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ABSTRACT

Tofu is becoming a popular food in Nigeria because of the various nutritional and medicinal attributes associated with soybean products. In Nigeria, *tofu* a coagulated product of soymilk is usually produced at household level using various types of coagulants such as calcium chloride, alum and steep water (effluent from pap produced from maize). The effect of the various types of locally used coagulants on the proximate, mineral, energy composition, digestibility (*In vitro and In vivo*) and biochemical response (serum enzymes and lipids) of rats fed tofu for 14days was assessed. The result of the study revealed that there was no significant difference ($P > 0.05$) in the tofu yield by each of the coagulants (17.5 - 18.3 %), however alum coagulation gave the highest yield, while calcium chloride gave the lowest yield. The protein (17.6 %), fat (6.2%), Mn (0.3), Mg (34.2), energy (6.6 cal/g) and *In vitro* multienzyme protein digestibility (75.8 - 77.6%) of steep water coagulated *tofu* was significantly higher ($P < 0.05$) than that of other coagulants. While *tofu* produced with alum had a significantly higher ($P < 0.05$) Fe (1.6), Ca (23.5), K (33.9) and Na (21.1) than the tofu produced by other coagulants. The *tofu* produced by calcium chloride had the highest Zn (0.6) content but the lowest energy content (5.3 cal/g) and *In vitro* multienzyme protein digestibility (61.6 - 63.5 %). Feeding albino rats with *tofu* and water *ad libitum* for 14days caused a decrease in the serum aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (ALP), cholesterol and low-density lipoproteins when compared with the control, while there was no significant difference ($P > 0.05$) in the average daily feed intake, average weight gain and feed: gain ratio of the rats feed *tofu*. Conversely, there was a significant increase ($P < 0.05$) in the serum high-density lipoproteins when compared with the control. However, rats fed steep water coagulated tofu had the lowest serum

level of cholesterol and LDL followed by those fed $CaCl_2$ and alum coagulated tofu respectively, while those fed with calcium chloride coagulated *tofu* had the highest serum HDL level, and closely followed by those fed steep water coagulated *tofu*. It could therefore be concluded that of all the locally used coagulants in Nigeria for *tofu* production steep water (effluent from pap produced from maize) which is considered to be waste appears to be better for the production of tofu with high nutritional value and hypocholesterolemic effect, however it has the least acceptability, further research will be carried out on how to improve its sensory quality.

CHAPTER ONE

1.0 INTRODUCTION

Soybean is an important source of dietary protein and it has been widely used in a variety of dishes by oriental people for many centuries. Soya milk and Tofu are the most important non-fermented Soya bean products. They are digestible and nutritive foods (Byun and Kang, 1995). Soybeans have long been staple of the human diet in Asia, especially as soymilk or tofu, which is prepared from soybeans (Liu, 1997; Wantambe, 1997). They are inexpensive, high quality protein source, soymilk and tofu consumption is increasing in North America due to an increase in Asia immigrants, greater acceptance of soy foods by the general population, and increase recognition of the health benefits of soy foods, especially by those who wish to reduce their consumption of animal products (Murphy *et al*., 1997).

Tofu is a good source of proteins; carbohydrates low in fat and rich in mineral contents. The incorporation into western diet is principally to prevent and treat chronic diseases, such as cancer and cardiovascular diseases as supported by epidemiological studies (Troll, *et al.*, 1980; Lee, *et al.*, 1991). Tofu can be made from any soybeans; soy food processors prefer large seeded, high protein cultivars. Only limited specific information is available, however, on which soybean traits contribute to superior food production.

Soy foods may be divided generally into fermented (Tempe, miso, and Soy sauce etc) and non-fermented (Soy milk, okara, Tofu etc). The recent increase in soymilk and Tofu consumption especially in western countries is due to an increase in acceptance of Soy foods by the general and recognition of the health benefits of Soy foods. For instance consumption of 25g of Soybean protein per day can contribute to the lowering of serum cholesterol level and the prevention of heart disease, (Jackson

et al., 2002). This health claim places soy foods among a selected category of functional foods possessing unique medicinal as well as nutritional value (Jackson *et al.*, 2002).

The potential role of dietary Soya in the prevention and treatment of chronic disease, in particular, heart disease and cancer, has been recognized for a long time. Soybean protein, Isoflavonoids, phospholipids, Saponins, and phytate, have been investigated in a search for the active component responsible for the anti-atherogenic effect of Soyabean. Some experiments suggested that the amino acid profile of proteins and other non-protein components present in Soyabeans may be partially responsible for the hypocholesterolaemic effect (Huff *et al*., 1977; Potter *et al*. 1996).

1.1 FOOD AND THE COMPOSITION

Food is any substance, usually composed primarily of carbohydrates, fats, water and/or proteins, that can be eaten or drunk by an animal or human for nutrition or pleasure. Items considered food may be sourced from plants, animals or other categories such as fungus or fermented products like alcohol. Although many human cultures sought food items through hunting and gathering, today most cultures use farming, ranching, and fishing, with hunting, foraging and other methods of a local nature included but playing a minor role (McGee, 2004).

Most traditions have a recognizable cuisine, a specific set of cooking traditions, preferences, and practices, the study of which is known as gastronomy. Many cultures have diversified their foods by means of preparation, cooking methods and manufacturing. This also includes a complex food trade which helps the cultures to economically survive by-way-of food, not just by consumption (McGee, 2004).

1.11 Food sources

Almost all foods are of plant or animal origin, although there are some exceptions. Foods not coming from animal or plant sources include various edible fungi, such as mushrooms. Fungi and ambient bacteria are used in the preparation of fermented and pickled foods such as leavened bread, alcoholic drinks, cheese, pickles, and yogurt. Many cultures eat seaweed, a protist, or blue-green algae (cyanobacteria) such as Spirulina. Additionally, salt is often eaten as a flavoring or preservative, and baking soda is used in food preparation. Both of these are inorganic substances, as is water, an important part of human diet (McGee, 2004).

1.12 Plants

Many plants or plant parts are eaten as food. There are around 2,000 plant species which are cultivated for food, and many have several distinct cultivars.

Seeds of plants are a good source of food for animals, including humans because they contain nutrients necessary for the plant's initial growth. In fact, the majority of foods consumed by human beings are seed-based foods. Edible seeds include cereals (such as maize, wheat, and rice), legumes (such as beans, peas, and lentils), and nuts. Oilseeds are often pressed to produce rich oils, such as sunflower, rapeseed (including canola oil), and sesame. One of the earliest food recipes made from ground chickpeas is called hummus, which can be traced back to Ancient Egypt times (McGee, 2004).

Fruits are the ripened ovaries of plants, including the seeds within. Fruits, therefore, make up a significant part of the diets of most cultures. Some botanical fruits, such as tomatoes, pumpkins and eggplants, are eaten as vegetables (McGee, 2004).

Vegetables are a second type of plant matter that is commonly eaten as food. These include root vegetables (such as potatoes and carrots), leaf vegetables (such as spinach and lettuce), stem vegetables (such as bamboo shoots and asparagus), and inflorescence vegetables (such as globe artichokes and broccoli). Many herbs and spices are highly-flavorful vegetables (McGee, 2004).

1.13 Animals

Animals can be used as food either directly, or indirectly by the products they produce which include milk produced by mammals, which in many cultures is drunk or processed into dairy products such as cheese or butter. In addition birds and other animals lay eggs, which are often eaten, and bees produce honey, a popular sweetener in many cultures. Some cultures consume blood, some in the form of blood sausage, as a thickener for sauces, a cured salted form for times of food scarcity, and others use blood in stews such as civet (McGee, 2004).

1.14 Carbohydrates: Substances that provide energy

Carbohydrates may be classified as monosaccharides, disaccharides, or polysaccharides by the number of monomer (sugar) units they contain. They are found in large proportion in foods such as rice, noodles, bread and other grain-based products. Carbohydrates are classified by their number of sugar units: monosaccharides (such as glucose and fructose) they contain 1 sugar unit, disaccharides contain 2 sugar units (such as sucrose and lactose), and polysaccharides contain 3 or more. Polysaccharides are often referred to as complex carbohydrates because they are long chains of sugar units, whereas monosaccharides and disaccharides are simpler. The difference is important because complex carbohydrates

take longer to digest and absorb since their sugar units are processed one-by-one off the ends of the chains; the spike in blood sugar levels caused by substantial amounts of simple sugars is thought to be at least part of the cause of increased heart and vascular disease associated with high simple sugar consumption. Simple carbohydrates are absorbed quickly and thus raise blood sugar levels more rapidly (McGee, 2004).

1.15 Protein

Proteins are organic compounds that consist of the amino acids joined by peptide bonds. The body cannot manufacture some of the amino acids (termed essential amino acids); the diet must supply these. In nutrition, proteins are broken down through digestion by proteases back into free amino acids (McGee, 2004).

Proteins are the basis of animal body structures (eg, muscles, skin, hair etc.). They are composed of amino acids, sometimes many thousands, which are characterized by inclusion of nitrogen and sometimes sulphur. The body requires amino acids to produce new body protein (protein retention) and to replace damaged proteins (maintenance). Amino acids not needed are discarded, typically in the urine. In animals, amino acid requirements are classified in terms of essential (an animal cannot produce them internally) and non-essential (the animal can produce them from other nitrogen containing compounds) amino acids (McGee, 2004). Humans use about 20 amino acids, and about ten are essential in this sense. Consuming a diet that contains adequate amounts of essential (but also non-essential) amino acids is particularly important for growing, pregnant, nursing, or injured animals, all of whom have a particularly high requirement. Protein nutrition which contains the essential

amino acids is a complete protein source, one missing one or more is called incomplete. It is possible to combine two incomplete protein sources (eg, rice and beans) to make a complete protein source. Dietary sources of protein include meats, tofu and other soy-products, eggs, grains, legumes, and dairy products such as milk and cheese. A few amino acids from protein can be converted into glucose and used for fuel through a process called gluconeogenesis. The remaining amino acids are discarded (McGee, 2004).

1.16 Fat

Fats consist of a glycerin molecule with three fatty acids attached. Fatty acids are unbranched hydrocarbon chains, connected by single bonds alone (saturated fatty acids) or by both double and single bonds (unsaturated fatty acids). Fats are needed to keep cell membranes functioning. Fat has an energy content of 9 kcal/g (~37.7 kJ/g); proteins and carbohydrates 4 kcal/g (~16.7 kJ/g). Ethanol (grain alcohol) has an energy content of 7 kcal/g (~29.3 kJ/g).Fats are composed of fatty acids (long carbon/hydrogen chains) bonded to a glycerol; they are typically found as triglycerides (three fatty acids attached to one glycerol backbone). Certain fatty acids are essential. Fats may be classified as saturated or unsaturated. Saturated fats have all of their carbon atoms bonded to hydrogen atoms, whereas unsaturated fats have some of their carbon atoms double-bonded in place of a hydrogen atom. In humans, multiple studies have shown that unsaturated fats are to be preferred for health reasons, particularly mono-unsaturated fats (McGee, 2004). Saturated fats, typically from animal sources, Saturated and trans fats are typically solid at room temperature (such as butter or lard), while unsaturated fats are typically liquids (such as olive oil or flaxseed oil). Unsaturated fats may be further classified as monounsaturated (one

double-bond) or polyunsaturated (many double-bonds). Trans fats are saturated fats but are typically created from unsaturated fat by adding the extra hydrogen atoms in an industrial process called hydrogenation; they are also called hydrogenated fat. They are very rare in nature, but have properties useful in the food processing industry (McGee, 2004).

Fats consist of a wide group of compounds that are generally soluble in organic solvents and largely insoluble in water. Chemically, fats are generally triesters of glycerol and fatty acids. Fats may be either solid or liquid at normal room temperature, depending on their structure and composition. "Oils" is usually used to refer to fats that are liquids at normal room temperature, while "fats" is usually used to refer to fats that are solids at normal room temperature. "Lipids" is used to refer to both liquid and solid fats, along with other related substances. The word "oil" is used for any substance that does not mix with water and has a greasy feel, such as petroleum (or crude oil) and heating oil, regardless of its chemical structure (McGee, 2004).

Fats form a category of lipid, distinguished from other lipids by their chemical structure and physical properties. This category of molecules is important for many forms of life, serving both structural and metabolic functions. They are an important part of the diet of most heterotrophs (including humans). Fats or lipids are broken down in the body by enzymes called lipases produced in the pancreas (McGee, 2004).

Examples of edible animal fats are lard (pig fat), fish oil, and butter or ghee. They are obtained from fats in the milk, meat and under the skin of the animal. Examples of edible plant fats are peanut, soya bean, sunflower, sesame, coconut, olive, and

vegetable oils. Margarine and vegetable shortening, which can be derived from the above oils, are used mainly for baking. These examples of fats can be categorized into saturated fats and unsaturated fats (McGee, 2004).

1.17 Micronutrients: Substances that support metabolism

Micronutrients are nutrients needed for life in small quantities. The **Microminerals** or **trace elements** include iron, cobalt, chromium, copper, iodine, manganese, selenium, zinc and molybdenum. They are dietary minerals needed by the human body in very small quantities (generally less than 100micrograms/day) as opposed to macrominerals which are required in larger quantities.

Vitamins are organic chemicals that a given living organism requires in trace quantities for good health, but which the organism cannot synthesize, and therefore must obtain from its diet (McGee, 2004).

More than two billion people (i.e. one in three persons worldwide) suffer from micronutrient deficiency, a form of malnutrition. The most common deficiencies can have devastating consequences:

- Vitamin A deficiency: Nearly 3 million preschool children in developing countries are blind because of vitamin A deficit.
- Iron-deficiency Anemia results in one out of four maternal deaths in the developing world.
- Iodine deficiency is the world's leading cause of mental retardation -- more than 2 billion children suffer from lowered IQ and retardation due to Iodine

deficiency. The costs of providing iodized salt are estimated at 10 cents per person per year (McGee, 2004).

Vitamin E, zinc and Manganese deficiencies are also serious causes for concern.

- Dietary minerals are generally trace elements, salts, or ions such as copper and iron. Some of these minerals are essential to human metabolism.
- Vitamins are organic compounds essential to the body. They usually act as coenzymes or cofactors for various proteins in the body.
- Water is an essential nutrient and is the solvent in which all the chemical reactions of life take place.

- Many cultures study the dietary analysis of food habits. While humans are omnivores, religion and social constructs such as morality often affect which foods they will consume. Food safety is also a concern with foodborne illness claiming many lives each year (Lieberman and Bruning, 1990).

1.2 SOY: The Magic Bean

More than five thousand years ago early Chinese farmers discovered and began cultivating a legume that would eventually become an essential food for much of the world. This plant is known today as the ‘greater bean’ in China, or as it is known in the west, the soybean. During the subsequent millennia, soybean use spread across China, Korea, Japan, and Southeast Asia. (Gissen, 1996). Soybeans yield more protein per acre than any other crop, it has become a staple for many societies. Numerous food products have been developed from soybeans including soymilk, tofu (curdled soymilk), and meat substitutes. Developed since two thousand years ago,

today tofu is the world's most popular soy food product. In fact, the average Japanese person eats approximately a pound of tofu every week. (Liu, 1997).

Since the introduction of soybean as a food, it has gained a reputation for having medicinal properties. The Chinese believe that soybeans are effective for treating the common cold, skin diseases, beriberi, diarrhea, and toxemia of pregnancy, constipation, anemia, and leg ulcers (Gissen, 1996). The soft like cheese curd, known as tofu (toufu) or tubu and other local names has a bland taste and can be flavored with seasonings or blended with other foods. Soybeans curd is made into a variety of products by frying, drying and freezing and is consumed daily in the same manner as high-protein foods in western cultures. Soybeans are also prepared by fermentation with microorganisms to form highly flavored foods and seasoning such as Soy sauce and fermented Soybean taste. AAFC (2008)

1.21 Antioxidants

Antioxidants are a recent discovery. As cellular metabolism/energy production requires oxygen, potentially damaging (e.g. mutation causing) compounds known as free radicals can form. Most of these are oxidizers ie, (acceptors of electrons) and some react very strongly. For normal cellular maintenance, growth, and division, these free radicals must be sufficiently neutralized by antioxidant compounds. Some are produced by the human body with adequate precursors (glutathione, Vitamin C) and those that the body cannot produce may only be obtained through the diet through direct sources (Vitamin C in humans, Vitamin A, Vitamin K) or produced by the body from other compounds (Beta-carotene converted to Vitamin A by the body, Vitamin D synthesized from cholesterol by sunlight). Phytochemicals and their subgroup polyphenols are the majority of antioxidants; about 4,000 are known. Different

antioxidants are now known to function in a cooperative network, e.g. vitamin C can reactivate free radical-containing glutathione or vitamin E by accepting the free radical itself, and so on. Some antioxidants are more effective than others at neutralizing different free radicals. Some cannot neutralize certain free radicals. Some cannot be present in certain areas of free radical development (Vitamin A is fat-soluble and protects fat areas, Vitamin C is water soluble and protects those areas). When interacting with a free radical, some antioxidants produce a different free radical compound that is less dangerous or more dangerous than the previous compound. Having a variety of antioxidants allows any byproducts to be safely dealt with by more efficient antioxidants in neutralizing a free radical (Berg *et al.* 2002).

1.22 Phytochemicals

Phytochemicals are collections of trace chemicals that have positive effect upon human health. These nutrients are typically found in edible plants, especially colourful fruits and vegetables, but also other organisms including seafood, algae, and fungi. The effects of phytochemicals increasingly survive rigorous testing by prominent health organizations. One of the principal classes of phytochemicals are polyphenol antioxidants, chemicals which are known to provide certain health benefits to the cardiovascular system and immune system. These chemicals are known to down-regulate the formation of reactive oxygen species, key chemicals in cardiovascular disease (Berg *et al.* 2002).

Perhaps the most rigorously tested phytochemical is zeaxanthin, a yellow-pigmented carotenoid present in many yellow and orange fruits and vegetables. Repeated studies have shown a strong correlation between ingestion of zeaxanthin and the prevention

and treatment of age-related macular degeneration (AMD). Less rigorous studies have proposed a correlation between zeaxanthin intake and cataracts. A second carotenoid, lutein, has also been shown to lower the risk of contracting AMD. Both compounds have been observed to collect in the retina when ingested orally, and they serve to protect the rods and cones against the destructive effects of light (Berg *et al.* 2002).

Another carotenoid, beta-cryptoxanthin, appears to protect against chronic joint inflammatory diseases, such as arthritis. While the association between serum blood levels of beta-cryptoxanthin and substantially decreased joint disease has been established, neither a convincing mechanism for such protection nor a cause-and-effect have been rigorously studied. Similarly, a red phytochemical, lycopene, has substantial credible evidence of negative association with development of prostate cancer. The correlations between the ingestion of some phytochemicals and the prevention of disease are, in some cases, enormous in magnitude (Berg *et al.* 2002).

