KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI
COLLEGE OF SCIENCE
DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

MICROBIAL QUALITY, PHYTOESTROGEN LEVELS AND NUTRITIONAL CONTENT OF LOCALLY PRODUCED SOYMILK/POWDER IN THE KUMASI METROPOLIS, ASHANTI REGION, GHANA

BY

GLORIA OWUSU-ANSAH

JULY, 2016

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A THESIS SUBMITTED TO THE DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY, COLLEGE OF SCIENCE, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF PHILOSOPHY IN BIOLOGICAL SCIENCES

BY

GLORIA OWUSU-ANSAH

JULY, 2016

DECLARATION

I hereby declare that this submission is my own work towards the degree of Master of Philosophy in Biological Sciences and that, to the best of my knowledge, it contains no materials which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

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ABSTRACT

The use of soy-containing food is increasing as the public has been made aware of the health promoting properties of soybean. The seed, as a whole, processed is used to produce soymilk, soya powder and other products. Production and sale of soymilk and powder in Ghana is largely on small scale, unsophisticated, unmonitored and unrestrained. This study assessed the microbial quality, phytoestrogen levels, processing stages and the nutritional content of locally produced soymilk and soy powder in the Kumasi metropolis of the Ashanti region. Soymilk and soya powder samples were collected from five different sampling sites within the study area. Coliform counts, phytoestrogens levels and nutrient content were determined using standard methods. The results obtained showed that soya powder samples had high concentrations of most of the investigated parameters than the soymilk samples. Faecal coliform numbers in both soymilk end product (1.98×10^5 MP/100ml) and soya powder (1.67×10^3- 4.25×10^4 MPN/100ml) for all but one sampling location were above the WHO permissible limit for food. Faecal coliforms and *E. coli* were high in the soya powder products obtained from C. and T. hospitals respectively. No faecal coliform were detected in soya powder obtained from K. hospital. The end product of soymilk and soya powder from K. hospital and C. market had no *E. coli.* Soymilk end product contained 28.49%, 0.20%, and 0.13% protein, calcium and magnesium respectively whereas soya powder contained 39%42.10% protein, 0.24%-0.26% calcium and 0.27%-0.28% magnesium. Recommended Dietary allowance (IOM, 2005) for children between the ages of one and three are protein 13% (13g/100kg), calcium 0.7% (700mg/100g) and magnesium 0.08% (80mg/100g). Soymilk end product had total Isoflavone content of 69.32µg/g whereas isoflavone content in soya powder ranged from 180.06 µg/g to 208.75 µg/g.

The results of the study generally indicated that, soymilk and powder could serve as a vehicle for pathogens transmitted by the faecal-oral route and an unhealthy nutritional profile for children

between the ages of one and three. Considering the body weight of children between the ages of one and three, it is conceivable that consumption of soya powder on regular basis could affect endogenic hormone production of infants.

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GLOSSARY

ANOVA	:	Analysis of variance
AOAC	:	Association of Official Analytical Chemist
APHA	:	American Public Health Association
ASTM	:	American Society for Testing and Materials
MPN	:	Most Probable Number
COT	:	Committee-on-Toxicity
EBT	:	Erichrome Black T
EDCs	:	Endocrine Disrupting Chemicals
EDTA	:	Ethylene diamine tetra-acetate
FAO	:	Food and Agriculture Organization
H_2SO_4	:	Sulfuric acid
HPLC	:	High-performance liquid chromatography
IOM	:	Institute of Medicine.
IPCS	:	International Programme on Chemical Safety
KCN	:	Potassium cyanide:
KOH	:	Potassium hydroxide
NaOH	:	Sodium hydroxide
NAS	:	National Academy of Science
NIEHS	:	National Institute of Environmental Health Sciences
NIH	:	National Institute of Health
RPM	:	Revolution per minute
SD	:	standard deviation
TEA	:	Triethanolamine
µg	:	microgram(s)
USDA	:	United States Department of Agriculture
USFDA	:	United States Food and Drug Administration
WHO	:	World Health Organization

CHAPTER ONE

INTRODUCTION

The soybean (*Glycine max*) plant belongs to the family Leguminosae (Wang *et al.*, 1979). It is a crop of global importance and has the highest output of vegetable oil and protein content among the cultivated crops in the world (FAO, 2009). Soymilk which is a watery extract of whole soybean is rich in water soluble protein, oil and carbohydrate (AdebayoTayo *et al.*, 2008). Soymilk is prepared by soaking soybeans in water, wet milling and sieved while soy powder is roasted and milled. The process if not carried out under hygienic conditions, could lead to microbial contamination. The milk is a whitish or creamy suspension which bear a resemblance to cow milk in consistency and appearance (Iwe, 2003). It is generally characterized to have a soy flavor (grassy), beany, which apparently can be enhanced by lactic acid fermentation, just like yoghurt products (Jimoh and Kolpo, 2007). Besides the extra protein and fiber, the growing acceptance of soymilk and soy powder globally is accredited to its health benefits e.g. low cholesterol, prevention and reduction of heart diseases and certain cancers, its capability to reduce bone loss and menopausal symptoms, (Anderson and Garner, 1997).

Soymilk and soy powder can be used in place of cow milk in mashed potatoes, sauces, gravies, cakes, puddings or any dish that demand for milk. Because milk from soy beans lacks lactose unlike cow's milk, lactose intolerant people can certainly use soy milk or powder on a daily basis. Soya has all the amino acids essential to make a complete protein similar to meat or eggs. It is possibly the finest protein form of all non-animal sources, and healthier than animal source protein for individuals who of a necessity must lower their cholesterol levels.

At present, contrasting perspectives on the health benefits of soya products have surfaced. Health problems such as infertility, malnutrition, thyroid dysfunction, digestive distress, reproductive disorders, cognitive decline, birth defect, cancer and immune system breakdown have been associated with soya by some studies (Doerge *et al.,* 2002).

The first recognition was in the 1940s when estrogenic effect were caused by some plantderived compounds (Bennetts *et al*., 1946). Multiple fertility problems on sheep that grazed on red clover pastures. Signs of estrus were shown by juvenile animals, ewes were unable to get pregnant and those that were pregnant often miscarried. Isoflavone formononetin and biochanin A levels were high in clover pastures, which were among the first phytoestrogens discovered (Bennetts *et al.,* 1946).

Phytoestrogens are compounds present naturally in countless foodstuffs of plant source and include coumestans, isoflavones, and lignans. These compounds and their metabolites have estrogenic properties which is similar to the human sex hormone 17b-estradiol but generally less potent to it (Setchell, 1995). Biological effects have been revealed when tested in the laboratory and this has provoked research on how food phytoestrogen may affect humans. Isoflavones are a class of chemical compounds found naturally in a variety of plants such as soybeans, which contain relatively high levels. Daidzein, glycitein and genistein are the three parent isoflavone found in soy. Daidzin, glycitin, and genistin are the glucosides forms of the parent Isoflavone. They also occur as the glucoside esters of the parent isoflavones (Chiarello *et al.*, 2005).

Soy drink and powder made locally in Ghana are common products because of the health benefits, its affordability and the availability of the grains and other ingredients used for their production and packaging. Furthermore, the processing methods are simple and cheap as no sophisticated equipment and expertise are required, thus the beverage and powder are

largely produced on small scale using indigenous methods with unquantified amounts of ingredients.

If the processing methods and packaging conditions tend to be unhygienic, soy drink and powder may be contaminated with microorganisms. The type and number of microorganism existing on the product are major determinants of quality deterioration. Under favourable conditions, these microbes could multiply leading to spoilage of the products rendering them uneatable or make them channels of food poisoning or infection.

1.1 JUSTIFICATION

The public has been exposed to the health promoting benefits of soya which has increased the use of soy in infant foods. Anti-nutritive factors are also present in soy but these are removed by the processing methods used or by supplementation for infant feeding.

However, daidzein and genistein which are isoflavones found primarily in raw soybeans as daidzin and genistein demonstrate considerable carry-over through processing because they are heat stable (Irvine *et al*., 1998).

Recently, concerns have been shown that phytoestrogen exposure through soya may result in a developmental hazard particularly to the reproductive system of infants and also alter the reproductive hormones of adults (Irvine *et al*., 1998). Perturbations of the sex steroid milieu by phytoestrogens are poorly tolerated by neonates. Additionally humans and livestock excrete phytoestrogens through food consumption. Therefore it is imperative to evaluate their concentrations in food. The ingredients, equipment used, the various processing stages, packaging and retailing conditions of the soy drink and powder predispose the product to microbial contamination.

This study was therefore, designed to determine the microbial quality, phytoestrogens levels, processing stages and the nutritional content of locally produced soymilk and soy powder in the Kumasi metropolis of the Ashanti region.

1.2 Main objective;

To determine the microbial quality, phytoestrogen levels and nutritional content of locally produced soymilk and soy powder in the Kumasi metropolis.

1.3 The Specific Objectives were to;

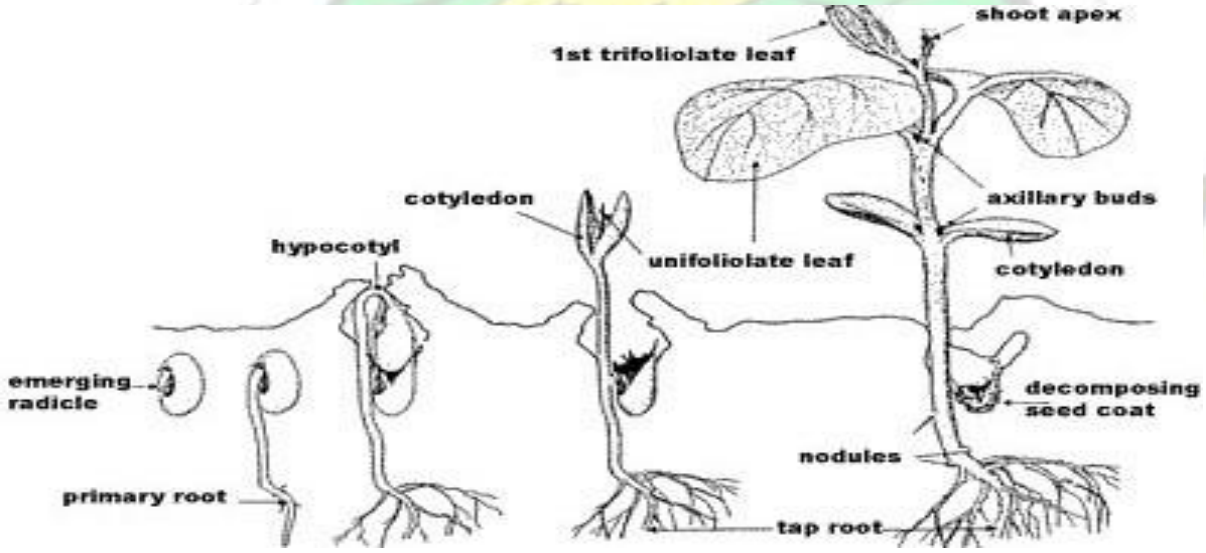
i. determine total coliforms, faecal coliforms and *E. coli* in the soymilk/powder in the study area.
ii. determine protein, ash, calcium and magnesium content in soymilk/powder.
iii. determine the pH, and levels of Isoflavones (genistein, daidzein and glycitein) in soymilk/powder.
iv. identify the different processing stages in the preparation of soymilk/powder locally.

CHAPTER TWO

LITERATURE REVIEW

2.1 SOYBEAN

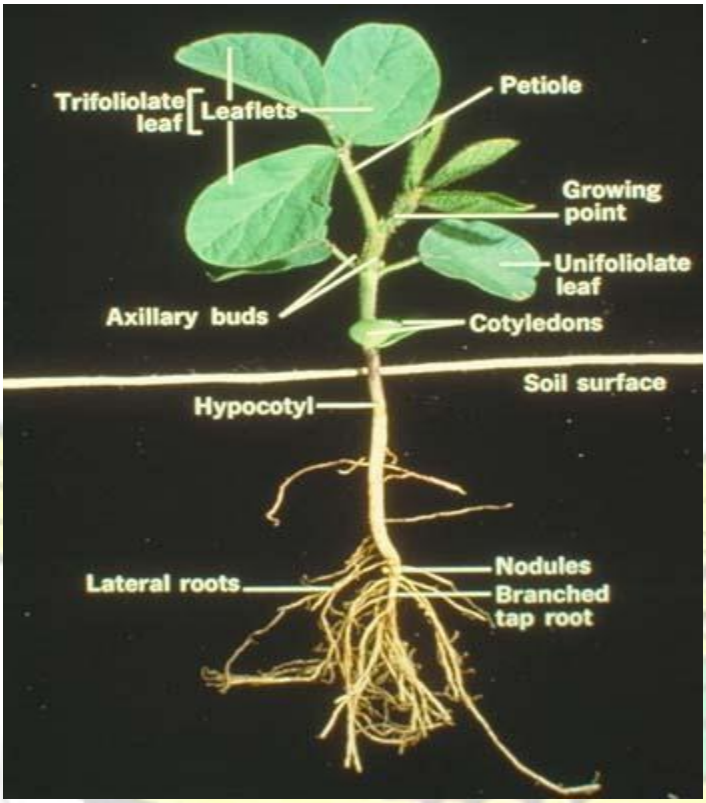
The soybean (*Glycine max*) belonging to the Family Leguminosae, is a universally essential oilseed crop and a high-quality source of protein (FAO, 2009). In many parts of the world soybean has in recent years become popular as a major protein and oil source for consumption by humans and animals (Manyong *et al*., 1996) and an important component in improved crop rotation systems. Soybean is a promising crop that will help agricultural scientists and other stakeholders to achieve the goal of sustainable agricultural and food production for the world's ever increasing population (Oyekanmi and Fawole, 2007). Anochili (1984) states that Eastern Asia is the originator of soybean. It has different colors, such as green, yellow and red, however the most common ones are yellow. The UN Food and Agricultural Organization describes the plant as an oilseed rather than a pulse by (FAO, 2009).The soybean plant (Figure 2.1) is dicotyledonous that exhibits epigeal (above the surface) emergence.



(Bennett *et al.*, 1999)

Figure 2.1: Soybean and its growth stages

The cotyledons are pushed through the soil to the surface by an elongating hypocotyl (Figure 2.2) during germination. Afterwards, the developing leaves are supplied with stored energy by the green cotyledons which opens and capture light energy in smaller quantities. First to develop are the unifoliolate leaves in which two of these (unifoliolate leaves) emerge direct opposite one another on top the cotyledons. Subsequently, trifoliolate leaves comprised of 3 leaflets appear afterwards (Bennett *et al.*, 1999).



(McWilliams *et al*., 1999)

Figure 2.2: Soybean plant

2.2. PHYTOESTROGENS IN SOY

Phytoestrogens are a group of non-steroidal polyphenolic compounds that occur naturally in a wide range of plants and induce biological responses based on their ability to bind to estrogen receptors. Legumes such as clover, soy, peas, alfalfa and beans usually have high concentrations of phytoestrogens (Ferrer and Thurman, 2009). Phytoestrogens can be divided in different sub-families according to their chemical structure and they include

isoflavones, coumestans and lignans which occur in either plants or seeds. Isoflavones (e.g. genistein and daidzein) found in soybean and Ligands (e.g. enterolactone and enterodiol) found in flaxseed, cereal bran, legumes and fruit are the most important groups of phytoestrogens. (Price and Fenwick, 1985).

Setchell (1995) showed that these compounds and or their metabolites have estrogenic properties similar to but generally less potent than the human sex hormone, 17β-estradiol. Isoflavones are active biological composites naturally present in many plant varieties, with soybean having higher levels relative to others (Klump *et al.*, 2001). Three parent isoflavones are found in soy: daidzein, glycitein, and genistein called aglycones. The isoflavones also occurs as the glucosides (daidzin, glycitin, and genistin) and the glucoside esters of the parent isoflavones. The acetyl and malonyl isoflavone glucoside esters are the most abundant forms found in soybeans, with either an acetyl or a malonyl group attached to the isoflavone glucoside at the 6^2-O position (Klump *et al.*, 2001).

In plants, Isoflavones generally occur as inactive glucoconjugates but by the action of intestinal bacteria in humans, they are hydrolyzed to active forms aglycones (Tsuchihaschi *et al.* 2008). In humans, genistein and daidzein are regarded as the most important biologically active forms of Isoflavones; they arise from hydrolysis of biologically inactive forms of glucoconjugates and also by metabolism from formononetin and biochanin A.

Isoflavones are similar to estrogen in structure and seem to have activity like estrogen and bind to estrogen receptors (Umphress *et al.*, 2004). Many potential health benefits of isoflavones in soy products have been investigated, including effects on cancer, vascular disease, osteoporosis, menopausal symptoms and cognitive function (Anderson and Garner, 1997). Data signifying potential of isoflavones in preventing both prostate, breast cancer, lowering cholesterol levels, bone loss attenuation in postmenopausal women and alleviating menopausal

symptoms is the basis for the interest in soy isoflavones (Hendrich and Murphy, 2001). Dry soybeans contain about 1.2-4.2 mg/g isoflavones (Wang and Murphy, 1994). Factors such as the soil in which they are grown, climate, stage of their maturity, level of processing and many others affect the exact concentration of Isoflavones in the dry soybean.

Conditions such as soybean variety, conditions during processing and dilution with nonsoy ingredients determines the Isoflavone profile in commercial soya products. Processing conditions such as heat, fermentation and enzymatic hydrolysis alters significantly the Isoflavone constituents in soy foods (Wang and Murphy, 1994). Isoflavone profile changes due to temperature as a result of processing is a well-established fact (Kudou *et al.,* 1991). Heat processing decreases the amount of the malonyl forms, which are heat sensitive (Park *et al*. 2002), and contents of aglycones (genistein and daidzein) forms are increased by enzymatic hydrolysis with b-glycosidades (Park *et al*., 2001) and fermentation with bifidobacteria (Tsangalis *et al*. 2003). Lower content of Isoflavone is generally associated with higher degree of processing. Only 6-20 % of the total amounts of isoflavone found in unprocessed soybeans are contained in the second generation of soy products [e.g." tofu"] (Duncan *et al.,* 2003).

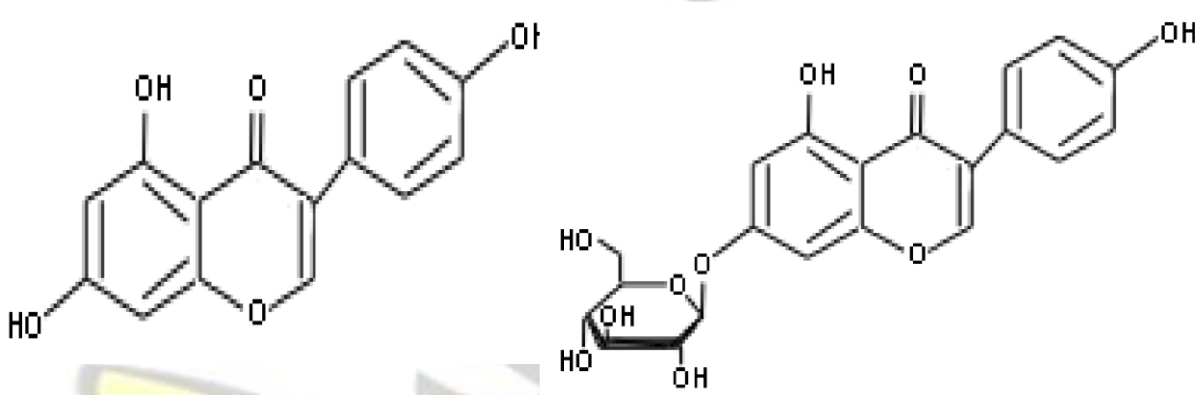
2.2.1 MODE OF EXPOSURE

Ingestion is the source of human exposure to isoflavones. Exposure occurs principally through foods made with soybeans and soy protein, baby formulations as well as dietary supplements prepared with soy protein and soybeans, but not soy oils (Drugstore.com, 2004).

