EFFECT OF SOME PROCESSING METHODS ON HEMAGGLUTININ ACTIVITY OF LECTIN EXTRACTS FROM SELECTED GRAINS (CEREALS AND LEGUMES)

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ABSTRACT

Lectins was extracted from processed and unprocessed black-eyed peas, green beans, soybeans, peanuts, and red kidney beans by grinding using attrition mill and then soaked in distilled water. The proteins in the supernatant were precipitated by dissolving 10g of ammonium sulphate in 100ml of the supernatants. The lectin extracts were evaluated for hemagglutinin activity using human erythrocytes from blood group A^+, B^+, AB^+, and O^+, respectively. Result revealed that lectins from unprocessed soybean, black-eyed pea and green bean are blood group selective and may be classified as blood type specific lectins. While lectins from kidney beans and peanuts agglutinated all blood groups and may be classified as panhemagglutinin lectins, i.e. they agglutinated erythrocytes from all blood groups. Result shows that blood group O is less affected by hemagglutinin activity of lectin. Reduction/loss of hemagglutinin activity was observed with reduction in concentration of the crude lectin extracts in all unprocessed legumes. Boiling (at 100^0C) for 30minutes completely inactivated hemagglutinin activity of lectin in Black eyed peas, soybeans, green beans and red kidney beans. Slight hemagglutinin activity was seen in lectin from roasted peanuts, indicating that dry heating may be very effective method to inactivate lectin completely.

Also, in this study, lectins were extracted from rice (Oryza sativa), maize (Zea mays), sorghum (Sorghum bicolor), Acha (Digitaria excells), millet (Panicum maniceum); partial purification of the extract assayed from hemugglutinin activity using 4% of human erythrocyte in lectin buffer. Extract from these selected cereals showed positive and negative agglutination reaction with blood group A^+, B^+, O^+ and AB^+ depending on the concentration (dilution) and the blood group. Lectin extract from maize showed no agglutination reaction when diluted in blood group A^+, B^+, and AB^+ and even when processed and diluted. While crude lectin extract from rice showed no activity in blood group A^+, B^+, and AB^+ and when unprocessed and showed lectin agglutination activity when processed by boiling (cooking) showing that heat may encourage its activity. Blood group B^+, and AB^+ showed no activity in all dilution. Also acha showed high agglutination activity in blood group A^+, B^+, AB^+ and O^+ but losses activity in dilution a_2 and a_3. Crude lectin extract from processed sorghum showed

high hemagglutinin activity in the blood groups. The effect of high concentration on hemagglutinin activity revealed the activity decreased with dilution of the extract.

1.0 INTRODUCTION

Legumes are plants in the family *Fabaceae (*or *leguminosae)* or the fruit or seed of such plant. They have seed pods that split into two halves. Edible seeds from plants in the legume family include soybeans, beans, peas, lentils, peanut. Legumes are inexpensive, nutrient dense, rich source of protein that can be substituted for dietary animal protein (Smith et al., 1999). However legumes contain anti-nutrients which may affect health.

A cereal is a grass, a member of the monocot family proceae cultivated for the edible component of its grain; composed of the endosperm, germ and bran. Cereals are grown in the greater quantity and provide more food energy worldwide than other types of crops. They are therefore staple crops.

In their natural forms they are rich source of vitamins, minerals, carbohydrates, oils and fats and protein. However, when refined by the removal of bran and germ the remaining endosperm is mainly carbohydrate and lacks the majority of other nutrients. In some developed countries, cereal consumption is moderate and varied but still substantial

Anti-nutrients are natural synthetic compounds that interfere with the absorption of nutrients (Oxford Dictionary, 2006). Anti-nutrients are found at some in almost all foods for a variety of reasons. However, most of these anti-nutrients play an important role in the defense mechanism of plants against the attack of microorganism, pests, and insects. Consequently, the large fraction of modern diet that comes from a few crops has raised concerns about the effects of the anti-nutrient in these crops on human health (Cordian, 1999).

Lectin also called phytohemagglutinin is one of the anti-nutrients found in human food (Krispin, 1999). Other examples of these anti-nutrients include protease inhibitors in plants, such as soybean, lipase inhibitor, phytic acids, amylase inhibitors, oxalates, etc. (Preuss et al, 2009).

High levels of lectins may be found in cereal grains, night shades (e.g. tomatoes, potato, and egg plant), legumes, and vegetables and fruits. Many other foods contain lectins but are less well studied and the amount of lectin present are not thought to be high or as potentially toxic (Krispin, 1999). Many legume seeds have been proven to contain high lectin activity termed as “hemagglutinin activity” (Komath et al, 2006).