Even when the evidence is obtained, translating it to practical dietary advice can be difficult and counter-intuitive. Lutein, for example, occurs in many yellow and orange fruits and vegetables and protects the eyes against various diseases. However, it does not protect the eye nearly as well as zeaxanthin, and the presence of lutein in the retina will prevent zeaxanthin uptake. Additionally, evidence has shown that the lutein present in egg yolk is more readily absorbed than the lutein from vegetable sources, possibly because of fat solubility (Berg *et al.* 2002).

TABLE 1: PHYTOCHEMICAL GROUPS AND SOURCES ARRANGED BY FAMILY

Family	Sources	Possible Benefits
Flavonoids	berries, herbs, vegetables, wine, grapes, tea	general antioxidant, oxidation of LDLs, prevention of arteriosclerosis and heart disease
Isoflavones (phytoestrogens)	soy, red clover, kudzu root	general antioxidant, prevention of arteriosclerosis and heart disease, easing symptoms of menopause, cancer prevention[17]
Isothiocyanates	cruciferous vegetables	cancer prevention
Monoterpenes	citrus peels, essential oils, herbs, spices, green plants, atmosphere	cancer prevention, treating gallstones
Organosulfur compounds	chives, garlic, onions	cancer prevention, lowered LDLs, assistance to the immune system
Saponins	beans, cereals, herbs	Hypercholesterolemia, Hyperglycemia, Antioxidant, cancer prevention, Anti-inflammatory
Capsaicinoids	all *capiscum* (chile) peppers	topical pain relief, cancer prevention, cancer cell apoptosis

Source: AAFC (2008)

1.23 FUNCTIONAL FOODS

They are conventional foods that are consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions, i.e. they contain bioactive compound (AAFC, 2008).

Bioactive Compounds are the naturally occurring chemical compounds contained in, or derived from, a plant, animal or marine source, that exert the desired health/wellness benefit (e.g. omega-3 fatty acids in flax or fish oils and beta-glucans from oats and barley) (AAFC, 2008).

The Institute of Medicine's Food and Nutrition Board 1 has defined functional foods as "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains." While most research and food product development efforts have focused on plant foods, several physiologically-active components in foods from animal sources also are being studied. Thus, functional foods can be from either plant or animal sources (AAFC, 2008).

The concept of functional foods has somewhat different connotations in different countries. In Japan, for example, functional foods are defined based on their use of natural ingredients. In the United States, however, the functional foods concept can include ingredients that are products of biotechnology.

Functional Ingredients are the standardized and characterized preparations, fractions or extracts containing bioactive compounds of varying purity, that are used as ingredients by manufacturers in the food (human and pet) and preparations, fractions or extracts containing bioactive compounds of varying purity, which are used as

ingredients by manufacturers in the cosmetics and pharmaceutical sectors (AAFC, 2008).

Herbs are the leaves, roots and flowers of plants grown and processed for culinary, cosmetic, industrial, medicinal, landscaping, decorative and fragrant purposes. Much of the early interest in functional foods and nutraceuticals was based on the medicinal uses of herbs (AAFC, 2008).

1.24 Functional Foods From Plant Sources

Oats. Oat products are a widely studied dietary source of the cholesterol-lowering soluble fiber b-glucan. There is now significant scientific agreement that consumption of this particular plant food can reduce total and low density lipoprotein (LDL) cholesterol, thereby reducing the risk of coronary heart disease (CHD) (AAFC, 2008).

Soy. Soy has been in the spotlight during the 1990s. Not only is soy a high quality protein, as assessed by the FDA's "Protein Digestibility Corrected Amino Acid Score" method, it is now thought to play preventive and therapeutic roles in cardiovascular disease (CVD), cancer, osteoporosis, and the alleviation of menopausal symptoms (AAFC, 2008).

Flaxseed. Among the major seed oils, flaxseed oil contains the most (57%) of the omega-3 fatty acid, a-linolenic acid. Others include Tomatoes, Garlic.(Allium sativum), Broccoli and other Cruciferous Vegetables, Citrus Fruits, Cranberry, Tea, Wine and Grapes (AAFC, 2008).

1.25 Functional Foods From Animal Sources

Although the vast number of naturally occurring health-enhancing substances are of plant origin, there are a number of physiologically-active components in animal products that deserve attention for their potential role in optimal health.

Fish. Omega-3 (n-3) fatty acids are an essential class of polyunsaturated fatty acids (PUFAs) derived primarily from fish oil (AAFC, 2008).

Dairy Products. There is no doubt that dairy products are functional foods. They are one of the best sources of calcium, an essential nutrient which can prevent osteoporosis and possibly colon cancer. In view of the former, the National Academy of Sciences recently increased recommendations for this nutrient for most age groups. In addition to calcium, however, recent research has focused specifically on other components in dairy products, particularly fermented dairy products known as probiotics (AAFC, 2008).

A ***nutraceutical*** is a product isolated or purified from foods that is generally sold in medicinal forms not usually associated with foods. A *nutraceutical* is demonstrated to have a physiological benefit or provide protection against chronic disease.

It is a product isolated or purified from foods that is generally sold in medicinal forms not usually associated with foods. A *nutraceutical* is demonstrated to have a physiological benefit or provide protection against chronic disease (AAFC, 2008). It may also be considered a food or part of a food that provides medical or health benefits, including the prevention and treatment of disease. Under this broad definition, nutraceuticals might be isolated nutrients like vitamin E; dietary

supplements of any kind; processed foods; herbal products; or genetically engineered foods. While some individuals and groups use nutraceutical as an umbrella term, others restrict the meaning to isolated active compounds or dietary supplements (McGee, 2004).

Natural Health Products (NHP) includes homeopathic preparations; substances used in traditional medicines; minerals or trace elements; vitamins; amino acid; essential fatty acids; or other botanical, or animal or microorganism derived substances. These products are generally sold in medicinal or "dosage" form to diagnose, treat, or prevent disease; restore or correct function; or to maintain or promote health. As a product group, NHPs include nutraceuticals (McGee, 2004).

Novel Foods are defined by Health Canada as: products that have never been used as food; foods that result from a process that has not previously been used for food; or, foods that have been modified by genetic manipulation (AAFC, 2008).

Spices are seeds, root, bark and flowers of plants that are grown, harvested and processed for use as food or beverage flavouring. Examples include caraway, coriander, dill and mustard. Recently there has been interest in bioactive compounds identified in spices AAFC (2008).

TABLE 2: FUNCTIONAL COMPONENTS, SOURCES AND POTENTIAL BENEFITS OF FOOD

Carotenoids		
FUNCTIONAL COMPONENTS	**SOURCE**	**POTENTIAL BENEFITS**
Alpha-carotene/Beta-carotene	Carrots, Fruits, Vegetables	Neutralize free radicals, which may cause damage to cells
Lutein	Green vegetables	Reduce the risk of muscular degeneration
Lycopene	Tomato products (ketchup, sauces)	Reduce the risk of prostate cancer
Dietary Fibre		
Insoluble Fibre	Wheat Bran	Reduce risk of breast or colon cancer
Beta-Glucan	Oats, barley	Reduce risk of cardiovascular disease. Protect against heart disease and some cancers; lower LDL and total cholesterol
Soluble Fibre	Psyllium	Reduce risk of cardiovascular disease. Protect against heart disease and some cancers; lower LDL and total cholesterol
Fatty Acids		
Long chain omega-3 Fatty Acids-DHA/EPA	Salmon and other fish oils	Reduce risk of cardiovascular disease. Improve mental, visual functions
Conjugated Linoleic Acid (CLA)	Cheese, meat products	Improve body composition. Decrease risk of certain cancers

Phenolics		
Anthocyanidins	Fruits	Neutralize free radicals; reduce risk of cancer
Catechins	Tea	Neutralize free radicals; reduce risk of cancer
Flavonones	Citrus	Neutralize free radicals; reduce risk of cancer
Flavones	Fruits/vegetables	Neutralize free radicals; reduce risk of cancer
Lignans	Flax, rye, vegetables	Prevention of cancer, renal failure
Tannins (proanthocyanidines)	Cranberries, cranberry products, cocoa, chocolate	Improve urinary tract health. Reduce risk of cardiovascular disease
Plant Sterols		
Stanol ester	Corn, soy, wheat, wood oils	Lower blood cholesterol levels by inhibiting cholesterol absorption
Prebiotics/Probiotics		
Fructo-oligosaccharides (FOS)	Jerusalem artichokes, shallots, onion powder	Improve quality of intestinal microflora; gastrointestinal health
Lactobacillus	Yogurt, Other dairy	Improve quality of intestinal microflora; gastrointestinal health
Soy Phytoestrogens		
Isoflavones: Daidzein Genistein	Soybeans and soy-based foods	Menopause symptoms, such as hot flashes Protect against heart disease and some cancers; lower LDL and total cholesterol

AAFC (2008)

1.26 FOOD ADDITIVES

Food additives are substances added to food for preserving flavors, or improving taste or appearance. These are generally listed by "E number" in the European Union or GRAS ("Generally recognized as safe") by the United States Food and Drug Administration (Fennema, 1996).

Despite modern-day associations food additives have been used for centuries. Food preservation began when man first learned to safeguard food from one harvest to the next and by the salting and smoking of meat and fish. The Egyptians used colours and flavourings, and the Romans used saltpetre (potassium nitrate), spices and colours for preservation and to improve the appearance of foods. Cooks regularly used baking powder as a raising agent, thickeners for sauces and gravies, and colours, such as cochineal, to transform good-quality raw materials into foods that were safe, wholesome and enjoyable to eat. The overall aims of traditional home cooking remain the same as those prepared and preserved by today's food manufacturing methods (Fennema, 1996).

A food additive is defined as "any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods" (Council Directive 89/107/EEC).

Foods are subjected to many environmental conditions, such as temperature changes, oxidation and exposure to microbes, which can change their original composition. Food additives play a key role in maintaining the food qualities and characteristics that consumers demand, keeping food safe, wholesome and appealing from farm to fork. Food additives are very carefully regulated and the general criteria for their use is that they perform a useful purpose, are safe and do not mislead the consumer (Fennema, 1996).

Colours

Reactions to tartrazine (E 102, a yellow food colour) and carmine (E 120 or red cochinille) have been reported occasionally in sensitive individuals. Symptoms include skin rashes nasal congestion and hives, although the incidence is very low (estimated to be 1-2 persons per 10,000) and very rare. IgE-mediated allergic reactions have been reported for carmine. Tartrazine has also been reported to cause asthma in sensitive individuals although the incidence is extremely low (Fennema, 1996).

Colour is one of the first and most important sensory qualities and it helps us to accept or reject particular foods. Whilst adding colour may appear to some to be purely cosmetic, there is no doubt that colour is important in consumer perception of a food and it is often associated with a specific flavour and intensity of flavour. Some colours are used purely for visual decoration on cakes and confectionery items. Masking or disguising inferior quality, however, are unacceptable uses of colours.

The primary reasons for adding colours to foods include:

- To offset colour loss due to exposure to light, air, extremes of temperature, moisture and storage conditions
- To compensate for natural or seasonal variations in food raw materials or the effects of processing and storage to meet consumer expectations (Masking or disguising inferior quality, however, are unacceptable uses of colours.).
- To enhance colours that occur naturally but at levels weaker than those usually associated with a given food (Fennema, 1996).

Sulphites

One group of additives that can cause problems in sensitive individuals is the sulfiting agents. This group includes several inorganic sulphite additives (E 220-228), including sodium sulphite, potassium bisulphite and metabisulphite containing sulphur dioxide (SO_2). These preservatives are used to control microbial growth in fermented beverages and they have been widely used in wines, beers and fruit products for over 2000 years. In sensitive (asthmatic) individuals, sulphites may trigger asthma characterised by breathing difficulties, shortness of breath, wheezing and coughing (Fennema, 1996).

Monosodium glutamate (MSG) and aspartame

MSG is made up of sodium and glutamic acid. Glutamic acid is an amino acid found naturally in high protein foods such as meats and dairy products like Camembert cheese. MSG is also a flavour enhancer used in prepared meals, some Chinese food, certain sauces and soups. MSG has been "blamed" for a variety of side effects including headaches and body tingling, however scientific studies show no link between MSG and these reactions suggesting that some other component of the meal,

or even psychological responses, may be responsible for any adverse effects (Fennema, 1996).

Similarly, the high-intensity sweetener aspartame (another substance made from naturally occurring amino acids, aspartic acid and phenylalanine) has been blamed for a wide variety of adverse effects, none of which have been validated by scientific studies (Fennema, 1996).

- **Additives that maintain freshness and prevent deterioration**

Some food additives help to keep foods fresh and safe. They help increase shelf-life by protecting foods against deterioration caused by oxidation or by micro-organisms. They can be divided into two categories based on their principal function (Fennema, 1996).

- **Antioxidants**

Antioxidants prevent the oxidation of foods that results rancidity or discoloration. They are used in baked foods, cereals, fats, oils and salad dressings. The major fat soluble antioxidants are:

- Tocopherols (E 306-309), BHA (butylated hydroxyanisole or E 320) and BHT (butylated hydroxytoluene or E 321) - these protect edible fats, vegetable oils and salad dressings from turning rancid.
- Ascorbic acid (E 300) and citric acid (E 330) - which preserve the colour of freshly cut fruits and vegetables.

➢ **Preservatives**

Preservatives limit, retard or arrest the growth of micro-organisms (e.g. bacteria, yeast, mould) that are present in or gain entry to the food, preventing spoilage or food poisoning. They are used in baked foods, wine, cheese, cured meats, fruit juices and margarine among others. Examples include:

- Sulphur dioxide and sulphites (E 220-228) - these help to prevent colour changes in dried fruits and vegetables. Sulphites also inhibit the growth of bacteria in wine and fermented foods, some snack foods and baked goods. Sulphites also have antioxidant properties.
- Calcium propionate (E 282) - prevents bread and baked foods from turning mouldy.
- Nitrates and nitrites (sodium and potassium salts) (E 249-252) - are used as a preservative in processed meats such as ham and frankfurters to keep the products safe by preventing the growth of botulinum bacteria, Clostridium botulinum, which is highly pathogenic (Fennema, 1996).

❖ **Additives that amplify or promote sensory qualities**

Additives are also useful for imparting certain characteristics to foods, improving texture or helping in food processing.

➢ **Taste and texture modifiers**

Examples are: Emulsifiers and stabilisers - The purpose of these food additives is to maintain consistent texture and to prevent the separation of ingredients in such products as margarine, low-fat spreads, ice cream, salad dressings and mayonnaise.Examples include lecithin, mono- and diglycerides (Fennema, 1996).

- Thickeners - these substances help increase the viscosity of foodstuffs. They are added to foods such as salad dressings and flavoured milk. Gelatin or pectin are often used as thickening agents.
- Sweeteners - Both "bulk" and "intense" sweeteners impart a sweet taste to foodstuffs and are useful in low-calorie products and for special dietary products, such as those for diabetics. Intense sweeteners, such as acesulfam K (E 950), aspartame (E 951) and saccharin (E 954) are 130-200 times, 200 times and 300-500 times sweeter, respectively, than sugar-and they have zero calories. Thaumatin (E 957), a naturally sweet protein extracted from the fruit of the plant Thaumatococcus danielli, is 2500 times sweeter than sugar and is used at very low levels for its flavouring properties. Bulk sweeteners include sorbitol (E 420), isomalt (E 953) and maltitol (E 965) and these can be incorporated into "table-top" sweeteners and in energy-reduced foods, in which they provide volume and mouth feel. These substances have reduced caloric value, providing 2.4 kcal/gram compared with 4 kcal/gram for other carbohydrates (Fennema, 1996).

Flavors

- Flavor in food gives food good smells and tastes to the consumer, especially in sensory analysis. Some of these products occur naturally like salt and sugar, but flavor chemists (called a "flavorist") develop many of these flavors for

food products. Such artificial flavors include methyl salicylate which creates the wintergreen odor and lactic acid which gives milk a tart taste.

- Flavour enhancers - Probably the best known is monosodium glutamate (MSG; E 621), which is used to bring out and enhance the flavours in the foods to which it is added. It is used mainly in savoury products and in a wide variety of oriental dishes (Fennema, 1996).
- Others - this group includes acids, acidity regulators (used to control acidity and alkalinity in various types of food products), anti-caking agents (used to keep powders flowing freely), anti-foaming agents (reduce foams, e.g. when jams are boiled), and packaging gases (used in certain types of sealed packages, such as for meat, fish, seafood and ready-prepared vegetables and salads found in chill cabinets) (Fennema, 1996).

1.3 SOY COMPOSITION

Soybean has recently undergone a transformation from food to medicine confirming the beliefs of ancient Chinese doctors on the medicinal value of soy bean. This is primarily due to the discovery of a number of phytochemicals that are found in it. These phytochemicals include protease inhibitors, phytate, saponins and isoflavones (Liu ,1997).

1.31 PROTEASE INHIBITORS

These are traditionally viewed as antinutrients, as they prevent the proper functioning of the enzymes responsible for digesting protein in the diet (Gissen, 1996). This is the reason why animals fed on soybeans do not grow properly. Heating and processing of soybean into its products prevents the problem of protease inhibitors since they are broken down or removed (Gissen, 2004). It was recently

discovered that soybeans has the potential of inhibiting cancer in animals. The protease inhibitors appear to have an anti-carcinogen mechanism of action whereby they prevent normal functioning of proteins involved in the activation of certain cancer related genes. Large amounts prevent proper digestion of soy; however, processed soy foods contain only 5% or less of the naturally occurring protease inhibitors. Since small amounts of protease inhibitors do not cause growth suppression but do have anticancer effects in animals, soy foods can be consumed safely without fear of growth suppression (Gissen, 2004).

1.32 PHYTATES

Phytate, a compound of inositol, is the storage form of mineral phosphorus in plants. They are also considered as antinutrients due to the fact that they bind iron and calcium preventing their proper absorption (Oboh *et al.* 2003). Phytates are quite high in soybean and are considered beneficial due to its anticancer properties and capability of preventing heart diseases. Inositol phosphates are biochemicals that are in our cells and play a part in controlling cell growth by their role as intracellular messenger. Dietary phytate are now thought of as being able to alter the body's pool of intracellular inositol phosphates and may prevent cancer by controlling proliferic cell growth. It is also considered to have antioxidant properties due to its ability to chelate iron, preventing both iron's reactivity and absorption (Gissen, 2004).