The most astounding amounts of isoflavones and their glycosides are found in soy flour and soybeans (COT, 2003). Very low concentrations of Isoflavones are contained in soy sauces whiles soy oil contain trace levels of Isoflavones (Setchell, 1998).

Food processing is known to influence with the conjugation state (conjugated vs. aglycones) of isoflavones in soy foods. Non fermented soy foods (e.g. tofu) contain higher levels of glucosides, while fermented soy foods (e.g. tempeh) contain higher levels of aglycones, as a result of enzymatic hydrolysis during fermentation (Wang and Murphy, 1994).

2.2.2. CHEMICAL STRUCTURES OF ISOFLAVONES DAIDZEIN, GENISTEIN AND GLYCITEIN



(NTP-CERHR-GENISTEIN-06)

Figure 2.3:A- Genistein and B- Genistin (genistein glucoside)

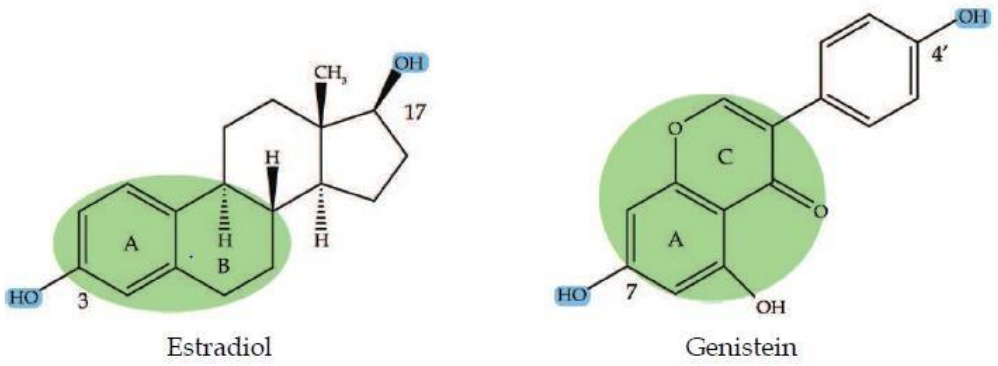
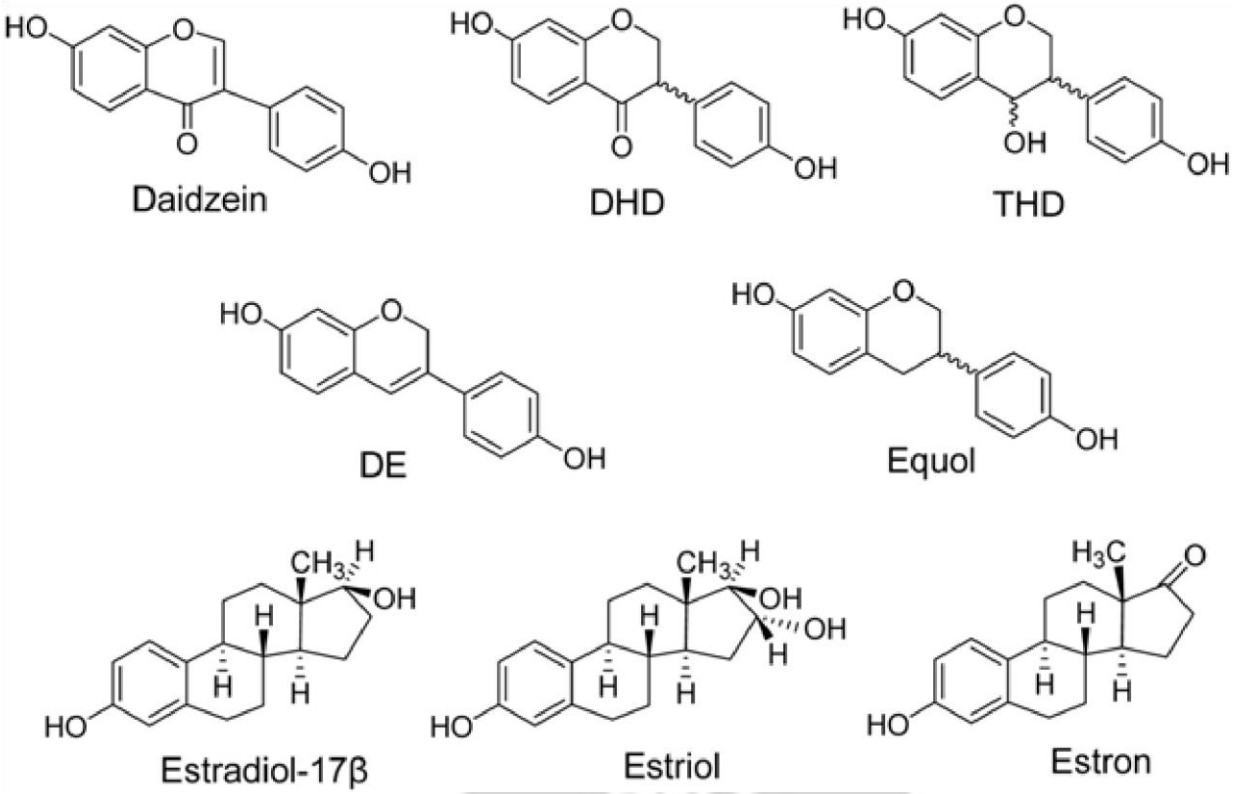


Figure 2.4: Structures of estradiol and genistein **(Tepavcevic*et al.*, 2011)**



(Kim *et al.*, 2008) Figure 2.5: Chemical structures of Daidzein, its metabolites, and three human estrogens

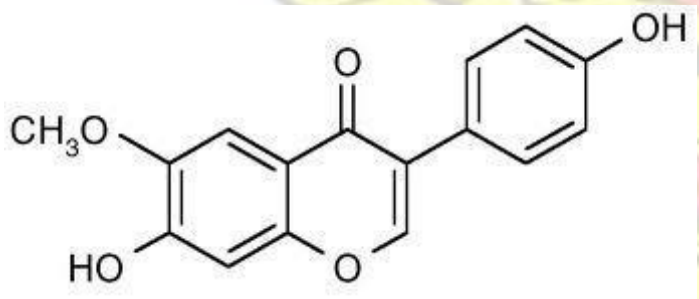


Figure 2.6: Chemical structure of Glycitein (Kim et al., 2008)

2.2.3. ISOFLAVONE GENISTEIN

The Chemical Abstracts Service registry number for genistein is 446-72-0 (ChemIDplus, 2004). Synonyms for genistein include 4', 5, 7-trihydroxyisoflavone, 5, 7, 4'trihydroxyisoflavone, genisterin, prunetol, and sophoricol. Genistein glucoside is called genistin (Figure 2.3). The term "total genistein" refer to genistein aglycone and its conjugates (ChemIDplus, 2004).

The molecular formula for genistein is $C_{15}H_{10}O_5$, and the molecular mass is 270.241 (Chemfinder, 2004). Genistein has a hydroxy group in the 5 position, giving it three hydroxy groups. Isoflavone genistein is comparable in structure to human estrogens therefore usually performs its genetic functions by estrogenic receptor s (ER) modulations (Cederroth *et al.,* 2011). From (Figure 2.4), it can be observed that genistein and estradiol have similar chemical structures and that genistein rings A and C are comparable to rings A and B of estradiol and also in both molecules, the interval amid the hydroxyl groups is virtually the same. The attraction of Isoflavone to the receptors of estrogen brings about several changes to estrogens regulated functions which includes central nervous, reproduction, skeleton, cardiovascular and metabolic systems

A major trait of Isoflavones is the ability to connect equally to the two types of estrogen receptors thus ER α and ER β particularly to receptor β. Such precise linkage to recptors of estrogen gives room for it to execute estrogen and anti- estrogen fuctions based on the nature of the cells and the underlying levels of estrogen (Kupier *et al*., 1997). Isoflavones in assessment with biological estrogens, are extremely feeble estrogens that have from 10^{-2} to 10^{-4} functions of 17 β-estradiol on molar basis (Song *et al*., 1999).Therefore, phytoestrogens can go about as feeble estrogen agonists, incomplete agonists, or as adversaries to endogenous estrogens, (for example, estradiol) and xenoestrogens with estrogen receptors in both animals and humans.). In this manner, filling in as estrogen mimics, these plant phytoestrogens could both have the same results as estrogen or block its effects (Barlow *et al*., 2007).

2.2.4. ISOFLAVONE DAIDZEIN

Daidzein [7, 4'-dihydroxyisoflavone] (Figure 2.5) and its glycosides are one of the major isoflavones in soybean with a molecular formula of $C_{15}H_{10}O_4$ and molar mass of

254.23g/mol. According to data from USDA (2002), total Isoflavone levels generally in soybeans are 6 percent glycitein, 57 percent genistein and 37 percent daidzein. 41.7 percent daidzein is found in soy germ (Zhang *et al*., 1991). Genistein, daidzein and their ßglucoside conjugates are found at high concentrations up to 3mg/g in soy beans (Coward *et al*., 1993). Estrogenic action of daidzein is known to be less vital than genistein (Verma and Goldin, 1998). Conversely, daidzein, in the course of the reductive metabolism,is transformed into its metabolites, including DHD (dihydrodaidzein), cis- and trans-THDs (tetrahydrodaidzeins), O-desmethylangolensin, DE (dehydroequol), and equol, by the intestinal microflora after ingestion (Kelly *et al*., 1995).Fascinatingly, amongst the metabolites of daidzein, (3S)-equol has about 100 times elevated estrogenic activity than the daidzein itself (Hwang *et al*., 2006).The molecular structures of daidzein, its metabolites, and three human estrogens are given in Figure 6.

2.2.5. ISOFLAVONE GLYCITEIN

The soybean is the most abundant source (Kudou *et al*., 1991) of isoflavones in nature. Soybean contains three types of isoflavone aglycone daidzein, genistein and glycitein. Daidzein, genistein and their glycosides contribute to >90% of total isoflavone; whereas glycetein and its glycoside are present as minor component <10% (Wang and Murphy, 1994).

Glycitein [4‘,7-dihydroxy-6-methoxyisoflavone] (Figure 2.6) accounts for 5−10% of the total isoflavones in soy food products with a chemical formula $C_{16}H_{12}O_5$ and molecular weight of 284.3g/mol (Song *et al*., 1999).

2.2.6. BIOLOGICAL ROLES OF PHYTOESTROGENS

The roles of phytoestrogens biologically are plant protection from hassle and to act as defensive mechanism for the plant. Some ecologists hypothesize that phytoestrogens may

have been developed to interfere with the reproductive system of grazing animals in an attempt to protect the plants (Hughes, 1998).

Phytoestrogens were first associated with undesirable effects on mammalian development and fertility from observations of animals consuming phytoestrogen-rich plants. Ewes feeding on Australian clover developed abnormal plasma concentrations of endogenous hormones with subsequent loss of fertility (Bennett *et al.*, 1946), a syndrome termed as "Clover Disease".

Soy isoflavones examines in vitro have uncovered that they can tie to estrogen receptors and go about as aggressive agonists or enemies to endogenous estrogens relying upon relative focuses and affinities (Axelson *et al*., 1984). Moreover, they can control endogenous steroid digestion system by hindering 17β – hydroxysteroid oxidoreductase Type 1, which is the protein in control for changing over generally inept estrone to the more powerful estradiol and, to a lesser degree, androstenedione to testosterone.

2.2.7 ENDOCRINE DISRUPTING CHEMICALS

Different sorts of endocrine disrupting chemicals (EDCs) continuously bombard the general human population. Concerns about the long haul results of introduction to these chemicals have heightened because of the possibility to disturb the hormonal framework and subsequently result in adverse health effects (IPCS/WHO, 2002). Endocrine disrupting chemicals are extremely diverse compositionally and incorporate manufactured natural mixes, for example, pesticides (e.g. methoxychlor, organophosphates, dichlorodiphenyl-trichloroethane or DDT), fungicides (vinclozolin) pharmaceutical operators (diethylstilbestrol, DES), dioxins, plasticizers (phthalates), plastics (bisphenol An or BPA), polychlorinated biphenyls (PCBs), fire resistant polybrominated diphenyl ether (PBDE),

and antifoulant paint added substance (tributyltin), and also common plantdetermined EDCs termed phytoestrogens (Cederroth *et al*., 2012).

With the definition of EDCs, phytoestrogens and isoflavones fit well into it since they have the capacity to tie and actuate estrogen receptors (ERs). EDCs substances are "exogenous agents that get in the way with metabolism, transport, secretion, transport, restricting activity, or elimination of intrinsic hormones in the body that are in charge for synthesis, reproduction, homeostasis, development and behavior". Once expose to EDCs, fertility could be altered by disturbing some reproduction phases including gamete production, onset of puberty, sex-dependent manners, sexual development, pregnancy, lactation and testicular and ovarian endocrine functions (Cederroth *et al*., 2012).

2.3. SOYBEAN NUTRITIONAL PROPERTIES

Proteins, lipids, minerals and some vitamins are the main essential nutrient constituents of soybeans. Collectively, about 60% of dry soybeans by weight is accounting for soybean oil and protein content (Oil at 20% and protein at 40%). On average the protein content in commercial cultivars is approximately 40% (ranging from 34% to 48%) depending on the genotype, growing environment and cultural practices of the crop. The remainder consists of 35% carbohydrate and nearly 5-6% ash which is its mineral concentration index. Potassium normally commended for the treatment of hypertension is in the uppermost concentration of (2.3%) in soybean. Moreover, other key minerals – Magnesium (0.3%), Calcium (0.2%) and Phosphorous (0.6%) are also found in the soybean (Kumar *et al*., 2010). Dietary fibre, omega-3 essential fatty acids, vitamins B and some important food components are rich in whole soy foods. Nutritionally, carbohydrates play a minor role, even though they are major components quantitatively. This is as a result of the fact that soybeans are consumed mainly for its rich levels of protein as opposed to the carbohydrate they contribute to the human diet (Lokuruka, 2010).

Foodstuffs from animals having whole proteins yet have a tendency to contain more fat, particularly immersed fat-without requiring significant conventionalities somewhere else in the eating regimen can be replaced by soy protein because they offer a complete profile of protein. Moreover, with the exception of methionine which cannot be synthesized by the human body, all the essential amino acids are contained in soybeans (Lokuruka, 2010).

2.3.1. THE PROTEIN CONTENT OF SOYBEAN

Protein is one of the most important components of soybean, mainly because is composed of amino acids which supplements the amino acids of cereals. Amino acids containing sulphur are very essential for fairly all animal species as well as humans but are limiting in soybean, however, soybean have adequate lysine to overpower the lysine deficiency of cereals (Potter *et al*., 1995). Mixture of soybean-rice can be used to complement methionine and lysine, and this could help clarify the thriving use of soy protein foodstuffs such as soybean curds associated with rice eating cultures of Asia. Soybeans contain 3844% proteins whereas other legumes have 20-30% protein content, and even much better than the 8-15% protein content of cereals (Synder *et al*., 1998). This high content of protein in soybeans builds their worth as edible food items and is the main basis for the economic advantage of soybeans over some oil seeds.

2.3.2. SOYBEAN MINERAL

Ash is the inorganic residue obtained by burning a sample at 500 $^{\circ}$C -600°C. Ashing of a feed sample burns off all organic constituents, leaving behind the non-volatile mineral elements. Dry-ashing is used to determine the total ash content as well as the concentration of the individual nutrient elements in plant materials (ASTM, 2003). Phosphates, sulphates and carbonates are the main forms of minerals in soybean. Minor minerals comprise of selenium, copper, molybdenum, cobalt, arsenic, lead, silicon, cadmium, iron, iodine, chromium, zinc, manganese, fluoride and mercury. They fall within the range of 0.01-140

ppm. Majority of these mineral deposits are held to meal as opposed to taking after the oil. Growing location, variety of soybean and season has influence on the mineral content of soybean (Kumar *et al.*, 2010). Soybean contains both water-soluble and oil soluble vitamins. The water- soluble vitamins (thiamin, riboflavin, pantothenic acid and niacin) are not lost during oil extraction. Pantothenic acid and niacin are generally prescribed for controlling high blood pressure. Mature soybean contains almost negligible amount of vitamin C (ascorbic acid). Above all, the newly discovered vitamin pyroloquinoline quinine, a water-soluble vitamin that is being judged as a new member of the vitamin B family performs the most important role in lysine metabolism and has been reported to be present in some soy foods such tofu and natto (Kumar *et al.*, 2010). However, bioavailability of minerals from utilization of animal foods is healthier than from plant foods (Cook *et al.*, 1981).There is a very much interaction of zinc, phytate and calcium in soy foods to form an insoluble compound, thereby reducing the assimilation of zinc to a larger extent than phytate alone. Again, the iron haeme in animal diets is easily accessible than the non-haeme iron like that in soybean (Cook *et al.*, 1981).

2.3.2.1. CALCIUM

Calcium is mainly the most plentiful mineral in the body and sums up 1.9% of the total body weight. Almost all (about 99%) can be found in the skeleton. The remaining can be located in the extracellular fluid (0.06%), the plasma (0.03%), teeth (0.6%) and the soft tissues (0.6%). Rigidity thus structure and strength of the skeleton is offered by calcium. Hydroxyapatite [$Ca_{10}(OH)_2(PO4)_6$] crystals which are entrenched in collagen fibrils is a form of calcium phosphate providing this firmness (Nordin, 1997).A large ion exchanger can be formed as a result of an interaction between calcium ions on bone surfaces and those ions in body fluids.

This is vital in relation to the function of bone as a reservoir of calcium to help maintain a stable concentration of calcium in the blood (Gurr, 1999). Calcium in blood performs an important task to control vital body processes such as muscle contraction, blood coagulation, nerve mediation and transmission and a few hormonal activities across cell membranes. Foods rich in calcium besides dairy products and milk are beans and its products including "tempeh" and "tofu" (fermented soybeans), yellow dhal, fish with edible bones such as anchovies and canned sardines. Processed foods locally as shrimp paste as well as vegetables like mustard leaves, spinach, broccoli, tapioca leaves and watercress also contains calcium (Tee *et al.*, 1997). However, calcium is not entirely absorbed when taken with foods high in fiber such as cereal bran's.

Presently, producers of food have also made accessible in the market products which are calcium fortified such as breakfast cereals, high-calcium milk, biscuits, yogurt and also rice. The concentration of calcium needed by individuals depends mostly on their age. Children require higher calcium content for the development of bones and teeth properly. As adult's advances in age, calcium is imperative to prevent osteoporosis and sustain bone health, hence intake of soy could avert osteoporosis by providing a high-quality supply of calcium. Poor absorption and insufficient intake of calcium result in calcium exclusion from bones to be used for various vital functions in the body (Gurr, 1999). Table 2.1 beneath shows levels of calcium based on age.

Table 2.1: Dietary Reference Intake Values for Calcium by Life Stage Groups

Life Stage Group	Adequate Intake(mg/day)
Children (4-8 years)	800 mg/day
Children (9-18 years)	1300 mg/day
Adults (19-50 years)	1000 mg/day
Older Adults (51-70 years)	1200 mg/day

Senior Adults (> 70 years)	1200 mg/day

(IOM, 1997)

2.3.2.2. MAGNESIUM

Magnesium is naturally present in many foods and abundant mineral in the body. It is added to other food products, available as a dietary supplement, and present in some medicines such as antacids and laxatives. Magnesium is a cofactor in more than 300 enzyme systems that regulate diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control, and blood pressure regulation (IOM, 1997).