The toxicity of lectin has been identified by consumption of food with high lectin content which can lead to diarrhea, nausea, bloating, vomiting and even death. Hemagglutinin activity of lectin extracts from different legumes differ in theory potency (Lierier, 1975). Foods with high concentration of lectins may be harmful if consumed in excess in uncooked or improperly processed form. Adverse effects may include nutritional deficiencies and immune reactions (BJN, 2000).

Lectin can cause damage to microvilli, shedding of cells, reduction in the absorption capacity of the intestine, increased turnover of epithelial cells, interference with the immune system, hypersensitivity reactions, interference with microbial ecology of gut and selective

overgrowth (Krispin, 1999). Crude extract of lectin agglutinates the red blood cells of human beings and animals if injected directly into the blood stream (Enwere, 1998).

Most effect of lectins is due to gastrointestinal distress through interaction of the lectins with the gut epithelial cells. A recent in vitro study has suggested that mechanism of lectin damage may occur by interfering with the repair of already damaged epithelial cells (Miyake, 2007). Lectin damage to gut wall may allow other non-lectin proteins to cross undigested into general circulation and cause allergic reactions including anaphylaxis (Pusztai, 1991).

There has been information that lectins may be inactivated, destroyed or drastically reduced by some processing operation, such as boiling, soaking, malting (germinating) or fermenting. Soaking legumes overnight, draining the water, rinsing and draining again, does not seem to remove many of the lectins (Pusztai, 1991). The large concentration of lectins in plant seeds decreases with growth and suggests a role in plant germination and perhaps in the seed's survival itself. Heat processing can reduce the toxicity of lectins, but low temperature or insufficient cooking may not completely eliminate their toxicity, as some plant lectins are resistant to heat. Other conventional processing methods such as cooking, blanching, soaking, sun drying etc. have been applied in processing of foods. Their effects on anti-nutritional properties in food have constantly featured in food and nutritional literature.

However, moist heating has been found to be effective than dry heating (Komath et al, 2006).

1.1 PROBLEM STATEMENT

The potency and lethality of lectins when ingested by animals, especially human, demands that proper attention be given to study through research. It is recognized that lectins constitute one of the main physiologically active components of food of plant origin and some of them are anti-nutrients. The consumption of food containing them can have serious consequences on growth and health.

The toxic effect of lectins has been observed in previous works. However, many have being victims of lectins toxicity due to consumption of uncooked or improperly cooked foods high in lectins content (Pusztai, 1991).

Pusztai (1991) indicated that the presence of lectins in foods has not been systematically investigated. However, most edible plants are known to contain lectins. The observation by Nachbar et al (1980) that processed foods tested even contain lectins, demands for a serious insight on lectins.

Many of legume and cereal lectins have been implicated in health problem, which calls for proper research and attention on anti-nutritive properties of legume and other foods with anti-nutritive properties. This has raised quest for ways to destroy, inactivate or deactivate presence of lectins in these foods or better reduce them to a level tolerable by the organism.

1.2 JUSTIFICATION

This research will give concise information on the toxicity of lectins found in various foods of legume family (*Fabaceae*) and cereal family as well as explain how some processing methods can be used to deactivate, destroy and/or reduce the amount of lectins in these foods.

Furthermore, it will also expand knowledge on area of lectin specificity for different blood groups. Information generated will be significant in advising food processors, consumers,

vendors and other comestibles handler on effective and efficient processing technique to adapt during food preparation and what food needs to be avoided by various blood groups.

1.3 OBJECTIVES OF THE STUDY

- To extract and purify lectins from selected legumes and cereals.

- To determine hemagglutinin activity of lectins from selected cereals and legumes on erythrocytes.

- To determine effects of some processing methods on hemagglutinin activity of lectin from selected legume

- To evaluate the specificity of the lectin towards human blood groups

2.0 LITERATURE REVIEW

Legumes (beans, peas and lentils) are among the most versatile and nutritious foods available. Legumes are typically low in fat, contain no cholesterol, and are high in folate, potassium, iron and magnesium. They also contain beneficial fats and soluble and insoluble fiber. A good source of protein, legumes can be a healthy substitute for meat, which has more fat and cholesterol (Smith et al, 1999).

However, legumes contain various anti-nutrients which have adverse effect on health and may induce immune reaction (Pusztai, 1991).

A cereal is a grass, a member of the monocot family proceae cultivated for the edible component of its grain, composed of the endosperm, germ and bran. Cereals are grown in the greater quantity and provide more food energy worldwide than other types of crops.

In their natural forms they are rich source of vitamins, minerals, carbohydrates, oils and fats and protein. However, when refined by the removal of bran and germ the remaining endosperm is mainly carbohydrate and lacks the majority of other nutrients. In some developed countries, cereal consumption is moderate and varied but still substantial.