1.33 SAPONINS

Plant saponins have been long considered as toxic. However, some human foodstuffs have been found to contain significant amounts of saponins that possess little toxicity (Oboh *et al.* 1999; Oboh *et al.* 2002), soybean is one of such foods. Saponins have been suggested as possible anti carcinogens having a mechanism of

action which include cytotoxicity immune modulatory effects, bile acid binding and normalization of carcinogen induced cell proliferation (Oboh *et al.* 1998; Oboh *et al.* 1999; Potter *et al*., 1993). Soy saponins particularly have been shown to inhibit human cancer cell growth despite its low toxicity. Recent research has also suggested that saponins have cholesterol lowering properties and diets high in saponins are associated with lower rates of cardiovascular diseases (Oboh *et al.*1997; Gissen, 1996).

1.4 *TOFU*

Tofu is one of the most important and popular food products in east and south eastern state of Asian countries and is gaining an increasing popularity in western countries as well. Developed some two thousand years ago; it has become the world's most popular soy food product (Gissen, 2004). There are many ways of preparing soymilk and tofu (Watambe *et al.*, 1997; Liu, 1997). Soy milk is commonly made by soaking soybeans in excess water, draining, grinding, with additional water extracting the raw soymilk from the pulp residue (okara) and cooking the soymilk (Liu, 1997).

Tofu is an unfermented soy product, also known as soy bean curd and is a soft, cheese-like food made by curdling fresh hot soy milk with a coagulant which is either a salt ($CaCl_2$, $CaSO_4$) or an acid (glucuno-d-lactone). Traditionally the curdling agent used to make Tofu is nigari-a compound found in natural ocean waters or Calcium Sulfate ($CaSO_4$) (Prestamo *et al.*, 2002).The coagulant produce a soy protein gel which traps water, soy lipids and other constituents in the matrix forming curds. The curds are then pressed into solids cubes. The yield and quality of tofu are influenced by soy bean varieties, soy bean quality (growth and storage environment dependent) and processing conditions of the coagulants) (Prestamo *et al.,* 2002).

The yield and quality of tofu is affected by soy bean varieties, soybean quality (growth and environmental dependent), and processing conditions. Coagulation is the most difficult to master, since it relies on complete interrelationship of the following variable: soybean chemistry, soymilk cooking temperature, volume, solid content and PH; coagulant type, amount, concentration and the method of adding and mixing; and coagulation temperature and time (Shurtleff and Aoyagi, 1990; Cai and Chang, 1997). The variations in controlling all these variables greatly affect tofu yield quality. Increasing coagulation temperature, stirring speed, and coagulant concentration increased tofu hardness but decrease tofu yield (Watambe *et al*., 1964; Hu *et al*., 1997). Stirring speed and time also had significant influence on tofu yield and quality (Hu *et al.,* 1997).

Most studies available in literature (Kamel and de Man, 1982) used small manual laboratory scale method to prepare tofu from 5 to 300g soybeans. None of them have applied a production scale method for determining suitability of soybeans for tofu making. It does not depend only on processing parameters but also on researcher's skills in making *tofu*, particularly when a small manual laboratory scale method is used. Laboratory scale methods using a small quantity of beans are difficult to reproduce manually because tofu making involves a complex interaction of many variables. Variations in tofu making procedures and new soybean materials can cause difficulties in comparing results among laboratories (Murphy *et al*, 1997) suggested that the evaluation of soybean variety for suitability as a *tofu* cultivars tofu preparation on a preparation scale.

A small production scale method has been developed to evaluate the quality of soybean variety for tofu making using an automatic tofu machine (Cai and Chang, 1998). The use of the automatic machine reduces variability of the manual laboratory

procedures. The tofu machine is an effective means of evaluating suitability of existing commercial soybean varieties and soybean cultivars in the final phases of breeding selection for tofu making. With the rapid development of tofu market and new soybean varieties in the world, the need for more reproduce able quantitative data on Soya milk coagulation in tofu production with a production scale method has become necessary (Prestamo *et al*, 2002).

Coagulation of soymilk is the most important step in the tofu process and the most difficult to master since it relies on the complex interrelationship of the following varieties: soy bean chemistry; soymilk cooking temperature, volume, solid content and pH; coagulant type, amount; concentration and the method of adding and mixing; and the coagulation temperature and time (Cai and Chang, 1999). Each coagulant produces Tofu with different textural and flavor properties (Shurtleff and Aoyagi, 1990; Watanabe, 1997; Poysa and Woodrow, 2002).The texture of Tofu should be smooth, firm and coherent but not hard and rubbery. Since Tofu is a soy protein gel, the amount of soy protein used to make the soy milk is critical for Tofu yield and quality (Poysa and Woodrow, 2002).

Tofu is available as three (3) major types which are firm, soft and silken tofu. Firm *Tofu* is dense and solid and has a higher protein and fat content than other forms of *Tofu*. Firm *tofu* also contains higher calcium content. Soft *Tofu* is solid but not firm while silken *Tofu* is a creamy product (Shurtleff and Aoyagi, 1990).

1.41 *TOFU* COAGULANTS

Natural calcium sulfate (gypsum) and magnesium chloride (nigari) are the most common *tofu* coagulants used. They have been used for hundreds years in Japan and China. Nigari is composed mainly of magnesium chloride, but also contains other

minerals found in seawater, with the exception of sodium chloride (sea salt). Gypsum is a naturally occurring calcium sulfate. (Shurtleff and Aoyagi, 1990).

Salt coagulants

- **Calcium sulfate** (gypsum): The traditional and most widely used coagulant to produce Chinese-style *tofu*. It produces a tofu that is tender but slightly brittle in texture. The coagulant itself has no perceivable taste. Use of this coagulant also makes a tofu that is rich in calcium. As such, many *tofu* manufacturers choose to use this coagulant to be able to market their *tofu* as a good source of dietary calcium (Troll, *et al.*, 1980; Lee, *et al*., 1991).

- Chloride-type Nigari salts or Lushui - Magnesium chloride and calcium chloride: Both of these salts have a high solubility rate in water and affect soy protein in the same way, whereas gypsum is only very slightly soluble in water and acts differently in soy protein precipitation, the basis for *tofu* formation. These are the coagulants used to make *tofu* with a smooth and tender texture. In Japan, a white powder called *nigari*, which consists primarily of magnesium chloride, is produced from seawater after the sodium chloride is removed and the water evaporated. Depending on its production method, *nigari/Lushui* may also contain small quantities of magnesium sulfate (Epsom salt), potassium chloride, calcium chloride, and trace amounts of other naturally occurring salts. Although the term *nigari* is derived from *nigai*, the Japanese word for "bitter," neither *nigari* nor pure magnesium chloride imparts a perceivable taste to the finished tofu. Calcium chloride is a common coagulant for tofu in North America. Fresh clean sea water itself can also be used as a coagulant (Shurtleff and Aoyagi, 1990)

Acid coagulants

- **Glucono delta-lactone** (GDL): A naturally occurring organic acid also used in cheese making, which produces a very fine textured *tofu* that is almost jelly-like. This coagulant is used especially for "silken" and softer *tofus*, and confers an almost imperceptible sour taste to the finished product. (Liu, 1997) Commonly used together with calcium sulfate to give soft tofu a smooth tender texture.

- Other edible acids: Though they can affect the taste of the *tofu* more, and vary in efficacy and texture, acids such as acetic acid (vinegar) and citric acid (such as lemon juice), can also be used to coagulate soy milk and produce *tofu* (Troll, *et al.*, 1980; Lee, *et al.*, 1991).

Enzyme coagulants

- Among enzymes that have been shown to produce *tofu* are papain, and alkaline and neutral proteases from microorganisms. In the case of papain, the enzyme to substrate ratio, by weight, was held constant at 1:400. An aliquot of 1% crude papain was added to "uncooked" soy milk at room temperature and heated to 90–100 °C.

Contemporary *tofu* manufacturers may choose to use one or more of these coagulants, since they each play a role in producing a desired texture in the finished tofu. Different textures result from different pore sizes and other microscopic features in tofus produced using each coagulant. The coagulant mixture is dissolved into water, and the solution is then stirred into boiled soy milk until the mixture curdles into a soft gel (Shurtleff and Aoyagi, 1990).

The curds are processed differently depending on the form of tofu that is being manufactured. For soft silken tofu or tofu flower the soy milk is curdled directly in the *tofu*'s selling package. For standard firm Asian *tofu*, the soy curd is cut and strained of excess liquid using cheese cloth or muslin and then lightly pressed to produce a soft cake. Firmer tofus, such as Asian dry tofu or Western types of *tofu*, are further pressed to remove even more liquid. In Vietnam, the curd is strained and molded in a square mold and the end product is called molded bean. The *tofu* curds are allowed to cool and become firm. The finished *tofu* can then be cut into pieces, flavored or further processed (Troll, *et al.*, 1980; Lee, *et al.*, 1991).

Although tartness is sometimes desired in dessert tofu, the acid used in flavoring is usually not the primary coagulant since it is not desirable to the flavor or texture of the resulting tofu to add it in a sufficiently high concentration so as to induce coagulation. A sour taste in tofu and a slight cloudiness in its storing liquid is also usually an indication of bacterial growth and, hence, spoilage.

Each type of coagulant produces different textures and subtle flavours, and commercial *tofu* manufacturers often mix up blends of coagulants. Food intolerance sufferers might find they are more sensitive to certain types of coagulants as compared to other, this is one of the reasons this work was carried out (Shurtleff and Aoyagi, 1990).

1.42 VARIETIES OF *TOFU*

There is a wide variety of *tofu* available in both Western and Eastern markets. Despite the daunting variety, *tofu* products can be split into two main categories: fresh *tofu*, which is produced directly from soy milk, and processed tofu, which is produced from

fresh *tofu*. *Tofu* production also creates important side products which are often used in various cuisines.

Fresh *tofu*

Depending on the amount of water that is extracted from the *tofu* curds, fresh *tofu* can be divided into three main varieties. Fresh *tofu* is usually sold completely immersed in water to maintain its moisture content (Shurtleff and Aoyagi, 1990).

FIRM *TOFU*

These types of firm *tofu* are produced with seawater instead of nigari (magnesium chloride), or using concentrated soy milk. Some of them are squeezed of excess moisture using heavy weights. These products are produced in areas where travelling is inconvenient, such as remote islands, mountain villages, heavy snowfall areas, and so on (Gissen, 1996).

CHINESE "DRY *TOFU"*

Tofu is dried but is rather an extra firm variety of tofu with a large amount of liquid pressed out of it. Dòu gān contains the least amount of moisture of all fresh tofu and has the firmness of fully cooked meat and a somewhat rubbery feel similar to that of paneer. When sliced thinly, this *tofu* can be crumbled easily. The skin of this form of tofu has the pattern of the muslin used to drain and press it. Western firm *tofu* is milled and reformed after the pressing and sometimes lacks the skin with its cloth patterning (Troll, *et al.,* 1980; Lee, *et al*., 1991).

Processed *tofu*

Many forms of processed *tofus* exist, due to the varied ways in which fresh *tofu* can be used. Some of these techniques likely originate from the need to preserve *tofu*

before the days of refrigeration, or to increase its shelf life and longevity. Other production techniques are employed to create tofus with unique textures and flavors (Gissen, 1996).

Fermented

- **Pickled tofu:** "preserved *tofu*" or "fermented *tofu*," this food consists of cubes of dried tofu that have been allowed to fully air-dry under hay and slowly ferment from aerial bacteria. The dry fermented tofu is then soaked in salt water, Chinese wine, vinegar, and minced chiles, or a unique mixture of whole rice, bean paste, and soybeans. In the case of red pickled tofu red yeast rice (cultivated with *Monascus purpureus*) is added for color. And in Japan, pickled tofu with miso paste is called '*tofu* no misodzuke', which is a traditional preserved food in Kumamoto. In Okinawa, there is a pickled and fermented *tofu* called *tofuyo* (Troll, *et al.*, 1980; Lee, *et al*., 1991).
- **Stinky tofu**: A soft *tofu* that has been fermented in a unique vegetable and fish brine. The blocks of *tofu* smell strongly of certain pungent cheeses, and are described by many as rotten and fecal. Despite its strong odor, the flavor and texture of stinky tofu is appreciated by aficionados, who describe it as delightful. The texture of this tofu is similar to the soft Asian tofu that it is made from. The rind that stinky tofu develops from frying is said to be especially crisp, and is usually served with soy sauce, sweet sauce, and/or hot sauce (Shurtleff and Aoyagi, 1990).

Flavored

Flavors can be mixed directly into curdling soy milk while the tofu is being produced.

- **Sweet**: Common sweet dessert tofus include peanut *tofu*, almond tofu, mango *tofu*, coconut *tofu* and longan *tofu*. In order to produce these forms of *tofu*, sugar, fruit acids, and flavorants are mixed into soy milk prior to curdling. Most sweet tofus have the texture of silken *tofu* and are served cold (Troll, *et al.*, 1980; Lee, *et al.*, 1991).
    - Products called "almond *tofu*" in some cases are actually not *tofu* but are instead gelatinous mixtures including agar or gelatin and whitened with milk or coconut milk. In Japan these are canned with syrup and sold as sweet desserts (Gissen, 1996).
- **Savory**: Egg tofu is the main type of savory flavored *tofu*. Whole beaten eggs are filtered and incorporated into the soy milk before the coagulant is added. The mixture is filled into plastic tubes and allowed to curdle. The *tofu* is then cooked in its packaging and sold. Egg *tofu* has a pale golden color that can be attributed to the addition of egg and, occasionally, food coloring. This *tofu* has a fuller texture and flavor than silken *tofu*, which can be attributed to the presence of egg fat and protein (Shurtleff and Aoyagi, 1990).

Dried *tofu*

Two kinds of dried *tofu* are produced in Japan. They are usually rehydrated (by being soaked in water) prior to consumption. In their dehydrated state they do not require refrigeration.

- *Koya tofu* is made using nigari.
- *Kori tofu* is freeze-dried.

Fried

- With the exception of the softest *tofus*, all forms of *tofu* can be fried. Thin and soft varieties of tofu are deep fried in oil until they are light and airy in their core "bean bubble," describing the shape of the fried tofu as a bubble).
- *Tofu*s such as firm Asian with their lower moisture content, are cut into bite-sized cubes or triangles and deep fried until they develop a golden-brown, crispy surface. These may be eaten on their own or with a light sauce, or further cooked in liquids; they are also added to hot pot dishes or included as part of the vegetarian dish called *luohan zhai* (Shurtleff and Aoyagi, 1990).

1.43 BYPRODUCTS OF *TOFU* PRODUCTION

Tofu production creates some edible byproducts. Food products are made from the protein-oil film, or "skin," which forms over the surface of boiling soy milk in an open shallow pan. The leftover solids from pressing soy milk are called *okara*.

Tofu skin

Tofu skin is produced through the boiling of soy milk, in an open shallow pan, thus producing a film or skin composed primarily of a soy protein-lipid complex on the liquid surface. The films are collected and dried into yellowish sheets known as **soy milk skin**. Its approximate composition is : 50–55% protein, 24–26% lipids (fat), 12% carbohydrate, 3% ash, and 9% moisture (Gissen, 1996).

The skin can also be bunched up to stick form and dried into something known as "tofu bamboo", or myriad other forms. Since tofu skin has a soft yet rubbery texture, it is folded or shaped into different forms and cooked further to imitate meat in vegan

cuisine. Some factories dedicate production to tofu skin and other soy membrane products (Shurtleff and Aoyagi, 1990).

Okara

Okara (おから) (雪花菜, *xuěhuācaì*, lit. "snowflake vegetable"; 豆腐渣, *dòufuzhā*, lit. "tofu sediment/residue"; *kongbiji*, 콩비지 in Korean), sometimes known in the west as "soy pulp" or "tofu lees", is the fibre, protein, and starch left over when soy milk has been extracted from ground soaked soybeans. Although it is mainly used as animal feed in most tofu producing cultures, it is sometimes used in Japanese and Korean cuisines. It is also an ingredient for vegetarian burgers produced in many western nations (Shurtleff and Aoyagi, 1990).

Non-tofu "tofus"

Due to their Asian origins and their textures, many food items are called "tofu" even though their production processes are not technically similar. For instance, many sweet almond tofus are actually gelatinous desserts made from agar or gelatin and whitened with milk or coconut milk more similar to Japanese anmitsu. As well, some foods such as Burmese tofu are not coagulated from the "milk" of the legume but rather set in a manner similar to soft polenta, Korean muk, or the jidou liangfen of Yunnan province of Southwest China (Troll, *et al.*, 1980; Lee, *et al*., 1991).

Burmese *tofu*

Burmese tofu (*to hpu* in Burmese) is a type of legume product made from *besan* (*chana dal*) flour; the Shan variety uses yellow split pea flour instead. Both types are

yellow in color and generally found only in Myanmar, though the Burman variety is also available in some overseas restaurants serving Burmese cuisine (Gissen, 1996).

Burmese *tofu* may be fried as fritters cut in rectangular or triangular shapes. Rice *tofu*, called *hsan to hpu* (or *hsan ta hpo* in Shan regions) is made from rice flour (called *hsan hmont* or *mont hmont*) and is white in color, with the same consistency as yellow Burmese *tofu* when set. It is eaten as a salad in the same manner as yellow *tofu* (Shurtleff and Aoyagi, 1990).

1.44 NUTRITIONAL COMPOSITION OF *TOFU*

Tofu is rich in high quality proteins, low in saturated fats and cholesterol free. It is also a good source of B-vitamins and minerals. *Tofu* also contains isoflavones which is a naturally occurring heterocyclic phenols found mainly in soy bean and its products (Gissen, 1996).

1.4.4.1 PROTEINS

Tofu is an excellent source of soy protein. (Liener, 1994) proteins are polypeptides which are formed as a result of condensation of amino-acids. of the 20 amino-acids utilized in protein synthesis, only 10 can be synthesized in the human system(non essential amino acids) while the other 10 must necessarily be derived from food products(essential amino acids) making the essential amino acids a very important component of human diet. Essential amino acids are isoleucine, leucine, lysine, methionine, phenyl alanine, threonine, tryptophan and valine (for adults) arginnine and histidine are added to list for infants. Tofu contains all of the above essential amino acids such as cysteine, tyrosine, alanine, aspartic acid, glutamic acid,

glycine, proline and serine. Due to the fact that Tofu as a food product contains all of the essential amino acids, it is regarded as a rich source of high quality proteins (Huff *et al*., 1976).

1.4.4.2 LIPIDS

Lipids are organic compounds that are poorly soluble in water but readily dissolved in organic solvents. They are polymers of fatty acids, and represent a concentrated energy supply in the diet as well as an efficient form of energy storage in the body (Montgomery *et al.*, 1990). Fatty acids can be grouped into saturated and unsaturated fatty acids. Saturated fatty acids do not contain double bonds in their alkyl chains while unsaturated fatty acids contain one or more double bonds in their alkyl chain. Fatty acids having only one double bond are referred to as monounsaturated fatty acid while those containing two or more double bonds are referred to as polyunsaturated fatty acids (Montgomery *et al*., 1990).

