quality protein. Increasing the meat and eggs produced by rural poultry has been a major concern of the FAO for many years (FAO, 1998). Many constraints regarding the development of small-scale poultry production need to be addressed. These include disease control, protection against various predators and improvements in nutrition, genetics, marketing, training and management (Mack *et al.*, 2005).

Feed contributes up to 70% of the production costs in a commercial poultry system. Scavenging chickens rely less on purchased feed and supplements and nutrition remains a major constraint in rural systems. Legume seeds are an important source of energy and protein in many parts of the world, both for animal and human nutrition. However, their nutritional value may be limited in part by the presence of undesirable components known as antinutritional factors (ANFs). These factors include protease inhibitors, lectins, phenolic compounds, phytates and indigestible carbohydrates of the raffinose family. The content of these components vary for different legumes, and these differences may be reflected in the efficiency of nutrient utilisation (Donangelo *et al.*, 1995).

The processing of legumes with heat is a quick technique to decrease or eliminate ANFs. The application of this technique must follow strict guidelines and expensive facilities and equipment

are needed, putting this technique out of reach for the average small-scale farmer or lesser developed countries. An optimal processing temperature must be applied, as overheating can cause protein and amino acid damage (Kabeya & Kiatoko, 2004). Under-processing on the other hand does not destroy ANFs activity. This thermal processing method does not modify protein content as caused by processes such as dehulling, soaking and germination treatments of legumes (Kabeya & Kiatoko, 2004).

In spite of their good nutritional qualities, legume consumption is declining worldwide. This is mostly observed in developing countries and probably due to a lack of heat treatment facilities or inadequate heat treatment when applied. According to Iyer *et al.* (1980), the necessity of extensive preparation and cooking time, and the occurrence of gastro-intestinal distress after ingestion are also contributing factors to the abstention of the use of legumes. In addition, the presence of some nutritional problems in legumes, such as the interference with protein and carbohydrate digestibility, as well as the formation of complexes with minerals and protein which depresses the absorption of minerals, was also noted to have a negative impact on legume utilisation (Huisman & Tolman, 2001).

Globally, soybean (*Glycine max*) is probably the largest source of vegetable seed oil and protein as it contains about 40% crude protein and 20% oil. It is also a source of calcium, iron, carotene and ascorbic acid. Soybean meal has become the principal protein supplement for livestock in many countries (Ghaly & Sutherland, 1982). Soybean is one of the legumes mostly used in the poultry industry. However, soybeans contain undesirable components such as lipoxygenase and trypsin inhibitors which limit their utilisation. Trypsin inhibitor is an antinutritional factor that affects protein digestibility (Vineet *et al.*, 2005). A high level of trypsin inhibitors in a diet stimulates pancreatic juice secretion, causes pancreatic hypertrophy and poor growth performance in animals (Liener & Kakade, 1980, cited in Huisman & Tolman, 2001).

Traditional processing methods of soybeans such as germination, soaking, dehulling and roasting are sometimes used to reduce or eliminate the anti-nutritional factors that affect protein utilisation.

The aim of this study was to evaluate the efficacy of germination of soybeans as an alternative processing method in reducing the levels of antinutritional factors. If effective, germination can

be a valuable way to treat soybeans in rural areas where heating facilities are scarce and expensive. This method could be also profitable to people living in developing countries.

The *in vitro* protein digestibility (IVPD) of raw, roasted and germinated soybeans was also assessed using different proteases (pepsin, trypsin and papain), and the relationship between trypsin inhibitor activity and IVPD could therefore be determined.

CHAPTER 1: LITERATURE REVIEW

1.1. Origin and importance of soybeans in Africa

Nigeria is the largest producer of soybeans for food in West and Central Africa. The Democratic Republic of Congo (Ex. Zaire), Cameroon and Ghana also produce and consume soybeans (Root *et al.*, 1987). The commercial production of soybeans in Africa is significant. The average area cultivated with soybeans in Africa between 1982 and 1984 was 394,340 ha. Egypt and Zimbabwe led the way producing about 160,000 tons and 90,000 tons of soybeans respectively in the years 1982 to 1984. During this time, Nigeria produced about 67,000 tons, South Africa about 27,000 tons and Zaire about 15,000 tons yearly (Kolavalli *et al.*, 1987).

Like Nigeria, the Democratic Republic of Congo has a history of soybean production by indigenous farmers. Soybeans were introduced and promoted first by missionaries before the nation's independence. They are now considered as medicinal food to prevent and cure the wasting effects of kwashiorkor (Root *et al.*, 1987). Today, soybean is becoming the most important crop in the world since it is the most efficient and least costly source of protein as well as being an important source of oil (Botsford, 1980; Karl, 1987). For this reason, it is extensively used as a protein concentrate in feeds fed to livestock and poultry throughout the world (Wright & Staley, 1981). As poultry producers desire diets of high nutrient density, soybean meal is of superior value because no other plant protein feedstuff exceeds soybean meal in crude protein content. Soybean meal matches or exceeds all other common plant proteins in both total and digestible amino acid content. Soybean meal is almost an ideal protein supplement for all types of poultry as it contains most of the essential amino acids. However, like other legumes, it has marginal amounts of the sulphur containing amino acids, methionine and cystine, but high levels of lysine (Karl, 1987). During the early years of soy oil production, the meal was generally regarded as a by-product and its use was limited to cattle feed or occasionally as a fertilizer. The use of the protein for poultry, swine and other commercial animal feeds was not developed until the late 1930's (Smith & Circle, 1978).

Practically, all the soybeans produced in the Southern Africa region are used in edible oil, fats and meal for animal feed. However, soybeans are also used as an ingredient in baby foods, as a supplement in flour in some countries (Naik *et al.*, 1987) and as milk-replacement. Furthermore, soybean is not only an important source of food, but it also has numerous other uses in industry,

playing a part in the manufacturing of a large range of products, from explosives and insecticides to paint and plastics (Botsford, 1980).

In the Democratic Republic of Congo, raw soybeans are often used in poultry feeds (Kabeya & Kiatoko, 2004). This practice is probably used by a lack of effective heat treatment industries. Consequently, farmers are victims of poor quality control in the poultry production systems.

1.2. Antinutritional factors

The presence of protein and starch in adequate proportions in legumes, as well as fibre, vitamins and micro-elements, contributes towards the high nutritional value attached to legumes or soybeans. However, in these rich sources of vegetable protein, there are a series of compounds, generally known as antinutrients. These antinutrients impede the digestion of some of the components of legumes, among them protein. In some cases, antinutrients are simply toxic or cause undesirable physiological effects such as flatulence. On the other hand, it has recently been discovered that some antinutrients may have beneficial effects when ingested in small quantities and can even aid in the prevention of certain illnesses such as cancer and coronary disease. As a result, they are now called non-nutritional compounds, since they have no direct nutritional value, but are not always harmful. From a biochemical point of view, these non-nutritional compounds are very different. They do not appear equally in all pulses and vary in their physiological effects (Muzquiz, 2004). Due to the presence of antinutritional factors in soybeans, their utilisation has been limited. Antinutrients have been defined as substances which by themselves, or through their metabolic products arising in living systems, interfere with food utilisation and affect the health and production of animals (Makkar, 1993). The inclusion of raw soybeans in diets considerably inhibits the growth of young animals (Grant, 1989).

Antinutritional factors can be classified on the basis of their effects on the nutritional value of feedstuffs, and on the biological response to them in the animal. Huisman & Tolman (2001) divided the antinutritional factors into the following groups:

- Factors with a depressive effect on protein digestion and on the utilisation of protein, such as protease inhibitors, tannins, saponins and lectins;
- Factors with a negative effect on the digestion of carbohydrates, such as amylase inhibitors, phenolic compound and flatulence factors;

- Factors that affect mineral utilisation, which include phytates, gossypol pigments, oxalates and glucosinolates;
- Factors that inactivate vitamins or cause an increase in the animal's vitamin requirements;
- Factors that stimulate the immune system and may cause a damaging hypersensitivity reaction, such as antigenic proteins;
- Miscellaneous substances, such as mycotoxins, mimosine, cyanogens, nitrates, alkaloids, photosensitising agents, phyto-oestrogens and saponins.

The table below shows the distribution of antinutritional factors and their physiological effects in some legumes:

Table 1.1 Distribution and physiological effects of antinutritional factors found in vegetal protein meals (Adapted from Huisman & Tolman, 1992; 1994 cited in Thorpe & Beal, 2001)

Antinutritional factors	Distribution	Physiological effect
Protease inhibitors Lectins	Most legumes	-Reduction of trypsin and chymotrypsin activity -Impaired growth -Pancreatic hypertrophy Pancreas carcinogen
Lectins	Most legumes	-Gut wall damage -Immune response -Increased endogenous nitrogen loss
Antigenic proteins	Soybeans and kidney beans	-Immune response -Interference with gut wall integrity
Tannins	Most legumes	-Interference with protein and carbohydrate digestibility by formation of protein– carbohydrate complexes
Saponins	Soybeans	-Haemolysis & effects on intestinal permeability

Liener (1980) found antinutritional factors in soybeans as follow and classify them according to their reaction toward the application:

Table 1.2 Antinutritional factors in soybeans (Liener, 1980)

Heat-labile	Heat-stable
Trypsin inhibitors	Saponins
Heamagglutinins	Estrogens
Goitrogens	Flatulence factors
Anti-vitamins	Lysinoalanine
Phytate	

Many studies have been conducted on the effects of antinutrients on digestibility, absorption and the utilisation of nutrients. Huisman & Tolman (2001) distinguished two major classes of factors which are responsible for depressing digestibility: a lack of appropriate enzymes in the gastrointestinal tract, and the presence of antinutritional factors (ANFs) which hamper digestion, absorption or utilisation of nutrients. A number of alternative feedstuffs, particularly those regarded as good protein and energy sources often contain varying amounts of ANFs which can affect the energy value of the feed (Longland & Low, 2001).

New directions in research on ANFs over the last decade have led to major developments in our understanding of its role in nutrition. In this research, instead of carrying out simple mechanistic studies which measure the negative effects of various ANFs on the nutritional performance of laboratory and farm animals, as done in the past, the mode of action of ANFs in the gastrointestinal tract, and particularly their effects on the metabolism and the gut microflora, has been investigated. There is an increasing scientific interest in the possible useful and beneficial applications of ANFs as gut, metabolic and hormonal regulators and as probiotic/prebiotic agents. However, there is still much to learn about the role of ANFs in these applications (Pusztai *et al.*, 2004).