Magnesium again plays a function in the vigorous transport of potassium and calcium ions across cell membranes, a procedure that is important to muscle contraction, nerve impulse conduction and normal heart rhythm (Rude *et al*., 2012).

Magnesium is commonly circulated in beverages, animal and plant foods. Analyses from the 1989 Total Diet Study of the U.S. Food and Drug Administration indicated that approximately 45 percent of dietary magnesium was obtained from vegetables, fruits, grains, and nuts, whereas about 29 percent was obtained from milk, meat, and eggs (Pennington and Young, 1991).

Foodstuff processing methods which involve grains refining reduces the magnesium content significantly as a result of taken away the germ and bran which is nutritionally rich. Hence polished foods usually contain the lowest content of magnesium (IOM, 1997). Food items as unrefined nuts and grains comprise higher content of magnesium, while starches, meats and milk are mostly intermediary. Green leafy vegetables, such as spinach, legumes, nuts, seeds, and whole grains, are good source (Rude *et al*., 2012). Basically, foods with dietary fiber supply magnesium.

Symptoms of magnesium insufficiency owing to lower dietary consumption in hale and hearty people are rare since excretion of this mineral in urine is limited by the kidneys (Rude *et al*., 2012). Too much dietary magnesium in healthy folks does not have a health risk since the excess is eliminated by the kidneys in urine (Musso, 2009). Nevertheless, higher intake of magnesium from medications or food supplements usually leads to diarrhea which could be associated by abdominal cramping and nausea (IOM, 1997). The diarrhea and laxative effects of magnesium salts are due to the osmotic activity of unabsorbed salts in the intestines and colon and the stimulation of gastric motility (Natural Medicines Comprehensive Database, 2013).

2.3.2.3 pH

The composition and chemistry of a food dictates the microorganisms that will grow well on it. The chief compositional factors that influence microbial activity are pH, moisture, available oxygen, nutrients, and the presence of natural inhibitors (Rahman, 2007). pH is an expression of hydrogen ion concentration in water. Specifically, pH is the negative logarithm of hydrogen ion (H^+) concentration (mol/L) in an aqueous solution (Springer, 2006). The value of pH for neutral, acidity and alkalinity conditions are 7, less than 7 and more than 7, respectively (O'Mahony, 1998). Foods with a low pH (below 4.5) taste acidic and are not readily spoiled by bacteria. Thus adding vinegar to soymilk dressing will increase its shelf life (molds grow best in foods with low pH (Shurtleff and Akiko, 2000)). pH measurements are often used as acceptance tests for milk. Milk contains a large number of a weak acid or a weak base in its salt. So it behaves as a buffer solution. As milk is a buffer solution, considerable acid development may occur before the pH changes. pH values lower than 6.5 indicates considerable acid development has taken place. This normally is due to bacterial activity (O'Mahony, 1998).

2.4 MICROBIAL PROFILE OF SOY

Soymilk and powder is widely consumed owing to its high protein quality and content. Snack, like most ready to eat foods and drinks, can serve as common vehicle in transmission of infections (Ikuomola and Eniola, 2010).

Food inspectors classify foods into four groups: non-perishable, semi-perishable, perishable and potentially hazardous. Perishable foods have a shelf life of several weeks or less and are subject to biochemical change from natural enzymes or enzymes secreted by invading microorganism. Potentially hazardous foods are a subcategory of perishable. They can support rapid and continuous growth of pathogenic microorganisms and many are moist protein foods such as meat, eggs, milk products, and other foods labeled "Keep Refrigerated'. Soy milk is definitely perishable and probably will be classified as potentially hazardous (Jay *et al*., 2000).

The main causes of soy milk and powder contamination are microorganism of four basic types; bacteria, yeasts, molds and viruses. In soy milk and powder preparation, virtually all of the problems are caused by bacteria although underwater fungi can also cause spoilage (Chapra, 1997).

Bacteria are the smallest, oldest and most abundant group of microorganism in the world combined, despite their extremely small size. Bacteria can be grouped into three according to their deleterious activities. Those that produce toxins, which can cause food poisoning, those that that can cause infection in the human body, and those that simply cause food spoilage without ceating pathogens (Lynch and Hobbie, 1998). The main problem with microorganisms in soy milk is spoilage, rather than toxicity or infection. Moist foods like soy drink are usually spoiled by bacteria not yeast or molds. Spoilage can occur at any temperature between -5 and 70°C (Hurst *et al*., 1997).

2.4.1. TOTAL COLIFORMS

The coliform bacteria group consists of several genera of bacteria belonging to the family enterobacteriaceae. These mostly harmless bacteria live in soils, water and the digestive systems of animals (Leclerc *et al*., 2001). Coliforms or other bacteria in drinking water and food will not necessarily make a person ill. However, since these organisms are present, other disease-causing organisms may also be present (Rompre' *et al*., 2002). Total coliform is the collective name used for all coliform groups. A large group of rod-shaped, gram – negative, bacteria that have in common a number of characteristics are referred to as total coliforms. T The natural resources for individuals from this category fluctuate from being fecal precise, for example, E. coli to being broadly scattered in soil, water and vegetation (Leclerc *et al*., 2001). The group consists of bacteria of faecal origin, thermotolerants coliforms and also diverse bacteria that may be isolated from resources in the environmental (APHA, 2005).

Thermotolerant coliforms are sub group of the total coliforms group having the capability for the fermentation of lactose at 44-45°C formerly stated as faecal coliforms. They usually served as faecal pointers given that they were viewed most as faecal precise than total coliforms. By explanation, thermotolerant coliforms comprise the part of the total coliform pool that produces a blue colony on m-FC broth within 24hours at 44.5°C or able to form gas within 24hours at 44.5°C (APHA, 2005). This faction includes the faecal specific *Escherichia* genera and also organisms originating from faecal and non-faecal environs including *Enterobacter*, *Klebsiella* and *Citrobacter* (Edberg *et al*., 2000).

2.4.2. FAECAL COLIFORMS

Faecal coliforms are a subset of a larger group of organisms known as coliform bacteria. Faecal coliforms in general live in the intestinal tract of warm-blooded animals. Faecal

coliforms are short-lived once outside of a warm-blooded host as compared to the coliform bacteria that are free-living and not associated with the digestive tract of man or animals (Bergey and Holt, 1994).

The faecal group consist of both disease-causing (pathogen) and nonpathogenic bacteria. *Escherichia coli* or *E. coli* is an exemplar of faecal coliform bacteria grouping. The occurrence of faecal coliforms pinpoints fecal contamination. It also indicates the presence of possible enteric pathogens (disease causing organisms which originate in the digestive system), particularly bacterial pathogens (Blanch, 2007).

The term faecal coliforms indicate coliform organism which grow at 44 or 44.5°C and ferment lactose to produce acid and gas in water microbiology. Hence the existence of total coliforms might or might not specify faecal contamination. In severe cases, a high total coliform count could be linked with a lower, or zero count for the thermotolerant coliforms. Results of this nature might not even designate the existence of faecal coliforms. However the occurrence of thermotolerant coliforms almost at all times point to faecal contamination. The quantity of faecal coliform bacteria is an excellent sign of the amount of pollution presents (Bartram and Rees, 2000).

2.4.3. *Escherichia Coli*

Escherichia coli is a distinct form of faecal coliform, that thrives well in humans and other warm blooded animals intestines and in their waste. *E coli*, the existence of which is definite proof of faecal contamination and may indicate the possible presence of diseasecausing pathogens, such as bacteria, viruses, and parasites. Although most strains of *E. coli* bacteria are harmless, certain strains, such as *E. coli* 0157:H7 may cause severe disease and may be fatal in small children and the elderly (APHA, 2005). *E. coli* is a rod shaped bacteria that is distinguished by its inability to break down urease. These bacteria are a preferred indicator

for freshwater recreation and its presence provides direct evidence of faecal contamination from warm-blooded animals. Although usually harmless, *E. coli* can cause illness such as meningitis, septicemia, urinary tract infection and intestinal infections (Edberg *et al*., 2000). Figure 2.7 gives an illustration of coliforms.

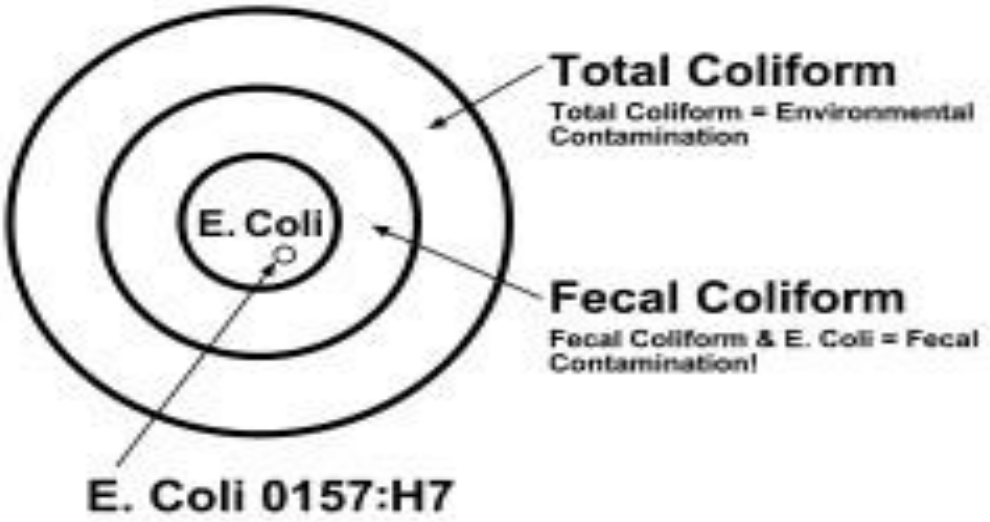


Figure 2.7: Illustration of Coliform bacteria (Health, 2013)

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY AREA

The study was conducted in the Kumasi Metropolis, the capital of the Ashanti Region of Ghana which is third largest of the ten administrative regions in Ghana, occupying a total land surface of 10.2 per cent of the total land area of Ghana (Ghana Statistical Service, 2012). According to the 2000 census, it is the most populated region accounting for 19.1 per cent of Ghana's total population (Ghana Statistical Service, 2012). Kumasi is the second largest city in Ghana and located in the middle belt of the country. The city lies within the tropical rainforest zone between the northern and southern savanna zones of Ghana (Latitude 6°30' and 7°00' North and Longitude 1° 30' and 2° 00' West).It is humid with relative humidity ranging between 53% and 93%. The vegetation is primarily of tropical moist semi-deciduous forest type. The climate is marked by a fairly high incidence of solar radiation with temperature ranging from a minimum of 20.2°C to a maximum of 37.1°C on the average. The rainfall pattern is seasonal (the rainy (wet) season and the dry (Harmattan) period) with an annual rainfall varying between 127cm and 165cm. (Meteorological Services Department, 1994).

It's gorgeous outline and vegetation has given it the great compliment of being the "Garden City of West Africa". The metropolis is hastily on the increase with an annual growth rate of 5.47 per cent (Ghana Statistical Service, 2012). It comprises of about 90 suburbs, a lot of which were engrossed into it as a consequence of the process of physical expansion and growth.

The major occupation in all the districts is Agriculture, Animal Husbandry, Forestry, except in the Kumasi metropolis, where Sales work predominate. Residents in the rural areas are mostly in Agriculture whereas those in urban areas are mainly in industry, commerce and the service sector (Ghana Statistical Service, 2012). The Kumasi metropolis alone accounts for nearly one-third of the region's population. The proportion of the economically active population varies from 71.4 to 85.2 per cent. Majority of the economically active population are self-employed, mainly in the private informal sector, which provides job opportunities, particularly for females with little or no formal education (Ghana Statistical Service, 2012).

The economic status of women on the average is relatively low hence they face the challenge of malnutrition during and after pregnancy.

The availability of locally produced soy drink and powder which is believed to be nutritious, affordable and economical has provided some tentative solution to most women in the metropolis.

3.2. SELECTION OF SAMPLING SITES

The areas of interest to this study were vendor/processing outlet at Kumasi hospitals as C. Hospital, T. Hospital, K. Hospital, also market as C. market, and an eatery as Veg. diet Restaurants. The three hospitals were chosen on purpose because they have maternal and child health care units where mothers and pregnant women are introduced to soy products, its health benefits and various delicacies of the product. C. market and the two outlets of Veg. diet restaurants were also selected because they are known for the sale of soymilk, powder and other soy product.

3.3. SAMPLING DESIGN AND SAMPLE COLLECTION

a. Sampling of Soy drink.

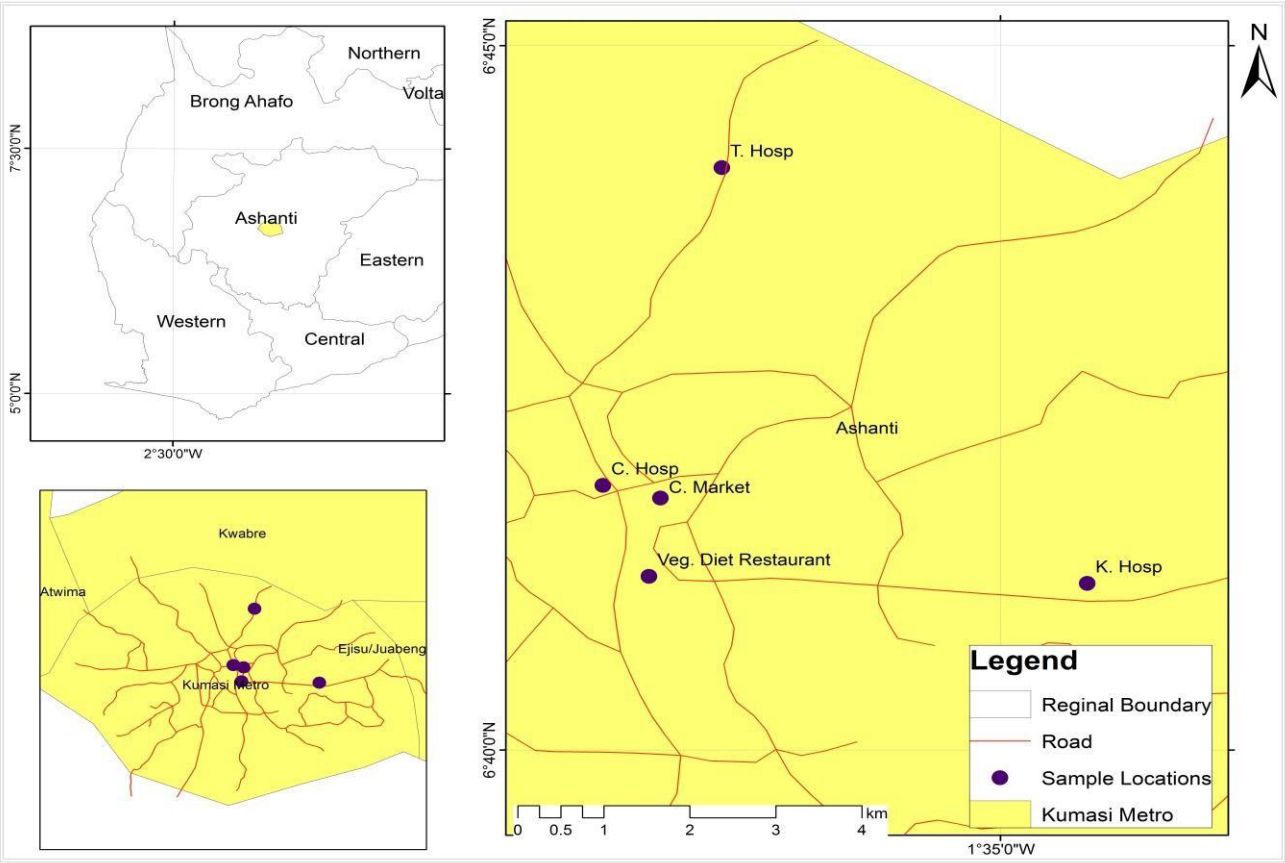
From the preparation of soy drink, three sampling points where taken along the production line. The sampling points were;

i. **Before boiling**; thus after milling when it is pressed to decant, the liquid obtained.
ii. **After boiling**; the liquid obtained after sieving is boiled for 35 or 40 minutes at 90100°C while stirring and the suspension of a liquid layer that forms on the surface is taken off. iii. **End product**; when it is served with sugar, honey and other flavours depending on the customer's choice.

These three sampling stations were selected along the production line to give adequate spatial coverage and to represent the variety of conditions in the soy drink and the production premises. At each sampling point, two samples were collected into sterile plastic bottles with a ladle (300ml each) twice in a month over a six month period of the study. The samples were labeled and placed on ice and sent to the laboratory for analysis. The samples were used for analysis on phytoestrogen levels, microbial profile and the nutritive value of the soy drink.

b. Sampling of Soy Powder

From the various hospitals and sale center, two samples were randomly purchased twice a month for a six month period. Samples were labeled and sent to the laboratory for storage and analysis on phytoestrogen levels, microbial profile and the nutritive value.



(Source; Wildlife Department, 2016)

Figure 3.1 A map showing the Sampling locations at the study site.

3.4. LABORATORY ANALYSIS

The soy drink and powder samples collected from the various sampling locations were analyzed for the chemical parameters (pH, genistein, daidzein and glycitein), nutritional value (ash, calcium, magnesium and protein) and microbial parameters (total and faecal coliforms and *E coli*) using suitable standardize methods (APHA, 2005). The laboratory analyses were done at Department of Chemistry, The Faculty of Renewable Natural Resources Laboratory and the Microbiological Laboratory at the Department of Theoretical and Applied Biology, all of Kwame Nkrumah University of Science and Technology (KNUST).

3.5. CHEMICAL ANALYSIS OF SOY DRINK/POWDER

The samples were analyzed for pH, genistein, daidzein and glycitein using the pH/Cond 3400i/SET pH meter and the Varian Prostar HPLC using the appropriate standard method.

3.5.1 pH

The pH of the samples was measured using the pH/Cond 3400i/SET pH meter. The pH meter was first calibrated using standardized buffer 4, 7 and 10 solutions respectively. Enough soy drink was poured into a beaker so that the tip of the probe could be submerged in the sample. The probe was left in the sample for the meter to attain equilibrium before the pH readings were taken (APHA, 2005). Five (5) grams of the soy powder was weighed into a 100ml beaker and 25 ml distilled water was added. The suspension was stirred vigorously for 20 minutes. Soy powder – water suspension was left to stand for 30 minutes after which it was stirred and the pH electrode inserted to read the pH values (APHA, 2005). 3.5.2 DETERMINATION OF GENISTEIN, DAIDZEIN AND GLYCITEIN CONTENT OF SOY (AOAC 2001.10).

Extraction and Saponification

Soy drink

Five milliliters of each sample was measured into 250 mL Erlenmeyer flask and extracted with 40 mL extraction solvent (Methanol: water =80:20). The neck of the flask was covered with aluminum foil and flask shaken in a 60°C water bath for 2 hours. It was cooled to room temperature and 3mL of 2M NaOH solution added. The aluminum foil was replaced and flask shaken at room temperature on an orbital shaker for 10 minutes. Five milliliters of the extraction solvent was added to the extract, mixed well and filtered through a quantitative-grade filter paper into a 250mL beaker

Soy Powder

One gram of each sample was weighed and 40mL of extraction solvent added. After extracting for 2 hours at 60°C, the mixture was then allowed to cool to room temperature and 3mL of 2 M NaOH solution added. It was shaken for 10 minutes on an orbital shaker after which 5mL of the extraction solvent added. The flask was swirled to suspend its content and filtered through a quantitative-grade filter paper into a 250mL beaker.