Humans can biosynthesize many fatty acids including the saturated and unsaturated varieties but can not synthesize all of the necessary types of the polyunsaturated fatty acids which implies that these polyunsaturated fatty acids must necessarily be sourced from the diet. Those polyunsaturated fatty acids are called essential fatty acids (Goldberg, 1995).

Tofu contains low saturated fatty acids and has relatively higher polyunsaturated fatty acid content. This makes *Tofu* a good nutritional source of the polyunsaturated fatty acids. Also *Tofu* is cholesterol free. Although cholesterol is the major sterol in the body and is a structural component of cells membranes and plasma lipoproteins elevated levels of serum cholesterol results in a higher risk of cardiovascular disease. Hence the fact that Tofu is cholesterol free reduces the occurrence of such diseases (Huff *et al*, 1977; Potter *et al*., 1996).

1.4.4.3 CARBOHYDRATES

Tofu contains carbohydrates and sugars. Sugar are often called carbohydrates, the word carbohydrate (hydrate of carbon) with general formula $(CH_2O)_n$ n=3 or more. Carbohydrates are polymers of monosaccharide and disaccharides they serve as the immediate source of the energy in the body (Troll *et al.*, 2000). The simplest sugar is monosaccharide or "single sugar" such as fructose and glucose, $C_6H_{12}O_6$. Disaccharides ("double sugar") are formed by the joining of two monosaccharide unit. Also polysaccharides are polymers made up of thousands of glucose units. Glycogen is the polysaccharide form in which Carbohydrates are stored in our bodies. It is vitally important because it is a store house of energy in molecular form.

1.4.4.4 VITAMINS

Vitamins are small organic molecules in the diet that either cannot be synthesized in humans or are synthesized at a rate less than that consistent with health (Montgomery *et. al*, 1990). They generally serve as precursors of certain cofactors/coenzymes necessary for enzymatic reactions in the body. Tofu contains a number of vitamins which includes ascorbic acid (Vitamin C), thiamin, riboflavin, niacin, panthothenic acid, Vitamins A, B_{12} and folate (folic acid).

1.4.4.5 MINERALS

Minerals are the inorganic components of the body. The major minerals are Na, K, Ca, Mg, Fe, P, Cl, and S which are referred to as Macro elements. The microelements or trace elements include Cu, Mn, Zn, Se, I, Fl etc. To a large extent, *Tofu* contains quite a lot of the of the macro elements. The mineral composition of *tofu* includes Ca, Fe, Mg, P, K, Na, Zn, Cu, Mn and Se. The Ca content however

depends on the coagulant used. There is usually an increase in Ca content when a Ca salt coagulant is used. (Montgomery *et. al*, 1990).

1. 5 ISOFLAVONE CONTENT OF TOFU

Isoflavones are a group of naturally occurring heterocyclic phenols found in soybean and its products (Jackson *et al.*, 2002). Isoflavones such as diadzen, genisten, glycetein and their derivatives (glucosidic conjugates which are 9 in numbers) have been isolated from soybeans and products. They are also referred to as soy phytoestrogens, and have been credited for performing several health promoting functions. These phytoestrogens have effects on cardiovascular and menopausal health and are noted for cancer prevention. (Jackson *et al*., 2002).

Several investigators have suggested that soy food consumption may contribute to lower rates of certain diseases such as hormone dependent cancers and osteoporosis (Yeung and Yu, 2003). Because of resemblances to human estrogen and the observations that Asian population which consume more isoflavones (especially in soy products) compared with women in western countries have less menopausal symptoms isoflavones are postulated as natural products that may be beneficial to postmenopausal women in cardiovascular health (Yeung and Yu, 2003). The lower incidence of certain diseases has been reported in Asian countries where soybean consumption is high with the average intake of isoflavones being about 40-80 mg per day (Jackson *et al*, 2002). For instance, genistin has been shown to play a protective role in hormonally induced cancers (breast cancer) by acting as an antiestrogen (Goldbe's, 1995)

Tofu also contains these isoflavones but not in quantities as high as those contained in the raw soy bean or soy beverage (soy milk). The reduction in isoflavone

content of *Tofu* is as a result of the loss during the processing of soy beans into Tofu. Despite the loss in isoflavones, it still contains some amount of isoflavones, which is better than not occurring at all. Recent investigation shows that the total recovery of isoflavones in tofu was about 36% based on dry matter (Jackson *et al.*, 2002).

1.6 SERUM LIPIDS

Serum Lipids are fats found in the blood which are used to determine coronary risk profile i.e. the serum level of these lipids are indicators of risk for heart disease. Batteries of blood tests are carried out to evaluate serum lipids such as cholesterol, triglycerides, High density lipoproteins, Low density lipoproteins (Agbedama, 1997).

1.6.1 CHOLESTEROL

Cholesterol is a critical fat and is a structural component of all membrane and plasma lipoproteins. It is also crucial in the synthesis of steroid hormones, glucocorticoids and bile acids. It is mostly synthesized in the liver although some are also absorbed through the diets, especially diets high in saturated fats. Elevated cholesterol has been seen in atherosclerosis, diabetes, hypothyroidism and pregnancy while low levels of these lipids are seen in depression, malnutrition, liver insufficiency and malignancies etc (Agbedama, 1997).

1.6.2 LOW DENSITY LIPOPROTEINS (LDL)

Lipoproteins are proteins in the blood which transports other cholesterol, triglycerides and other lipids to various tissues. LDL is the cholesterol rich remnants of the lipid transport vehicle VLDL (Very Low Density Lipoproteins). Elevated plasma cholesterol concentration particularly the form resulting from elevation in LDL class of plasma lipoproteins increases the tendencies towards atherosclerosis (hardening of the arteries) where the plasma LDLs enter the arterial wall and deposit

their lipid content thereby causing the accumulation of cholesteryl esters (Pasternak, 1979). There have been many studies to correlate the association between high levels of LDL and atherosclerosis. Therefore, an elevation in the LDL content of serum is indicative of a disease state.

1.6.3 HIGH DENSITY LIPOPROTEINS (HDL)

High density lipoprotein (HDL) is the cholesterol carried by the alpha lipoproteins. HDL appears to function mainly in carrying excess cholesterol (and probably offer phospholipids and proteins) to the liver or excretion in the bile. High serum levels of HDL is indicative of a healthy metabolic system if there is no sign of liver disease or intoxication thus HDL is sometimes referred to as ‘good’ cholesterol. Two mechanisms that explain how HDL offers protection against chronic heart disease are, firstly, that HDL inhibits cellular uptake of LDL and secondly, that HDL serves as a carrier that removes cholesterol from the peripheral tissues and transports it back to the liver for catabolism and excretion.

1.6.4 TRIGLYCERIDES

Triglycerides are stored in adipose tissues as glycerol, fatty acids and monoglycerides and are reconverted as triglycerides by the liver. They are a storage form of energy and are gradually released and metabolized between meals according to the energy requirement of the body.

Large portions of the fat in the diet are in form of triglycerides. Immediately after a meal, triglycerides appear in the blood as the major constituents of chylomicrons. In normal conditions, triglycerides in chylomicrons loose their fatty acids as they move through various tissues leaving the chylomicrons to be taken up by the liver. the triglycerides remaining, together with those synthesized in the liver are repackaged as very low density lipoproteins (VLDL) and secreted into the blood from

the liver .Increased level of triglycerides is indicative of atherosclerosis, hypothyroidism, liver disease, pancreatisis etc while a decreased level may be present in chronic obstructive pulmonary diseases, hyperthyroidism, malnutrition amongst others. (Jackson *et al.*, 2002).

1.6.5 TRANSAMINASES

Transaminases are enzymes which are involved in the intertransfer of amino groups and ketone groups between amino acids and keto acids in transamination reactions. Two transaminases are used majorly as marker enzymes in the liver function tests to detect/indicate damage to the liver and they are serum glutamate oxaloacetate transaminase SGOT (Alanine transaminase-ALT) and serum glutamate Pyrruvate transaminase SGPT (Aspartate transaminase-AST). Both of these enzymes are found in large concentrations in liver cells (hepatocytes) and muscle cells and in lesser amounts in the heart, pancreas and kidney. They are released from the liver when hepatocytes are damaged, which results in a greater amount in the serum in the case of liver damage. Serum GPT is more specific for liver damage than GOT (Gissen, 2004).

Transaminases may be increased in the serum in all kinds of acute and chronic liver diseases. GPT levels fluctuate in response to the extent of cellular necrosis (cell death) and therefore may be temporarily and minimally elevated early in the disease process and extremely elevated during the most acute phase. Maximum elevations are associated with certain diseases and conditioning e.g. very high elevations may be indicative of either acute viral hepatitis, severe skeletal muscle trauma; high levels may indicate severe myocardial infarction, severe infectious mononucleosis, and alcoholic cirrhosis; moderate –high levels may indicate chronic hepatitis and other

conditions; low-moderate levels may be indicative of metastasis hepatic tumors, acute pancreatisis, pulmonary emboli, fatty liver.

SGOT is an enzyme found primarily in the liver, heart, kidney, pancreas and muscle. GOT is found in the serum not only in liver damage, it is also found in elevated amounts in the cardiac muscle damage. However, in Vitamin B deficiency and pregnancy, there is a decrease in GOT level.

1.6.6 ALKALINE PHOSPHATASE

Alkaline phosphatase is produced in the cell of the bone and liver, with some activities in the kidney, intestine and placenta. It is mostly found in an alkaline state with a pH of 9. It is a metal enzyme and some bivalent metal ions such as $Mg^{2+,}$ Ca^{2+} and Ba^{2+} increase its activity, but Ag^{+}, Cu^{2+} and Zn^{2+} inhibits the activity of this enzyme (Prestamo *et al.*, 2002).

Levels of the enzymes are raised in many types of liver diseases. Normally it is secreted into bile and excreted with the bile into the intestine. Biliary obstruction stimulates the production of ALP in the hepatocytes.Once the elimination route is blocked, significantly raised plasma levels of enzymes appear where there is intrahepatic or extra hepatic cholestaris.

Alkaline Phosphatase is not specific to the liver and it is raised in many other conditions including bone disease, bone healing and pregnancy. If the source of elevation of Alkaline Phosphatase in the plasma is not due to liver damage or a physiological process, then a bone disease is usually indicated e.g. osteomalacia. It is also used extensively as a tumour marker (Gissen,2004).

1.7 METHODS OF FOOD ANALYSIS

The analytical methods for protein, fat and carbohydrate are discussed below.

1.7.1 ANALYTICAL METHODS FOR PROTEINS IN FOODS

The **Kjeldahl method** or **Kjeldahl digestion** analytical chemistry is a method for the quantitative determination of nitrogen in chemical substances developed by Johan Kjeldahl in 1883 (Cohen,1910).

The protein content of foods has been determined on the basis of total nitrogen content, while the Kjeldahl (or similar) method has been almost universally applied to determine nitrogen content (AOAC, 2000). Nitrogen content is then multiplied by a factor to arrive at protein content. This approach is based on two assumptions: that dietary carbohydrates and fats do not contain nitrogen, and that nearly all of the nitrogen in the diet is present as amino acids in proteins. On the basis of early determinations, the average nitrogen (N) content of proteins was found to be about 16 percent, which led to use of the calculation N x 6.25 (1/0.16 = 6.25) to convert nitrogen content into protein content (FAO, 1964).

Method

The method consists of heating a substance with sulfuric acid, which decomposes the organic substance by oxidation to liberate the reduced nitrogen as ammonium sulfate. In this step potassium sulfate is added to increase the boiling point of the medium (from 337°F to 373°F / 169°C to 189°C). Chemical decomposition of the sample is complete when the medium has become clear and colorless (initially very dark).

The solution is then distilled with sodium hydroxide (added in small quantities) which converts the ammonium salt to ammonia. The amount of ammonia present (hence, the

amount of nitrogen present in the sample) is determined by back titration. The end of the condenser is dipped into a solution of boric acid. The ammonia reacts with the acid and the remainder of the acid is then titrated with a sodium carbonate solution with a methyl orange pH indicator.

Degradation: Sample + $H_2SO_4 \rightarrow (NH_4)_2SO_4(aq) + CO_2(g) + SO_2(g) + H_2O(g)$

Liberation of ammonia: $(NH_4)_2SO_4(aq) + 2NaOH \rightarrow Na_2SO_4(aq) + 2H_2O(l) + 2NH_3(g)$

Capture of ammonia: $B(OH)_3 + H_2O + NH_3 \rightarrow NH_4^+ + B(OH)_4^-$

Back-titration: $B(OH)_3 + H_2O + Na_2CO_3 \rightarrow NaHCO_3(aq) + NaB(OH)_4(aq) + CO_2(g) + H_2O$

Nowadays, the Kjeldahl method is largely automated and makes use of specific catalysts (mercury oxide or copper sulfate) to speed up the decomposition.

Applications

The Kjeldahl method's universality, precision and reproducibility have made it the internationally-recognized method for estimating the protein content in foods and it is the standard method against which all other methods are judged. It does not, however, give a measure of true protein content, as it measures non protein nitrogen in addition to the nitrogen in proteins. This is evidenced by the 2007 pet food incident and the 2008 Chinese milk powder scandal, when melamine, a nitrogen-rich chemical, was added to raw materials to fake high protein contents. Also, different correction factors are needed for different proteins to account for different amino acid sequences. Additional disadvantages, such as the need to use concentrated sulfuric acid at high temperature and the relatively long testing time (an hour or more), compare

unfavorably with the Dumas method for measuring crude protein content. (*FAO*, 2004; McClements,2007)

Conversion factors

Conversion factors for common foods range from 6.38 for dairy and 6.25 for meat, eggs, corn (maize) and sorghum to 5.83 for most grains, 5.70 for wheat flour and 5.46 for peanuts (FAO, 1964; FAO, 1973).

TABLE 3: SPECIFIC (JONES) FACTORS FOR THE CONVERSION OF NITROGEN CONTENT TO PROTEIN CONTENT (SELECTED FOODS)

Food	Factor
Animal origin	
Eggs	6.25
Meat	6.25
Milk	6.38
Vegetable origin	
Barley	5.83
Corn (maize)	6.25
Millets	5.83
Oats	5.83
Rice	5.95
Rye	5.83
Sorghums	6.25
Wheat: Whole kernel	5.83
Bran	6.31
Endosperm	5.70
Beans: Castor	5.30
Jack, lima, navy, mung	6.25
Soybean	5.71
Velvet beans	6.25
Peanuts	5.46

Source: FAO, 2004

Recommendations

1) It is recommended that protein in foods be measured as the sum of individual amino acid residues (the molecular weight of each amino acid less the molecular weight of water) plus free amino acids, whenever possible. This recommendation is made with the knowledge that there is no official Association of Analytical Communities (AOAC) method for amino acid determination in foods. Clearly, a standardized method, support for collaborative research and scientific consensus are needed in order to bring this about.

2) Related to the previous recommendation, food composition tables should reflect protein by sum of amino acids, whenever possible. Increasingly, amino acid determinations can be expected to become more widely available owing to greater capabilities within government laboratories and larger businesses in developed countries, and to the availability of external contract laboratories that are able to carry out amino acid analysis of foods at a reasonable cost for developing countries and smaller businesses.

3) To facilitate the broader use of amino acid-based values for protein by developing countries and small businesses that may lack resources, FAO and other agencies are urged to support food analysis and to disseminate updated food tables whose values for protein are based on amino acid analyses.

4) When data on amino acids analyses are not available, determination of protein based on total N content by Kjeldahl (AOAC, 2000) or similar method x a factor is considered acceptable.

5) A specific Jones factor for nitrogen content of the food being analysed should be used to convert nitrogen to protein when the specific factor is known. When the specific factor is not known, N x the general factor 6.25 should be used. Use of the general factor for individual foods that are major sources of protein in the diet introduces an error in protein content that is relative to the specific factors and ranges from -2 percent to +9 percent. Because protein contributes an average of about 15 percent of energy in most diets, the use of N x 6.25 should introduce errors of no more than about 1 percent in estimations of energy content from protein in most diets ([-2 to +9 percent] x 15) (FAO).

6) It is recommended that only amino acid analysis be used to determine protein in the following:

- foods used as the sole source of nourishment, such as infant formula;
- foods/formulas designed specifically for special dietary conditions;
- novel foods (FAO).

1.7.2 ANALYTICAL METHODS FOR FATS IN FOOD

The determination of fat content in foods is as varied as the sample that is desired to be analyzed. The following is an overview of a few methods applicable or adaptable to a wide variety of foods. The various fat determination methods lead to different definitions of total fat content.

- **Infrared Spectrometry**

Method : In this method the fat is extracted from a homogeneous sample by a chloroform-methanol solvent containing an internal standard. The internal standard found to be most suitable for this content of the analyte (Cronin and McKenzie, 1990)

Caviezel MethodMethod

The homogenized sample and the internal standard (IS, tridecanoic acid) are added to the *n*-butyl alcohol solvent. Potassium hydroxide is used to saponify and extract the fats simultaneously. An acidic aqueous solution is added to convert the fatty acids salts to fatty acids, producing a two phase system where the fats and internal standard are contained in the top layer. This is injected into the fat determination system where it is separated by gas chromatography. The peak areas of the IS and fatty acids are used to determine the fat content which is then converted to triglyceride content with a predetermined factor. The fat determination unit is a commercial instrument which is based on gas chromatography and is geared specifically to this analysis method. This company also produces mixers and extraction units which are designed for this analysis (Pendl *et al.*, 1998). The accuracy of this method is very good.

- **Simplified Gravimetric after Chloroform-Methanol Extraction**

Method

Samples of 1-5 g are massed into a polypropylene centrifuge bottle. Sodium acetate is added so that the total volume of solution is 32 ml. Aliquots of methanol and chloroform are added, the bottles capped and shaken for 2 hours. Another aliquot of chloroform is added, and the bottles shaken again for 30 minutes. Finally, an aliquot of water is added, and the bottles are shaken a final time for 30 min. Centrifuge tubes which had been dried and massed are used to hold 20 ml aliquots of the chloroform layer. These are then centrifuged for 10 min, and allowed to set in 25_C water bath for 15 minutes. The samples are then evaporated to dryness under a nitrogen blanket, heated in a drying oven for 30 minutes, and cooled in a dessicooler for at least 30 minutes. Finally, they are massed and the total lipid content determined by:

total lipid (g/100 g wet weight) = (W2-W1)*Vc*100/(VA*SW)

where W2 is the weight of glass tube and dried extract (g), W1 is the weight of empty dried glass tube (g), Vc is the total volume of chloroform (ml), VA is the volume of extract dried (ml), and SW is the weight of food sample assayed (g) (Phillips,1997).