However, the inherent toxic factors or anti-nutritional component in plants have been major obstacles in harnessing the full benefits of the nutritional value of plant food, cereal inclusive.

2.1 ANTI-NUTRIENTS

Anti-nutrients are natural or synthetic compounds that interfere with the absorption of nutrients (Oxford Dictionary, 2006). Nutrition studies focus on those on anti-nutrients commonly found in food sources and beverages.

2.1.1 EXAMPLES OF ANTI-NUTRIENTS

Protease inhibitors are substances that inhibit the action of trypsin, pepsin and other proteases in the gut, preventing the digestion and other subsequent absorption of protein in soybeans (Tan-Wilso et al, 1987).

Lipase inhibitors interfere with enzymes such as human pancreatic lipase that catalyze the hydrolysis of some lipids, including fats. Amylase inhibitors prevent the action of enzymes

that break the glycoside bonds of starches and other complex carbohydrates, preventing the release of simple sugars and absorption by the body (Preuss et al, 2009). Phytic acid has a strong binding affinity to minerals such as calcium, magnesium, iron, copper and zinc. Thus results in precipitation, making the minerals unavailable for absorption in the intestines (Ekholm et al, 2003). Phytic acids are common in the hulls of nuts, seeds and grains.

Oxalates present in many plant, bind to calcium and prevent its absorption in the human body. Glucosinolates prevent the uptake of iodine, affecting the function of the thyroid and thus considered goitrogens. They are found in broccoli, brussel sprouts and cabbage and cauliflower. (Preuss et al, 2009).

Excessive intake of required nutrients can also result in them having an anti-nutrient action. Excessive intake of fibre can reduce the transit time through the intestines to such a degree that other nutrients cannot be absorbed. Because calcium, iron, zinc and magnesium share the same transporter within the intestine, excessive consumption of one of these minerals can lead to saturation of the transport system and reduced absorption of the other minerals (Pearson, 2007).

Some proteins can also be anti-nutrients, such as the trypsin inhibitors and lectins found in legumes. (Cockell et al, 2005).

Another particularly widespread form of anti-nutrients are the flavonoids, which are a group of polyphenolic compounds that include tannins (Beecher, 2003). These compounds chelate metal such as iron and zinc and reduce the absorption of these nutrients, but they also inhibit digestive enzymes and may also precipitate proteins.

There are many examples of anti-nutrients which were not mentioned here.

2.1.2 OCCURRENCE AND REDUCTION

Anti-nutrients are found at some level in almost all foods for a variety of reasons. However, their levels are reduced in modern crops, probably as an outcome of the process of domestication. The possibility now exists to eliminate anti-nutrients entirely using genetic engineering. Many traditional methods of food preparation such as fermentation, cooking and malting increase the nutritive quality of plant foods through reducing certain anti-nutrients such as phytic acids, polyphenols, and oxalic acid (Gibson et al, 2007). Such processing methods are widely used in societies where cereals and legumes form a major part of the diet (Phillips, 1993). An important example of such processing is the fermentation of cassava to produce cassava flour: thus fermentation reduces the level of both toxins and anti-nutrients in the tuber (Oladunmonye and Oboh, 2007).

2.2 HEMAGGLUTININ

Hemagglutinin refers to a substance that causes red blood cells to agglutinate. This process is called hemagglutination. Antibodies and lectins are commonly known hemagglutinins (Dorland's Medical Dictionary, 2001).

Types of hemagglutinin include:

- Inflenza hemagglutinin
- Measles hemagglutinin
- Parainfluenza hemagglutinin – neuramindase.

- Mumps hemagglutinin – neuraminidase.
- The PH-E form of phytohemagglutinin.

The terms phytohemagglutinins, phytagglutinins and lectins are used interchangeably to refer to most purified plant hemagglutinin are carbohydrate – containing proteins. Hemagglutinins may be pure proteins or glycoproteins (Maurice et al, 1998).

2.3 LECTINS

Lectins are carbohydrate-binding proteins (not to be confused with glycoproteins, which are proteins containing sugar chains or residue) that are highly specific for sugar moieties, particularly, the high specificity of plant lectins for foreign glycoconjugates (Van Damme et al, 1998).

They are glycoproteins of 60,000-100,000 MW that are known for their ability to agglutinate (clump) erythrocytes in vitro. There are over 40,000 estimated binding sites for kidney bean agglutinin on the surface of each erythrocyte (Barondes, 11981). Lectins may be disabled by specific mono and oligosaccharides, which bind to ingested lectins from grains, legumes, nightshade plants and diary; binding can prevent their attachment to the carbohydrate within the cell membrane (Krispin, 1999).

These lectins selectively bind carbohydrate moieties of the glycoprotein that decorate the surface of most of the animal cells.