3.5.2 DETERMINATION OF GENISTEIN, DAIDZEIN AND GLYCITEIN BY HPLC

Ten milliliters of each filtrate (extract) from soymilk and soy powder were measured into a 15mL centrifuge tubes and centrifuged at 4000RPM (Revolution per minute) for 10 minutes. The clear supernatant was transferred into a high performance liquid chromatography (HPLC) sample vial. Each centrifuged extract was diluted as follows; 0.2mL of the extract was taken and diluted with 3mL of the extraction solution. Isoflavone (genistein, daidzein and glycitein) were separated on a C18 reversed-phase column with a methanol–water – acetic acid (186:10:4) mobile phase at a constant flow rate and determined by UV detection at 260 nm. The system was allowed to equilibrate by running 1 complete gradient with no injection. The systems performance was verified by injecting 20µLof each working standards and the baseline separation of daidzein, genistein and glycitein peaks were also verified. 20µL of each test extract was injected into the HPLC using the syringe. The area and concentration of each isoflavone peak was determined. Results were expressed in aglycon units by summing the concentrations of the aglycon isoflavones genistein, glycitein, and daidzein (AOAC, 2002).

3.5.2.1 CALCULATION FOR AMOUNT OF ISOFLAVONEAGLYCONS

Soy drink (µg/mL)

Total amount of sample taken from the start is 5 mL of which 0.2mL of the initial (5mL) was taken for analysis. 3mL of the extraction solution was added to 0.2mL sample.

Therefore 0.2mL of the sample is contained in 3mL extraction solvent.

Total volume used for analysis= 3.2mL

Hence to get the concentration in 5mL;

$C_1V_1 = C_2V_2$ (Harvey, 2000)

$$C_2 = \frac{C_1 V_1}{V_2} \text{ (µg/mL)}$$

Where C_1 = Concentration in 0.2mL sample used in the analysis,

V_1= Total volume used for the analysis (3.2mL), and

V_2= Initial volume of the sample (5mL)

C_2= Concentration in the initial sample (5mL)

Soy powder (µg/g)

Total amount of sample taken from the start is 1 g and 0.2mL was used for analysis out of the initial 1g.3mL of the extraction solution was added to 0.2mL sample. Therefore 0.2mL of the sample is contained in 3mL extraction solvent.

Total volume used for analysis= 3.2mL .

The concentration obtained from the analysis is based on the 0.2mL used.

Therefore to get the actual concentration for the initial 1g used

$$C_2 = \frac{C_1 V_1}{V_2} \text{ (µg/mL)}$$

Where C_1 = Concentration in 0.2mL sample used in the analysis,

V_1= Total volume used for the analysis (3.2mL),

V_2= initial volume of the sample (1g), and

C_2= Concentration in the initial sample (1g)

3.6. NUTRITIONAL ANALYSIS OF SOY DRINK AND POWDER

Total Nitrogen was determined using the Kjeldahl digestion method (Okelabo *et al*., 1993), calcium and magnesium by EDTA titrimetric method (Moss *et al*., 1961). Ash content was also determined by the dry ashing method (AOAC, 1990).

3.6.1 SAMPLE PREPARATION

a. Soy Drink;

The soy drink samples were oven dried at 60 ℃ for 48 hours making it into solid form. Hence the nutritional analysis was done based on dry matter for the determination of Total nitrogen and ash.

b. Oven dried soy drink/Soy powder

Dry-ashing was used to determine the total ash content as well as the concentration of calcium and magnesium in soy drink and powder. This procedure involved ashing of the material to destroy the organic matter component leaving the various elements in the ash. One gram of the soy powder and oven dried soy drink were weighed into a porcelain crucible. The crucible was placed in a muffle furnace and heated at 500°C for four hours, after which it was removed from the furnace and cooled in a dessicator. The ignited residue was moistened with 2ml distilled water and slowly and carefully, 5ml of 8N HCl was added. The crucible was covered and placed on a steam water bath for 20mins. It was filtered through what man No. 42 filter paper, catching the filtrate in a 100ml volumetric flask. The crucible was washed well with distilled water, the washing going through the filter. It was made up to the 100ml mark with distilled water and shaken vigorously to ensure complete

mixing. The solution was then used for both the determination of calcium and magnesium by EDTA titrimetric method (Moss, 1961).

3.6.2 DETERMINATION OF CALCIUM AND MAGNESIUM IN SOY DRINK/POWDER

Calcium and magnesium determination by EDTA titration involves addition of several reagents (Moss, 1961). One ml of 0.02 N EDTA = 0.4 mg Ca and 0.24 mg Mg. EDTA complexes with Ca^{2+} and removes it from solution giving a blue end point in the presence of Ca^{2+}.

Determination of calcium

Five milliliter of sample solution from 3.7b was transferred into a 100 ml Erlenmeyer flask. 10 ml of 10 % KOH solution was added followed by 1 ml of 30% TEA. Three drops of 10 % KCN and few drops of EBT indicator solution were also added. The mixture was shaken to ensure homogeneity. The mixture was titrated with 0.02 N EDTA solution from a red to blue end point.

Calcium in mg = Titre value of EDTA x 0.40

$$\% \text{ Calcium} = \frac{\text{mg Calcium}}{\text{Sample weight}} \text{X } 100$$

Determination of magnesium

Five milliliter sample solution from 3.7b was emptied into a 100 ml Erlenmeyer flask. 5 ml of ammonium chloride – ammonium hydroxide buffer solution was added followed by 1 ml 30% TEA. Three drops of 10 % KCN and a few drops of EBT indicator solution. The mixture was shaken to ensure homogeneity. The mixture was titrated with 0.02 N EDTA solution from a red to blue endpoint.

Magnesium in mg = Titre value of EDTA x 0.24 (Moss, 1961).

$$\% \text{ Mg} = \frac{\text{mg Magnesium}}{\text{Sample wt}} \text{ x } 100$$

3.6.3 DETERMINATION OF THE ASH CONTENT OF SOY DRINK/POWDER

The ash content was determined using the dry ashing method. Five grams of soy sample was weighed into porcelain crucible in duplicate. It was put into the muffle furnace for 4 hours at 550^{o}C. After combustion, the furnace was allowed to cool below 200^{o}C and maintain for 20 minutes. The crucibles were removed from the furnace, placed in desiccator, cooled and weighed (AOAC, 1990).

Calculations

(A + B) – A = B

(A + C) - A = C

% Ash = C/B x 100 where A = crucible weight, B = sample weight, C = ash weight.

3.6.4 DETERMINATION OF TOTAL NITROGEN IN SOY DRINK/POWDER

Total Nitrogen was determined using the Kjeldahl digestion method. Two (2) grams of the sample was weighed into a 500ml Kjeldahl digestion flask and10ml of distilled water added. One digestion tablet (acts as catalyst) and 20 ml of concentrated H_2SO_4 were also added to the contents in the digestion flask. The mixture was heated strongly to digest the sample to a clear colour. The digest was cooled and transferred to a 100ml volumetric and made up to the mark with distilled water. A 10ml aliquot of the digest was transferred into a distillation flask and 90ml distilled water added. 20ml of 40% NaOH solution was added and placed in the distillation unit for distillation. The ammonium distilled (100 ml) was collected into a 250ml flask containing 10ml of 4% boric acid with mixed indicator of bromocresol green and methyl red. The distillate was titrated with 0.1 N HCl solution. A blank digestion, distillation and titration were carried out as a check against traces of nitrogen in the reagents and water used (Okelabo *et al.*1993)

Calculation

The N content of the sample can be calculated by the formula:

$$\% N = \frac{(a - b) \times 1.4 \times M \times V}{s \times t}$$

Where a= ml of HCl used for

sample b= ml of HCl used

for blank

1.4= $14 \times 10^{-3} \times 100\%$ (14=atomic weight of N)

M= molarity of HCl

V= total volume o digest

S= weight of sample taken for digestion in grams (2g)

t=volume o aliquot taken for distillation hence

% Crude Protein (CP) = Total Nitrogen (N_T) x 6.25(Protein factor)

3.7 DETERMINATION OF TOTAL AND FAECAL COLIFORMS OF SOY DRINK/POWDER

Total and faecal coliforms in the soy drink and powder samples were determined by using MPN (the Most Probable Number) method (APHA, 2005). Soy powder was prepared by adding one gram soy powder to 9 mls of sterile distilled water. One milliliter of soymilk and soy powder samples were pipetted into 9 mls of sterile distilled water to form 10^{-1} dilution. 1 ml aliquots were again pipetted from 10^{-1} dilution to form 10^{-2}serial dilution. 1 ml was also taken from 10^{-2} serial dilution to form 10^{-3}dilution and finally 10^{-4} serial dilutions was done by pipetting 1ml aliquot from 10^{-3} and 9 mls of distilled water (sterile) added.

One milliliter aliquots from each of the dilutions were inoculated into three different test tubes containing 5 mls of MacConkey Broth and incubated at 35^0C for total coliforms and another set was incubated at 44^0C for faecal coliforms both for 24 hours. Test tubes showing

colour change from purple to yellow were identified as positive for both total and faecal coliforms. Counts per 100 ml were calculated from Most Probable Number (MPN) tables (APHA, 2005).

3.7.1 *E coli* (Thermotolerant coliforms)

The Most Probable Number (MPN) was used to determine *E .coli* in the samples. From 3.7.0, each of the positive tubes identified from faecal coliform was used to determine *E. coli* in soymilk and soy powder. A drop was transferred from each positive tube into a 5 ml test tube of trypton water and incubated at 44^0C for 24 hours. A drop of Kovacs' reagent was then added to the tube of trypton water and the sample. All tubes showing red ring colouration development after gentle agitation indicated the presence of indole recorded as presumptive for themotolerant coliforms (*E. coli*). Counts per 100 ml were calculated from Most Probable Number (MPN) tables (APHA, 2005).

3.8. STATISTICAL ANALYSIS

The data were organized in Microsoft excel and the one way analysis of variance (ANOVA) was used to determine the significant differences ($p<0.05$) among treatments followed by Turkey's Multiple Comparison test to identify differences among groups. The results are presented as graphs and tables, and given as means ± SD. The GraphPad Prism 5.01 statistical software for Windows was used to execute all analysis whiles graphs were developed with Microsoft Excel® 2007.

CHAPTER FOUR

4.0 RESULTS

Generally, production of soymilk goes through five main stages of production whereas the powder undergoes three main processing stages. The means and standard deviations of the chemical, nutritional and biological parameters in soymilk are represented in Table 4.1. The protein content (dry matter basis) in the soymilk was relatively lowered along the production line ranging from 56.08 % ± 2.24 before boiling to 28.49 % ± 3.33 at the end of production. Isoflavone daidzein and genistein also increased from the beginning to the end of soy milk preparation ranging from 1.59 µg/g ±0.79 to 10.81 µg/g ±6.48 for daidzein and 40.13 µg/g ±7.04 to 58.51 µg/g ± 14.56 for genistein whiles calcium reduced by 64.71% after boiling but saw 15% increase at the end product. Magnesium was increase by 28.57% after boiling but was lowered by 61.57% at the end of soymilk production. Total and faecal coliforms were lowered by 94 and 97% after boiling but saw an increase (39 and 51%) at the end product respectively. However, *E. coli* count of 8.33 x 10^3±9.31 x 10^3 MPN/100ml before boiling was reduced to 0 after boiling and also in the end product. Soy powder recorded relatively higher protein content ranging from 39.99 % ±4.81 to 42.10 % ± 1.17. Again, soy powder recorded higher values for daidzein (25.42 ug/g±1.29-38.12 ug/g±14.15) and extremely higher values for genistein (154.64 ug/g ±6.96-170.63±40.68 ug/g). Protein, calcium, magnesium, daidzein and genistein content at the end product of soymilk were relatively lower compared to soy powder (Table 4.1).

Table 4.1: Means and Standard Deviations of the Chemical, Nutritional and biological parameters in soymilk

Chemical/Nutrition/ Microbial parameters	VEG. DIET-SOY DRINK				
	Before boiling	**(%)↑↓[#]**	**After boiling**	**(%)↑↓**	**End product**
Daidzein (µg/g)	1.59±0.79	**+50.31**	3.20± 1.10	**+70.40**	10.81±6.48
Genistein (µg/g)	40.13±7.04	**+18.37**	49.16±13.68	**+15.98**	58.51±14.86
Total (µg/g)	41.72±0.41	**+20.32**	52.36±20.26	**+24.47**	69.32±11.85
pH	6.85±0.11	**+2.00**	6.99±0.12	**-0.4**	6.96±0.06
Ash (%)	3.80±1.16	**+5**	4.00±0.89	**-45.45**	2.75±0.52
Protein (%)	56.08±2.24	**-4.74**	53.54±3.31	**-87.93**	28.49±3.33
Calcium (%)	0.28±0.09	**-64.71**	0.17±0.04	**+15**	0.20±0.06
Magnesium (%)	0.15±0.05	**+28.57**	0.21±0.14	**-61.53**	0.13±0.03
T C (MPN/100ml)	$3.38 \times 10^6 \pm 1.95 \times 10^6$	**-94.25**	$1.74 \times 10^6 \pm 2.40 \times 10^6$	**+38.95**	$2.85 \times 10^6 \pm 1.85 \times 10^6$
F C (MPN/100ml)	$1.90 \times 10^5 \pm 0.505 \times 10^5$	**-97.1**	$0.963 \times 10^5 \pm 1.05 \times 10^5$	**+51.36**	$1.98 \times 10^5 \pm 1.13 \times 10^5$
***E.coli*(MPN/100ml)**	$8.33 \times 10^3 \pm 9.30 \times 10^3$		0		0

N=6. RDA is the average daily intake level sufficient to meet the nutrient requirement of nearly all (97-98%) of healthy individuals in a group. RDA for Children between 13years**.** Protein -13% (13g/100Kg); Calcium- 0.7% (700mg/100g); Magnesium- 0.08 (80mg/100g); pH- 6.5-8.5; TC (Total coliform) -0; FC (Faecal coliform) -0; *E. coli0*WHO (2011) food quality guideline.

[#] ↓- Percentage reduction;↑-Percentage increase

4.1. PROCESSES INVOLVED IN SOY DRINK AND SOY POWDER PRODUCTION

a. Soymilk

Locally in Ghana, the processing method involves cleaning and picking of stones from the beans (stage one). It is soaked in water overnight in household utensils such as bowls, buckets and earthenware vessels (stage two). The soybeans are then thoroughly washed with clean water. Water is added to the beans and blended (Stage three). The slurry is poured into cheesecloth and pressed manually to sieve (stage four). The liquid obtained after sieving is boiled for 30 minutes while stirring. After boiling, it is left to cool and settle. A suspension of a liquid layer that forms on the surface is taken off [stage five] (Fig 4.1).

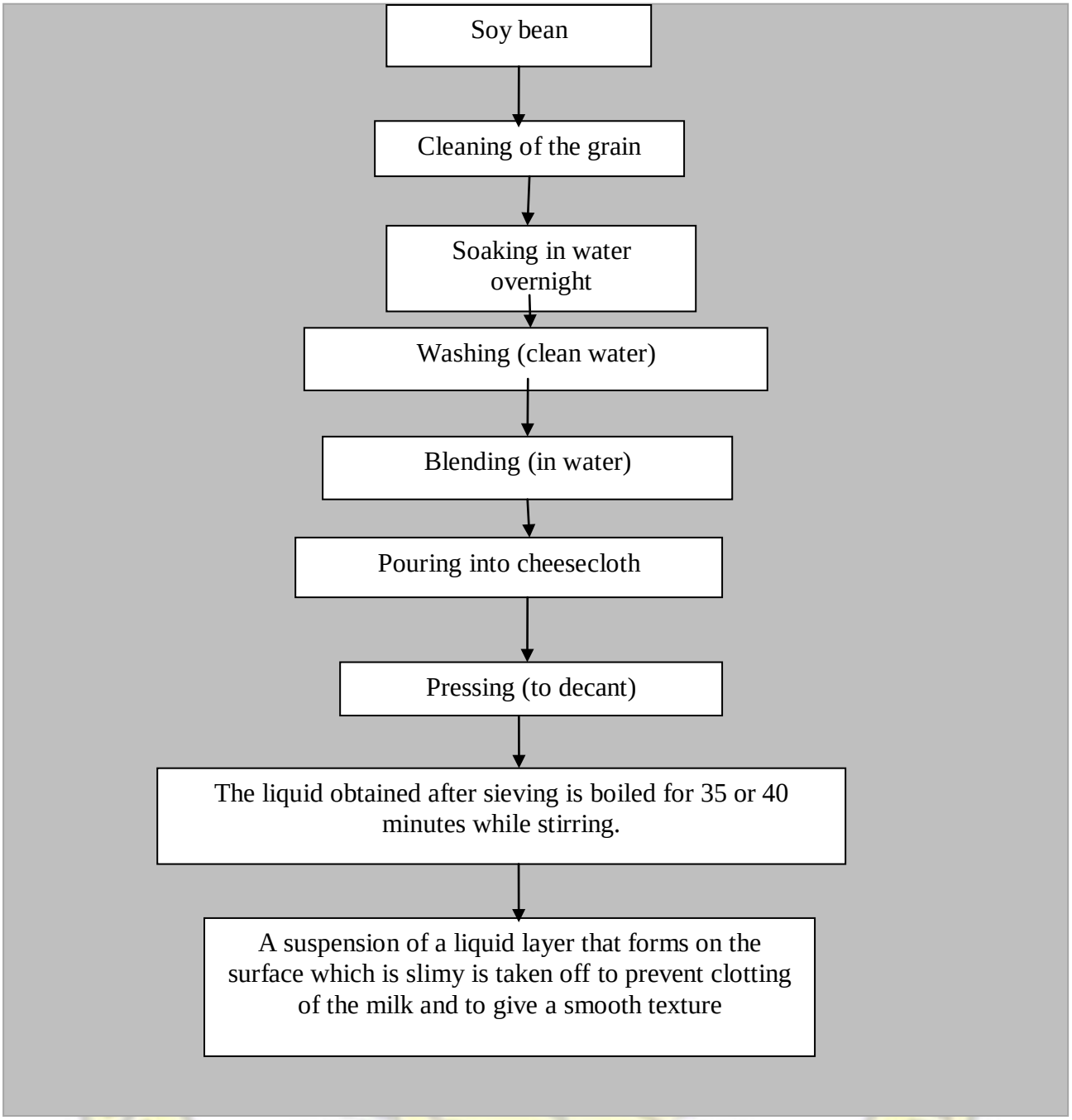


Figure 4.1: Schematic flow sheet for soymilk processing **(source, local producers)**

b. Soy powder

Initially (stage one) the soybean is cleaned and stones picked out from it. The beans are roasted and air blown to remove any chaff and particles (Stage two) after which it's milled dry [Stage three] and then allowed to cool overnight [Plate 4.1] (Figure 4.2).