Supercritical CO_2 Extraction Method

The finely ground sample is massed and placed in an extraction cartridge and mixed with an equal amount of ISCO (ISCO Inc, Lincoln, NE) wet support. SFC/SFE grade CO_2 is used with a commercial instrument at pressures up to 10,000 psi, temperatures of about 80^{o}C and approximately a 4 mL/min flow of liquid CO_2 for about 30 minutes to extract fat content. The extraction parameters vary depending on the sample matrix. After extraction the collection vial is heated for 20 minutes at 120^{o}C. The collection vial is massed before and after extraction. The variable conditions include extractor temperature, pressure, modifiers of ethanol or ethanol and water mixture, restrictor temperature, flow rate, and extraction time (Dionisi *et al*., 1999; Weisshaar and McConville, 2012).

There are accepted AOAC gravimetric methods for crude fat, which includes phospholipids and wax esters, as well as minor amounts of non-fatty material (AOAC, 2000). Total fat can be expressed as triglyceride equivalents determined as the sum of individual fatty acids and expressed as triglycerides. This method is satisfactory for the determination of fat in a wide variety of foods (FAO, 1994).

Recommendations

1) For energy purposes, it is recommended that fats be analysed as fatty acids and expressed as triglyceride equivalents, as this approach excludes waxes and the

phosphate content of phospholipids, neither of which can be used for energy (James, Body and Smith, 1986).

2) A gravimetric method, although less desirable, is acceptable for energy evaluation purposes (AOAC, 2000).

The methods reviewed all had acceptable figures of merit. Supercritical CO_2 extraction is the simplest and quickest method, however the capital equipment cost may be prohibitive. The Caviezel method is also rapid and largely automated, but again the cost of special equipment may be undesirable. If a large capital expenditure and method development expense is acceptable, the supercritical CO_2 method is advantageous in the elimination of the use of halogenated hydrocarbons. The gravimetric method is unfavorable due to the errors introducible in the mass and volume measurements. Although the IR method uses the unfavorable halogenated hydrocarbon, it has the advantage of some automation with the use of no specialized equipment. Assuming an IR spectrometer with integration capabilities is available, this method would be preferred.

1.7.3 ANALYTICAL METHODS FOR CARBOHYDRATES IN FOODS

Total carbohydrate content of foods are calculated by difference, rather than analysed directly. Under this approach, the other constituents in the food (protein, fat, water, alcohol, ash) are determined individually, summed and subtracted from the total weight of the food. This is referred to as *total carbohydrate by difference* and is calculated by the following formula:

100 - (weight in grams [protein + fat + water + ash + alcohol] in 100 g of food)

It should be clear that carbohydrate estimated in this fashion includes fibre, as well as some components that are not strictly speaking carbohydrate, e.g. organic acids (Merrill and Watt, 1973). Total carbohydrate can also be calculated from the sum of the weights of individual carbohydrates and fibre after each has been directly analysed (FAO, 1998).

Available carbohydrate represents that fraction of carbohydrate that can be digested by human enzymes, is absorbed and enters into intermediary metabolism. (It does not include dietary fibre, which can be a source of energy only after fermentation - see the following subsections.) Available carbohydrate can be arrived at in two different ways: it can be estimated by difference, or analysed directly. To calculate available carbohydrate by difference, the amount of dietary fibre is analysed and subtracted from total carbohydrate, thus:

100 - (weight in grams [protein + fat + water + ash + alcohol + dietary fibre] in 100 g of food)

This yields the estimated weight of available carbohydrate, but gives no indication of the composition of the various saccharides comprising available carbohydrate. Alternatively, available carbohydrate can be derived by summing the analysed weights of individual available carbohydrates (AOAC, 2000). In either case, available carbohydrate can be expressed as the weight of the carbohydrate or as monosaccharide equivalents. The summary of all the methods are seen in the table below (FAO, 1998).

Dietary fibre is a physiological and nutritional concept relating to the carbohydrate components of foods that are not digested in the small intestine. Dietary fibre passes

undigested from the small intestine into the colon, where it is fermented by bacteria (the microflora), resulting to variable quantities of short-chain fatty acids and several gases such as carbon dioxide, hydrogen and methane. Short-chain fatty acids are an important direct source of energy for the colonic mucosa; they are also absorbed and enter into intermediary metabolism (Cummings, 1981).

TABLE 4: TOTAL AND AVAILABLE CARBOHYDRATE

Total carbohydrate:
By difference: 100 - (weight in grams [protein + fat + water + ash + alcohol] in 100 g of food) By direct analysis: weight in grams (mono- + disaccharides + oligsaccharides + polysaccharides, including fibre)
Available carbohydrate:
By difference: 100 - (weight in grams [protein + fat + water + ash + alcohol + fibre] in 100 g of food) By direct analysis: weight in grams (mono- + disaccharides + oligosaccharides + polysaccharides, excluding fibre)*

* May be expressed as weight (anhydrous form) or as the monosaccharide equivalents (hydrous form including water).

Chemically, dietary fibre can comprise: cellulose, hemicellulose, lignin and pectins from the walls of cells; resistant starch; and several other compounds. . As more has been learned about fibre, a variety of methods for analysis have been developed. Many of these measure different components of fibre, and thus yield different definitions of, and values for, it. Three methods have had sufficient collaborative testing to be generally accepted by such bodies as AOAC International and the Bureau Communautaire de Reference (BCR) of the European Community (EC) (FAO, 1998): the AOAC (2000) enzymatic, gravimetric method - Prosky (985.29); the enzymatic, chemical method of Englyst and Cummings (1988); and the enzymatic, chemical method of Theander and Aman (1982). Monro and Burlingame (1996) have pointed out, however, that at least 15 different methods are applied for determining the dietary fibre values used in food composition tables. Their publication, and the FAO/WHO report on carbohydrates in human nutrition (FAO, 1998), discuss these issues in more detail. The effect of having such a variety of methods for dietary fibre, each giving a somewhat different value, affects not only the values in food composition tables for dietary fibre *per se*, but also those for available carbohydrate by difference (FAO, 2004).

Recommendations

1) Available carbohydrate is a useful concept in energy evaluation and should be retained. This recommendation is at odds with the view of the expert consultation in 1997, which endorsed the use of the term “glycaemic carbohydrate” to mean “providing carbohydrate for metabolism” (FAO, 1998). The current group expressed

concerns that “glycaemic carbohydrate” might be confused or even equated with the concept of “glycaemic index”, which is an index that describes the relative blood glucose response to different “available carbohydrates”. The term “available” seems to convey adequately the concept of “providing carbohydrate for metabolism”, while avoiding this confusion.

2) Carbohydrate should be analysed by a method that allows determination of both available carbohydrate and dietary fibre. For energy evaluation purposes, standardized, direct analysis of available carbohydrate by summation of individual carbohydrates (Southgate, 1976; Hicks, 1988) is preferred to assessment of available carbohydrate by difference, i.e. total carbohydrate by difference minus dietary fibre. This allows the separation of mono- and disaccharides from starches, which is useful in determination of energy content.

3) Determination of available carbohydrate by difference is considered acceptable for purposes of energy evaluation for most foods, but not for novel foods or food for which a reduced energy content claim is to be made. In these cases, a standardized, direct analysis of available carbohydrate should be carried out.

4) “Dietary fibre” is a useful concept that is familiar to consumers and should be retained on food labelling and in food tables. Because the physical characteristic of solubility/insolubility does not strictly correlate with fermentability/non-fermentability, the distinction between soluble and insoluble fibre is not of value in energy evaluation, nor is it of value to the consumer (FAO, 2004).

5) The AOAC (2000) analysis - Prosky (985.29) or similar method should be used for dietary fibre analysis.

6) Because dietary fibre can be determined by a number of methods that yield different results, when the Prosky method is not used the method used should be stated and the value should be identified by INFOODS tagnames[7] (Klensin *et al*., 1989). In addition, the method should be identified with the tagname in food composition tables.

7) Further research and scientific consensus are needed in order to develop standardized methods of analysis of resistant starch.

1.7.4 CALCULATION OF THE ENERGY CONTENT OF FOODS - ENERGY CONVERSION FACTORS

Determining the energy content of foods depends on the following: 1) the components of food that provide energy (protein, fat, carbohydrate, alcohol, polyols, organic acids and novel compounds) should be determined by appropriate analytical methods; 2) the quantity of each individual component must be converted to food energy using a generally accepted factor that expresses the amount of available energy per unit of weight; and 3) the food energies of all components must be added together to represent the nutritional energy value of the food for humans. The energy conversion factors and the models currently used assume that each component of a food has an energy factor that is fixed and that does not vary according to the proportions of other components in the food or diet (FAO, 2004).

1.7.5 JOULES AND CALORIES

The unit of energy in the International System of Units (SI) is the joule (J). A joule is the energy expended when 1 kg is moved 1 m by a force of 1 Newton. This is the

accepted standard unit of energy used in human energetics and it should also be used for the expression of energy in foods. Because nutritionists and food scientists are concerned with large amounts of energy, they generally use kiloJoules (kJ = 10^3 J) or megaJoules (MJ = 10^6 J). For many decades, food energy has been expressed in calories, which is not a coherent unit of thermochemical energy. Despite the recommendation of more than 30 years ago to use only joules, many scientists, non-scientists and consumers still find it difficult to abandon the use of calories. This is evident in that both joules (kJ) and calories (kcal) are used side by side in most regulatory frameworks, e.g. Codex Alimentarius (1991). Thus, while the use of joules alone is recommended by international convention, values for food energy in the following sections are given in both joules and calories, with kilojoules given first and kilocalories second. In tables, values for kilocalories are given in italic type. The conversion factors for joules and calories are: 1 kJ = 0.239 kcal; and 1 kcal = 4.184 kJ (FAO, 2004).

1.8 OBJECTIVE OF THE STUDY.

The general objective of this project is to assess the effect of coagulants on the nutritional quality of tofu.

The specific objectives are to determine the effects of coagulants on:

a. tofu yield, sensory quality, energy content and other nutritional indices of tofu
b. the protein and dry matter digestibility (*In vivo* and *In vitro)* of tofu
c. the serum lipids, transaminases and alkaline phosphatase of rats fed with tofu

CHAPTER TWO

EXPERIMENTALS

2.1 MATERIALS

Soy beans used in the tofu production was obtained from Erekesan Market, Akure, Ondo State, Nigeria. The steep water was obtained from the process of pap from maize. The chemical used were analytical grades, while the water was glass distilled. The albino rats (3 weeks old) used were of the same litter origin obtained from the rat colony of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

2.2 METHODS

2.2.1 SAMPLE PREPARATION

The *Tofu* was prepared using the method reported by Cao and Chang, 1999. Soybeans were soaked in water overnight. The water was removed and the soaked soybeans was blended to obtain the slurry. Soymilk or soy beverage was then extracted from the slurry using a sieve leaving the okara. The okara was sieved again to further extract any more of soy beverage remaining. The raw soymilk was divided then transferred into a cooker where it was heated to about $98^{o}C$ with constant stirring which was held at the same temperature for about two minutes. Coagulant solutions (2g of $CaCl_2$ in 20mls of water; 2g of alum in 20mls of water; steep water) were mixed with the first second and third divisions of the heated soymilk preparations respectively and the three mixtures were allowed to solidify to tofu. The tofu was removed from the cookers and further compressed to remove water to make firm tofu. Afterwards the tofu was cut into small pieces and further grated into smaller sizes. The tofu was then dried on a heat chamber to form dry pellets.

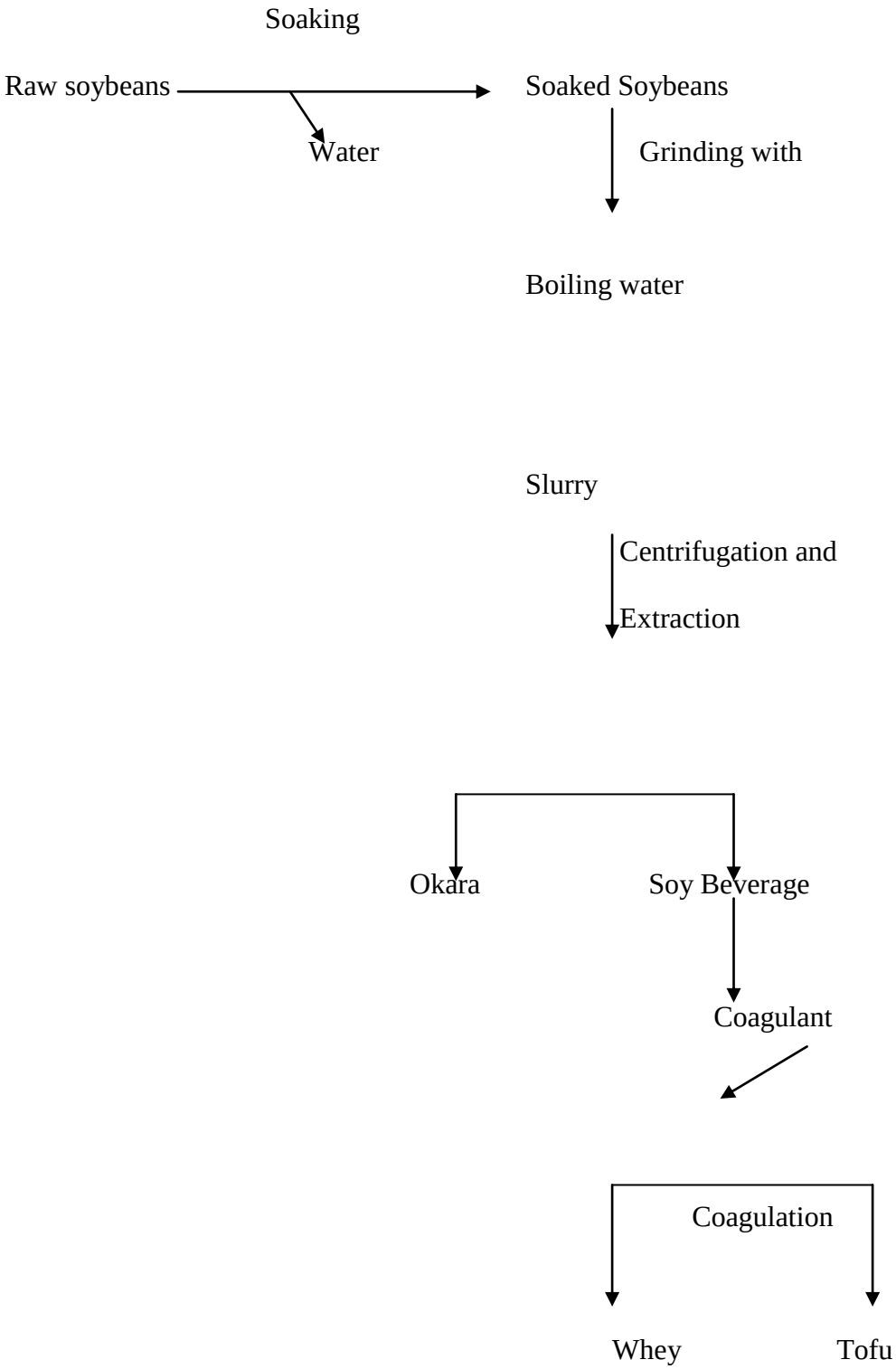

FIGURE 1: PRODUCTION CHART OF TOFU SAMPLE ANALYSIS

2.2.2 YIELD OF TOFU

The yield was expressed in volume of fresh tofu obtained from 100g of Soya bean. The yields of total dry matter and protein in tofu were calculated as follows (Chang et. al. 1990)

$$\text{Total dry matter yield (\%)} = \frac{\text{dry matter in tofu x 100}}{\text{dry matter in Soya bean}}$$

2.3 PROXIMATE COMPOSITION

Proximate composition of each tofu prepared from different coagulants was determined using the standard (AOAC, 1990) methods.

2.3.1 MOISTURE CONTENT DETERMINATION

About 2g of each tofu made from different coagulants was weighed into pre-weighed oven-dried Petri-dishes which were later transferred into oven and dried at 70-80^{0}C to constant weight. The dried samples were cooled in the desiccators and the loss in weight was experienced as percent moisture (AOAC, 2000).

2.3.2 ASH CONTENT DETERMINATION

About 0.5g of each tofu coagulants was weighed into pre-weighed oven-dried crucibles. The crucibles were placed in the Muffle furnace, and the temperature was increased gradually between 200^{0}C and 450^{0}C and each of the samples were ached until they became white. Thereafter, the crucibles were removed and cooled in the dedicator, after which their weights were taken and percentage subsequently determined (AOAC, 2000).

2.3.3 CRUDE FAT

Crude fat quantification was by soxhlet extraction. 10.0 g of oven-dried sample was accurately weighed into a cellulose thimble and placed in a soxhlet extractor. The oil was extracted by a continuous soxhlet extraction with petroleum ether (40-60 ^{0}C) for 12 hours. The solvent was evaporated at 400C, the percentage crude was calculated (AOAC, 2000).

2.3.4 LIPID DETERMINATION

About 0.5g of the oven-dried of each samples were weighed into fitter paper of known weight. Then extraction was carried out using soxhlet extractor. About 400ml petroleum ether (40-60) in a 500ml round bottom flask was used for the extraction. After the fitting of the soxhlet extractor with reflux condenser, the heat source was adjusted so that the solvent would boil gently. It was then left to siphon over several hours (4hours). The filter paper was detached after the extraction and it was placed in an oven at 50^{0}C to dry to constant weight. Thereafter, the filter paper was cooled in the desiccators and the weight was determined and percentage lipids was subsequently determined (AOAC, 2000).

2.3.5 CRUDE FIBRE DETERMINATION

200ml of boiling 1.25% H_2SO_4 was added to about 1.5g of the samples. This was followed by boiling for 30minutes. This was then filtered through poplin cloth by suction using buchner funnel. Thereafter, it was rinsed with boiling water. The samples were then returned into the flask, and 200ml boiling water. The samples were then returned into the flask and 200ml boiling 1.25% NaOH was added. This was boiled for 30minutes, and then the alkaline-boiled samples were filtered through the poplin cloth, and then washed with distilled water and twice with methylated spirit, and three times with petroleum ether. The residue was transferred into oven-dried

crucible of known weight, then placed in the oven and dried at 105^0C. It was cooled in a desiccator, and the weight was determined. Thereafter, the samples were transferred into muffle furnace at about 450^0C for about 3hours. This was followed by cooling to room temperature and their weight and percentage were subsequently determined (AOAC, 2000).

2.3.6 NITROGEN DETERMINATION

About 0.5g oven dried of each sample was transferred into 50ml microkjeldal flask. Then 0.5ml of concentrated H_2SO_4 with half Kjeldal catalyst tablet were added, after which the samples were digested by heating until the digest was clear, that is, from light green to grey white. The heating was allowed to continue for another 2 minutes to ensure complete digestion .then they were coded and their volume were made up to 50ml each with distilled water.