Antinutritional factors in velvet bean seeds (*Stizolobium deringianum*) have been studied. Chemical analysis of the seeds showed that their total phenol content was 3.45%, as measured by spectrophotometry, while condensable tannins were about 0.03% and cyanogenic glucosides were 0.42 mg of HCN/100 g dry matter. A study with Wistar rats from the same litter and of about the same weight was also conducted. Each rat was given a dose of 4.2 and 5.4 mL of

glucosides mixed in water. The feeding experiment lasted for 10 and 21 days. Each day the rats were weighed and food consumption was recorded. At the end of the experiment, rat liver damage was analysed. The results of the chemical analysis and the rat study showed that the presence of ANFs in velvet bean seeds does not present a problem for human and animal consumption, provided that beans are processed before consumption. In conclusion, Barrientos *et al.* (2004) suggested that this wild legume species can be proposed as alternative non-traditional food source.

A trial conducted by Diaz *et al.* (2004) showed the variability in ANF concentrations among legume species and varieties. Soybeans contained the highest trypsin (15.86 g/100 g for Incasoy 27 and 13.29 g/100 g in Duocrop) and chymotrypsin inhibitor levels (6.40 g/100 g in Duocrop), followed by dolichos.

1.2.1. Protease Inhibitors

Trypsin inhibitors are probably the best known, and certainly the most studied, of all the antinutritional factors known to be present in soybeans. It was observed that the thermal inactivation of these inhibitors is accompanied by a marked enhancement in the nutritive quality of the protein. Liener (1980) concluded that trypsin inhibitors are the main cause of the poor growth of animals which are fed with inadequately heated soybeans. However, there are several lines of evidence which indicate that trypsin inhibitors are only partially responsible for the poor nutritive value of raw soybeans. For example, when rats were fed heated soybeans with added isolated inhibitors to provide the same level of antitryptic activity which is present in raw soybeans, the reduction in the growth of these rats were less than the reduction in growth noted when fed raw beans (Liener, 1980).

Armour *et al.* cited by Diaz *et al.* (2004), reported that soybeans had higher levels of trypsin inhibitors than dolichos, mucuna and jackbean. More than 6% of soybean protein is comprised of two powerful protease inhibitors namely Kunitz and Bowman Birk inhibitor. The latter family occurs widely in legume seeds, while Kunitz trypsin inhibitor is found mainly in soybeans. In most instances, the inclusion of protease inhibitors in the diet of an animal reduces its growth rate and feed-utilisation. Trypsin inhibitors are employed as tools in studies of the diet-induced stimulation of pancreatic enzyme secretion. When ingested trypsin/chymotrypsin inhibitors reach the duodenum, they neutralise the proteases present. Therefore, without a further influx of

proteases from the pancreas, food digestion would stop. The reduction in the duodenal protease level is a signal for the release of cholecystokinin from the duodenal epithelial endocrine cells, which, after reaching the exocrine pancreas, stimulates the secretion of more serine proteases into the duodenum. Thus, in a negative feedback loop, protease levels in the small bowel are regulated by the protease inhibitors consumed in the diet (Pusztai *et al.*, 1997b cited in Diaz *et al.*, 2004). According to Karl (1987), trypsin inhibitors irreversibly bind trypsin, making the enzyme incapable of performing its role in the breakdown of proteins. This causes the intestine to release cholecystokinin to stimulate the pancreas to enlarge. The amino acids present in trypsin cannot be reabsorbed and thus are lost when the trypsin combines with the trypsin inhibitor.

These inhibitors were noted to decrease growth and to cause pancreatic hypertrophy, hyperplasia, and adenomatous lesions in the pancreas of a monogastric animal fed with raw soybean seed. Not all animals exhibited an enlarged pancreas after a diet of raw soybeans. How strongly an animal was affected was directly related to the animal's age and time of exposure to the diet (Diaz *et al.*, 2004). Adult animals do not lose weight when fed raw soybean diets because they have a lower amino acid requirement (Rackis & Gumbmann, 1981). Young animals fed raw soybeans exhibit growth inhibition and an enlarged pancreas. This is caused by the trypsin inhibitor. Growth inhibition in young animals is caused by excessive losses of protein, excreted in the faecal matter. It has been suggested that the loss of the amino acids contained in trypsin is responsible for inhibiting growth (Liener, 1981).

Trypsin contains a large amount (15 to 22%) of the sulphur containing amino acids, methionine and cystine (Hwang *et al.*, 1978). Soybeans are a poor source of these amino acids. Therefore, when raw soybeans are used as feed, the small quantity of sulphur-containing amino acids ingested does not offset the large losses caused by trypsin inhibitor. Thus, trypsin inhibitor decreases the protein quality of soybeans more than it decreases the protein quality of foods with large quantities of sulphur-containing amino acids (Karl, 1987).

1.2.2. Polyphenols

Polyphenols are one of the most numerous and widely distributed groups of substances in the plant kingdom (Pusztai *et al.*, 2004). Tannins (tannic acid) are water-soluble polyphenols, which are present in many plant foods. They are considered nutritionally undesirable because they form

complexes with proteins, starch and digestive enzymes and reduce the nutritional value of foods. However, it is now believed that the most important dietary effect of condensed tannins in the digestive tract is their formation of less digestible enzymes (Pusztai *et al.*, 2004).

1.2.3. Phytate

Cereal grains, oilseed meal and grain by-products are major ingredients in commercial pig and poultry diets. Approximately 60 to 80% of phosphorus in these ingredients is in the form of phytates (Pusztai *et al.*, 2004). Phytates are generally located in different parts of seeds. In legumes, phytate accumulates in the cotyledon, but in soybean it is located in protein bodies. Phytate is among the antinutritional factors which have a negative effect on the utilisation of minerals. The phosphate groups are capable of forming complexes, resulting in a reduced availability of Ca, Mg, Zn, Cu and Fe (Reddy *et al.*, 1985 cited in Pusztai *et al.*, 2004). Phytic acid has been shown to interfere with the basis residues of proteins. Liener (1989) suggested that this may be a reason why phytates inhibit a number of digestive enzymes such as pepsin, pancreatin and α -amylase. A suboptimal mineral status increases morbidity, poor pregnancy outcomes and growth impairment. It also decreases the immune competence and cognitive function (Pusztai *et al.*, 2004).

1.2.4. Lectins

It has been recognised for many years that soybeans and most other legumes contain lectins or hemagglutinins (Liener, 1989). Lectins are proteins which are mostly present in the form of glycoproteins (Jaffé, 1980). They are characterized by their unique capability to bind sugar components. The affinity to sugar components may differ among the various lectins. The primary effect of lectins is related to the fact that they bind with the mucosa of the intestinal wall. This binding can result in damage to the intestinal epithelial cells, which can result in a decreased absorption of nutrients; a change in the activity of brush border enzymes and the hypersecretion of endogenous protein; an increased production of mucins and a loss of plasma proteins to the intestinal lumen (Jaffé, 1980; Pusztai, 1989). These effects may cause a decreased nutrient digestibility, decreased nitrogen retention and sometimes scouring. They also lead to reduced weight gain and a less efficient feed conversion. There is a broad variation in toxicity among the lectins of the various seeds. Jaffé (1980) reported that soybean lectins are less toxic than those of kidney beans. Liener (1989) stated that it appears that the soybean hemagglutinin

does not play any role as a determinant of the nutritional quality of soybeans. It is important to study the toxicity of each type of lectin or hemagglutinin separately.

1.2.5. Saponins

Legumes are a major source of saponins (Pusztai, 2004). Saponins are glycosides, which are present in many plants. They are characterized by a bitter taste, foaming properties in aqueous solution and have the ability to haemolyse red blood cells. They are poorly absorbed from the gut (Pusztai, 2004). Various reports (reviewed by Birk & Peri, 1980) showed that poultry are much more sensitive to saponins than other monogastric animals and ruminants. Levels of 20% lucerne meal in chick diets (equal to about 0.3% saponins) resulted in a growth depression which was attributed entirely to the saponin content. When the same levels of lucerne were incorporated into pig diets no negative effects were found (Birk & Peri, 1980). The effects of saponin in soybeans are negligible. Generally, saponins can be considered as a less important antinutritional factor because their levels are low in most common feed ingredients for monogastric animals (Huisman & Tolman, 2001).

1.2.6. Goitrogens

Raw soybeans have been reported to cause a marked enlargement in the thyroid glands of rats and chicks (Liener, 1979).

1.2.7. Estrogens

Liener (1980) stated that genistein, daidzein and coumestrol are present in soybeans. These substances inhibit growth and interfere with reproductive performance.

1.3. Treatment of soybeans

Heat treatment has been employed for some considerable time to denature the heat labile antinutritive factors present in raw soybeans. Different technological processes have been developed, but all are essentially based upon heating for a certain amount of time, with some using additional moisture, added occasionally as steam (ASA, 1996).

Raw and treated cowpea, pea and kidney bean seeds have been investigated for their content of ANFs, including tannins, phytic acid, trypsin inhibitors and oligosaccharides. Treatments applied included water soaking, boiling, roasting, microwave cooking, autoclaving, fermentation and

micronization. All treatments conducted caused significant decreases in tannins, phytic acid, trypsin inhibitor activity (TIA) and oligosaccharides as compared to raw seeds. Boiling caused the highest reduction in tannins, followed by autoclaving and microwave cooking. Heat treatments (boiling, roasting, microwave cooking and autoclaving) brought about a total removal of the trypsin inhibitors in all of the samples (Khatab & Arntfield, 2009).

Heat treatment is the most common method used to reduce any antinutritional factor present in raw soybeans and other pulses. Nevertheless, if a protein source overheats, its amino acid availability, in particular that of lysine, drops. On the other hand, inadequate treatment diminishes the oxidising stability of the fat contained within the soybeans (Gonzalo *et al.*, 2006). One of the extra advantages of using heat treatments on soybeans is that the process improves the taste of the end product. The heat applied to the beans releases additional aromas and flavours which encourage both piglets and other domesticated mammals to increase feed intake (Gonzalo *et al.*, 2006).

Attempts to increase the utilisation of legumes have employed a wide range of processing techniques, such as germination, dehulling, cooking, roasting, autoclaving, fermentation and recently extrusion cooking (Alonso *et al.*, 1998). Dehulling, soaking and germination were found to increase the biological value of legumes. According to Hammond *et al.* (1951) as cited in Howell (1963), the total nitrogen in the soybean plant nearly triples during seed development. The seed accumulation of nitrogen far exceeds that which is lost from the stem and the leaves.