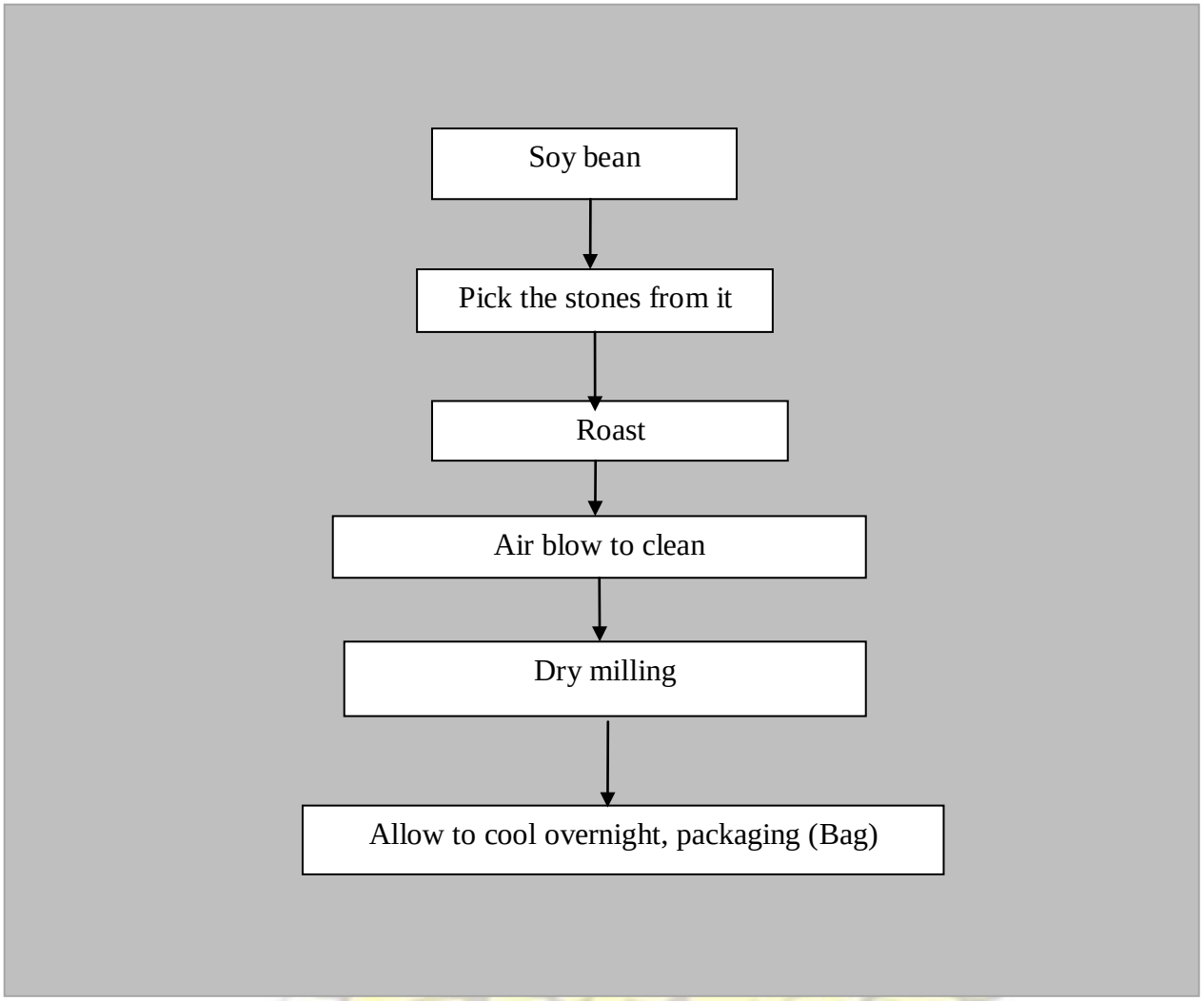


Figure 4.2: Schematic flow sheet for soy powder processing (source, local producers)

Plate 4.2: Soy powder, indicated by the arrows, allow to cool over night after milling.

4.2. Nutritional Quality Soy Powder and Soymilk

4.2.1 Calcium

The mean calcium values from soymilk production line ranged from 0.17% to 0.28%, the highest recorded mean value was observed before boiling whereas the least was observed after boiling (Figure 4.3).

The mean calcium values from the various sampling locations of soy powder ranged from 0.24% to 0.26%. The highest recorded mean value of 0.26% was observed at K. hospital whiles the lowest value was recorded at C. hospital, T. hospital and C. market respectively. The mean value of soymilk end product as compared with mean value of soy powder from the various locations, it was observed that end product of soymilk recorded the least value of 0.20% as against the highest mean value of soy powder (0.26%) which was observed at

K. hospital (Figure 4.3). There was no significant difference among the soy powder from the various locations. However, a significant difference occurred in the soy milk production line between before boiling and after boiling of soy milk (P= 0.0316)

[Appendix 2e]. All the mean values from the various sampling locations were below the Recommended Dietary allowance (IOM, 1997) of 0.7% (700mg/100g/dy) for children between the ages of one and three as indicated by the reference line (Figure 4.3).

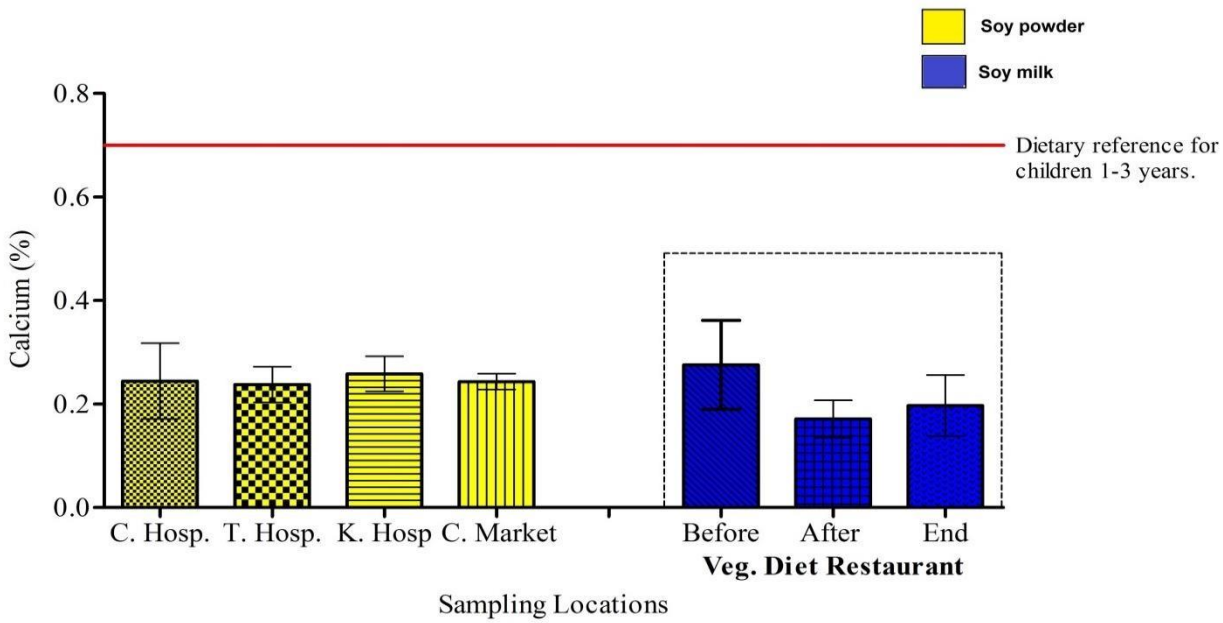


Figure 4.3: Calcium (%) content in soy powder and soymilk from the various sampling locations

4.2.2. Magnesium

The mean values recorded for magnesium from soymilk production stages ranged from 0.13% to 0.21%. The highest recorded mean value of 0.21% was observed after boiling whereas the least mean value of 0.13% was recorded at the end product (Figure 4.4). Soy powder from the sampling locations observed mean magnesium values ranging from 0.27% to 0.28%. The highest mean value of 0.28% was recorded at C. and T. hospitals whereas the lowest was observed at K. hospital and C. market respectively (Figure 4.4).

The mean values for soymilk end product and soy powder from the various locations

ranged from 0.13% to 0.28%. End product of soymilk recorded the least value of 0.13% whereas C. and T. hospitals recorded the highest mean value of 0.28% respectively (Figure 4.4).

There was no significant difference (P> 0.05) among the various powder locations and along the production line of the soymilk. All the mean values from the various sampling locations were above the Recommended Dietary allowance (IOM, 1997) of 0.08% (80mg/100g/dy) for children 1-3years as indicated by the reference line (Figure 4.4).

Figure 4.4: Magnesium (%) content in soy powder and soymilk from the various sampling locations

4.2. Protein

The mean protein (%) was found to vary from 28.49 % to 56.08% at the soymilk processing stages. The highest recorded mean value of 56.08 % was recorded before boiling whilst the lowest of 28.49% was also recorded at end product (Figure 4.5). Also, the mean protein (%) value from the various vendor locations of soy powder ranged from 39% to 42.10% with

the highest value observed at K. hospital and the least was recorded at T. hospital respectively.

End product of soymilk and soy powder from the various vendor locations observed mean values fromn28.49% to 42.10%. The highest mean value of 42.10% was recorded at K. hospital whereas the least was observed at the end product of soymilk. There was a significant difference along the soymilk production line ($P < 0.0001$) whereas no significant difference occurred among the powdered locations. The significant difference occurred between before boiling and end product of soymilk and also between after boiling and end product (Appendix 1h). All the mean values from the various sampling locations were above IOM's Recommended Dietary allowance of 13% (13g/100g)/day for children between 1-3 years of age (IOM, 2002; IOM, 2005)[Figure 4.5].

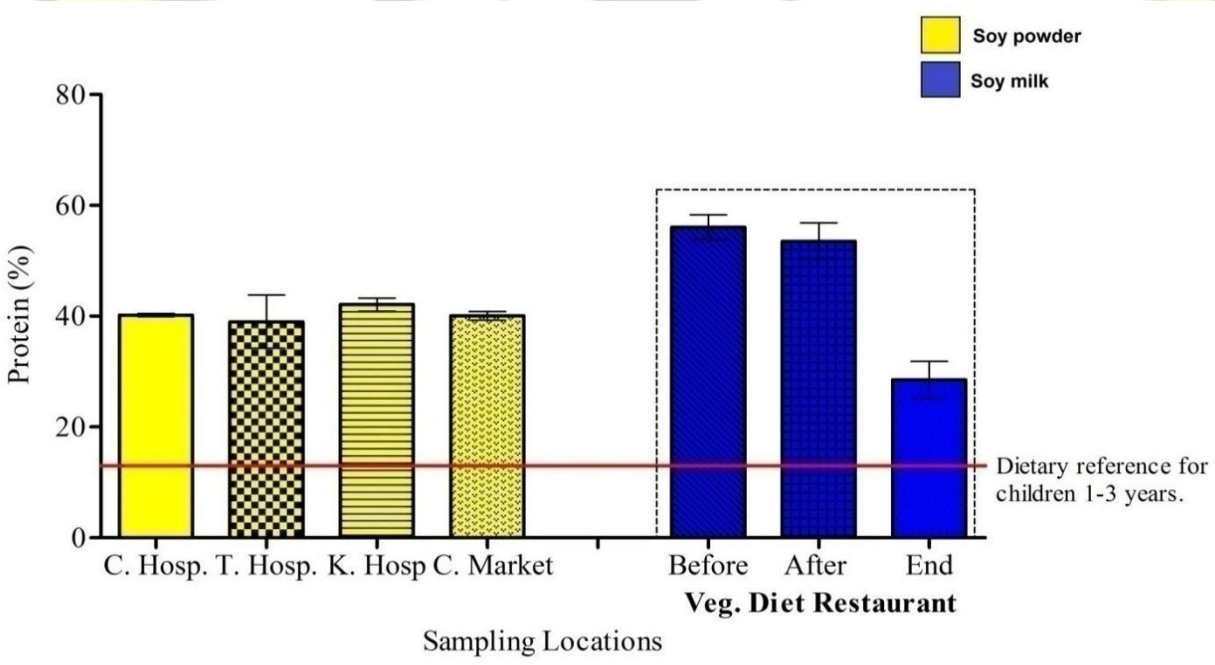


Figure 4.5: Protein (%) content in soy powder and soymilk from the various sampling locations

4.2.4 Ash

Figure 4.6 represents results of the ash content of soymilk processing stages in the study. The highest value recorded was after boiling (4.00 %) and the lowest mean value of 2.75% was observed in the end Product of soymilk. The mean values from soy powder sampling points recorded the highest value of 6.17% at K. hospital and the lowest mean value 3.63% was also observed at C. hospital.

The mean values of soy powder from the vendor locations and the end product of soymilk ranged from 2.75% to 6.17% with the highest value recorded at K. hospital and the least at the end product of soymilk. Significant differences occurred among the various sampling locations for the soy powder ($P < 0.0001$). The significant difference occurred between C. Hospital and T. Hospital, C. Hospital and K. Hospital, C. Hospital and C. Market, T. Hospital and K. Hospital and also T. Hospital and C. Market (appendix 2d).However, no significant difference was observed in the soymilk producing stages ($P>0.05$).

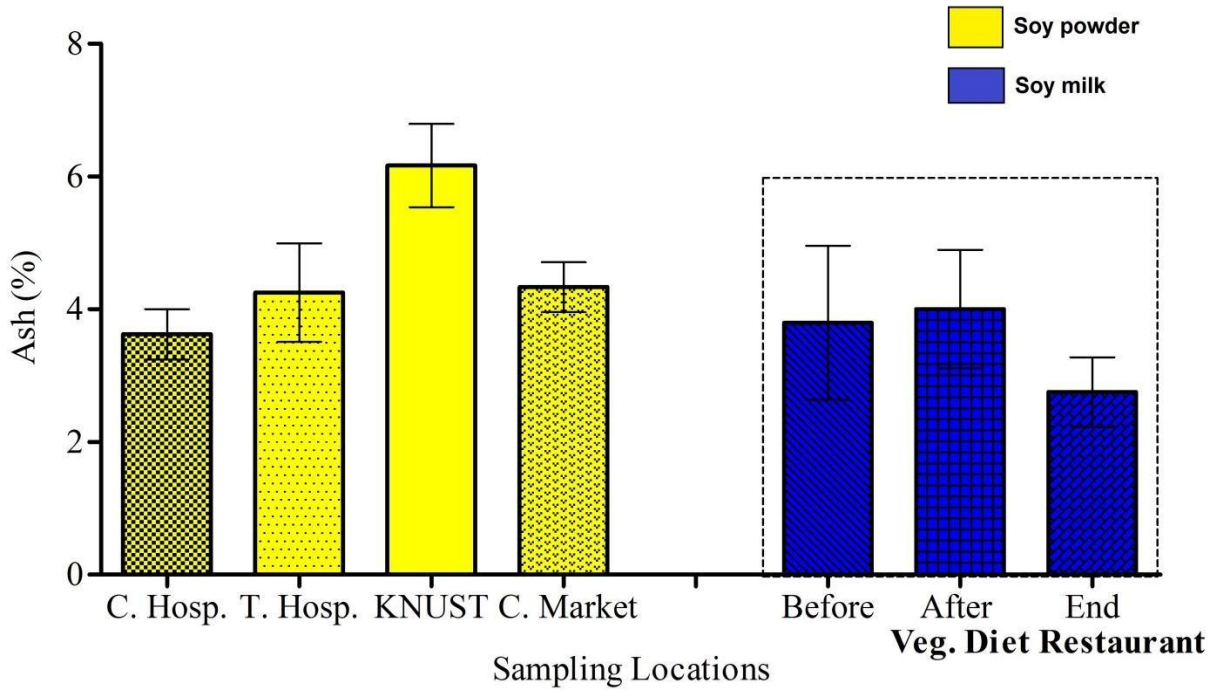


Figure 4.6: Ash (%) content in soy powder and soymilk from the various sampling locations

4.3 MICROBIOLOGICAL QUALITY OF SOYMILK AND SOY POWDER

4.3.1 Total Coliform

Production line of soymilk observed a decrease in total coliform from a high mean value of 3.38×10^6 MPN/100 ml before boiling to 1.74×10^6 MPN/100 ml after boiling which represents 94.25% reduction. Total coliform levels recorded in soy powder sampling locations ranged from 3.48×10^4MPN/100 ml to 4.82×10^6 MPN/100 ml. The highest mean value of 4.82×10^6 was observed at C. Hospital whereas the lowest value of 3.48×10^4 MPN/100 ml was recorded at K. Hospital (Figure 4.7). The mean total coliform values of soymilk end product and soy powder from the various vendor outlets ranged from 3.48×10^4 MPN/100 ml to 4.82×10^6 MPN/100 ml. K. Hosp. recorded the least value whiles C. hospital recorded the highest mean value. Significant difference (P=0.0268) occurred in the mean total coliform at the various sampling locations of soy powder, however, soymilk production did not observe significant difference. The differences were recorded between C. Hospital and T. Hospital and also C. hospital and C. market respectively (Appendix 2h). The mean total coliform values in the soymilk and soy powder were high above the WHO guideline of zero (0) counts/100ml MPN for food (WHO, 2011) as indicated by the reference line (Figure 4).

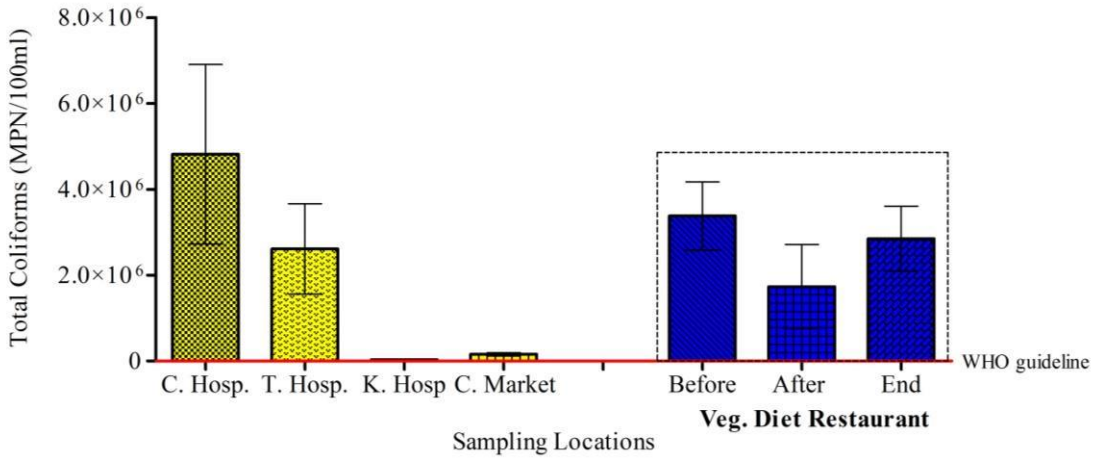


Figure 4.7: Total Coliform (MPN /100ml) in soy powder and soymilk from the various sampling locations

4.3.2 Faecal Coliform

The mean faecal coliform in soymilk production line was found to vary from 9.63×10^4 MPN/100ml to 1.98×10^5 MPN/100 ml. The highest recorded mean value of 1.98×10^5 MPN/100ml was at end product whiles the lowest mean of 9.63×10^4 MPN/100 was recorded after boiling condition (Table 4.1). Faecal coliform in soy powder from the vendor outlets recorded mean values ranging 0 to 4.25×10^4 MPN/100 ml. The lowest mean value was observed at K. hospital whereas the highest was recorded at C. hospital. The mean faecal coliform values of soymilk end product and soy powder from the various vendor outlets ranged from 0 to 1.98×10^5 MPN/100 ml (Figure 4.8). The highest recorded mean value of 1.98×10^5 MPN/100 ml was at the end product of soymilk whilst K. hospital recorded the least value of 0 respectively. There was no significant difference along the production line of soymilk production and soy powder from the various vendor locations ($P>0.05$). The mean values of Faecal coliform in the soymilk and soy powder were above the WHO guideline of zero (0) counts/100ml MPN for food (WHO, 2011) as indicated by the reference line (Figure 4.8).