5ml of boric acid was transferred into 100ml conical flasks (as receiving flask) and 3 drops of mixed indicators were added to each of them. Then the receiving flask was placed is such a way that the tip of the condense tube is below surface of boric acid. Thereafter, 5ml of the digested sample was transferred in to the mark ham distiller. This was followed by the addition of 10ml of 40% NaOH. 0 50ml of the distillate was collected into the receiving flask and titrated against 0.025M H_2SO_4. The blank was titrated against the acid as well. The percentage of Nitrogen was subsequently determined (AOAC, 2000).

2.3.7 CARBOHYDRATE DETERMINATION

Percentage of soluble carbohydrates was determined by subtracting the sum of percentage ash, crude fibre, crude nitrogen, fat and moisture content from one hundred (AOAC 1984).

2.4 MINERAL COMPOSITION DETERMINATION

The Zn, Ca, Fe, Mn and Mg contents were determined on aliquot of the solution of the ash by using a Perkins Elmer absorption spectrometer (model 732) (Perkins Elmer, 1982), while Na and K was determined using flame photometer.

2.5 SENSORY ANALYSIS

The organoleptic properties of Alum, $CaCl_2$ and steep water coagulated tofu prepared based on traditional method described in detail by Murphy et al., (1997). Each samples for sensory evaluation were prepared from freshly made tofu by draining of the surface water and keeping them in an incubator (30^0L) until served. Tests on overall acceptability, such as colour, texture, odor, taste and structure were conducted in duplicate by panel of ten members. Using 7 point scale, the sensory scores were, 7: Excellent; 6: Very good; 5: Good; 4: Average; 3: Fair; 2: Poor; 1: Very poor: The result obtained were computed by analysis and attribute mean score calculated (Larmond , 1973).

2.6 *IN-VITRO* MULTIENZYME PROTEIN DIGESTIBILITY ASSAY

In-vitro protein digestibility was carried out using the method of Hsu *et. al.*1977. Sample suspension was prepared by dissolving 1.75 g in 50 ml of distilled water. The suspension was adjusted to pH 8.0 with 0.1 M HCl or 0.1 M NaOH, while stirring in a 37 ^oC water bath. The multienzyme solution consisting of 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase ml^{-1} was maintained in an ice bath and adjusted to pH 8.0 (with 0.1 M HCl or 0.1 M NaOH). 5 ml of the multienzyme solution was added to the sample suspension with constant stirring at 37 ^oC. The pH of the suspension was recorded 15 min after the addition of the multienzyme solution and

the in vitro digestibility was calculated using the regression equation of Hsu *et. al.* 1977.

$$Y = 210.46 – 18.10X$$

Where Y is in vitro digestibility (%), and X is pH of the sample suspension after 15min digestion with the multienzyme solution.

2.7 DETERMINATION OF ENERGY VALUES

A 0.1 g sample each of the tofu was ignited electrically in a Ballistic Bomb Calorimeter (Gallemkamp, CBB– 330–010 L) and burned in excess oxygen (25 atm.) in the bomb. The rise in temperature obtained was compared with that of benzoic acid to determine the calorific value of the sample material (Akindahunsi and Oboh, 2003).

2.8 BIOASSAY

The Bioassay was carried out based on the method reported by Prestamo *et al.* (2002). Wistar strain albino rats weighing 85-100g were purchased from Biochemistry Department, University of Ilorin, Nigeria, and acclimatized for 2 weeks during which period they were maintained *ad libidum* on commercial diet. The rats were subsequently divided into four treatment groups. Animals in group 1 were fed the commercial diet (16.0% proteins), while animals in group 2 were fed Calcium chloride coagulated tofu, animals in group 3 were fed alum coagulated tofu, while animals in group 4 were fed steep water coagulated tofu *ad libidum.* The experiment lasted two weeks. Average daily feed intake, body weight gain, dry matter and protein digestibility were monitored during the experiment. At the end of which the rats were sacrificed by decapitation after an 18-hour fast and the blood were collected and the serum was subsequently prepared. Serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), Cholesterol, Low-density

lipoproteins (LDL) and High-density lipoproteins (HDL) were determined by the aid of an automated machine called Hitachi 705.

2.9 ANALYSIS OF DATA

The results of the analysis were pooled and expressed as mean±standard error (S.E.). A one-way analysis of variance (ANOVA) and the Least Significance Difference (LSD) were carried out (Prestamo *et al*., 2002). Significance was accepted at $P \leq 0.05$.

CHAPTER THREE

RESULTS

The tofu yield is shown in figure 2, the results revealed that there was no significant difference ($P > 0.05$) between the tofu yield by the various coagulants, however $CaCl_2$ gave the highest tofu, while alum gave the least tofu. The result of the proximate composition of the tofu produced using various coagulants are presented in table 1. The protein content ranged from 15.1 % for $CaCl_2$ coagulated tofu (CH) to 17.6 % for steepwater (SW) coagulated tofu, while SW had the highest 6.2 %, the ash content of the tofu ranged from 0.5 - 0.7 %. Alum coagulated tofu (AM) had the highest carbohydrate content.

The results of the mineral content of the tofu are presented in table 2, $CaCl_2$ coagulated had the highest amount of Zn (0.6 mg/100 g), while steep water coagulated tofu had the highest amount of Mn (0.3 mg/100 g) and Mg (34.2 mg/100 g), while alum coagulated tofu had the highest amount of Fe (1.7 mg/100 g), K (33.9 mg/100 g) and Ca (23.5 mg/100 g) content.

The results of the sensory evaluation are shown in table 3. The result revealed that steep water coagulated tofu had a significantly lower ($P < 0.05$) general acceptability than alum and calcium chloride coagulated tofu as typified by the taste, structure, texture, odour and colour. The energy content has determined with bomb calorimeter is presented in figure 3, there was no significant difference ($P < 0.05$) in the energy content of the tofu. However steep water and alum coagulated (6.6 cal/g) tofu had higher energy content than $CaCl_2$ coagulated tofu (5.3 cal/g). As shown in figure 4, steep water coagulated tofu had higher *in vitro* digestibility than either alum or $CaCl_2$ coagulated tofu.

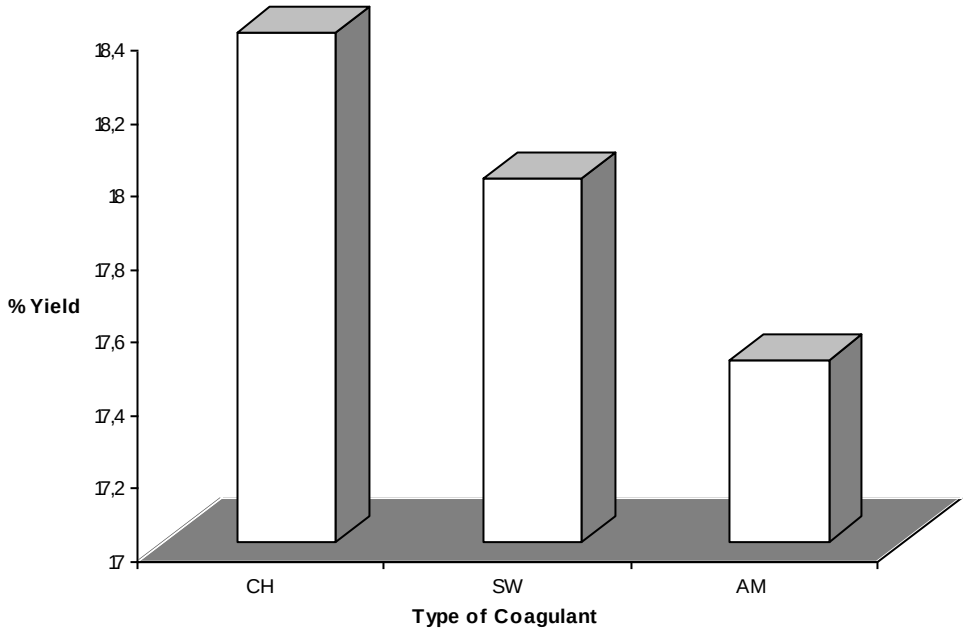

FIGURE 2: THE TOFU YIELD OF VARIOUS COAGULANTS

CH- Calcium chloride coagulated tofu;

SW- Steep water coagulated tofu;

AM- Alum coagulated tofu.

TABLE 5: PROXIMATE COMPOSITION OF TOFU PRODUCED WITH VARIOUS COAGULANTS (%)

Sample	CH	SW	AM
Protein	15.1±0.3^{b}	17.6±0.2^{a}	13.3±0.4^{c}
Fat	5.2±0.2^{b}	6.2±0.2^{a}	4.9±0.3^{b}
Ash	0.7±0.2^{a}	0.5±0.1^{a}	0.7±0.0^{a}
Carbohydrate	5.1±0.2^{b}	3.8±0.1^{c}	7.2±0.1^{a}

Value represents mean of triplicate readings

Values with the same superscript along the same row are not significantly different CH- Calcium chloride coagulated tofu; SW- Steep water coagulated tofu; AM- Alum coagulated tofu.

TABLE 6: MINERAL COMPOSITION OF TOFU PRODUCED WITH VARIOUS TYPE OF COAGULANTS (MG/100G)

Sample	CH	SW	AM
Fe	0.5±0.1^{c}	0.9±0.1^{b}	1.7±0.2^{a}
Zn	0.6±0.0^{a}	0.2±0.0^{b}	0.3±0.1^{b}
Mn	0.2±0.1^{a}	0.3±0.1^{a}	0.2±0.0^{a}
Mg	30.2±0.3^{b}	34.2±0.1^{a}	29.2±0.1^{b}
K	23.1±0.1^{a}	26.3±0.1^{b}	33.9±0.3^{a}
Ca	15.9±0.1^{a}	19.0±0.3^{a}	23.5±0.1^{a}

Value represents mean of triplicate readings

Values with the same superscript along the same row are not significantly different

CH- Calcium chloride coagulated tofu; SW- Steep water coagulated tofu; AM- Alum coagulated tofu.

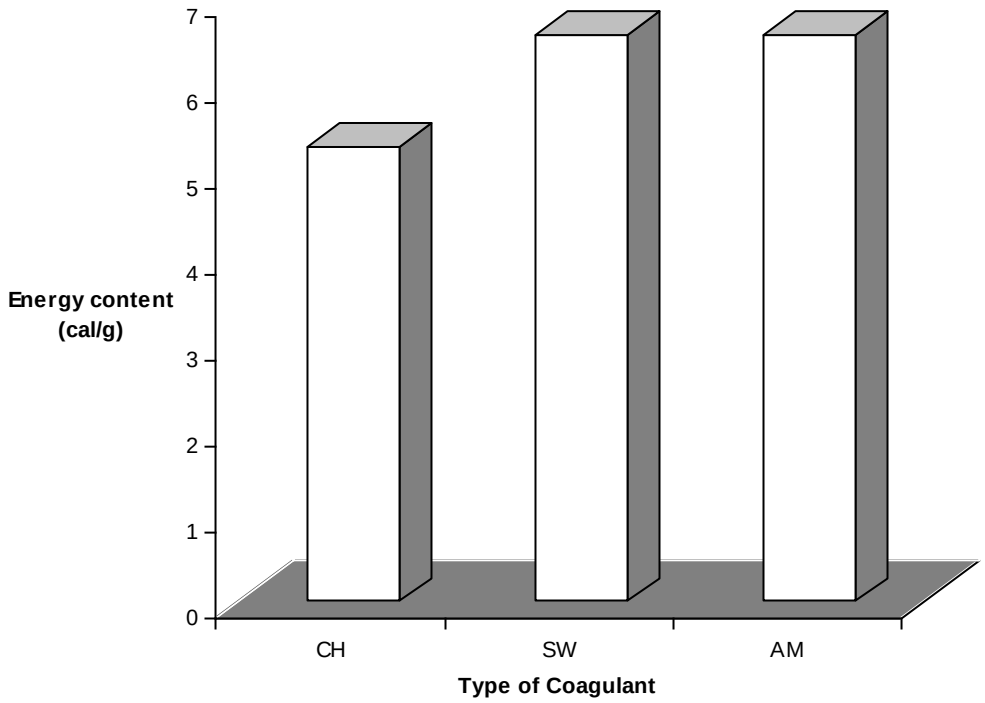

FIGURE 3: THE ENERGY CONTENT OF TOFU PRODUCED USING LOCALLY SOURCED COAGULANTS

CH- Calcium chloride coagulated tofu;

SW- Steep water coagulated tofu;

AM- Alum coagulated tofu.

TABLE 7: SENSORY EVALUATION OF TOFU PRODUCED WITH VARIOUS COAGULANTS

Sample	CH	SW	AM
Taste	6.4±0.1^{a}	4.4±0.3^{b}	5.2±0.6^{a}
Structure	6.8±0.2^{a}	4.8±0.2^{b}	6.2±0.4^{a}
Colour	6.5±0.3^{a}	3.2±0.1^{a}	6.7±0.2^{a}
Odour	6.2±0.3^{a}	2.5±0.1^{a}	6.4±0.2^{a}
Texture	6.1±0.3^{a}	4.8±0.5^{b}	5.8±0.3^{a}
General Acceptability	6.5±0.2^{a}	4.1±0.4^{b}	6.3±0.1^{a}

Value represents mean of triplicate readings

Values with the same superscript along the same row are not significantly different

CH- Calcium chloride coagulated tofu; SW- Steep water coagulated tofu;

AM- Alum coagulated tofu; GA-General Acceptability

7-excellent; 6- very good; 5-good; 4-average; 3-fair; 2-poor; 1-very poor

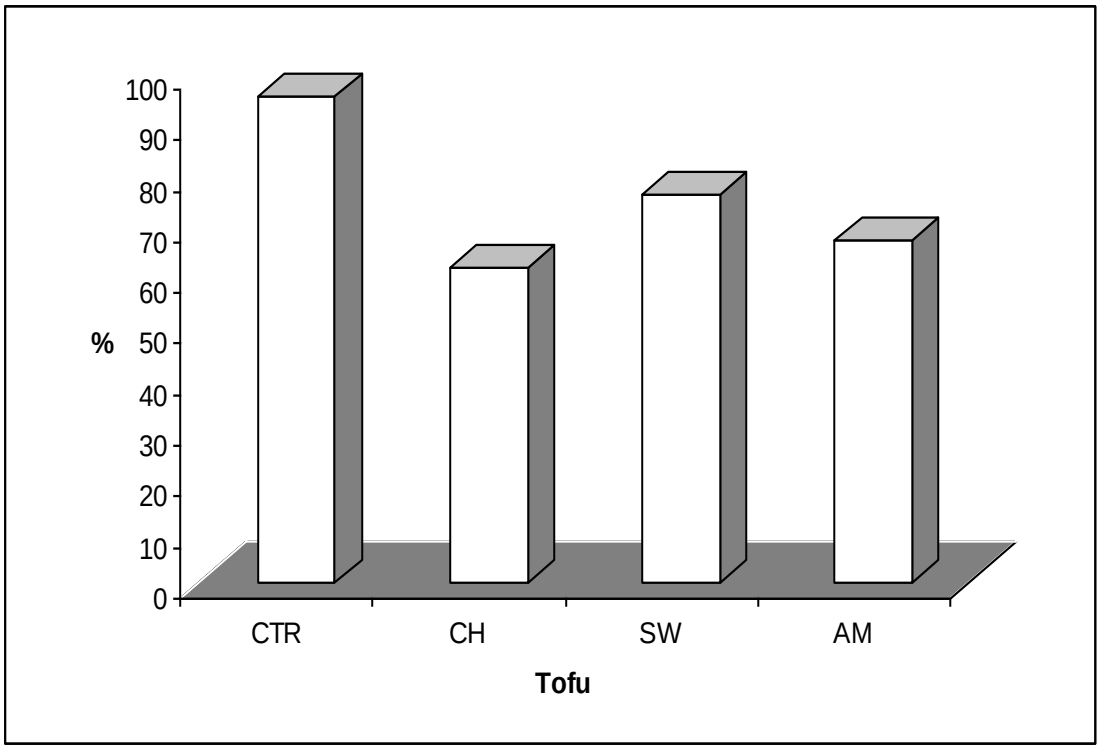

FIGURE 4: *IN VITRO* MULTIENZYME PROTEIN DIGESTIBILITY OF TOFU

Where AM = Alum coagulated tofu

SW = Tofu coagulated with effluent from pap

CH = Calcium coagulated tofu

CTR= Control

The result of the growth performance of the tofu is shown in table 4, there was no significant difference ($P > 0.05$) in the average daily feed intake, weight gain and the feed:gain ratio. Likewise there was no significant difference in the apparent digestibility (Figure 5) and dry matter digestibility (Figure 6) of the various tofu, however $CaCl_2$ coagulated tofu had the highest apparent and dry matter digestibility.

The results of the serum alkaline phosphatase (ALP), glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT) are shown in figures 7, 8 and 9, respectively. The alanine transaminase (ALT) and aspartate transaminase (AST) were previously called the *serum glutamate-pyruvate transaminase* (SGPT) and the *serum glutamate-oxaloacetate transaminase* (SGOT) respectively. There was a decrease ($P < 0.05$) in the serum ALP, GOT and GPT of rats fed tofu in comparison to the control. However, rats fed $CaCl_2$ coagulated tofu had the lowest serum levels of ALP and AST, while those fed alum coagulated tofu had the lowest serum levels of ALT.

The results of the serum cholesterol, low-density lipoprotein (LDL) and high-density lipoproteins (HDL) are shown in figures 10, 11 and 12 respectively. The results revealed that there was a significant decrease ($P < 0.05$) in the serum cholesterol (figure 10) and LDL (figure 11), while there was a significant increase ($P < 0.05$) in the serum HDL levels of rats fed tofu (figure 12). However, those rats fed steep water coagulated tofu had the lowest serum cholesterol and LDL levels; while those fed $CaCl_2$ coagulated tofu had the highest serum HDL levels.

TABLE 8: GROWTH PERFORMANCES OF TOFU PRODUCED WITH VARIOUS COAGULANTS

Sample	CTR	CH	SW	AM
Feed intake (g/rat/day)	7.2	7.0	6.2	6.8
Weight gain (g/rat/day)	2.4	2.2	2.0	2.0
Feed: Gain	3.0	3.2	3.1	3.4

Value represents mean of triplicate readings

Where CTR = Control

AM =Alum coagulated tofu

SW =Tofu coagulated with effluent from pap

CH= Calcium coagulated tofu.