A brief description of the major methods which are used today to process soybeans is provided below:

1.3.1. Cooking

Cooking is a relatively simple process to use. For this process, raw beans are cleaned and poured into a drum of boiling water. The beans are allowed to cook for 15 to 30 minutes, after which they are dried mechanically, or alternatively spread out on the ground (Kaankuka *et al.*, 1996). These beans are eaten whole, ground, rolled or crushed (ASA, 1996). Cooking is the more traditional method used by local soya producers (Gonzalo *et al.*, 2006). This method might be ineffective in terms of capacity and/or production outputs, but it seems effective in reducing tannins.

1.3.2. Autoclaving

Autoclaving involves the cooking of soybeans in hot (121°C) and pressurised steam (15 atmospheres) for approximately 10 minutes in distilled water (Mohamed *et al.*, 2011). This process is a variation of the cooking method. It is the process which has been studied the most by research centres (ASA, 1996).

1.3.3. Hydrothermal reactor

The hydrothermal reactor method essentially involves treating the soybeans in a container subject to a certain level of pressure and other conditions that vary depending on the size and capacity of the machine. The system comprises of elements such as the conditioning unit, the cooking reactor, the expansion system and the drier/cooler (ASA, 1996).

1.3.4. Roasting

Several models for roasting of soybeans exist, including conventional dry systems, similar to those used to dry out cereals and moist heat systems. The heat used for the latter can be generated by an oven, a coal burner or directly by a flame. The temperature reached varies between 110 and 170°C depending on the equipment used (ASA, 1996). The most commonly used roasting systems are the rotating drum, fluidized bed models, cascade roasting, jet-sploding, micronizing and microwave treatments. The simplest roasting method is carried out in a common drier. Other straightforward but less common methods use heating on solid carriers such as sand, salt and ceramic tiles (ASA, 1996). The key differences between roasting methods are the manner in which heat is applied (dry or moist) and the use of subsequent lamination or expansion.

1.3.5. Extrusion

Extrusion involves preparing and treating the given product using pressure and hot steam. Alonso *et al.* (1998) found that extrusion is the best method to abolish trypsin, chymotrypsin and α -amylase inhibitors, as well as haemagglutinating activity, without modifying protein content. Furthermore, this thermal treatment was found to be more effective in improving protein and starch digestibility than dehulling, soaking and germination.

1.3.6. Other methods

Other systems that have been studied, but are rarely used in practice to process soybeans, are gamma-radiation and radio frequencies. Alternative methods that do not rely on the use of heat, but are successful in improving the nutritional value of soybeans also exist. Of these, the most noteworthy methods are germination, soaking, dehulling, shelling, enzyme addition, reducing the level of oligosaccharides, and reducing the content of kunitz and other essential antinutritional factors through genetic enhancement (Gonzalo, 2006). Under-processing, as well as over-processing of soybeans can damage the availability of the protein fraction. Under-processing may not destroy all of the antinutritional factors, which may lead to a reduction in the utilisation of the amino acids. In the case of over processing, reactions are produced between the amino groups of some of the amino acids and free sugars contained inside the beans (Maillard reactions), leading to a reduction in the digestibility of the protein fraction (Gonzalo, 2006).

1.3.6.1. Germination

Germination is a natural biological process of all superior plants. In this process, the seed comes out of its latency stage. During germination, some quantitative and qualitative changes occur within the seed. These changes depend on the type of vegetable, the seed variety and the conditions of germination. Different researchers have developed the process of germination as an alternative tool to defeat undesirable tastes and smells, as well as the presence of antinutrients in legumes. Reihaneh & Jamuna (2007) reported that germination caused significant increases in the protein and thiamine levels; *in vitro* iron and calcium bioavailability; and *in vitro* starch and protein digestibility of all the legume samples. Further increases in the mentioned parameters were observed after dehulling of the germinated legumes. There was a negative correlation between the bioavailability and digestibility of nutrients and the presence of ANFs. Chen & Thacker (1978) showed that after 5 days of germination, there was a slight increase in total nitrogen, a slight decrease in protein nitrogen, and a marked increase in both total non-protein nitrogen and free-amino acid nitrogen, when compared on a weight basis with ungerminated grains. Germinated grains are good sources of ascorbic acid, riboflavin, choline, thiamine, tocopherols and pantothenic acid (Sangronis & Machado, 2007).

Sangronis & Machado (2007) evaluated the changes in trypsin inhibitor activity, phytic acid, tannins, ascorbic acid, thiamine, protein digestibility, and minerals in germinated black beans

(*Phaseolus vulgaris L.*), white beans (*Phaseolus vulgaris L.*) and pigeon beans (*Cajanus cajaan L.Millsp.*). The ungerminated legumes were analysed as a control. A significant decrease in the TIA of pigeon beans (19.2%), white beans (52.5%) and black beans (25%) was observed. The reduction in phytic acid was more than 40% in all three types of the germinated beans. Tannins were reduced by 14.3% in pigeon beans. Germination increased protein digestibility by between 2% and 4%, and increased ascorbic acid by 300% in white beans, by 33% in black beans and by 208% in pigeon beans. The thiamine content increased with 12.8%, 26.5% and 7.4% respectively in white, black and pigeon beans. Germination affected mineral content erratically, depending more on the grain and the type of mineral. These variations in the content of nutrients and antinutrients in the germinated grains were attributed to the joint effect of the germination and previous soaking which the grains were subjected to. Germination has also been reported to increase the availability of minerals such as copper, sodium, potassium, iron, phosphorus, calcium, manganese and magnesium (Youssef *et al.*, 1987).

The effect of germination on the nutritive value of seed has been reported by several researchers (Khan & Ghafoor, 1978; Kaushik *et al.*, 2010). During sprouting of dry beans, the storage materials are converted into other forms that are more usable to both plants and humans (Kaushik *et al.*, 2010; Peñas *et al.*, 2010). Germination may improve the nutritive value of legumes by inducing the formation of enzymes, including α -galactosidase which eliminates or reduces antinutritional factors. During soybean seed germination, the α -galactosidase was partially purified from germinating seeds by partition in an aqueous two-phase system and ion-exchange chromatography (Guinaraes *et al.*, 2001; Viana *et al.* 2005). Germination was shown to be a good process to increase the phenolic content of lupin seeds as well as their antioxidant activity (Dueñas *et al.*, 2009). Wang *et al.* (1996) reported that 24 hours of germination led to a considerable reduction in the oligosaccharides in legumes, 17 – 70% in raffinose, 35 – 75% in stachyose and 66 – 91% in verbascose. It was found that raffinose in soybeans disappeared completely after 96 hours of germination (East *et al.*, 1972).

The effects of some processing methods, such as soaking, cooking, roasting, autoclaving and germination, on the nutrient composition, antinutritional factors and *in vitro* digestibility in *Dolichos lablad* seeds have been investigated (Osman, 2007). Germination was found to significantly increase the protein and moisture content in the seeds, while roasting and

autoclaving decreased their contents. Crude lipid content was significantly reduced by various processing methods. Ash content varied significantly between raw and processed samples. Trypsin inhibitor activity and phytic acid content were significantly decreased by different processing methods. The cooking of pre-soaked seed appeared to be the most effective method for reducing trypsin inhibitor activity. The reduction in phytic acid content was found to be greater in roasted samples than others (Osman, 2007). Osman (2007) found that germination significantly increased tannins compared to the other traditional methods, and that it was the most effective method for improving protein digestibility when compared to soaking and cooking. In a similar experiment with mung bean seeds, Mubarak (2005) found that germination and cooking processes caused significant decreases in fat, carbohydrates fractions, antinutritional factors and total ash contents. All processes also decreased the concentration of lysine, tryptophan, threonine and sulphur containing amino acids. In spite of this, all treated seeds had higher total concentrations of the branched-chain amino acids, leucine, isoleucine and valine than those prescribed by the FAO/World Health Organization (WHO). Dehulling, soaking and germination processes were found to be less effective than cooking processes in reducing trypsin inhibitor, tannins and hemagglutinin activity contents. Germination was the most effective method for reducing phytic acid, stachyose and raffinose. Germination also resulted in a greater retention of all minerals compared to other processes. *In vitro* protein digestibility and protein efficiency ratio were improved by all processes. In addition, the decrease in total carbohydrates and reducing sugar contents were attributed to their role as a source of energy during the germination process (Marian *et al.*, 1985; Naveeda & Jamuna, 2005).

Significant differences have been observed in the content of saponin and trypsin inhibitor activity in five varieties of cowpea (*Vigna unguiculata*). The dry seeds were given different treatments, including soaking, cooking and germination, and the changes in the levels of these ANFs were estimated. There was significant variation in the content of these two antinutrients as a result of the different treatments. Soaking for 18 hours resulted in a 34% and 23% reduction in the saponin content and TIA, respectively. The reductions were higher with longer periods of soaking. Losses of antinutrients were greater when soaked versus unsoaked seeds were cooked. Pressure cooking had a more beneficial effect than ordinary cooking. Germination had the most pronounced saponin lowering effect, followed by the pressure cooking of dehulled seeds. The processing method involving pressure cooking completely eliminated TIA, while soaking and

germination partly removed the activity (Khokhar & Chauhan, 1986; Sinha *et al.*, 2005). However, in a study by Oloyo (2004) on pigeon peas (*Cajanus cajan*), it was concluded that the increased contents of tannins, total phenolics and trypsin inhibitory activity in the seed during progressive germination might limit its nutritive quality.

1.3.6.2. Soaking

The effects of dehulling, soaking and germination on changes in the general chemical composition of legumes were found to be significant in different studies which have been conducted (Youssef *et al.*, 1987). The raw legume seeds were found to contain ANFs such as total free phenolics (2.75 g/100g); tannins (2.35 g/100g); phytic acid (692 mg/100g) and flatulence factors; raffinose (0.54 g/100g); stachyose (1.17 g/100g) and verbascose (0.95 g/100g). Soaking the seeds in distilled water caused maximum reduction in the phytic acid content, whereas soaking in NaHCO₃ solution reduced phenolics and tannins by significant levels (72% and 78%, respectively) (Vijayakumari *et al.*, 2007). Soaking faba beans for 9 hours produced losses of between 0 and 15% thiamine and 0 and 11% riboflavin, and caused no changes in niacin content (Prodanov *et al.*, 2004).