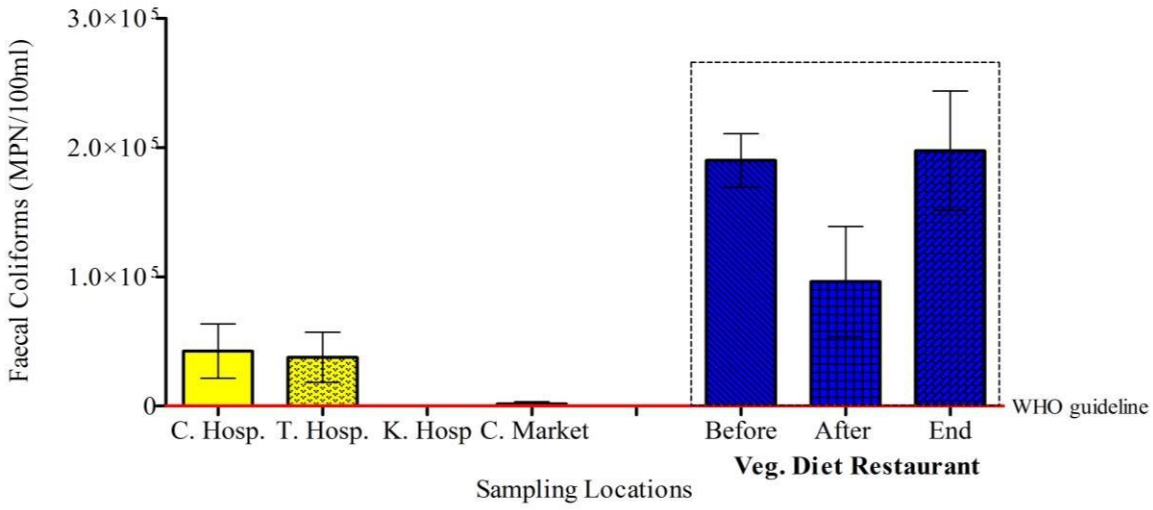


Figure 4.8: Faecal Coliform (MPN /100ml) in soy powder and soymilk from the various sampling locations

4.3.3. *Escherichia Coli*

From Table 4.1, *E. coli* levels in soymilk production line ranged from 0 to 8.33×10^3 MPN/100ml. The highest mean value of 8.33×10^3 MPN/100 was recorded before boiling whereas after boiling and end product recorded the lowest mean value of 0 MPN/100ml. The mean values of *E. coli* in soy powder from the vendor locations ranged from 0 to 1×10^4 MPN/100ml. T. hospital recorded the highest mean value whereas K. hospital and C. market recorded the least. The mean *E. coli* values of soymilk end product and soy powder from the various vendor outlets ranged from 0 to 1×10^4 MPN/100ml (Figure 4.9). The highest mean was observed at T. hospital and the lowest recorded at end product, K. hospital and C. market respectively. There was no significant differences among the soy powder at the various locations whereas soymilk had differences between the treatments (P= 0.0244). The differences were observed before and after boiling and also before boiling and end product (Appendix 2j).

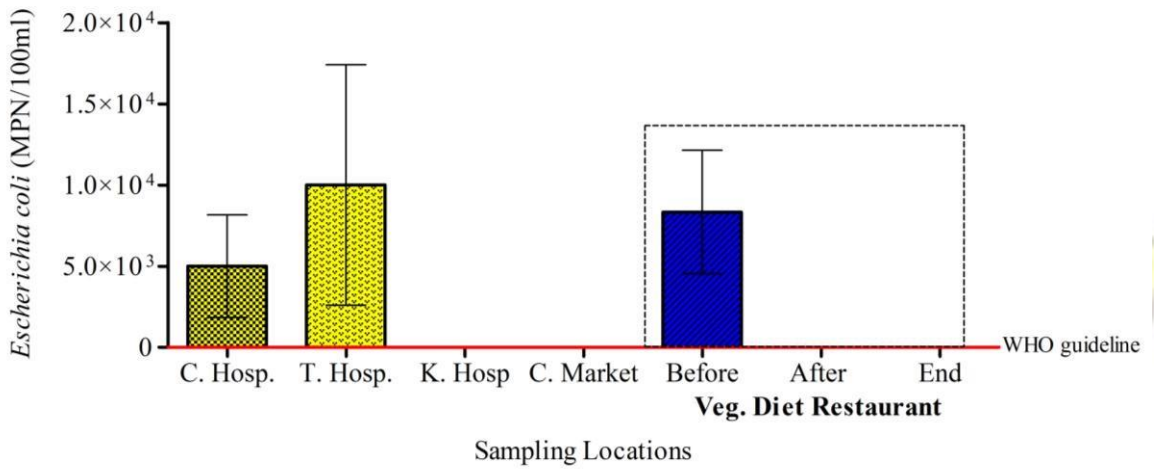


Figure 4.9*: E. coli (*MPN/100ml) in soy powder and soymilk from the various sampling locations

4.4. CHEMICAL ANALYSIS ON SOYMILK AND SOY POWDER

The chemical parameters analyzed in the study were pH, Daidzein, Genistein and Glycitein.

4.4.1. pH pH along soymilk production saw an increase from a mean value of 6.85 before boiling to 6.99 after boiling. The mean pH values in soy powder from the various vendor outlets ranged from 6.78 to 7.08, the highest value was observed at C. hospital (7.08) and the lowest at T. hospital (6.78). The mean pH from the sampling locations of soy powder and soymilk end product ranged from 6.78 to 7.08 with C. Hospital recording the highest mean value (7.08) whereas T. Hospital recorded the lowest mean value of 6.78 (Figure 4.10). There was no significant difference among treatments in the soymilk, however, significant differences were observed among the powder samples ($P<0.0001$). C. Hospital was significantly different from T Hospital, K. Hospital and also C. market. Moreover T. Hospital was also significantly different from K. Hospital and C. market (Appendix 2c).

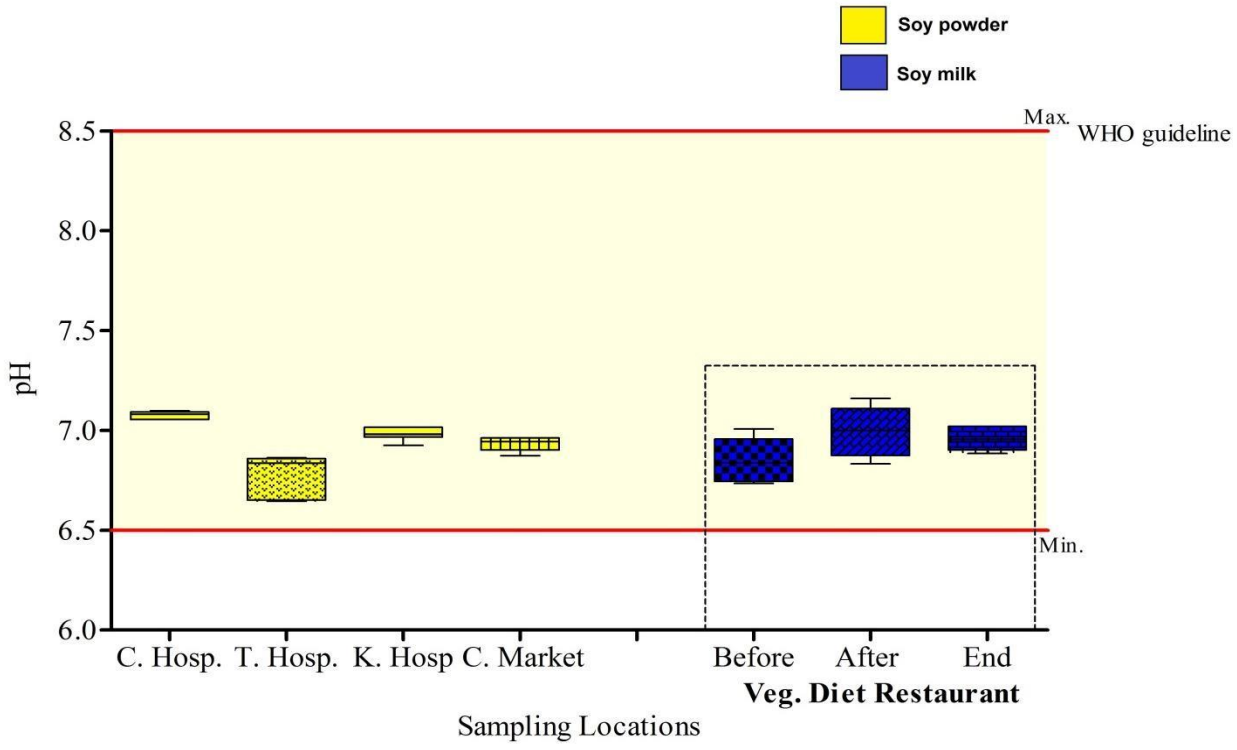


Figure 4.10: pH of soy powder and soymilk from the various sampling locations

4.4.2 Total Isoflavones (Daidzein+ Genistein)

Total Isoflavones increased from a mean value of 41.72µg/g before boiling to 52.36µg/g after boiling and of soymilk which is 20.32% increment (Table 4.1). The mean value of total

isoflavone in soy powder from the vendor outlets ranged from 180.06 µg/g to 208.75µg/g; the highest mean value was observed at T. hospital and the least occurred at C. market. Total Isoflavones in soymilk (end product) and soy powder at the various sampling locations ranged from 69.32µg/g to 208.75µg/g (Figure 4.11). The highest value was obtained at T. Hospital (208.75µg/g) and the least was recorded at end product of soymilk (69.32µg/g). Significant difference occurred along the production line of soymilk (P=0.0081). The differences were seen between samples before boiling and end product. There were no significant differences between the soy powders from the various sampling locations

(Appendix 2K, 2L). The mean total Isoflavone in the soy powder and soymilk were below 45mg/60g administered to premenopausal women which significantly altered their reproductive hormones (Cassidy *et al.*, 1994) as shown by the reference line (Figure 4.11).

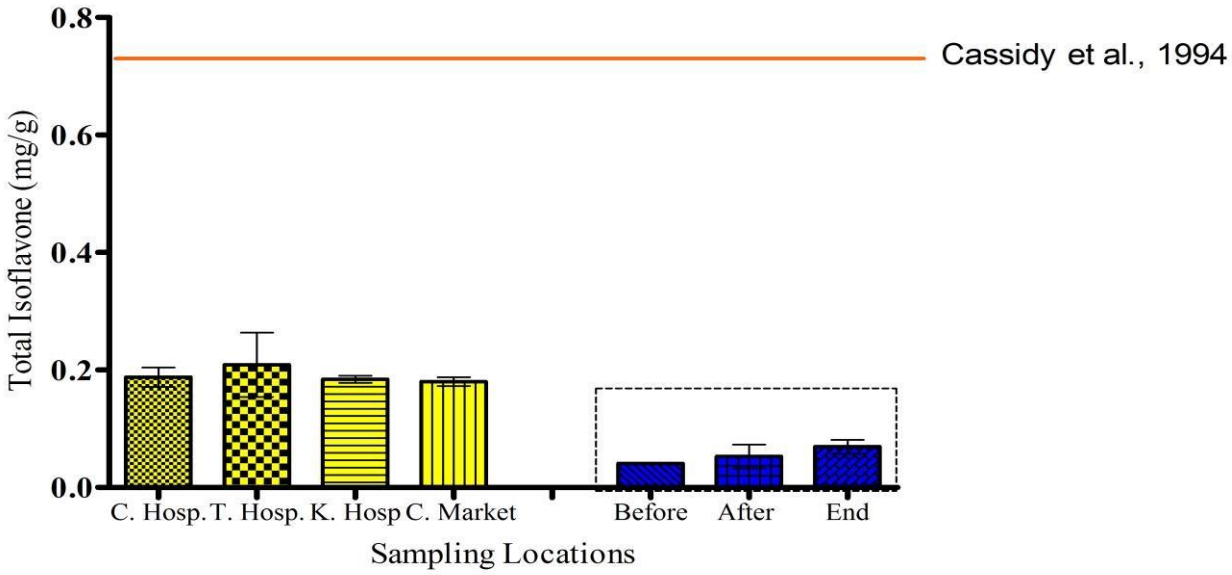


Figure 4.11: Total Isoflavones (µg/g) in soy powder and soymilk at the various sampling locations

4.4.3 Genistein

Genistein increased along the production of soymilk form a mean value of 40.13µg/g before boiling to 49.16µg/g after boiling representing 18.37% increase (Figure 4.12). Soy powder from vendor outlets observed mean genistein values from 154.64µg/g to 170.63µg/g. The

highest mean value of 170.63µg/g was observed at T. hospital whereas the least was recorded at C. market respectively. The mean genistein levels among soy powder from vendor outlets and soymilk end product ranged from 58.51µg/g to 170.63µg/g. The highest mean (170.63µg/g) was observed at T. Hospital whereas end product of soymilk recorded the least value of 58.51µg/g. There was no significant difference in the powdered samples; however the soymilk observed significant difference along the production line (P=0.0017). The differences were seen between before boiling and end product and also after boiling and end product (Appendix 2a).

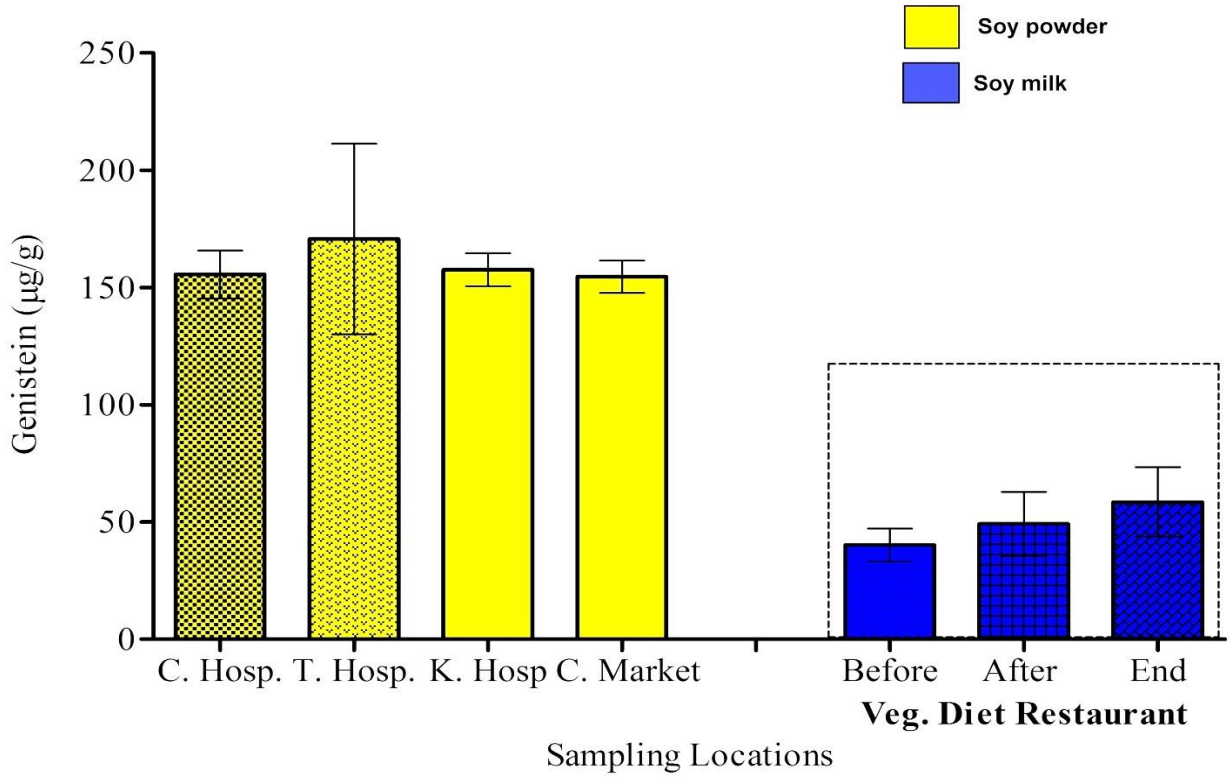


Figure 4.12: Genistein (µg/g) in soymilk and powder at the various sampling locations

4.4.4. Daidzein

There was an increase in daidzein (50.31%) along the production line of soymilk from a low mean value of 1.59µg/g before boiling to 3.20µg/g after boiling and again 70.40% increment at the end product (10.81 µg/g) respectively. Daidzein from soy powder sampling locations had a low mean value of 25.42µg/g at C. market and a high mean value of 38.12µg/g at T. hospital. From Figure 4.13, the mean values of daidzein from soy powder vendor outlets and soymilk

end product ranged from10.81µg/g to 38.12µg/g. The highest mean value of 38.12µg/g was recorded at T. Hospital whilst end product of soymilk recorded the least mean value of 10.81µg/g. There was no significant difference among the soy powder from the various sampling locations and also along the production line of soymilk production.

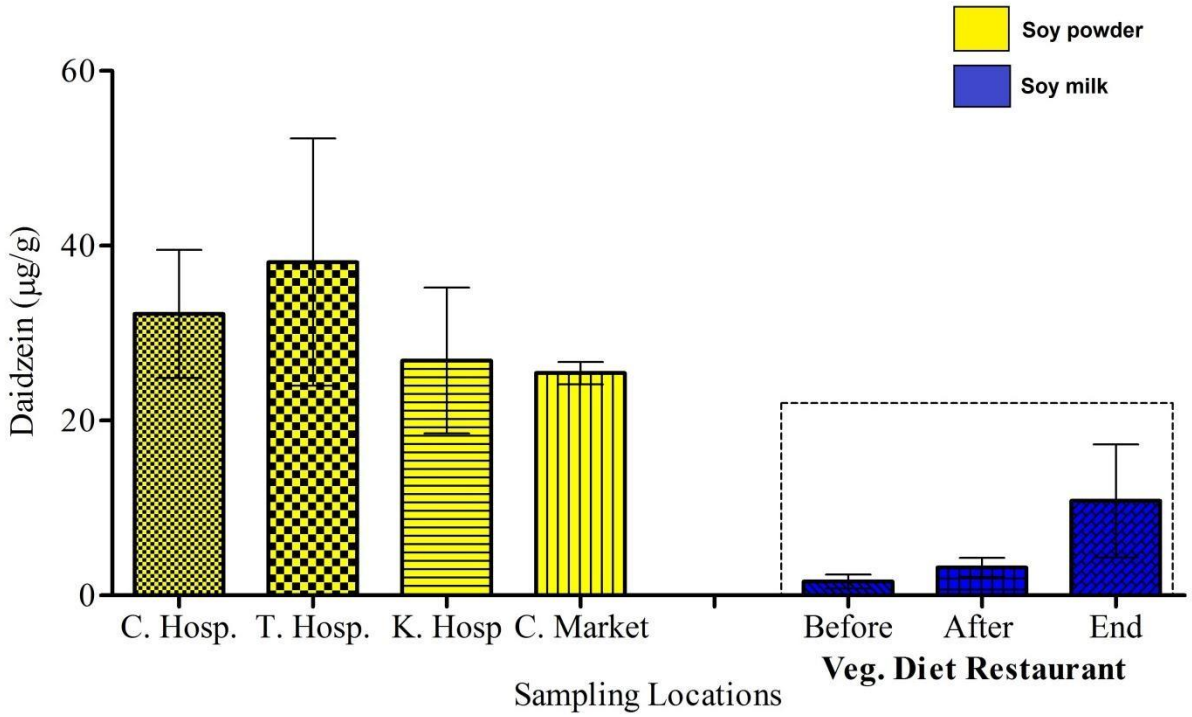


Figure 4.13: Daidzein (µg/g) in soy powder and soymilk from the various sampling locations

4.4.5 Glycitein

Isoflavone glycitein concentrations in the soymilk and soy powder at sales point (Plate 4.2) were less than the detection limit (< 0.1 µg/g) of the analytical method.