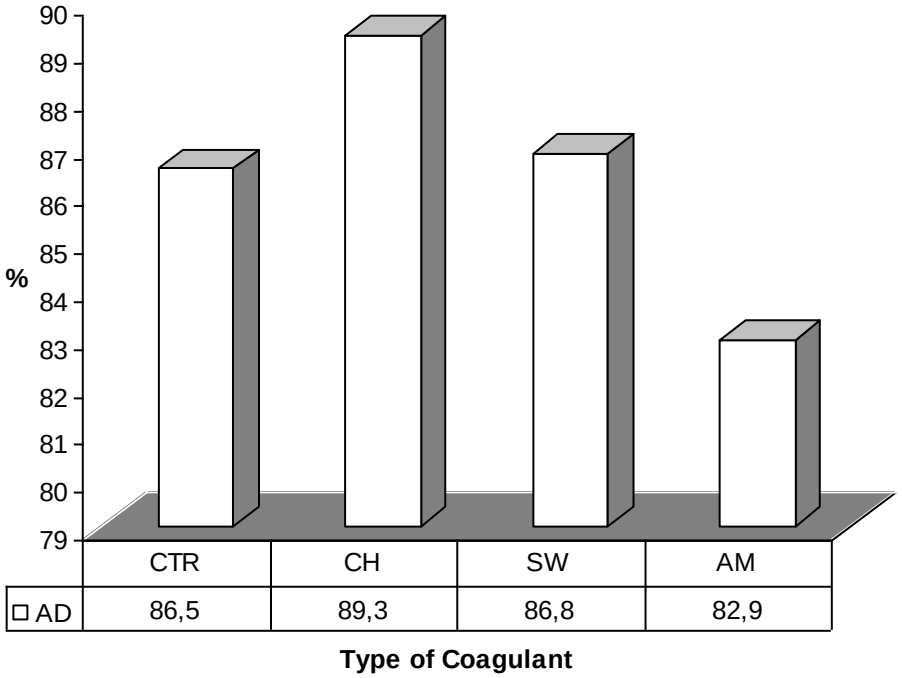

FIGURE 5: APPARENT DIGESTIBILITY OF TOFU

Where AM = Alum coagulated tofu

SW = Tofu coagulated with steep water

CH = Calcium coagulated tofu

CTR = Control

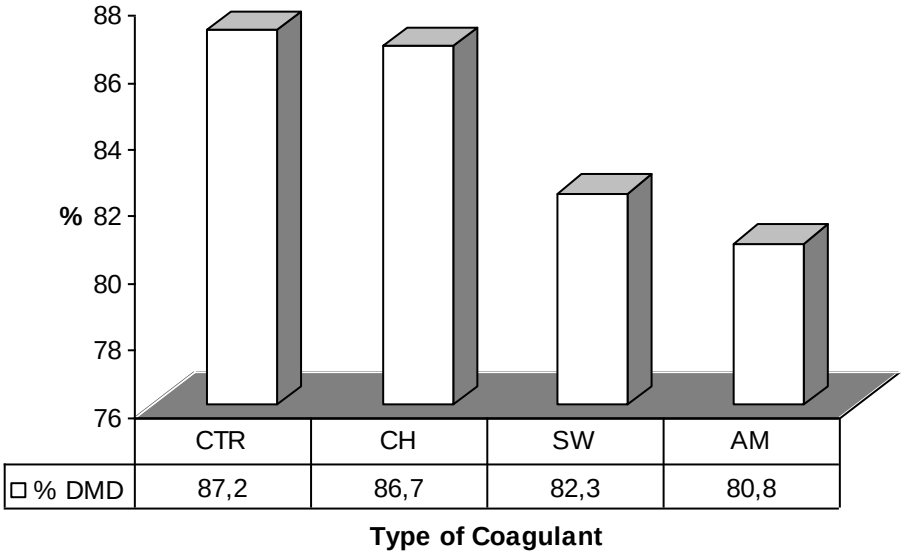

FIGURE 6: DRY MATTER DIGESTIBILITY OF TOFU USING VARIOUS COAGULANTS

Where AM = Alum coagulated tofu

SW=Tofu coagulated with steep water

CH = Calcium coagulated tofu

CTR = control

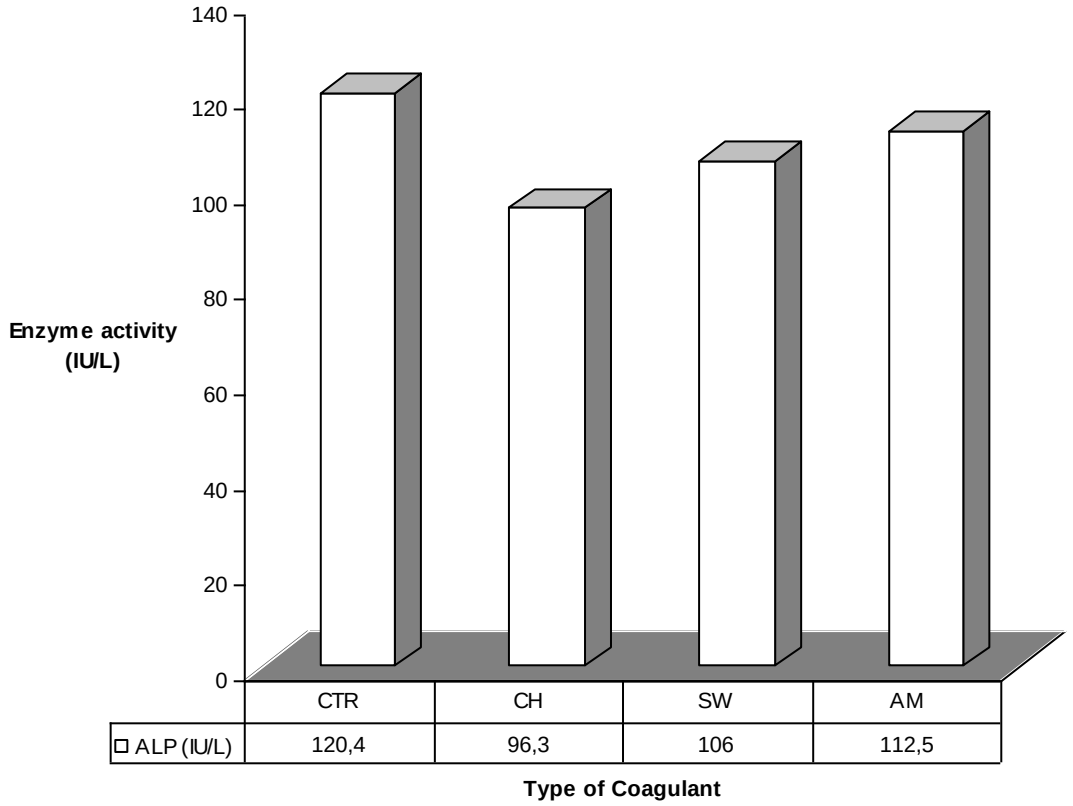

FIGURE 7: SERUM ALKALINE PHOSPHATASE ACTIVITY OF ALBINO RATS FED TOFU

Where AM = Alum coagulated tofu

SW = Tofu coagulated with steep water

CH = Calcium chloride coagulated tofu

CTR = Control

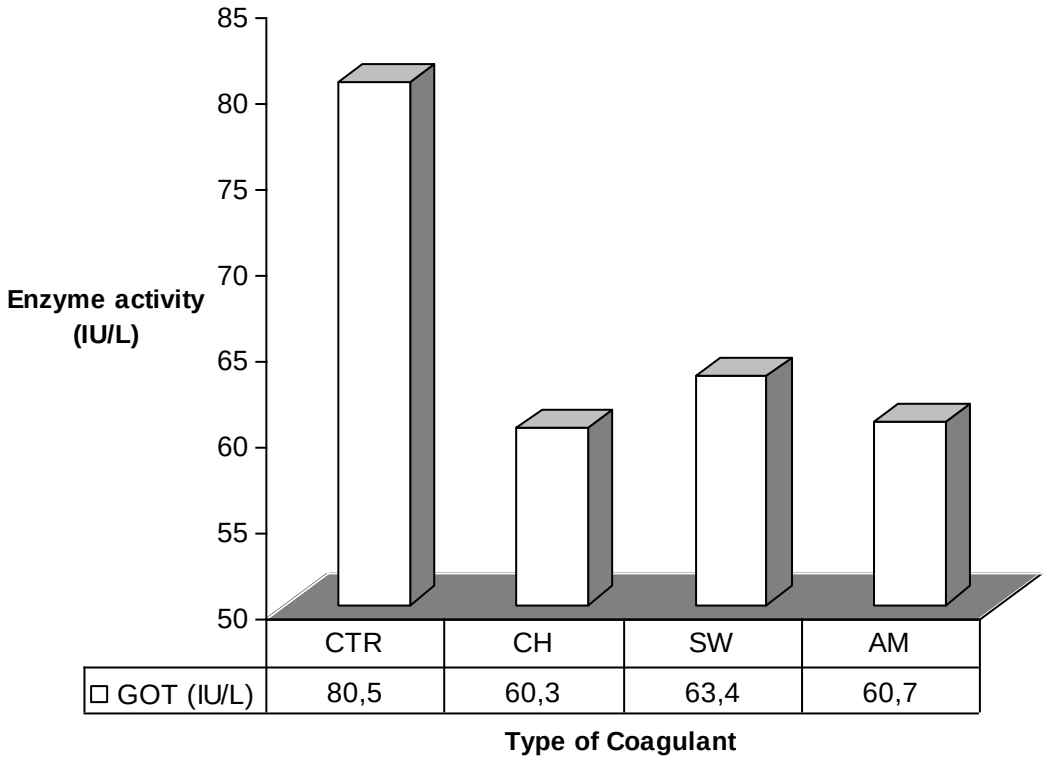

FIGURE 8: SERUM ASPARTATE TRANSAMINASE ACTIVITY OF ALBINO RATS FED TOFU

Where AM = Alum coagulated tofu

SW = Tofu coagulated with steep water

CH = Calcium chloride coagulated tofu

CTR = Control

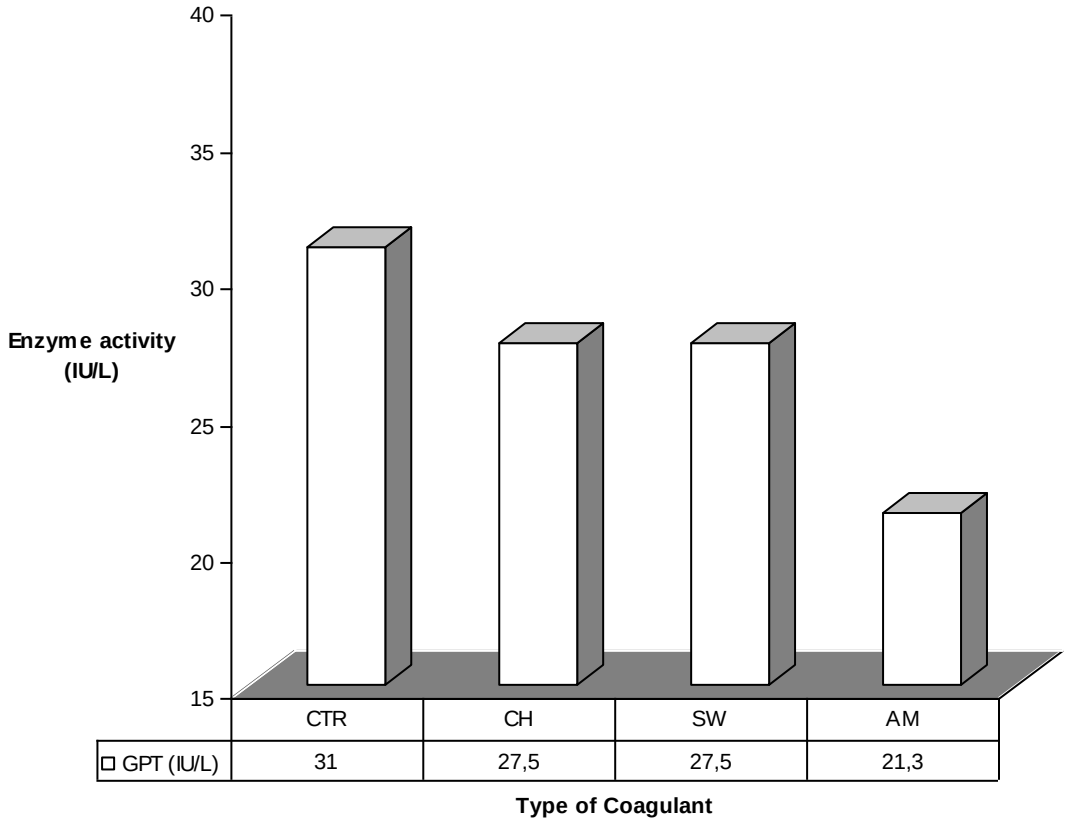

FIGURE 9: SERUM ALANINE AMINO TRANSAMINASE ACTIVITY OF ALBINO RATS FED TOFU

Where AM = Alum coagulated tofu

SW = Tofu coagulated with steep water

CH = Calcium chloride coagulated tofu

CTR = Control

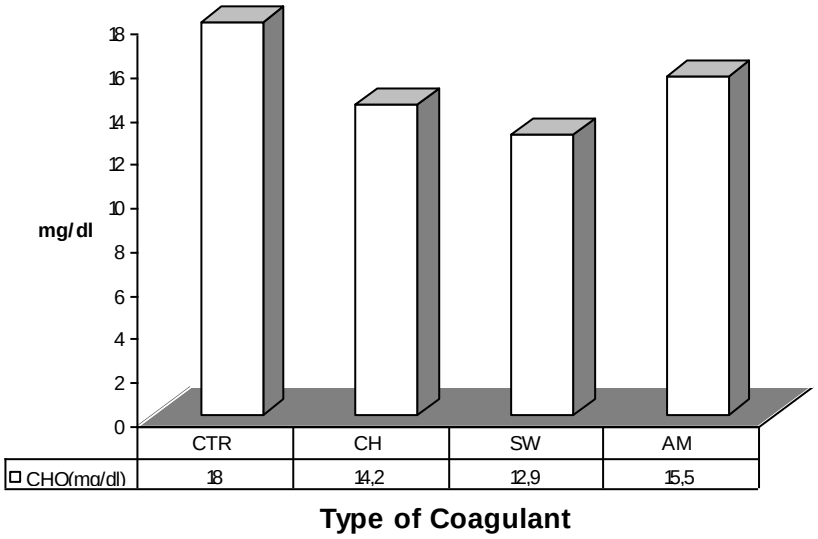

FIGURE 10: SERUM CHOLESTEROL CONTENT OF ALBINO RATS FED TOFU

Where AM = Alum coagulated tofu

SW = Tofu coagulated with steep water

CH = Calcium coagulated tofu

CTR = Control

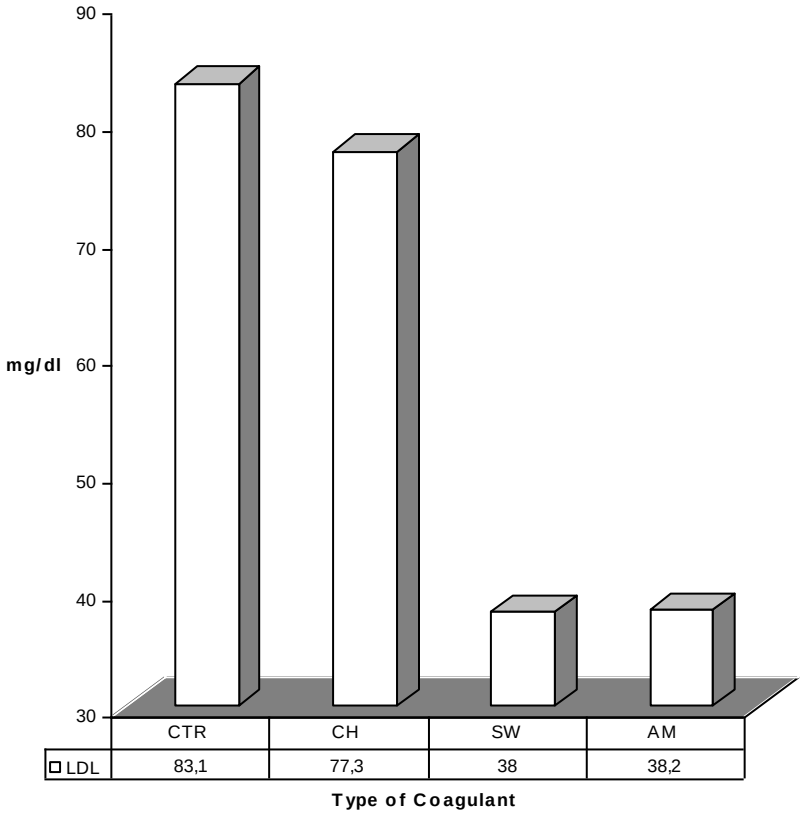

FIGURE 11: SERUM LOW DENSITY LIPOPROTEIN (LDL) CONTENT OF ALBINO RATS FED TOFU

Where AM = Alum coagulated tofu

SW = Tofu coagulated with steep water

CH = Calcium coagulated tofu

CTR = Control

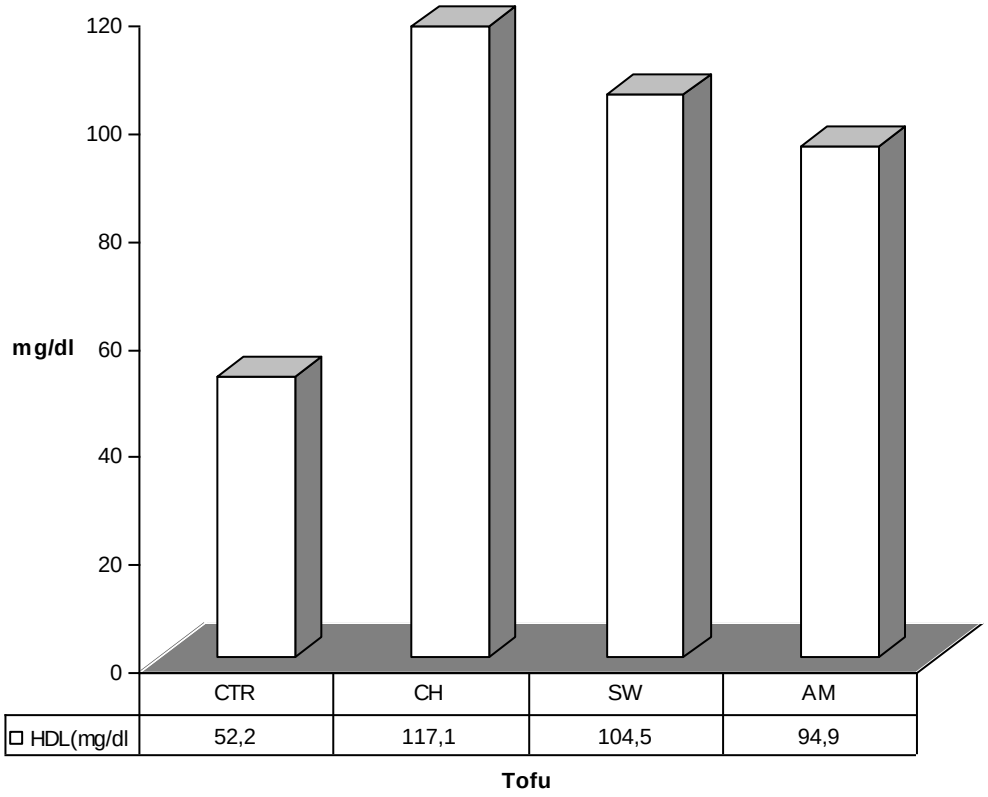

FIGURE 12: SERUM HIGH DENSITY LIPOPROTEIN (HDL) CONTENT OF ALBINO RATS FED TOFU

Where AM = Alum coagulated tofu

SW = Tofu coagulated with steep water

CH = Calcium coagulated tofu

CTR = Control

CHAPTER FOUR

4.0 DISCUSSION

Soya milk and tofu are the most important non-fermented soybean products (Liener, 1994). Soybean (Glycine Max) a legume is a major source of vegetable protein and oil for human an animal consumption and for industrial usage. It contains about 40% protein and 20% oil on a dry-weight beans (McGraw-Hill Encyclopedia of Science and Technology 2002). They are digestible and nutritive products, which also serve as inexpensive protein and unsaturated fatty acids sources for man. Soybean products are a good source of proteins, carbohydrates, low in fat and rich in mineral contents, they are part of principal meals in eastern countries. The incorporation of soybean food (*tofu*) into a western diet could be an important means of preventing and treating chronic diseases, such as cancer and cardiovascular diseases (Prestamo, *et al.*, 2002). Tofu is made by coagulation of soymilk with salt or acid to produce a soy protein gel, which traps water, soy lipids and other constituents in the matrix.