1.3.6.3. Dehulling

Dehulling significantly increased the phytic acid content of beans (from 1.63 to 3.67%). Dehulling also increased the trypsin, chymotrypsin, and α -amylase inhibitory activities of the beans (Deshpande *et al.*, 1982). The process was also noted to increase the contents of copper, zinc and potassium in beans, while it significantly decreased the amounts of iron, manganese, phosphorus, calcium, magnesium and sodium (Youssef *et al.*, 1987). Whole beans soaked for 12 hours, followed by dehulling, had higher amounts of iron, copper, zinc, calcium and sodium, and a lower level of potassium, than unsoaked dehulled beans (Youssef *et al.*, 1987).

Although the various chemical methods used for the quality control of soybean products offer a useful guide as to their subsequent nutritive value, animals remain the most reliable final judges of product quality (ASA, 1996).

1.4. Economic and practical considerations of germination of soybeans in small scale poultry farming

Attempts to increase the utilisation of legumes have employed a wide range of processing techniques such as germination, cooking and microbial α -galactosidase treatment. Of these techniques, the germination process has been studied the most. Many researchers have reported that this method enhances the nutritional value of legumes by increasing essential amino acids, protein digestibility, amino acid availability and the bioavailability of vitamins and minerals.

Germination is a natural process that causes important changes in the biochemical, nutritional and sensory characteristics of legumes. It is considered to be a suitable procedure for improving the nutritional value of legume seeds by reducing levels of antinutritional factors (Donangelo *et al.*, 1994). Germination is one of the methods that do not rely on the use of heat for improving the nutritive value and decreasing the level of antinutritional factors in soybeans. It is also an appropriate low cost, low technology option for household processing in rural areas or lesser developed countries.

1.5. Conclusion

Currently, the entire world is facing the distressing situation of financial recession and a high rate of unemployment, which contributes to poverty and increases crime. This recession is strongly pronounced in the mining sector and agriculture could be one of the sustainable tools used to alleviate this situation. Some constraints blocking the development of the poultry sector, such as feed procurement, disease and the need for low cost techniques, were listed in this review. Feed, which accounts for about 70% of the production costs in a commercial poultry system, is the first factor which should be considered before deciding on any further measures.

Soybean is rich in protein, energy, minerals, crude fat and amino acids, but it contains various antinutritional factors which limit its use. These antinutrients are destroyed with heat treatment, but the process is very expensive for small-scale farmers. Researchers have found that traditional methods such as germination, soaking and dehulling can be used by these farmers as alternative techniques. These techniques are useful as they cause reductions in trypsin inhibitor activity, tannins, and polyphenols. They have also been noted to increase the nutrients in soybeans, such as their protein content, amino acids and vitamins. However, animals remain the most reliable final judges of product quality (ASA, 1996). In terms of profitability, traditional techniques are a

low cost, low technology option for smallholders living in rural areas or developing “poor countries”.

CHAPTER 2: MATERIALS AND METHODS

2.1. Source of soybeans

Twenty bags of 50 kg soybeans (*Glycine max*, L.) were procured from a local South African farmer in Brits (North West Province). These seeds were harvested on the 28th of April 2008 and belonged to the variety RR 5409 Monsanto. After collection, a representative sample was taken from each bag and sorted visually. Dust, foreign materials and damaged seeds were removed.

2.2. Processing methods

The study focused on traditional methods for the processing of soybeans, such as soaking, dehulling and germination. Roasted soybeans were regarded as the positive control, while raw soybeans were the negative control in this trial. All treatments were conducted four times and independently from one another in order to justify the repeatability. The trial was conducted during summer time on the Hatfield experimental farm of the University of Pretoria in South Africa.

2.2.1. Soaking

For soaking tests, 500 g of the soybean samples were weighed in quadruplicate into 5000 mL plastic containers, and filled to the mark with tap water. Two different time periods of soaking were observed respectively for 24 and 48 hours at room temperature. The soaked seeds were then spread on hessian sacks for 48 to 72 hours under sunlight until dry. After drying, soybeans were milled with a 1mm sieve and kept in plastic containers at room temperature for further analyses.

2.2.2. Dehulling

Hulls were removed manually by rubbing the grains between the palms after soaking the soybean seeds for 24 hours in tap water (1:10, w/v) (Sangronis, 2007). After dehulling, the hulls were removed by floatation and the cotyledons were dried under sunlight. Dehulled soybeans were milled with a 1mm sieve, and then kept at room temperature for further analyses.

2.2.3. Germination

Soybeans were soaked for 24 hours, spread on hessian sacks on the floor indoors, and covered with aluminum foil to exclude light. Groups of seeds were kept at room temperature (22 - 26°C) for 24 up to 120 hours, respectively. Water was poured once daily to provide moisture during

sprouting. Thereafter, germinated beans were dried for 48 to 72 hours under sunlight, ground with a 1mm sieve, and then kept at room temperature for further analyses.

2.2.4. Roasting

Seeds were roasted in an oven for 30 min at 130°C. After cooling down, soybeans were milled by a 1 mm sieve and stored into plastic bottles at room temperature for further analyses. Roasting served as positive control in this trial.

2.3. Analytical methods

Samples were mixed several times and divided to ensure a homogenous and representative sample for analysis. This sampling procedure was very important and carefully conducted to avoid variations of results.

Determination of moisture content, crude protein, starch, ash, minerals, condensed tannins, total polyphenols, trypsin inhibitor, crude fat and *in vitro* protein digestibility were performed on each sample in duplicate at Nutrilab of the Department of Animal and Wildlife Sciences, University of Pretoria, while amino acids analysis was performed at the Department of Biochemistry, University of Pretoria.

2.3.1. Moisture content

Approximately 2 g of soybeans were weighed into a dry pre-weighed 30 mL porcelain crucible, and dried in an oven at 105°C for 16 hours. Thereafter, samples were weighed again after removing from the oven and allowed to cool in a desiccator (AOAC, 2000).

Dry matter (DM) was determined as follows:

$$\% \text{ DM} = (\text{dry weight} / \text{wet weight mass}) \times 100, \text{ then } \% \text{ Moisture} = (100 - \% \text{ DM})$$

2.3.2. Determination of ash content

Approximately 2 g of soybeans were weighed into a dry pre-weighed 30 mL porcelain crucible, and dried in an oven at 105°C overnight. After determining the dry matter content, the crucible and contents were placed in a cool muffle furnace at 250°C for 1 hour and the temperature was increased to 600°C for 4 hours. Crucibles were transferred into a desiccator and were allowed to cool. Samples were weighed as soon as possible after cooling to prevent moisture absorption (AOAC, 2000). The ash content of the samples was calculated as follows:

$$\% \text{ Ash} = (\text{Ash weight} / \text{Wet weight}) \times 100$$

2.3.3. Determination of trypsin inhibitor activity (TIA)

TIA was measured according to the method of Kakade *et al.* (1974). In this trial the dilutions were modified as illustrated in Table 2.1.

Table 2.1 Summary of the method used for determining trypsin inhibitory activity in the different soybean samples

	Reagent Blank (Aa)	Standard (Ab)	Sample Blank (Ac)	Sample (Ad)
Distilled water. Add	2.0 mL	2.0 mL	1.0 mL	1.0 mL
Standard trypsin solution	-	2.0 mL	-	2.0 mL
Diluted sample	-	-	1.0 mL	1.0 mL
Mix and place in water bath at 37°C for 10 minutes. Add				
BAPNA solution (37°C)	5.0 mL	5.0 mL	5.0 mL	5.0 mL
Mix and after exactly 10 minutes, stop the reaction by adding				
Acetic acid solution	1.0 mL	1.0 mL	1.0 mL	1.0 mL
Mix thoroughly after each addition of acetic acid solution. Add				
Standard trypsin solution	2.0 mL	-	2.0 mL	-
Mix and filter the solutions through whatman No. 542 filter paper				
Read the absorbance of filtrate solutions at 410 nm using water as the blank				

Soybean sample of 1 g pre-milled to a particle size of 1 mm sieve was extracted with 50 mL NaOH solution overnight at 4°C. The pH of the suspension was between 9.4 and 9.6. One mole of NaOH or 1 M HCl was used to adjust the pH where necessary. Samples were diluted in order to bring the inhibition in the range of 40% to 60%.

Modification in dilution factors (D) were made as follows:

1:25 mL dilution for roasted soybeans;

1:15 mL dilution for soybeans that were soaked, germinated for 6 days, dehulled and raw;

1:10 mL dilution for soybeans that were germinated for 1 - 5 days

Calculations:

The change in absorbance (A_1) due to trypsin inhibition per mL diluted sample extract was calculated as $(A_b - A_a) - (A_d - A_c)$.

$$\% \text{ inhibition} = \frac{100 A_1}{(A_b - A_a)} \quad : \text{ range must be } 40 - 60\%$$

$$\text{TIA} = \frac{2.632 \times D \times A_1}{\text{Sample mass}} \quad \text{mg pure trypsin inhibited per g sample}$$

One trypsin unit (TU) is defined as an increase of 0.01 absorbance units at 410 nm per 10 mL of the reaction mixture under the conditions used by Kakade *et al.* (1974).

Trypsin inhibitor activity is expressed in terms of trypsin inhibited units (TIU) per mg of protein, on a dry matter basis.

2.3.4. Determination of tannins

The dried treated and untreated samples were ground to pass through a sieve size of 1 mm diameter. A sample (400 mg) was measured in a conical flask and 40 mL of diethyl ether containing 1% acetic acid (v/v) was mixed to removed pigments and lipid material. After 5 minutes, the supernatant was carefully discarded and 20 mL of 70% aqueous acetone was added. The flask was sealed with a cotton plug covered with aluminum foil and kept in an electrical shaker for 2 hours for extraction. Then it was filtered through Whatman filter paper No 1 and the samples were kept in a refrigerator at 4°C until analysis (Makkar, 1993).

2.3.4.1. Estimation of total phenols and tannins

Total phenols and tannins were estimated according to the standard procedures described by Makkar (1993). An extract of 50 μL of tannin was measured in a test tube for each sample and the volume was adjusted to 1.0 mL with distilled water. Further, 0.5 mL of folin ciocalteu reagent (Sigma St Louis-USA) was added and mixed properly, after which 2.5 mL of 20% sodium carbonate solution was added, mixed and kept for 40 minutes at room temperature. Optical density was measured at 725 nm with a spectrophotometer (SPEKOL 1300) and the

concentration was estimated from the standard curve prepared from tannic acid solution. Tannin was estimated as tannic acid equivalents and expressed on a dry matter basis.