Plate 4.2: Soy powder at sales point

CHAPTER FIVE

5.0 DISCUSSION

The utilization of soy rich foods for infants is rising as the society has been made conscious of soy properties that promote health (Irvine *et al.*, 1998).The isoflavone genistein and daidzein consumption through soy may be a developmental hazard particularly to the reproductive system of infants and also alter the reproductive hormones of adults (Irvine *et al*., 1998). This study established that there were five and three main stages in the production chain of soymilk and soy powder respectively. Basically soy powder is made by dry milling of roasted soybean and bagged after cooling whiles soymilk is by wet milling, poured into cheesecloth to decant. It is boiled for thirty five to forty minutes. While stirring, a suspension of liquid layer that forms on the surface (Yuba) which is slimy is scraped off to prevent clotting of the milk giving it a smooth texture. The ingredients, crude processing method and packaging conditions predispose the products (soymilk and powder) to microbial contamination. Snack (soy milk), like most ready to eat foods and drinks, can serve as common vehicle for transmission of infections (Ikuomola and Eniola, 2010). This study revealed microbial contamination and higher isoflavone levels in soy powder and unhealthy nutrient profile. A healthy nutritional supply means the right amount, because overconsumption and excess intake of macronutrients can be just as problematic for infants as under-consumption and deficiency of nutrients (Andres *et al.*, 2012).

5.1 Total coliforms (TC)

The high levels of TC before boiling of soymilk reduced significantly after boiling, owing to the heat treatment (Schauer, 2007). The rise in TC of the final product of soymilk can be attributed to condiments added (flavours) and also unhygienic practices surrounding the packaging. Sales point at K. hospital observed the least count of TC followed by C. Market.

The ingredients, equipment used, the various processing stages and packaging conditions of the powder predispose the product to microbial contamination. Experiments by Ikuomola and Eniola, (2010) revealed that unhygienic processing and packaging conditions of soybeans and its products may contaminate the products with microorganisms. The number and type of microbes present on the produce are major determinant of quality deterioration.

The present results (support and augment these findings) show that the Total Coliform counts in the soymilk and soy powder were above the WHO guideline of zero (0) counts/100ml MPN for food (WHO, 2011). Presence of total coliform bacteria may not necessarily cause illness, but their presence (indicates that food items) may render the food vulnerable to contamination by more harmful microorganisms.

5.2 Faecal coliforms (FC)

FC in soymilk was reduced after boiling as a result of heat applied during processing but again increased at the end product presumably because other condiments were added to serve. FC at the end product of soymilk was relatively higher than all the soy powder samples, with the exception of K. hospital which had no faecal coliform. The relatively higher loads of FC in the soymilk could also be as a result of the milk serving as a very suitable media for the growth of bacteria populations (Sydner *et al.*, 1978). Contamination from the production site can account for FC at the various soy powder sampling locations with the exception of K. hospital. C. market had the least FC content which is an indication that production was carried out under some form of hygienic conditions. Products from C. market were well labelled, had batch number as well as production and expiring dates whereas products from the other sampling locations had no such description. This is an indication that production of soya powder on sale at C. market is monitored and follows hygienic processes. C. market is usually highly populated and often dusty, which is likely to be responsible for the contamination. FC in the soymilk and powder is an indication of contamination from both human and animal faecal matter resulting

from unhygienic processing and packaging. Large quantities of faecal coliform bacteria in food may indicate a higher risk of pathogens being present. Some pathogenic diseases include ear infection, dysentery, typhoid fever, viral and bacterial gastroenteritis, and hepatitis A (Chapra, 1997).

5.3 *Escherichia coli (E. coli)*

The detection of *Escherichia coli* is indicative of faecal contamination and of public health concern considering its role in food borne infection (Postgate, 2000). *E. coli* is present in very high numbers in animal and human faeces even though a few confirmation for growth is reported in tropical soils (WHO, 2011).

E. coli was present in the soymilk before boiling but was eliminated after boiling and in the end product owing to the heat application in the soymilk processing stages (Schauer, 2007). *E. coli* was absent in K. hospital and C. market and therefore conforms to WHO (2011) food quality guidelines but was detected in products from T. hospital and C. hospital. The production and packaging was done within the C. hospital premises hence contamination from the environment could account for the presence of *E. coli* in the soy powder. *E. coli* in the soy powder from T. hospital could be attributed to unhygienic processing and packaging by the personnel who have been authorized by the hospital for production and sale. *E. coli* contamination in the product can cause some pathogenic diseases (Postgate, 2000). E. coli can cause intestinal damages consisting of inflammation and ulceration, mucous diarrhea, stomach cramps and watery diarrhea. Also, they can cause serious illness including bloody diarrhea, blood clothing problems, kidney failure and death (Bad Bug Book, 2012).

5.4 Total Isoflavones (Genistein+ daidzein)

The total isoflavone content in the soymilk (69.32µg/g) was comparatively lower to the soy powder (180.06µg/g - 208.75µg/g). This is as a result of the different processing methods used in soymilk and powder preparation. Soymilk after wet milling was poured into cheesecloth to filter leaving behind the

okra (by-product) containing Isoflavones. The slurry is boiled to make soymilk whiles the whole soybean is roasted and milled to make soy powder (Jackson *et al*., 2002).

The total isoflavone content in the soy powder ranged from 180.06µg/g to 208.75µg/g, which is below the 450µg/60g administered to premenopausal women which significantly altered their reproductive hormones (Cassidy *et al.*, 1994). Considering the body weight of premenopausal women to children between the ages of 1-3 years, it is conceivable that the isoflavone intake by these children is relatively high based on their body weight. It is therefore possible that the consumption of a diet rich in plant-derived estrogens by children (1-3yrs) could affect endogenic hormone production (Cassidy *et al.*, 1995).

5.5 Isoflavones; Genistein and Daidzein

During soymilk production, there was an increase in genistein and daidzein along the production line. Heat treatment during soymilk production accounted for the increase in genistein and daidzein levels. Heat treatments does not destroy Isoflavone but subject to intra conversions among the various forms resulting in the increment of the aglycones forms (genistein and daidzein) [Chiarello *et al.*, 2006].

The isoflavone genistein and daidzein contents in the soy powder were relatively higher than the end product of soymilk. This is as a result of different processing methods used in the preparation of soymilk and soy powder. It is noticeable that every step in processing of soymilk production accounts for Isoflavones loss, resultant in a substantial sum of isoflavones being lost in the ensuing by-products (Jackson *et al.*, 2002). Isoflavones were fractionated into the by-product after the slurry was sieved to take out the soy beverage.

Measured losses of isoflavones in soymilk could also be understood to be the outcome of leaching into the water used to soak raw soybeans (Jackson *et al*., 2002). Defoaming during the heating process of soy beverage production may also remove isoflavones (Okubo *et al.,* 1983).The study has revealed that isoflavones composition can differ during various processing steps and the profile of circulation of

various forms of Isoflavones is affected by the technical procedure applied to products of soy (Wang and Murphy, 1996).

5.6 pH

The pH of the soymilk before boiling increased slightly after boiling attributable to heat processing (Ikya *et al.*, 2013). It lowered at the end product because all solutions will change their pH value with temperature. This is a result of the shifting of the chemical equilibrium of the components. C. hospital had a neutral pH (7.08) which presupposes encouragement of microbial growth (USFDA, 2012). Comparing the pH of soya powder from T. hospital with the other sample locations, it had very low pH. This could be the addition of preservatives to increase the shelf life of the soya powder.

However the pH range of the soymilk and powder were within the safe range of WHO (2011) food guideline of pH 6.5-8.5.

5.7 Protein

The protein content in soymilk was higher before boiling (56.08%) but reduced to 53.54% after boiling due to overheating. Overheating may destroy or reduce the availability of certain heat sensitive amino acids and reduce the nutritional value of soy protein (Caprita and Caprita, 2010). The protein content after boiling was lowered to 28.49% at the end product. This may be attributed to the removal of the concentrated protein-lipid surface film (called Yuba) which gradually formed when the soymilk was heated. The Yuba was taken off to prevent clotting of the milk to give a smooth texture.

The protein content in soymilk is lower as compared to the soy powders at the various sampling locations as a result of the different processing methods used. However, soy powder from different vendor outlets had protein levels closely related as a result of the same processing method used. The protein content in the soy powders ranged from 39.00 to 42.10% which is

similar to that obtained by Liener (1994) in which the crude protein of soybean ranged from 41 to 50% depending on the amount of hull that is removed, and the processing method used.

Protein content in both soymilk and soy powder were above IOM's Recommended Dietary allowance of 13% (13g/100g)/day for children between 1-3 years of age (IOM, 2002; IOM, 2005). The body uses protein to build up and repair tissues. It is an important building blocks of bones, muscles, cartilage, skin and blood.

According to IOM (2005), there is an increase in risk of obesity and the development of other negative side effects such as kidney problems in children who derive more than 20% of their calories from protein. Consuming excessive protein can lead to nausea, diarrhea, build-up of toxins in the blood and even death.

5.8 Calcium

The calcium content of the soymilk before boiling was higher but was reduced after boiling credence to heat. This agrees with Orhevba (2011) who stated that soymilk (which is a product of soybean) has nutritional parameters which may be affected by heat treatment with time. Calcium content at the end product of soymilk was increased as a result of the condiments (sugar, chocolate, vanilla) added after the processing to serve. Calcium content in soy powder from various locations were almost the same as a result of the same processing method used. However, calcium content at the end product of soymilk was lower compared to soy powder from different locations as a result of the different processing methods used.

Calcium content in both soymilk and powder were below the Recommended Dietary allowance (IOM, 1997) of 0.7% (700mg/100g/dy) for children between the ages of one and three.

The body uses calcium to keep bones and teeth strong, thereby supporting skeletal structures and function. The rest of the calcium in the body plays the role in cell signaling, blood clotting, muscle contraction and nerve function.

Insufficient intakes of calcium do not produce obvious symptoms in the short term because the body maintains calcium levels in the blood by taking it from the bones. Over the long period, intakes of calcium below the recommended levels may cause negative effects on bone health at all ages. Lack of calcium causes rickets in infants, tooth decay, retards acquisition of adequate bone mass during skeletal development in adolescents and finally responsible for accelerated bone loss in adulthood in both men and women. This results eventually in the development of osteoporosis (Peterlik *et al.*, 2009).

5.9 Magnesium

Magnesium content was increased after boiling credence to heat treatment (Orhevba (2011). Magnesium content was lowered at the end product of soymilk preparation probably as a result of the removal of the yuba. The soy powder had high magnesium content compared to soymilk which can be attributed to the different processing methods used in the preparation of soymilk and soy powder. The processing method of soymilk involves excessive use of water for washing and preparation may account for the loss of magnesium in soymilk (Jackson *et al.*, 2002).

Magnesium content in soymilk and powder were above the Recommended Dietary allowance (IOM, 1997) of 0.08% (80mg/100g/dy) for children between the ages of one and three.

Magnesium is a cofactor in enzyme systems that control differing biochemical respnses in the body including protein synthesis, nerve and muscle function and structural development of bones. Excess magnesium from foodstuff does not have healthiness risk in healthy individuals since the excess amount is eliminated by the kidneys in urine (Musso, 2009).

5.10 Ash

The ash value of soybean is about 5-6% indicating its mineral concentration. Soymilk production observed an increase in ash after boiling but lowered at the end product because of the removal of the Yuba.

Vendor outlets for the soya powder observed varying ash contents which were significantly different. Growing location, variety of soybean and season accounted for the different levels. The ash content in the soy powder was higher than the soymilk indicating the mineral composition in soy powder is higher than soymilk.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSIONS

Findings from the study indicate that soymilk and soy powder could serve as common vehicle for pathogens transmitted via the feacal-oral route to render the products inedible or make them vehicles of food poisoning or food infection. The mean level of faecal coliform in the end product of soymilk was 1.98×10^5 MPN/100ml whereas the FC in the soy powder ranged from zero (0) to 4.25×10^5 MPN/100m.Their distribution pattern showed that the products are contaminated during and after production.

Soymilk had a mean total Isoflavone content of 69.32µg/g in the end product. Also isoflavone content in soy powder ranged from 180.06 µg/g to 208.75 µg/g which is higher for children aged between one and three. Therefore consumption of soy powder on regular basis could affect endogenic hormone production of infants.

The study revealed that the mean nutritional values in Soymilk end product contained 28.49%, 0.20%, and 0.13% protein, calcium and magnesium respectively. On the other hand, soya powder contained 39%-42.10% protein, 0.24%-0.26% calcium and 0.27%-0.28% magnesium. Recommended Dietary allowance (IOM, 2005) for children between the ages of one and three are protein 13% (13g/100kg), calcium 0.7% (700mg/100g) and magnesium 0.08% (80mg/100g). This connotes unbalanced nutritional profile because protein and magnesium contents are above Recommended Dietary allowance whereas calcium is below Recommended Dietary allowance for children between the ages of one and three. Infants need adequate calories to support rapid growth and development and a healthy supply means the right amount. Over-consumption and excess intake of macro and micronutrients can be just as problematic for infants as under-consumption and deficiency (Andres *et al.*, 2012).

6.2 RECOMMENDATIONS

The following recommendations were derived from the present study:

- Education and monitoring programmes should be carried out to ensure proper food safety at the production and sale point of soymilk and powder
- Consumers should be provided with information on the benefits and side effects on soy products. This should be in the form of hand- out flyers, posters or organized educational forum.
- Soymilk is better to take than soy powder considering the Isoflavone intakes. However, soy powder can be use in moderation instead of a full meal.

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APPENDICES

APPENDIX 1: ANALYSIS OF VARIANCE OF TREATMENTS

Appendix 1-a: Results of one-way Anova of the various variables

	Sample Type			
	Soymilk		Soy Powder	
Parameters	P-value	Significant? P < 0.05	P-value	Significant? P < 0.05
Isoflavones				
Genistein	0.0017	Yes	0.5532	No
Daidzein	0.0637	No	0.0924	No
Chemical/Mineral elements pH	0.0668	No	< 0.0001	Yes
Ash	0.0623	No	< 0.0001	Yes
Calcium	0.0316	Yes	0.8688	No
Magnesium	0.2498	No	0.9115	No
Protein	< 0.0001	Yes	0.2268	No
Microbiological loads				
Total coliform	0.3994	No	0.0268	Yes
Faecal coliform	0.1478	No	0.0860	No
E. coli	0.0244	Yes	0.2691	No

Appendix 1-b: Results of One way ANOVA for Soymilk Genistein among the various treatments

Table Analyzed	Genistein Liquid				
One-way analysis of variance					
P value	0.0017				

P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	9.985				
R squared	0.5711				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	21.06				
P value	< 0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	291.3	2	145.7		
Residual (within columns)	218.8	15	14.59		
Total	510.1	17			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Before vs After	-1.618	1.037	No	ns	-7.346 to 4.111
Before vs End	-9.227	5.918	Yes	**	-14.96 to -3.498
After vs End	-7.610	4.880	Yes	**	-13.34 to -1.881

Appendix treatments

1-c: Results of One way ANOVA for Soymilk Daidzein among the various

Table Analyzed	Daidzein Liquid				
One-way analysis of variance					
P value	0.0637				
P value summary	Ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	3.326				
R squared	0.3072				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	2.524				
P value	0.2831				
P value summary	Ns				
Do the variances differ signif. (P < 0.05)	No				
ANOVA Table	SS	df	MS		
Treatment (between columns)	1014	2	507.1		
Residual (within columns)	2287	15	152.4		
Total	3301	17			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff

Appendix treatments

Before vs After	-9.030	1.791	No	ns	-27.55 to 9.489
Before vs End	-18.39	3.647	No	ns	-36.90 to 0.1340
After vs End	-9.355	1.856	No	ns	-27.87 to 9.164

1-d: Results of One way ANOVA for Soymilk pH among the various

Table Analyzed	pH Liquid				
One-way analysis of variance					
P value	0.0668				
P value summary	Ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	3.259				
R squared	0.3029				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	2.749				
P value	0.2529				
P value summary	Ns				
Do the variances differ signif. (P < 0.05)	No				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.06702	2	0.03351		
Residual (within columns)	0.1542	15	0.01028		

Appendix treatments

Total	0.2213	17			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Before vs After	-0.1442	3.482	No	ns	-0.2963 to 0.007928
Before vs End	-0.1062	2.567	No	ns	-0.2583 to 0.04584
After vs End	0.03792	0.9159	No	ns	-0.1142 to 0.1900

1-e: Results of One way ANOVA for Soymilk Ash among the various

Table Analyzed	Ash Liquid				
One-way analysis of variance					
P value	0.0623				
P value summary	Ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	3.359				
R squared	0.3093				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	2.638				
P value	0.2674				
P value summary	Ns				
Do the variances differ signif. (P < 0.05)	No				

Appendix treatments

ANOVA Table	SS	df	MS		
Treatment (between columns)	5.414	2	2.707		
Residual (within columns)	12.09	15	0.8058		
Total	17.50	17			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Before vs After	-0.2046	0.5582	No	ns	-1.551 to 1.142
Before vs End	1.048	2.858	No	ns	-0.2989 to 2.394
After vs End	1.252	3.417	No	ns	-0.09435 to 2.599

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1-f: Results of One way ANOVA for Soymilk Calcium among the various treatment

Table Analyzed	Calcium Liquid				
One-way analysis of variance					
P value	0.0316				
P value summary	*				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	4.388				
R squared	0.3691				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	3.157				
P value	0.2063				
P value summary	Ns				
Do the variances differ signif. (P < 0.05)	No				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.03561	2	0.01781		
Residual (within columns)	0.06087	15	0.004058		
Total	0.09649	17			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Before vs After	0.1046	4.021	Yes	*	0.009033 to 0.2001

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Before vs End	0.07875	3.028	No	ns	-0.01680 to 0.1743
After vs End	-0.02583	0.9933	No	ns	-0.1214 to 0.06972

1-g: Results of One way ANOVA for Soymilk Magnesium among the various treatments

Table Analyzed	Magnessium Liquid				
One-way analysis of variance					
P value	0.2498				
P value summary	Ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	1.524				
R squared	0.1689				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	9.659				
P value	0.0080				
P value summary	**				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.02254	2	0.01127		
Residual (within columns)	0.1110	15	0.007398		

Appendix

Total	0.1335	17			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Before vs After	-0.06125	1.744	No	ns	-0.1903 to 0.06776
Before vs End	0.02250	0.6408	No	ns	-0.1065 to 0.1515
After vs End	0.08375	2.385	No	ns	-0.04526 to 0.2128

1-h: Results of One way ANOVA for Soymilk Protein among the various treatments

Table Analyzed	Protein Liquid				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	154.5				
R squared	0.9537				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	0.8574				
P value	0.6514				
P value summary	ns				
Do the variances differ signif. (P < 0.05)	No				