The result of the tofu yield is shown in figure 2, the result revealed that there was no significant difference ($P > 0.05$) in the tofu yield by each of the coagulants, however, $CaCl_2$ gave the highest yield of tofu (18.4 %), while alum gave the least yield of tofu. The fact that there was no significant difference ($P > 0.05$) in the yield indicated that the various coagulants under consideration may not differ substantially in their coagulating ability, however, the slight difference could be as result of extraneous substance introduced by the coagulants. Conversely, there was a significant difference ($P < 0.05$) in the protein content of the tofu (13.3 - 17.6 %) as shown in table 1. The protein content of steep water (17.6 %) coagulated tofu is significantly ($P < 0.05$) higher than that of Calcium Chloride (15.1 %) and alum (13.3 %) coagulated tofu. The high protein content of steep water coagulated tofu could

possibly be attributed to the likelihood that the protein in the pap effluent might have been transferred into the tofu unlike Calcium chloride and alum, which are salt. Furthermore, it could also be speculated that acidic medium created by the steep water may have created a better coagulating environment for the protein present in the soymilk than the salts.

Furthermore, the protein content of the *tofu* produced using all these coagulants had higher protein content than that of the commercial *tofu* (12 %) reported by Prestamo *et al.* (2002), this variation could be as a result of the difference in the variety of soybean and the condition under which the coagulation was carried out. Furthermore, the protein content of the tofu was higher than that of some commonly consumed tropical plant foods such as yam (4-10 %), cassava products (4-12 %) and some commonly consumed green leafy vegetables in Nigeria (Akindahunsi and Oboh, 1998; Akindahunsi and Oboh, 1999; Akindahunsi, *et al.*, 1999; Oboh, *et al.*, 2002; Oboh and Akindahunsi, 2003).

The fat content of steep water coagulated (6.2 %) tofu was significantly higher ($P < 0.05$) than that of alum (4.9 %) and $CaCl_2$ (5.2 %) coagulated *tofu*. The higher fat content of steep water coagulated tofu could also be attributed to the fact that the coagulated protein in the milk might have trapped some of the fat in the heterogeneous solution of steep water and in addition, the acidic medium created by the steep water may have a higher ability to trap fat than the salty environment created by alum and $CaCl_2$. However, the fat content of the *tofu* was lower than the value (9 %) reported by Prestamo *et al*., 2002 for some commercially purchased *tofu*. Conversely, the fat content of the *tofu* produced by the various coagulants were generally high when compared with some commonly consumed plant foods in Nigeria (Akindahunsi and Oboh, 1998; Akindahunsi and Oboh, 1999; Akindahunsi, *et al*.

1999; Oboh, *et al.*, 2002; Oboh and Akindahunsi, 2003). However, there was no significant difference ($P > 0.05$) in the ash content of the tofu produced using the various types of *tofu*.

The result of the mineral composition is shown in table 2; Fe, K, Ca and Na were significantly higher ($P < 0.05$) in *tofu* produced using alum coagulant than the one produced using the other two coagulants, however, steep water coagulated *tofu* had the highest Mn and Mg content, while calcium chloride coagulated *tofu* had the highest Zn content (Table 2). The minerals were generally lower than that of some commonly consumed plant foods in Nigeria such as cassava products (Oboh and Akindahunsi, 2003), cultivated and wild yams (Akindahunsi and Oboh,, 1998) and green leafy vegetables (Akindahunsi and Oboh, 1999; Oboh, *et al.*, 2004). The low mineral content in the tofu could be attributed to the possible solubility of some of the salt of those minerals in the whey during tofu production, thereby preventing the trapping of the mineral in the protein matrix of the *tofu*.

Figure 3 shows the result of the energy content of the *tofu*. The energy content of the *tofu* produced using Calcium Chloride (5.3 cal/g) was significantly lower ($P < 0.05$) than the energy content of the tofu coagulated by steep water and alum (6.6 cal/g). However, the energy content of *tofu* is high when compared to that of cassava products (flour and gari) (3.1 - 3.9 cal/g) (Akindahunsi and Oboh 2003; Onwueme, 1978), which is considered as one of the main dietary energy source in Nigeria (Oboh and Akindahunsi, 2003; Oboh, *et al.*, 2002). The basis for the high energy content of the tofu could not be categorically stated, however, it could be attributed to the fact that *tofu* is very rich in protein, carbohydrate and fat which are energy-producing macromolecules. This is an indication that the coagulated *tofu* trapped a lot of energy producing macromolecules (Prestamo *et al.*, 2002).

The result of the sensory evaluation is shown in table 3. The result revealed that steep water coagulated *tofu* had a significantly lower ($P < 0.05$) general acceptability than alum and calcium chloride coagulated tofu as typified by the taste, structure, texture, odor and colour. This low general acceptability of steep water coagulated tofu compared to alum and calcium chloride coagulated *tofu*, could be attributed to the fact that the steep water is an heterogeneous mixture with characteristic taste, odour and colour; and might have imparted this taste, odor and colour on the tofu, which may have actually alter the taste and odour from the characteristic taste and odour of tofu, this may have reduce the acceptability of the tofu produced from the steep water despite its high nutrient content as highlighted earlier (Larmond, 1973).

Although the acceptability of the texture and structure was lower than that of the alum (6.3) and calcium chloride coagulated tofu (6.5). However, the differences were not significant ($P > 0.05$). The *tofu* produced by alum and calcium chloride had a very good general acceptability, while the general acceptability of steep water coagulated *tofu* was in the average of (4.1) (Potter, 1968).

In-vitro multienzyme protein digestibility of the tofu produced using the various coagulants are shown in Figure 5. The results clearly revealed that the digestibility of the tofu were significantly lower ($P < 0.05$) than that of casein, however, steep water coagulated tofu (75.8 %) had a significantly higher ($P < 0.05$) protein digestibility than those produced using either alum (66.9 %) or calcium chloride (61.6 %). The digestibility of steep water coagulated tofu was very close to that of maize (76.0%), pigeon pea (77.2 %) and African yam bean (77.0 %) as reported by Oshodi and Hall (1993); and palm wine yeast fermented cassava flour (79.1 %) reported by Akindahunsi and Oboh (2003). However, the digestibility of the

steep water coagulated tofu was higher than that of fermented and unfermented gari (a fried cassava product popularly consumed in Nigeria) (66.9 - 69.0 %), whose value compared well with that of alum (66.9) coagulated tofu. Calcium chloride coagulated tofu (61.6 %) had a digestibility far below that of maize (76.0 %), pigeon pea (77.2 %) and African yam bean (77.0 %) as reported by Oshodi and Hall (1993); and palm wine yeast fermented cassava flour (79.1 %) reported by Akindahunsi and Oboh, 2003.

The basis for the wide difference in the digestibility of *tofu* from the same soybean cannot be categorically stated. However, it could be speculated that the difference in the digestibility could be as a result of the difference in the type and amount of proteins, protease inhibitors such as trypsin inhibitor, chymotrypsin inhibitor and tannin (Aletor, 1993) trapped in the tofu matrix. Tannin, trypsin inhibitor and chymotrypsin inhibitors can interact with proteins or the digestive enzymes thereby reducing digestibility of the protein in the *tofu*, and the amount of inhibitors coagulated might have varied from one coagulant to another. However, the reduction in digestibility is more evident in calcium chloride and alum-coagulated *tofu* than that of steep water.

The results of the growth performance of the tofu on rats are shown in table 4, the results revealed that there were no significant difference ($P < 0.05$) in the average daily feed intake, average daily weight gain and feed: gain ratio, however, those rats fed $CaCl_2$ coagulated tofu had the highest average feed intake and body weight gain, while those fed alum coagulated *tofu* had the highest feed: gain ratio. It is worth noting that despite the fact that those rats fed steep water coagulated tofu had the lowest feed intake, same sets of rats had the lowest feed gain ratio, which is an indication that this *tofu* will support growth than the other two coagulants (Oboh and

Akindahunsi, 2005). This higher growth efficiency of steep water coagulated *tofu* could be attributed to its higher protein content as shown in table 1.

As shown in figure 5, there was no significant difference ($P > 0.05$) in the apparent digestibility of the control (86.5 %) and that of the various tofu (82.9 – 86.3 %); however, $CaCl_2$ coagulated tofu had the highest apparent digestibility, while alum coagulated *tofu* had the least apparent digestibility. The apparent digestibility of the tofu were within the same range with that of *Saccharomyces ceresisae* fermented cassava flour (86.5 %) (Oboh and Akindahunsi, 2005), higher than that of white beans (81.3 %), but below that of casein (95.2 %) (Bressani, 1999)

The results of the dry matter digestibility of the tofu are shown in figure 6; likewise there was no significant difference ($P < 0.05$) in the dry matter digestibility of the tofu (80.8 – 86.7 %) and that of the control (87.2 %), however, of all the *tofu*, $CaCl_2$ coagulated *tofu* had the highest dry matter digestibility while alum had the least dry matter digestibility. These values compared favourably with that of *Saccharomyces cerevisae* fermented cassava flour fed to rats (87.2 %) (Oboh and Akindahunsi, 2005).

Changes in the serum levels of transaminases, alkaline phosphatase, cholesterol and low-density lipoproteins are considered to be associated with various diseased states. An increase in the blood serum level of the transaminases and alkaline phosphatase especially glutamate pyruvate transaminase (GTP) is indicative of liver damage while an increase in serum levels of cholesterol and the low density lipoproteins is associated with hypercholesterolemia and atherosclerosis respectively (Jariwalla *et al.*, 1990).

Figure 7 shows that serum level of alkaline phosphatase (ALP) in rat fed with tofu were significantly lower ($P < 0.05$) than those fed the basal diet. However, those

rats fed alum-coagulated tofu had higher serum level of alkaline phosphatase than serum level of ALP in those rats fed tofu coagulated with other coagulants. ALP is a metal enzyme whose activity is increased in the presence of bivalent ions and is considered as the marker for bones (Osteoblasts) and liver diseases, but the relationship between ALP and diet is not clear (Prestamo *et al*, 2002). However, the general decrease in the serum ALP level of rats fed tofu may be attributed to the fact that the tofu had low minerals content, and this may affect ALP activity. The higher level of the serum ALP in rats fed alum coagulated could possibility be attributed to the fact that most of the minerals analyzed were higher in alum coagulated tofu (table 2). Furthermore the lower serum ALP levels of the *tofu* could possibly be due to the fact that the *tofu* may have protective effect on the liver, since elevated serum ALP could be attributed to possible liver damage (Oboh and Omotosho, 2005).

Furthermore, the serum GOT level of rats fed with *tofu* were significantly lower than those fed with the basal diet (figure 8), however there was no significant difference ($P > 0.05$) in the serum GOT of those rats fed the different tofu. Conversely, there was no significant difference ($P < 0.05$) in the serum GPT level of rats fed the basal with those fed tofu except in those fed alum coagulated tofu (figure 9). GPT and GOT are enzymes that are located in the liver cells and leak out and make their way into the general circulation when liver cells are injured (American Liver Foundation, 1995, 1997; David and Johnston, 1999). GPT is regarded to be a more specific indicator of liver inflammation, since GOT may be elevated in diseases of other organs such as heart disease or muscle disease (American Liver Foundation, 1995, 1997; David and Johnston, 1999). In acute liver injury, such as acute viral hepatitis, GPT and GOT levels may be very high, and sometimes over 1000U/L (American Liver Foundation, 1997). In chronic hepatitis or cirrhosis, the elevation of

these enzymes may be minimal (less than 2–3 times normal) or moderate (100–300U/L). Mild or moderate elevations of GPT or GOT are non-specific and may be caused by a wide range of liver diseases (American Liver Foundation, 1995, 1997; David and Johnston, 1999). This clearly indicates that that *tofu* would not cause liver damage; rather they could have a protective effect on the liver (Oboh, 2005; Jackson et al, 2002; Gissen, 1996).

Studies carried out by Prestamo *et al.* (2002) had established the fact that soy and its products effectively lower serum cholesterol and low-density lipoproteins (LDL). The results presented in figures 10 and 11 agree with the earlier finding by Prestamo *et al.,* (2002) to the extent that there was a significant decrease ($P < 0.05$) in the serum cholesterol and low-density lipoproteins in rats fed tofu coagulated with steep water, calcium chloride and alum respectively when compared with those fed with the commercial diet (control). Increase in serum cholesterol which is caused by LDL is due essentially to the presence of a mutant allele at the LDL receptor locus, which results in reduced ability to bind and to take up LDL (Agbedana, 1997), leaving LDL and cholesterol in the plasma because LDL is unable to transport cholesterol back to the Liver for biliary excretion or repackaging (Agbedana, 1997), this condition is a characteristic of hypercholesterolemia. However tofu was able to lower LDL cholesterol by stimulating the hepatic LDL receptor (Agbedana, 1997; Prestamo *et al.* 2002). The stimulation of the hepatic LDL receptor is related to the isoflavones, which resemble the estrogens but contain weak estrogenic activity. Higher levels of estrogens are associated with lower levels of cholesterol. One mechanism proposed for estrogenic effect is also through up regulation of LDL receptor (Agbedana, 1997; Georgi, 2002).

Although, there was a significant decrease in the serum cholesterol and low-density lipoproteins (LDL) level of rats fed tofu, however there was a marked variation in the serum cholesterol and LDL levels in the rats with the type of coagulant used in the production of the tofu. Rats fed with steep water coagulated tofu had the lowest serum cholesterol and LDL level, followed by those fed with calcium chloride coagulated tofu, while those fed alum coagulated tofu that had the highest serum cholesterol and LDL levels. This low cholesterol and LDL levels in rats fed steep water coagulated tofu could be as result of the high total phenol content (Oboh, unpublished data) when compared to alum and calcium chloride coagulated tofu that had significantly lower ($P < 0.05$) total phenol contents (Omotosho *et al.*, 2011).

The result of the serum levels of High-density lipoproteins (HDL) of rats fed tofu coagulated with steep water, calcium chloride and alum respectively is shown in figure 5. The result revealed that there was a significant increase ($P < 0.05$) in the serum HDL levels of rats fed tofu when compared with those fed the control diet. High serum HDL levels have been proven to protect LDL from oxidation, high serum levels of HDL is indicative of a healthy metabolic system, if there is no sign of liver disease or intoxication thus HDL is sometimes referred to as ‘good’ cholesterol. Two mechanisms that explain how HDL offers protection against chronic heart disease are: firstly, that HDL inhibits cellular uptake of LDL and secondly, that HDL serves as a carrier that removes cholesterol from the peripheral tissues and transport it back to the liver for catabolism and excretion (Agbedana, 1997; Georgi, 2002).

There was a marked difference in the serum HDL levels of rats fed *tofu* produced with steep water, alum and calcium chloride respectively. Rats fed calcium chloride-coagulated *tofu* had the highest serum HDL, followed closely by those fed steep water coagulated *tofu*, while those fed alum coagulated tofu had the least serum

levels of HDL. Even though steep water coagulated *tofu* showed the greatest effectiveness in lowering serum cholesterol and LDL compared with alum and calcium chloride coagulated tofu, this study also showed that calcium chloride coagulated *tofu* had the highest serum HDL levels, which is considered as good cholesterol. The basis for the highest serum HDL in rats fed $CaCl_2$ coagulated tofu when compared to others coagulant cannot be categorically stated. However, it can be inferred that the total phenols, specifically the isoflavone may not be responsible alone for the hypocholesterolaemic effect of the *tofu*; there may have been some other phytochemicals in the *tofu*, which were more in the $CaCl_2$ coagulated *tofu* that may have additive or synergistic effect on the hypocholesterolaemic of the total phenol.

4.2 CONCLUSION

The results of the present study indicated that

Steep water coagulated *tofu* when compared with other coagulants:

- has the highest protein
- has the highest Mn and Mg
- has the highest *In vitro* digestibility,
- has the highest energy content
- has the highest Zn bioavailability
- Caused a significant decrease ($P < 0.05$) in the serum AST, ALT, ALP, Cholesterol and low-density lipoprotein and at same time caused a significant increase in Serum High–density lipoproteins of rats fed the *tofu*.
- has high apparent and dry matter digestibility
- but has the lowest general acceptability

Therefore steep water (effluent from pap produced from maize) which is considered to be waste appears to be a better coagulant for the production of tofu with high nutritional value and hypocholesterolemic effect, however it has the least acceptability, further research will be carried out on how to improve the sensory quality of steep water coagulated *tofu*.

4.3 RECOMMENDATION

In view of the various health benefits associated with *tofu*, it should be introduced into Nigerian diet. Its intake should also be encouraged in homes.

Tofu can be used as a functional food to prevent oxidative stress due to its antioxidant properties and also in the treatment of liver damage, hypercholestorelemia and arteriosclerosis.

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