Non-tannin phenol was estimated by precipitating tannins with poly-vinyl-poly-pyrrolidone (PVPP) (Sigma St Louis-USA), which binds tannin. A weight of 200 mg PVPP was measured in a test tube; 2.0 mL of distilled water and 2 mL tannin extract were added. The solution was vortexed and kept in a refrigerator for 15 minutes at 4°C. The mixture was vortexed and filtered through Whatman filter paper No 1, measured into a test tube and distilled water was added to adjust the volume to 1 mL. Non-tannin phenol was estimated by the same method as described for total phenols (Makkar, 1993). Concentration of non-tannin phenol was calculated from a standard curve and expressed on DM basis.

Total tannins were calculated by subtracting non-tannin phenols from total phenols. The standard was prepared from the stock solution of tannic acid (0.5 mg/mL). From this 0, 10, 20, 30, 40 and 50 µL were measured in test tubes and the volumes were adjusted to 1 mL to obtain a tannic acid concentration of 0, 5, 10, 15, 20 and 25 µg, respectively. A solution of 0.5 mL of folin ceocaliteau reagent and 2.5 mL of 20% sodium carbonate were added. The whole content was mixed properly and after 40 minutes of mixing tannins were measured with spectrophotometer at 725 nm.

2.3.4.2. Estimation of condensed tannin (proanthocyanidins)

Condensed tannin was estimated according to Porter *et al.* (1986). 0.5 mL Tannin extract was measured in test tubes in triplicate. 3.0 mL of butanol HCl and 0.1 mL of ferric reagent were added. Tubes were vortexed to insure proper mixing. The mouth of the tube was covered with a glass marble and boiled for 60 minutes. Similarly, a blank for each sample was prepared without heating the reagent. The tube was cooled to room temperature and reading was taken at 550 nm using a spectrophotometer. Condensed tannin was expressed as leucocyanidin equivalent and was calculated as:

$$\% \text{ Condensed tannin} = (A_{550\text{nm}} \times 78.26 \times \text{dilution factor}) / (\% \text{DM})$$

2.3.5. Crude fat determination (Ether Extract)

According to the method of AOAC (2000), 2 g of sample was weighed onto a Whatman No.1 filter paper of 12.5 cm diameter. The filter paper was folded to completely contain the sample.

The samples were placed in Whatman extraction thimbles single thickness with an internal diameter of 30 mm and an external length of 100 mm. The samples were placed far enough down in the thimbles so that they were intermittently completely immersed in petroleum ether.

Flat bottom Soxhlet flasks that have been cleaned and dried overnight at 105°C, were weighed and connected to the extraction unit after filling with petroleum ether to three quarters of its volume. Extraction thimbles were placed in the extraction unit and the cooling water opened. Heat was adjusted to achieve a condensation rate of approximately 2 drops per second. The extraction procedure was continued for 16 hours (usually 8 hours per day). At the end of this period the thimbles were removed and the ether was distilled into a collection tube. The flat bottom flasks were removed from the heating element just before evaporating to dryness. The flat bottom flasks were placed in a cold explosion-proof oven which was then switched on and allowed to dry for 30 minutes from the time the oven have reached 105°C. The flasks were allowed to cool in a desiccator before weighing.

$$\text{Ether extract \%} = \frac{\text{Mass of flask plus residue} - \text{Mass of flask}}{\text{Mass of sample used}} \times 100$$

Values were expressed on a dry matter basis.

2.3.6. Determination of *in vitro* protein digestibility (IVPD)

The determination of *in vitro* protein digestibility was performed using the protease enzymes, pepsin, trypsin and papain. Pepsin (from porcine gastric mucosa, crystal lyophilized 10 FIP-U/mg) and trypsin (from porcine pancreas, crystal lyophilized 40 U/mg) were purchased from Merck (Darmstadt, Germany) while papain (from carica papaya, water-soluble 30000 USP-U/mg) was purchased from Sigma (St Louis-USA).

2.3.6.1 *In vitro* protein digestibility with pepsin

Soybeans (300 g) were weighed into a series of test tubes. A solution of 5 mL of 0.075 N HCl and 0.5mL of pepsin solution (2.0 mg/mL) in 0.075 N HCl were added to each tube. The tubes were incubated at 37°C and enzyme action was stopped after 24 hours by addition of 5 mL of 10% (w/v) trichloroacetic acid (TCA). Digestion was performed in duplicate. The digest was filtered through Whatman No. 2 filter paper, and the residue was washed with warm water on the

filter. Nitrogen in the residue was estimated by the micro-Kjeldahl method. IVPD was obtained by calculating the difference between the amount of total nitrogen in the sample before and after *in vitro* digestion with pepsin. Kjeldahl nitrogen was multiplied by the factor 6.25 to obtain crude protein.

2.3.6.2 *In vitro* protein digestibility with trypsin

IVPD with trypsin was conducted essentially as described for pepsin in paragraph 2.3.6.1., but the incubation was conducted in 0.1 M phosphate buffer at pH 7.6.

2.3.6.3 *In vitro* protein digestibility with papain

IVPD with papain was conducted according to the method used by Buchanan & Byers (1969) and modified by Nanda *et al.* (1977) as follow: The activator solution of 0.5 M thioglycolic acid was prepared by adding 4.6g (3.5 mL) laboratory grade thioglycolic acid to distilled water and making it up to 100 mL after having adjusted the pH to 6.6 with 0.45 M NaOH solution. A phosphate – citrate buffer solution of pH to 6.6 was made by mixing 7.7 parts of 0.2 M Na₂HPO₄ with 2.3 parts of 0.1 M citric. Quantities of substrate representing about 10 mg of protein N were accurately weighed in 75 mL centrifuge tubes. Into each digestion tube, 0.4 mL of papain solution was pipette together with 0.5 mL of 0.5 M thioglycolic acid and 9.1 mL citrate-phosphate buffer. Two drops of toluene were added as preservative, the tubes were stoppered and the contents mixed gently. Care was taken to ensure that all particles of the protein sample remained immersed in the digestion liquid. The samples were incubated in duplicate at 37°C for 24h. The control tubes contained only enzyme, activator and buffer. Immediately after incubation, the unhydrolysed protein fraction was precipitated by addition of 10 mL trichloroacetic acid (TCA) solution (100 g/L) to each tube. Thereafter the procedure was followed as described for pepsin.

In vitro protein digestibility was determined by calculating the difference between the amount of nitrogen in the sample before and after hydrolysis with pepsin, trypsin or papain (AOAC, 1965).

2.3.7. Determination of protein nitrogen

2.3.7.1 Kjeldahl method

The determination of protein nitrogen was done using Kjeldahl digestion technique. This method was applied as follows:

Samples were transferred in the special nitrogen digestion tube. One tea spoon salt (sodium sulphate), one drop of catalyst (selenium) and of 20 mL of sulphuric acid 98% were added in order to activate the digestion at 400°C for \pm 90 minutes. Once the digestion was complete, the solution was diluted to volume with distilled water and mixed well. The mixture was transferred in 50 mL bottle for analysis. A solution of 50 μ L standard, sample, control sample and 0.1NHCl (blank) were pipetted into test tubes in duplicate. Further, 2.5 mL phenol reagent was added and mixed. Furthermore, 2 mL hypochlorite reagent was also added and mixed. Tubes were placed in a 95°C waterbath for 5 minutes and cooled for 5 – 7 minutes in an ice bath. At about 25 - 30°C, samples were read on a spectrophotometer at 630 nm (SPEKOL 1300).

Below is the summary of the preparation of reagents and standard solution for the determination of protein nitrogen:

Reagents:

1. Phenol reagent

Sodium nitroferricyanide ($\text{Na}_2\text{Fe}(\text{CN})_5(\text{NO})2\text{H}_2\text{O}$)	0.05 g
Phenol, 90% w/v ($\text{C}_6\text{H}_5\text{OH}$)	11 mL

- The above items were dissolved in 800 mL of deionized water and adjusted to a volume of 1000 mL.

The solution (phenol reagent) was stored in *brown glass* bottle.

2. Hypochlorite Reagent

Sodium hydroxide (NaOH)	5 g
Disodium phosphate (Na_2HPO_4)	20.07 g

Or (Na_2HPO_4 Heptahydrate) (60.2 g)

- Sodium hydroxide was mixed to disodium phosphate and dissolved in 800 mL deionized water. Bleach was added and the volume adjusted up to 1000 mL.

The solution (hypochloride reagent) was stored in *brown polyethylene* bottle.

Phenol and hypochloride reagents were stable for two months at room temperature.

Standards

1. Stock Standard (100 mM)

A weight of 0.6607 g ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$) was weighed into 100 mL volumetric flask and filled to volume with 0.1 N HCl.

2. Working standard (at least 5)

<u>ml Stock / 100 mL</u>	<u>mM</u>
1	1
2	2
4	4
5	5

- The appropriate amount of stock was pipetted into 100 mL volumetric flasks and adjusted to volume with 0.1 N HCl.
- Stored capped in refrigerator and fresh standard was prepared weekly.
- Assay was linear through 10 mM when higher standard were needed.

Procedure

1. 50 μ L of standard sample were pipetted, control sample¹, and 0.1 N HCl (blank) into test tubes in duplicate.
2. 2.5 mL phenol reagent was added and mixed
3. 2 mL hypochlorite reagent was added and mixed.
4. Tubes were placed in a 95°C waterbath for 5 minutes.
5. Tubes were removed from an ice bath and cooled for 5 - 7 minutes or until 25 – 30°C.
6. Reading was done on a spectrophotometer at 630 nm.

2.3.7.2 Nitrogen and Protein analysis on the Leco FP-428

The Leco FP-428 is a microprocessor-based software controlled instrument that determines the nitrogen in a variety of materials. A sample of 200 mg was measured into a foil cup. There were 3 phases during an analyze cycle: In the sample drop purge phase the encapsulated sample was placed in the loading head, sealed and purged of any atmospheric gases. During the burn phase the sample was dropped into a hot furnace (950°C) and flushed with pure oxygen for very rapid combustion. The products of combustion, mainly CO₂, H₂O, NO_x and N₂ were passed through the thermo-electric cooler to remove most of the water then collected in the ballast volume. All the gas products in the ballast volume were allowed to become a homogenous mixture at a pressure of approximately 975 mm and a constant temperature. In the analyze phase, the piston was forced down and 10 mL aliquot of sample mixture was collected. The sample aliquot was swept through hot copper to remove oxygen and change NO_x to N₂, then through Lecosorb and Anhydrone to remove carbon dioxide and water, respectively. Nitrogen was measured by the thermal conductivity cell, and final product was displayed as percent nitrogen or protein according to Dumas' method (AOAC, 2000).