Appendix

ANOVA Table	SS	df	MS		
Treatment (between columns)	2790	2	1395		
Residual (within columns)	135.4	15	9.026		
Total	2925	17			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Before vs After	2.548	2.078	No	ns	-1.958 to 7.055
Before vs End	27.59	22.50	Yes	***	23.08 to 32.10
After vs End	25.04	20.42	Yes	***	20.54 to 29.55

Appendix1-i: Results of One way ANOVA for Soymilk Total Coliform among the various treatments

Table Analyzed	Total Coliform Liquid				
One-way analysis of variance					
P value	0.3994				
P value summary	Ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	0.9762				
R squared	0.1152				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	0.3627				
P value	0.8342				
P value summary	Ns				
Do the variances differ signif. (P < 0.05)	No				
ANOVA Table	SS	df	MS		
Treatment (between columns)	8455000000000	2	4228000000000		
Residual (within columns)	64960000000000	15	4331000000000		
Total	73420000000000	17			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Before vs After	1645000	1.936	No	ns	-1477000 to 4766000
Before vs End	530500	0.6244	No	ns	-2591000 to 3652000

After vs End	-1114000	1.311	No	ns	-4236000 to 2007000

Appendix 1-j: Results of One way ANOVA for Soymilk Faecal Coliform among the various treatments

Table Analyzed	Faecal Coliform Liquid				
One-way analysis of variance					
P value	0.1478				
P value summary	Ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	2.178				
R squared	0.2250				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	2.918				
P value	0.2325				
P value summary	Ns				
Do the variances differ signif. (P < 0.05)	No				
ANOVA Table	SS	df	MS		
Treatment (between columns)	38190000000	2	19100000000		
Residual (within columns)	131500000000	15	8770000000		
Total	169700000000	17			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Before vs After	93750	2.452	No	ns	-46710 to 234200

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Before vs End	-7500	0.1962	No	ns	-148000 to 133000
After vs End	-101300	2.648	No	ns	-241700 to 39210

1-k: Results of One way ANOVA for Soymilk E. coli among the various treatments

Table Analyzed	*E. coli* Liquid				
One-way analysis of variance					
P value	0.0244				
P value summary	*				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	4.808				
R squared	0.3906				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)					
P value					
P value summary	Ns				
Do the variances differ signif. (P < 0.05)	No				
ANOVA Table	SS	df	MS		
Treatment (between columns)	277800000	2	138900000		
Residual (within columns)	433300000	15	28890000		
Total	711100000	17			

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Before vs After	8333	3.798	Yes	*	271.6 to 16400
Before vs End	8333	3.798	Yes	*	271.6 to 16400
After vs End	0.0000	0.0000	No	ns	-8062 to 8062

APPENDIX 2

Appendix 2-a: Results of One way ANOVA for Soy powder Genistein among the various sampling location

Table Analyzed	Genistein Powder				
One-way analysis of variance					
P value	0.5532				
P value summary	Ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	0.7174				
R squared	0.09715				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	21.72				
P value	< 0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	999.9	3	333.3		
Residual (within columns)	9293	20	464.6		
Total	10290	23			

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Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
C. Hosp. vs Tafo Hosp.	-15.08	1.714	No	ns	-49.91 to 19.75
C. Hosp. vs KNUST	-2.063	0.2345	No	ns	-36.89 to 32.77
C. Hosp. vs Central Market	0.9108	0.1035	No	ns	-33.92 to 35.74
Tafo Hosp. vs KNUST	13.02	1.479	No	ns	-21.81 to 47.85
Tafo Hosp. vs Central Market	15.99	1.817	No	ns	-18.84 to 50.82
KNUST vs Central Market	2.974	0.3380	No	ns	-31.86 to 37.80

2-b: Results of One way ANOVA for Soy powder Daidzein among the various sampling location

Table Analyzed	Daidzein Powder				
One-way analysis of variance					
P value	0.0924				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	2.460				
R squared	0.2695				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	16.41				
P value	0.0009				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	599.6	3	199.9		
Residual (within columns)	1625	20	81.26		

Total	2225	23			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
C. Hosp. vs Tafo Hosp.	-5.926	1.610	No	ns	-20.49 to 8.639
C. Hosp. vs KNUST	5.338	1.450	No	ns	-9.228 to 19.90
C. Hosp. vs Central Market	6.775	1.841	No	ns	-7.791 to 21.34
Tafo Hosp. vs KNUST	11.26	3.061	No	ns	-3.302 to 25.83
Tafo Hosp. vs Central Market	12.70	3.451	No	ns	-1.865 to 27.27
KNUST vs Central Market	1.437	0.3905	No	ns	-13.13 to 16.00

Appendix 2-c: Results of One way ANOVA for Soy powder pH among the various sampling locations

Table Analyzed	pH Powder				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	27.20				
R squared	0.8032				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	15.15				
P value	0.0017				
P value summary	**				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.2743	3	0.09145		

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Residual (within columns)	0.06724	20	0.003362		
Total	0.3416	23			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
C. Hosp. vs Tafo Hosp.	0.2954	12.48	Yes	***	0.2017 to 0.3891
C. Hosp. vs KNUST	0.09375	3.960	Yes	*	0.00005833 to 0.1874
C. Hosp. vs Central Market	0.1438	6.073	Yes	**	0.05006 to 0.2374
Tafo Hosp. vs KNUST	-0.2017	8.519	Yes	***	-0.2954 to -0.1080
Tafo Hosp. vs Central Market	-0.1517	6.407	Yes	**	-0.2454 to -0.05797
KNUST vs Central Market	0.05000	2.112	No	ns	-0.04369 to 0.1437

2-d: Results of One way ANOVA for Soy powder Ash among the various sampling locations

Table Analyzed	Ash Powder				
One-way analysis of variance					
P value	< 0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	23.46				
R squared	0.7787				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	3.242				
P value	0.3557				
P value summary	ns				
Do the variances differ signif. (P < 0.05)	No				

ANOVA Table	SS	df	MS		
Treatment (between columns)	21.59	3	7.197		
Residual (within columns)	6.135	20	0.3068		
Total	27.73	23			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
C. Hosp. vs Tafo Hosp.	-0.6250	2.764	No	ns	-1.520 to 0.2700
C. Hosp. vs KNUST	-2.542	11.24	Yes	***	-3.437 to -1.647
C. Hosp. vs Central Market	-0.7083	3.133	No	ns	-1.603 to 0.1866
Tafo Hosp. vs KNUST	-1.917	8.476	Yes	***	-2.812 to -1.022
Tafo Hosp. vs Central Market	-0.08333	0.3685	No	ns	-0.9783 to 0.8116
KNUST vs Central Market	1.833	8.108	Yes	***	0.9384 to 2.728

Appendix 2-e: Results of One way ANOVA for Soy powder Calcium among the various sampling locations

Table Analyzed	Calcium Powder				
One-way analysis of variance					
P value	0.8688				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	0.2380				
R squared	0.03447				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	10.17				
P value	0.0171				
P value summary	*				

Appendix

Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.001408	3	0.0004694		
Residual (within columns)	0.03945	20	0.001973		
Total	0.04086	23			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
C. Hosp. vs Tafo Hosp.	0.006667	0.3677	No	Ns	-0.06510 to 0.07843
C. Hosp. vs KNUST	-0.01417	0.7813	No	Ns	-0.08593 to 0.05760
C. Hosp. vs Central Market	0.0008333	0.04596	No	Ns	-0.07093 to 0.07260
Tafo Hosp. vs KNUST	-0.02083	1.149	No	Ns	-0.09260 to 0.05093
Tafo Hosp. vs Central Market	-0.005833	0.3217	No	Ns	-0.07760 to 0.06593
KNUST vs Central Market	0.01500	0.8273	No	Ns	-0.05676 to 0.08676

2-f: Results of One way ANOVA for Soy powder Magnesium among the various sampling locations

Table Analyzed	Magnessium Powder				
One-way analysis of variance					
P value	0.9115				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	0.1758				
R squared	0.02569				
Bartlett's test for equal variances					

Bartlett's statistic (corrected)	3.668				
P value	0.2996				
P value summary	ns				
Do the variances differ signif. (P < 0.05)	No				
ANOVA Table	SS	df	MS		
Treatment (between columns)	0.0004979	3	0.0001660		
Residual (within columns)	0.01889	20	0.0009443		
Total	0.01938	23			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
C. Hosp. vs Tafo Hosp.	0.002083	0.1661	No	ns	-0.04757 to 0.05174
C. Hosp. vs KNUST	0.008333	0.6643	No	ns	-0.04132 to 0.05799
C. Hosp. vs Central Market	0.01125	0.8968	No	ns	-0.03840 to 0.06090
Tafo Hosp. vs KNUST	0.006250	0.4982	No	ns	-0.04340 to 0.05590
Tafo Hosp. vs Central Market	0.009167	0.7307	No	ns	-0.04049 to 0.05882
KNUST vs Central Market	0.002917	0.2325	No	ns	-0.04674 to 0.05257

Appendix

2-g: Results of One way ANOVA for Soy powder Protein among the various sampling locations

Table Analyzed	Protein Powder				
One-way analysis of variance					
P value	0.2268				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	1.575				
R squared	0.1911				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	31.48				
P value	< 0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	29.78	3	9.928		
Residual (within columns)	126.1	20	6.305		
Total	155.9	23			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
C. Hosp. vs Tafo Hosp.	1.198	1.169	No	ns	-2.859 to 5.256
C. Hosp. vs KNUST	-1.899	1.852	No	ns	-5.956 to 2.159

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C. Hosp. vs Central Market	0.1054	0.1028	No	ns	-3.952 to 4.163
Tafo Hosp. vs KNUST	-3.097	3.021	No	ns	-7.155 to 0.9603
Tafo Hosp. vs Central Market	-1.093	1.066	No	ns	-5.150 to 2.965
KNUST vs Central Market	2.004	1.955	No	ns	-2.053 to 6.062

2-h: Results of One way ANOVA for Soy powder Total Coliforms among the various sampling locations

Table Analyzed	Total Coliform Powder				
One-way analysis of variance					
P value	0.0268				
P value summary	*				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	4				
F	3.779				
R squared	0.3618				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	66.67				
P value	< 0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	93210000000000	3	31070000000000		
Residual (within columns)	164400000000000	20	8221000000000		
Total	257600000000000	23			

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Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
C. Hosp. vs Tafo Hosp.	2206000	1.884	No	ns	-2428000 to 6839000
C. Hosp. vs KNUST	4783000	4.086	Yes	*	149700 to 9416000
C. Hosp. vs Central Market	4660000	3.981	Yes	*	27040 to 9293000
Tafo Hosp. vs KNUST	2577000	2.202	No	ns	-2056000 to 7210000
Tafo Hosp. vs Central Market	2455000	2.097	No	ns	-2179000 to 7088000
KNUST vs Central Market	-122700	0.1048	No	ns	-4756000 to 4510000

2-i: Results of One way ANOVA for Soy powder Faecal Coliforms among the various sampling locations

Table Analyzed	Faecal Coliform Powder				
One-way analysis of variance					
P value	0.0860				
P value summary	Ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	2.533				
R squared	0.2754				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)					
P value					
P value summary	Ns				
Do the variances differ signif. (P < 0.05)	No				

Appendix

ANOVA Table	SS	df	MS		
Treatment (between columns)	9330000000	3	3110000000		
Residual (within columns)	24550000000	20	1228000000		
Total	33880000000	23			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
C. Hosp. vs Tafo Hosp.	4792	0.3350	No	ns	-51820 to 61410
C. Hosp. vs KNUST	42500	2.971	No	ns	-14120 to 99120
C. Hosp. vs Central Market	40830	2.855	No	ns	-15780 to 97450
Tafo Hosp. vs KNUST	37710	2.636	No	ns	-18910 to 94320
Tafo Hosp. vs Central Market	36040	2.520	No	ns	-20570 to 92660
KNUST vs Central Market	-1667	0.1165	No	ns	-58280 to 54950

Appendix 2-j: Results of One way ANOVA for Soy powder E. coli among the various sampling locations

Table Analyzed	*E. coli* Powder				
One-way analysis of variance					
P value	0.2691				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	1.410				
R squared	0.1746				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)					
P value					
P value summary	ns				
Do the variances differ signif. (P < 0.05)	No				
ANOVA Table	SS	df	MS		
Treatment (between columns)	412500000	3	137500000		
Residual (within columns)	1950000000	20	97500000		
Total	2363000000	23			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
C. Hosp. vs Tafo Hosp.	-5000	1.240	No	ns	-20960 to 10960
C. Hosp. vs KNUST	5000	1.240	No	ns	-10960 to 20960
C. Hosp. vs Central Market	5000	1.240	No	ns	-10960 to 20960

Tafo Hosp. vs KNUST	10000	2.481	No	ns	-5955 to 25960
Tafo Hosp. vs Central Market	10000	2.481	No	ns	-5955 to 25960
KNUST vs Central Market	0.0000	0.0000	No	ns	-15960 to 15960

Appendix 2-k: Results of One way ANOVA for Soymilk total Isoflavones among the various vendor outlets

Table Analyzed	Total Iso				
One-way analysis of variance					
P value	0.0081				
P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	3				
F	6.762				
R squared	0.4741				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	29.71				
P value	P<0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	2486	2	1243		
Residual (within columns)	2757	15	183.8		
Total	5242	17			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff

Before vs After	-12.00	2.168	No	ns	-32.33 to 8.334
Before vs End d	-28.66	5.178	Yes	**	-48.99 to -8.324
After vs End d	-16.66	3.010	No	ns	-36.99 to 3.676

Appendix 2-l: Results of One way ANOVA for Soy powder total Isoflavones among the various from the various vendor outlets

Table Analyzed	Row stats of Total Iso				
One-way analysis of variance					
P value	0.3494				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	1.161				
R squared	0.1483				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	26.30				
P value	P<0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	2927	3	975.7		
Residual (within columns)	16810	20	840.7		
Total	19740	23			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff

C. Hosp. vs T. Hosp.	-21.01	1.775	No	ns	-67.86 to 25.84
C. Hosp. vs K. Hosp	3.442	0.2908	No	ns	-43.41 to 50.29
C. Hosp. vs C. Market	7.685	0.6493	No	ns	-39.17 to 54.54
T. Hosp. vs K. Hosp	24.45	2.066	No	ns	-22.40 to 71.30
T. Hosp. vs C. Market	28.69	2.424	No	ns	-18.16 to 75.54
K. Hosp vs C. Market	4.243	0.3585	No	ns	-42.61 to 51.09

APPENDIX 3: CORRELATION ANALYSIS

Appendix 3-a: Results of Correlation analysis for soymilk samples indicating the "r" values and P values (in bracket). Bolded values are significantly different

	Genistein	Daidzein	pH	Ash	Calcium	Magnesium	Protein	Total coliforn	Faecal coliform
Daidzein	-0.07 (0.89)								
pH	-0.28 (0.59)	0.68 (0.14)							
Ash	-0.53 (0.28)	-0.49 (0.32)	0.04 (0.94)						
Calcium	0.34 (0.51)	0.51 (0.30)	0.41 (0.42)	-0.01 (0.99)					
Magnesium	-0.55 (0.26)	-0.46 (0.36)	0.08 (0.88)	0.37 (0.47)	-0.76 (0.08)				
Protein	0.25 (0.63)	0.26 (0.61)	-0.04 (0.93)	-0.85 (**0.03**)	-0.46 (0.34)	0.13 (0.80)			
Total coliform	-0.14 (0.79)	-0.40 (0.43)	-0.75 (0.09)	0.16 (0.76)	-0.39 (0.44)	-0.12 (0.82)	-0.09 (0.87)		
Faecal coliform	0.04 (0.94)	0.02 (0.97)	-0.57 (0.24)	-0.32 (0.53)	-0.31 (0.55)	-0.32 (0.53)	0.34 (0.51)	0.85 (**0.03**)	
E. coli	0.41 (0.42)	0.54 (0.27)	0.61 (0.20)	-0.62 (0.19)	0.23 (0.65)	-0.05 (0.93)	0.56 (0.25)	-0.82 (0.05)	-0.42 (0.40)

Appendix 3-b: Results of Correlation analysis for soy powder samples indicating the "r" values and P values (in bracket). Bolded values are significantly different

	Genistein	Daidzein	pH	Ash	Calcium	Magnesium	Protein	Total coliforn	Faecal coliform
Daidzein	0.83 (**0.04**)								
pH	-0.01 (0.98)	0.08 (0.88)							
Ash	-0.36 (0.49)	-0.81 (0.05)	-0.29 (0.58)						
Calcium	0.36 (0.49)	0.16 (0.77)	0.61 (0.20)	-0.07 (0.90)					
Magnesium	-0.37 (0.46)	-0.15 (0.78)	0.14 (0.79)	-0.04 (0.94)	-0.67 (0.15)				
Protein	0.26 (0.62)	-0.28 (0.60)	-0.22 (0.67)	0.74 (0.09)	0.31 (0.55)	-0.36 (0.48)			
Total coliform	0.63 (0.18)	0.55 (0.26)	-0.75 (0.08)	-0.17 (0.75)	-0.19 (0.71)	-0.43 (0.40)	0.19 (0.71)		
Faecal coliform	0.45 (0.38)	0.35 (0.50)	-0.82 (0.05)	0.02 (0.96)	-0.56 (0.24)	-0.02 (0.97)	0.12 (0.82)	0.85 (**0.03**)	
E. coli	0.14 (0.79)	-0.01 (0.98)	-0.65 (0.16)	0.28 (0.58)	-0.65 (0.16)	0.22 (0.68)	0.05 (0.92)	0.46 (0.35)	0.84 (**0.04**)

APPENDIX 4:DESCRIPTIVE STATISTICS

Appendix 4-a: Means and Standard Deviations of the Chemical and Nutritional parameters in soy powder from the various vendor outlets

Chemical/ Nutritional	SOY POWDER FROM VENDOR OUTLETS			
	C. Hosp.	T. Hosp	K. Hosp	C. Market
Daidzein (µg/g)	32.20±7.32	38.12±14.15	26.86±8.34	25.42±1.29
Genistein (µg/g)	155.5±10.25	170.63±40.68	157.61±7.08	154.64±6.96
Total (µg/g)	187.70±16.48	208.75±54.75	184.7±6.01	180.06±7.54
pH	7.08±0.02	6.78±0.10	6.98±0.03	6.93±0.04
Ash (%)	3.63±0.38	4.25±0.74	6.17±0.63	4.33±0.38
Protein (%)	40.20±0.30	39.00±4.81	42.10±1.17	40.10±0.78
Calcium (%)	0.24±0.07	0.24±0.03	0.26±0.03	0.24±0.02
Magnesium (%)	0.28±0.04	0.28±0.03	0.27±0.03	0.27±0.02

Appendix 4-b: Means and Standard Deviations of the microbial parameters in soy powder from the various vendor outlets

Soy powder vendor outlet	Total coliform (MPN/100ml)	Faecal coliform (MPN/100ml)	*E. coli* (MPN/100ml)
C. Hosp.	$4.82 \times 10^6 \pm 5.12 \times 10^6$	$4.25 \times 10^4 \pm 5.14 \times 10^4$	$5.00 \times 10^3 \pm 7.75 \times 10^3$
T. Hosp.	$2.61 \times 10^6 \pm 2.58 \times 10^6$	$3.77 \times 10^4 \pm 4.75 \times 10^4$	$10.00 \times 10^3 \pm 18.20 \times 10^3$
K. Hosp	$0.0348 \times 10^6 \pm 4.04 \times 10^6$	0	0
C. Market.	$0.158 \times 10^6 \pm 0.0925 \times 10^6$	$0.167 \times 10^4 \pm 0.408 \times 10^4$	0

WHO (2011)	**0**	**0**	**0**