¹ Note: Various sample preparation techniques have different effects on the assay. Therefore, it is necessary to run a spiked control sample that has been prepared in the same manner as the other samples in the assay. This will give a % recovery of ammonia from the samples which can then be used as a correction factor.

2.3.8. Determination of calcium, magnesium and iron by atomic absorption spectroscopy

An air dried sample of 1 g was weighed accurately in a porcelain crucible, placed in a cool muffle furnace and ashed at 550°C overnight. This was cooled, dissolved in 5 mL of HCl and evaporated to dryness in a water bath. After cooling down, 5 mL of HNO₃ was added. The solution was once more heated in a water bath to boiling temperature, and filtered into a 100 mL volumetric flask. The filter paper was washed with warm deionised, distilled water. The solution was diluted to volume with water and mixed well.

2.3.9. Determination of amino acids

Defatted soybeans (10 mg) were weighed into a hydrolysis flask. A solution of 1 mL of 6N HCl and 1% phenol was added. The flask was evacuated, blown with nitrogen to remove oxygen and sealed off under vacuum (0.01 mm Hg). Samples were placed in an oven for 24 hours at a temperature of 110°C. After cooling, deionised water was added up to 5 mL. Samples were derivatized, filtered and placed in WSIP (automatic loader). Amino acids were separated by pumping the solution through a reverse phase column. Two pumps were used to form gradient for optimum separation and system gold was used for calculations (Biddingmeyer *et al.*, 1984).

2.3.10. Determination of starch

The method entails the gelatinisation of all the starch in the sample by autoclaving followed by the enzymatic hydrolysis of the starch to glucose and the determination of the glucose content by the glucose oxidase method (McRae & Armstrong, 1968).

2.3.11. Statistical analysis

An analysis of variance with the GLM model (Statistical Analysis Systems, 2010) was used to determine the significance between treatments. Mean and standard deviations were calculated. Treatment means were separated using Fishers' protected least significant difference (LSD) at the 5% level of significance.

CHAPTER 3: RESULTS

3.1 Effect of soaking on chemical composition and in vitro digestibility of soybeans

The effects of soaking of soybeans on nutrient contents are shown in Table 3.1.1. The protein content of soybeans that was soaked for 1 day appeared to be similar to that of roasted soybeans. However, soaking for 2 days seemed to increase the protein content of soybeans significantly compared to raw and roasted soybeans. The ash content of soybeans that was soaked for 2 days was low compared to the other treatments. The fat content increased significantly for soybeans soaked for 2 days compared to the raw soybeans. Roasted soybeans were higher in fat contents when compared to soaked and raw soybeans. One day of soaking did not influence the starch content significantly as compared to that of raw soybeans. However, the starch content was significantly decreased in soybeans when soaking was prolonged to 2 days.

Table 3.1.1 Effect of soaking of soybeans on crude protein (%), ash (%), crude fat (%) and starch (%) contents on dry matter of soybean

Treatment	Crude protein	Ash	Fat	Starch
Raw	39.10 ^c ±0.37	4.84 ^c ±0.29	15.80 ^d ±1.34	1.24 ^{bc} ±0.11
Roasted	40.27 ^b ±0.57	4.99 ^c ±0.30	19.56 ^c ±1.03	1.38 ^c ±0.20
Soaking – 1 day	40.24 ^b ±0.10	4.35 ^b ±0.33	17.67 ^b ±0.82	1.17 ^b ±0.11
Soaking – 2 days	42.07 ^a ±0.25	3.72 ^a ±0.39	19.01 ^a ±0.40	0.96 ^a ±0.03

Mean (± SD) values within the same column with different superscripts differ significantly at P< 0.05.

The effects of soaking of soybeans on antinutrient contents are shown in Table 3.1.2. Soaking reduced the trypsin inhibitor activity (TIA) compared to raw soybeans, especially after soaking for 2 days. The TIA was reduced by approximately 7 and 20% when soybean was soaked for 1 and 2 days, respectively. However, it is important to note that roasting was the most effective of all treatments as the TIA was reduced by 37% in roasted soybean compared to raw soybean.

The total polyphenol content of soaked soybean was similar to that of roasted soybeans. The level of total polyphenols increased after soaking and roasting compared to raw soybean.

Soaking of soybeans decreased the level of condensed tannin. This reduction was ($P < 0.05$) pronounced when soaking was extended to 2 days. However, roasted soybeans did not show any significant difference.

Table 3.1.2 Effect of soaking of soybeans on trypsin inhibitor activity (mg/g), total polyphenols (%) and condensed tannins (%)

Treatment	Trypsin inhibitor activity	Total polyphenols	Condensed tannins
Raw	83.05 ^c ± 8.71	10.83 ^b ± 0.42	1.24 ^c ± 0.34
Roasted	52.26 ^b ± 14.55	12.36 ^a ± 1.07	1.50 ^c ± 0.46
Soaking – 1 day	77.29 ^a ± 9.67	12.38 ^a ± 0.20	1.04 ^{bc} ± 0.62
Soaking – 2 days	66.17 ^{ac} ± 5.31	12.42 ^a ± 1.56	0.71 ^a ± 0.21

Mean (± SD) values within the same column with different superscripts differ significantly at $P < 0.05$.

In vitro protein digestibility of soybeans was not significantly influenced by soaking, roasting, dehulling or germination. Although this analysis was done in 4 replicates, there was no clear trend within treatment. Consequently, the standard deviation was found high for all treatments. Subsequent researches are needed for investigation.

Table 3.1.3 Effect of soaking of soybeans on % of *in vitro* protein digestibility¹

Treatment	Pepsin digestion	Papain digestion
Raw	66.47 (±21.40)	62.65 (±13.01)
Roasted	44.61 (±19.79)	58.36 (±2.77)
Soaking – 1 day	65.38 (±24.44)	55.37 (±11.13)
Soaking – 2 days	80.03 (±7.03)	57.21 (±16.58)

¹Mean (± SD) reported on dry basis

3.2. Effect of germination on chemical composition and *in vitro* protein digestibility of soybeans

The protein contents of soybean appeared to increase with increasing days of germination. Six days of germination increased the protein content by 15% when compared to the raw soybean (Table 3.2.1). Ash, fat and starch contents were not ($P > 0.05$) affected by roasting of soybeans. The starch content decreased ($P < 0.05$) from 1 to 4 days then increased from 5 to 6 days of germination of soybeans.

Table 3.2.1 Effect of germination on crude protein (%), fat (%), ash (%) and starch (%) contents

Treatment	Crude protein	Ash	Fat	Starch
Raw	39.10 ^f ±0.37	4.84 ^c ±0.29	15.80 ^{cl} ±1.34	1.24(±0.11) ^b
Roasted	40.27 ^e ±0.57	4.99 ^c ±0.30	19.56 ^{ac1} ±1.03	1.38(±0.20) ^{ab}
Germination 1 day	41.28 ^{cl} ±0.19	4.51 ^{bc} ±0.10	18.12 ^{bc} ±0.89	0.99 (±0.89) ^a
Germination 2 days	42.17 ^c ±0.54	4.12 ^b ±0.30	19.08 ^b ±1.37	0.84 (±0.12) ^a
Germination 3 days	43.40 ^b ±0.20	4.20 ^b ±0.23	19.18 ^b ±0.99	0.75 (±0.10) ^a
Germination 4 days	43.80 ^b ±0.52	4.27 ^b ±0.29	19.22 ^b ±1.05	0.71 (±0.13) ^{ad}
Germination 5 days	44.10 ^b ±0.70	4.85 ^a ±0.09	18.54 ^b ±0.97	0.94 (±0.20) ^a
Germination 6 days	45.06 ^a ±0.30	4.98 ^a ±0.20	20.58 ^a ±0.60	0.86(±0.24) ^a

Mean (± SD) values within the same column with different superscripts differ significantly at P< 0.05.

The effects of germination of soybeans on different antinutritional factors are shown in Table 3.2.2. In general, the level of trypsin inhibitor activity decreased in germinated soybeans compared to raw soybeans. Again, roasting appeared to be the most effective treatment of soybeans to decrease TIA. The total polyphenol content and condensed tannins level did not show any clear trend. After 5 days of the germination of soybeans, total polyphenols increased slightly compared to raw soybean. Roasting also caused a (P<0.05) increase in the total polyphenols. Condensed tannin levels of soybeans decreased significantly when germination was increased from 2 to 4 days.

Table 3.2.2 Effect of germination of soybeans on trypsin inhibitor activity (mg/g), total polyphenols (%) and condensed tannins (%)

Treatment	Trypsin inhibitor activity	Total polyphenols	Condensed tannins
Raw	83.05 (\pm 8.71)	10.83 ^b \pm 0.42	1.24 ^b \pm 0.34
Roasted	52.26 (\pm 14.55)	12.36 ^a \pm 1.07	1.50 ^b \pm 0.46
Germination 1 day	63.96 (\pm 24.97)	11.77 ^b \pm 0.24	0.82 ^a \pm 0.29
Germination 2 days	67.91 (\pm 21.54)	11.17 ^b \pm 0.77	0.67 ^a \pm 0.14
Germination 3 days	64.37 (\pm 18.86)	10.63 ^b \pm 0.69	0.56 ^a \pm 0.22
Germination 4 days	68.96 (\pm 8.35)	10.38 ^b \pm 0.70	0.57 ^a \pm 0.28
Germination 5 days	63.94 (\pm 6.67)	12.37 ^a \pm 1.41	0.96 ^a \pm 0.39
Germination 6 days	67.31 (\pm 10.77)	12.78 ^a \pm 0.69	0.87 ^a \pm 0.26

Mean (\pm SD) values within the same column with different superscripts differ significantly at $P < 0.05$.

No significant differences were noted for any of the treatments on *in vitro* protein digestibility (Table 3.2.3).

Table 3.2.3 Effect germination on *in vitro* protein digestibility (%) of soybeans

Treatment	Pepsin digestion	Papain digestion
Raw	66.47 (\pm 21.40)	62.65 (\pm 13.01)
Roasted	44.61 (\pm 19.79)	58.36 (\pm 2.77)
Germination 1 day	64.56 (\pm 14.02)	55.43 (\pm 9.77)
Germination 2 days	63.78 (\pm 15.96)	52.60 (\pm 8.90)
Germination 3 days	59.82 (\pm 19.38)	52.47 (\pm 8.90)
Germination 4 days	57.78 (\pm 15.15)	49.52 (\pm 17.99)
Germination 5 days	58.09 (\pm 14.36)	46.82 (\pm 9.79)
Germination 6 days	62.75 (\pm 11.47)	50.67 (\pm 4.56)

¹Mean (\pm SD) reported on dry basis

3.3. Influence of dehulling on chemical composition and *in vitro* protein digestibility of soybeans

Table 3.3.1 shows the effect of dehulling of soybeans on the nutrient content of soybeans. Dehulling of soybeans led to an increase in protein and fat contents compared to raw and roasted samples. On the other hand, ash and starch contents were decreased for dehulled soybeans compared to the raw and roasted beans.

Dehulling increased ($P < 0.05$) the total polyphenols, while condensed tannins decreased. On the other hand, roasted soybeans had a significant lower trypsin inhibitor activity compared to dehulled and raw soybeans.

The effect of dehulling on *in vitro* protein digestibility seemed to be dependent on the type of enzyme used. The protein digestibility of dehulled soybean was high compared to raw and roasted when pepsin was used. The opposite was observed when papain was used. As shown in Table 3.3.3, neither roasting nor dehulling had a significant effect on IVPD.

Table 3.3.1 Effect of dehulling of soybeans on crude protein (%), fat (%), ash (%) and starch (%) contents of soybeans

Treatment	Crude protein	Ash	Fat	Starch
Raw	39.10 ^c ± 0.37	4.84 ^b ± 0.29	15.80 ^c ± 1.34	1.24 ^{ab} ± 0.11
Roasted	40.27 ^b ± 0.57	4.99 ^b ± 0.30	19.56 ^b ± 1.03	1.38 ^b ± 0.20
Dehulling	45.11 ^a ± 0.35	3.41 ^a ± 0.61	20.95 ^a ± 0.54	1.11 ^a ± 0.12

Mean (± SD) values within the same column with different superscripts differ significantly at $P < 0.05$.

Table 3.3.2 Effect of dehulling of soybeans on trypsin inhibitor activity (mg/g protein), total polyphenols (%) and condensed tannins (%)

Treatment	Trypsin inhibitor activity	Total polyphenols	Condensed tannins
Raw	83.05 ^{ab} ± 8.71	10.83 ^b ± 0.42	1.24 (± 0.34) ^{ab}
Roasted	52.26 ^a ± 14.55	12.36 ^a ± 1.07	1.50 (± 0.46) ^{cd}
Dehulling	68.72 ^a ± 9.20	12.77 ^a ± 1.35	0.64 (± 0.18) ^{bd}

Mean (± SD) values within the same column with different superscripts differ significantly at $P < 0.05$.

Table 3.3.3 Effect of dehulling of soybeans on *in vitro* protein digestibility (%)¹

Treatment	Pepsin digestion	Papain digestion
Raw	66.47 (±21.40)	62.65 (±13.01)
Roasted	44.61 (±19.79)	58.36 (±2.77)
Dehulling	71.87 (±24.69)	47.95 (±9.01)

¹Mean (± SD) reported on dry basis

3.4. Effect of processing method on amino acid and mineral composition of soybeans

The amino acid composition and mineral content of soybeans that were subjected to different treatments were determined and the results are shown in Table 3.4 and 3.5. These analyses were done only once on samples from the first replicate of the trial. In general, the amino acid profiles were similar for all the treatments. However, a slight increase was observed for some individual amino acids such as lysine and glycine for dehulled and soaked soybeans.

Table 3.4 Effect of soaking, dehulling and germination of soybeans on amino acid composition (%)

Amino acids	Treatment				
	Raw	Germination		Soaking	Dehulling
		(1 day)	(6 days)	(2 days)	
Aspartic acid	4.20	4.20	4.80	4.40	4.40
Glutamic acid	7.30	7.40	7.90	7.90	8.30
Serine	2.30	2.30	2.40	2.50	2.50
Glycine	1.98	1.96	1.97	2.05	2.08
Histidine	1.27	1.25	1.30	1.35	1.43
Arginine	3.19	3.12	3.31	3.44	3.50
Threonine	1.60	1.70	1.70	1.80	1.80
Alanine	2.00	2.02	2.18	2.08	2.16
Proline	2.23	2.28	2.53	2.35	2.51
Tyrosine	1.59	1.58	1.68	1.66	1.78
Valine	2.22	2.12	2.26	2.22	2.28
Methionine	0.66	0.64	0.70	0.69	0.76
Cysteine	0.15	0.13	0.16	0.15	0.16
Isoleucine	2.04	2.00	2.19	2.15	2.29
Leucine	3.35	3.29	3.47	3.40	3.64
Phenylalanine	2.28	2.20	2.38	2.39	2.45
Lysine	2.80	2.69	2.90	2.95	3.17



The iron content of soybeans was decreased for germinated soybeans compared to raw soybeans. There seemed to be a slight increase in sodium. In general, Ca, P, Mg, Fe and Na were not significantly affected by the length of the germination period. In the case of dehulling, only the iron content appeared to have decreased when compared to raw soybeans.

Table 3.5 Effect of traditional processing methods of soybeans on mineral content

Treatments	Minerals				
	Ca (g/100g)	P (g/100g)	Mg (g/100g)	Fe (mg/kg)	Na (g/100g)
Raw soybeans	0.25	0.52	0.20	121.2	0.04
Germination					
1 day	0.26	0.53	0.21	84.01	0.06
3 days	0.28	0.53	0.20	93.80	0.05
6 days	0.30	0.54	0.21	87.86	0.05
Soaking					
2 days	0.25	0.53	0.20	95.65	0.05
3 days	0.28	0.52	0.21	85.09	0.06
Dehulling	0.22	0.55	0.20	77.96	0.05

CHAPTER 4: DISCUSSIONS

Soybeans are an important source of protein and amino acids in the diets of monogastric animals (Huisman & Tolman, 2001). Despite the nutritional potential of soybeans as an economic source of significant amounts of proteins, amino acids, carbohydrates, vitamins and some minerals including trace minerals, the utilisation of this legume has been limited, due to the presence of certain antinutritional factors (Van der Poel, 1990). Among these are trypsin inhibitors, condensed tannins and total polyphenols which reduce the nutritional quality of the protein (Van der Poel, 1990). In addition, these antinutritional factors (ANFs) are responsible for the lost of production, due the interference with digestion, absorption and utilisation of nutrients (Huisman & Tolman, 2001). Attempts to increase the utilisation of soybeans have employed a wide range of heat treatment techniques and traditional processing methods, such as germination, dehulling, soaking, roasting and fermentation (Alonso *et al.*, 2000). These techniques were found to be effective in the lowering of ANFs levels of and the enhancement of the nutrient content of legumes seeds (Osman, 2007).

In this trial, germination was evaluated as a means of soybean processing, as this is described to be a versatile and low cost method compared to heat treatment methods. Germination is suitable for small scale chicken farmers in rural areas, or developing countries.

Soybeans were germinated for various time periods, from 1 to 6 days, in order to find the optimal germination period. Raw and roasted soybeans were used as negative and positive controls, respectively. Soaked and dehulled soybeans were also assessed for its nutritional value, levels of antinutritional factors and *in vitro* protein digestibility.

In the present trial, diverse changes of nutrients within the seeds were noted for all processing methods applied. The time of germination and the duration of soaking were important factors affecting measured parameters in the study. A combination of soaking and germination had a significant effect on crude protein and antinutrients levels such as condensed tannins, trypsin inhibitor activity and total polyphenols in the soybeans. In addition, a decrease in trypsin inhibitors and condensed tannins were observed.

4.1. Antinutritional factors

Among the different methods investigated, it appears that germination may be useful in removing/reducing certain unwanted heat labile components like condensed tannins and trypsin inhibitors.

Tannins reportedly inhibit digestive enzymes (Shimelis & Rakshit, 2007). Condensed tannins in the soybeans decreased significantly ($P < 0.05$) with 46%, 55% and 54%, respectively, after 2, 3 and 4 days of germination. This reduction in tannin content in soybeans is attributed to the tannin activity during germination (Mubarak, 2005) that leads to the formation of hydrophobic association of tannins with seed proteins and enzymes. Some loss of condensed tannins during germination may be due to the leaching of tannins into water. Reddy *et al.* (1985) found that overnight soaking of grains followed by germination for 2 days significantly reduced the tannin content by 50% in a wide variety of legumes. The decrease in condensed tannins after soaking, as observed in this study, is also in agreement with results reported by Alonso *et al.* (2000).

Dehulling also decreased the levels of condensed tannins. This finding confirmed those reported by Alonso *et al.* (1998). Tannins are mainly located in the seed coats (Matthäus & Angelini, 2003) that could explain its reduction after dehulling.

A 17-20% reduction of trypsin inhibitor activity (TIA) was noted after germination, soaking and dehulling of soybeans, while the TIA of roasted soybeans decreased with 37%.

Various researchers have reported losses in tannins, trypsin inhibitors and other antinutritional factors in pulses as a result of domestic processing, including soaking, dehulling and germination. Mwikya *et al.* (2000) found that the trypsin inhibitor activity decreased threefold, and also reported a significant decrease in tannins to undetectable level, in finger millet during sprouting. A 20 – 38% reduction of tannins was observed during the germination of green grams, cowpeas, lentils and chickpeas (Ghavidel & Prakash, 2007). Sangronis & Machado (2007) observed a significant decrease in the content of TIA in pigeon beans (19.2%), white beans (52.5%) and black beans (25%) after 5 days of germination, while Frias *et al.* (1995) found that six days of germination of lentils decreased TIA from 7% to 18%. Wang *et al.* (1997) reported that TIA decreased with shorter germination periods but slightly increased as the length of germination increased.

The levels of total polyphenols increased significantly ($P < 0.05$) after 5 days of germination, and also after roasting, soaking and dehulling of soybeans. Changes in phenolic compounds may occur depending on the type of seeds (López-Amorós *et al.*, 2006), processing conditions such as rinsing (Frias, 1995), presence of light and germination time (Frias, 1995; Oloyo, 2004; López-Amorós *et al.*, 2006 & Dueñas *et al.*, 2009;). Oloyo, (2004) and Dueñas *et al.* (2009) found that polyphenols of lupin increased during germination. Similarly, Satwadhar *et al.* (1981) found that the polyphenols levels in moth beans increased from 1.0% to 1.7% at 36 h of germination, while beans that germinated for 48 h had higher amounts of polyphenols than raw moth beans.

In this study, germination of soybeans for 3 to 4 days decreased the total polyphenol contents. However, the increase of total polyphenols observed in the soybeans that were germinated for a period of 5 to 6 days cannot be explained. However, these increases can perhaps be attributed to the presence of fungi as visible signs of fungal growth on the seeds were evident from the fifth day of germination (Wu *et al.*, 2011). Further research is needed before any definite conclusions can be made.

4.2. Proximate composition of germinated soybeans

There was a significant ($P < 0.05$) decrease in the starch content from 1 to 4 days of germination then an increase from 5 to 6 days of germination. The decrease in the starch content agrees with Mubarak (2005), who reported a similar decrease in starch content after 3 days of germination of mung bean seeds. This reduction was attributed to the hydrolysis of starch that supplies the developing seedling with energy during germination (Madden *et al.*, 1985). Mwikya *et al.* (2000) also found a gradual decrease in starch content of finger millet during the first 36 h of germination. However, the increase in starch content from the fifth day do correlate with the increase in total polyphenols.

In the present study, crude protein was found to increase as the number of days of germination of soybeans increased. Kaushik *et al.* (2010) also reported that the crude protein increased with germination of soybeans. Similar increases in protein have been reported for other legumes such as lablab beans (Osman 2007), mung beans (Mubarak, 2005), faba beans and kidney beans (Alonso *et al.*, 1998). Alonso *et al.* (1998) found a significant increase in protein content of faba and kidney bean after 48 h of germination compared to raw beans. The apparent increase in protein can be attributed to the utilisation of carbohydrates as an energy source for developing

sprouts (Donangelo *et al.*, 1995) and also due to the release of free amino acids after enzymatic hydrolysis for the synthesis of new protein (Echendu *et al.*, 2009). However, Bamdad *et al.* (2009) found a notable decrease in protein content after 1, 3 and 5 days germination of lentils.

The other processing methods that were included in this study, i.e. soaking, dehulling and roasting led to significant increases of soybean protein content. The removal of the seed coats causes an increase in the relative protein content, as proteins are characteristically present in the cotyledon fraction (Alonso *et al.*, 1998).

Dehulling of soybeans also caused a statistically significant ($P < 0.05$) increase in the fat and protein contents in the soybeans. The increase is due to the removal of the hull portion and the concentration of endosperm in the legume flours (Ghavidel & Prakash, 2007).

In the present study, germination of soybeans increased the fat contents. This agrees with the report of Echendu *et al.* (2009) on groundbean and Khattak *et al.* (2011) on chickpea seed. However, Ghavidel & Prakash (2007) found a decrease ($P < 0.05$) of fat content after germination of some legume seeds. This could be due to total solid loss during soaking prior to germination or use of fat as energy source during the sprouting process. Osman (2007) and Mubarak (2005) reported a significant ($P < 0.05$) decrease of fat content when mung bean seeds were allowed to germinate for 3 days. The increase in fat reported in this trial might be due to the microbial growth that occurred during the germination process (Penas *et al.*, 2010).

4.3. Amino acid composition

The amino acid composition of raw and treated soybean seeds was analysed. Soybean protein was rich in total essential amino acids such as valine, isoleucine, leucine, phenylalanine and lysine. Germination for 6 days caused a slight increase in total essential amino acids. These data agreed with the report of Mubarak (2005) who found a slight increase in total essential amino acids after 3 days of germination of mung bean seeds.

4.4. Mineral composition

Germination appeared to have increased the levels of Ca, P and Na in soybeans. Constancy was observed in Mg level while Fe decreased with germination. Findings on Na and P contents from this study agree with those reported by Echendu *et al.* (2009) in groundbean seeds. According to Kaushik *et al.* 2010, soybean germination was associated with an increase in Ca content. The loss of divalent metals was attributed to their binding to protein and formation of phytate-cation protein complex (Mubarak, 2005).

After an initial decrease of 30.7% in Fe content on day 1 there was an increase on day 3 followed by a decrease on day 6. Kaushik *et al.* (2010) reported similar fluctuations with an initial decrease of 6%. He also found a marginal increase in Mg during the germination of soybeans. These fluctuations of minerals are due to the leaching of soybeans in water (Mubarak, 2005).

4.5 *In vitro* protein digestibility (IVPD)

No significant differences were noted in IVPD between any of the treatments regardless of the type of enzyme used. There was no good trend within treatment and between different replicates. Consequently, the standard deviation was high than normal. Mubarak (2005) reported that dehulling, soaking and germination of mung bean seeds improved the IVPD thereof. A significant increase was observed in the IVPD of white beans, black beans and pigeon beans after germination (Alonso *et al.*, 2000; Sangronis & Machado, 2007).

CHAPTER 5: GENERAL CONCLUSIONS

Legume seeds are contributing significantly towards the diets of humans and livestock in both developed and developing countries, mainly as a source of protein.

However, raw legume seeds contain variable concentrations of antinutritional factors (ANFs), some of which reduce the bioavailability of trace elements and proteins. Different processing techniques were developed in order to facilitate the use of legume seeds in monogastric animals. Among these techniques, heat treatment appeared to be effective in reducing or eliminating ANFs and enhancing the nutritional value of legume seeds. Heat treatment requires appropriate equipment to monitor the process, as overheating leads to the alteration of protein quality, while under-heating is ineffective in reducing the ANFs in the beans. Therefore, this technique is costly, and it is not accessible to small-scale farmers or people living in developing countries.

This study focused on germination as a traditional processing method for soybeans in small-scale broiler farming. It was noted that germination led to significant increases in crude protein, fat and total polyphenols contents of soybeans. It was also observed th

at crude protein increased gradually with germination, while starch, trypsin inhibitor activity (TIA) and condensed tannins decreased. *In vitro* protein digestibility (IVPD) did not show any significant changes regardless of the enzyme used. Six days of germination increased the levels of amino acids such as aspartic acid, glutamic acid, arginine, proline, tyrosine, isoleucine, leucine, phenylalanine and lysine.

Germination led to an increase in Ca and marginal increases in P contents. Losses in Fe were noted, while Mg and Na stayed constant.

Germination was also compared to other traditional methods such as roasting, soaking and dehulling. Results showed that dehulled soybeans had the highest proximate composition which was significantly different from the other treatments ($P < 0.05$) because of the removal of hulls that contain the fibre, tannins etc.

Germination for a period of 3 to 4 days was effective in improving the nutritional value of soybeans and reducing ANFs. Longer periods of germination resulted in microbial growth that caused a significant increase ($P < 0.05$) in the level of total polyphenols. No statistical differences

were noted between the soybeans that were germinated for a period of 3 days compared to soybeans germinated for 4 days. However, the total polyphenols increased that may have negative effects on the digestion of carbohydrates. Furthermore, germination for 4 days will also be more time consuming. For these reasons, it is recommended that small scale farmers and farmers in developing countries use a germination period of 3 days for soybeans. This is effective to improve the nutritional value of soybeans and reduce the levels of condensed tannins and TIA. Cost for germinating soybeans is minimal as no expensive equipment and specialized facilities are needed.

CHAPTER 6: CRITICAL REVIEW AND RECOMMENDATIONS

Some of the findings in the present study are in conflict with results from previous studies. This could be attributed to differences in germination conditions, seed varieties and analytical methods used.

Generally, germination decreases the fat content and the total polyphenols of legume seeds, but the results of this study have shown an increase in the crude fat content with germination. Five to six days of germination has also increased the level of total polyphenols. These increases were in correlation with microbial growth that was visible during the germination of soybeans from around five days onwards. Microbial growth should perhaps be prevented by the use of microbial inhibitors such as sodium azide. For subsequent experiments, it is recommended that fungal growth must be inhibited during germination by rinsing the soybeans daily in distilled water and adding sodium azide to germinating beans. However, this might be impractical in rural conditions and therefore the ideal germination period recommended is up to a maximum of 3 days. Amino acid and mineral composition of soybeans should be repeated on different batches of soybeans in order to subject the results to statistical analyses.

As mentioned earlier, the germination of soybeans for 3 days was the optimal period to decrease ANFs and improve the nutritional value of soybeans. Extending the germination process for more than 3 days resulted in microbial growth and an increase of total polyphenols. Another *in vitro* study should follow this study prior to an *in vivo* trial in order to clarify certain findings. In a following study, soybeans will be subject to germination for 3 days. The soybeans will be divided into two groups. One group will be treated with distilled water and sodium azide will be added to prevent microbial growth. The second group will be treated with borehole or river water to simulate the scenario in rural areas. The main reason for this suggestion is that not all rural areas might have access to treated tap water, and since tap water that is supplied by municipalities, it is mostly treated with chemicals (Cl, F etc.) that might interfere with further chemical analysis of the seeds and consequently with results. Untreated soybeans will serve as a negative control. Samples will be tested for ANFs, proximate analyses and *in vitro* protein digestibility in order to validate the findings of the current study. A special attention will be paid for the digestibility of protein to avoid fluctuations and huge standard deviation. This experiment



will be followed by an *in vivo* study where the effects of germinated soybeans will be evaluated on the growth performance and mortality in broilers.

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LIST OF ABBREVIATIONS

ANFs	: Antinutritional factors
AOAC	: Association of Official Analytical Chemists
ASA	: American Soybeans Association
Ca	: Calcium
Cu	: Copper
C ₆ H ₅ OH	: Phenol
CO ₂	: Carbon Dioxide
DM	: Dry Matter Content
FAO	: Food and Agriculture Organization
Fe	: Iron
HCl	: Hydrochloric Acid
Hg	: Mercury
HNO ₃	: Nitric acid
H ₂ O	: Water
IVP	: <i>In vitro</i> protein digestibility
LSD	: Least Significant Difference
Mg	: Magnesium
Na ₂ HPO ₄	: Bicarbonate Hydrogenous Phosphate (Disodium Hydrogen Phosphate)
Na ₂ Fe(CN) ₅ (NO).2H ₂ O	: Sodium Nitroferricyanide
NaHCO ₃	: Sodium Hydrogen Carbonate
NaOH	: Sodium Hydroxide
(NH ₄) ₂ SO ₄	: Ammonium Sulphate
NO _x	: Nitrogen Oxides (NO ₂ /NO/N ₂ O)
N ₂	: Nitrogen
PVPP	: Poly vinyl poly pyrrolidine
TCA	: Trichloroacetic acid

TIA : Trypsin inhibitor activity
TIU : Trypsin inhibitor units
WHO : World Health Organisation
w/v : weight/volume (g/mL)
Zn : Zinc

CHAPTER 7: REFERENCES

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