Structurally, these lectins have a diverse class of proteins, which have the ability to bind carbohydrates with considerable specific (Rinijm, 1995). There are numerous studies which attempted to show the in-vivo and in-vitro effects of lectins. In-vitro, they have been shown to effect lymphocytes of gastro intestine tract being most susceptible. They possess the ability to aggregate immunoglobulins, to trigger the alternative pathway, to inhibit fungal growth and also to induce histamine release from basophils and mast cells. Lectins are relatively resistant to both heat (at 70^0C more than 30mins) and digestion some of the lectins are highly resistant to gastric acid and proteolytic enzymes (Rocca J.D., 2004).

2.3.1 ETYMOLOGY

The name “lectin” is derived from the Latin word legere, meaning, among other things “to select” (Komath et al, 2006).

2.3.2 HISTORY

Although they were first discovered more than 100 years ago in plants, lectins are now known to be present throughout nature. It is generally believed that the earliest description of lectin was given by Peter Herman Stillmark in his doctoral thesis presented in 1888 to the University of Dorpat. Stillmark isolated ricin, an extremely toxic hemagglutinin, from seeds of the castor plant (Komath et al, 2006). The legume lectins are probably, the most studied lectins.

2.4 LECTINS IN LEGUMES AND CEREALS

2.4.1 LEGUME AND CEREALS

Grain legumes are cultivated for their seeds, and are also called pulses. The seeds are used for human and animals consumption or for the production of oils for industrial uses. Grain

legumes include beans, lentils, lupins, peas and peanuts (Kurlovich et al, 1995). Farmed legumes and forage legumes also found their usefulness in provision of livestock feed and pharmaceutical industries.

A legume is plant in the family *Fabaceae* (or *leguminosae*), or the fruit or the seed of such a plant. Legumes are grown agriculturally, primarily for their food grain seed (e.g bean and lentils, or generally pulse), for livestock forage and silage and as soil-enhancing green manure. Well known legumes include alfalfa, clover, peas, beans, lentils, lupins, mesquite, carob, soybean and the woody climbing vine wisteria (Cirrus et al, 2003).

A cereal is any grass cultivated for the edible components of its grain (botanically, a type of fruit called a caryopsis), composed of the endosperm, germ, and bran. Cereal grains are grown in greater quantities and provide more food energy worldwide than any other type of crop and are therefore staple crops. Edible grains from other plant families, such as buck wheat (Polygonaceae), quinoa (Amaranthaceae) and chia (Lamiaceae), are referred to as pseudocereals.

In their natural form (as in *whole grain*), they are a rich source of vitamins, minerals, carbohydrates, fats, oils, and protein. When refined by the removal of the bran and germ, the remaining endosperm is mostly carbohydrate. In some developing nations, grain in the form of rice, wheat, millet, or maize constitutes a majority of daily sustenance. In developed nations, cereal consumption is moderate and varied but still substantial.

2.4.2 LEGUMES LECTINS

The legume lectins are family sugar binding proteins or lectins found in the seeds and, in smaller amounts, in the roots, stems, leaves and bark of plants belonging to the *Fabaceae* family (Loris et al, 1994). The exact function of the legume lectins in vivo is unknown, but they are probably involved in the defense of plants against predators. They have been used for decades as a model system for the study of protein-carbohydrate interactions, because they show an amazing variety of binding specificities and are easy to obtain and purify (Hamelryk et al, 1998). Well-studied members of this protein family include phytohemagglutinin and concanavalin A.

2.4.3 SUGAR BINDING BY LEGUME BY LEGUMES LECTINS

The legume lectins use ingenious framework for binding specific sugars. This framework consists of a conserved monosaccharide binding site in which four conserved residue from four separate regions in protein confer infirmity, a variable loop that confers monosacchandrise specificity, and a number of subsites around the monosaccharide binding site that harbor additional sugar residues or hydropholic groups (Hamelyrck, et al, 1998).

2.4.4 QUATERNARY STRUCTURE

The legume lectins are also interesting from the point of view of protein structure. Despite the conserved structure of the legume lectin subunit, they can adopt a wide range of questioning structures (Manoj et al, 2001) the reason behind this remarkable variability is probably to be found in the interaction with multivalent ligands (Moore et al, 2000).

2.5 BIOLOGICAL FUNCTIONS OF LECTINS

Most lectins do not possess enzymatic activity and not produced naturally by the immune system. Consequently, they are non-enzymatic in action and non-immune in origin (Goldstein et al, 1980). They typically agglutinate certain animal cells and/or precipitate glycol-conjugates. They bind to soluble carbohydrate or to carbohydrate moiety. That is part of glycoprotein or glycolipid (Goldstein et al, 1980).

2.5.1 FUNCTIONS IN ANIMALS

Lectins serve many different biological functions in animals from the regulation of cell adhesion to glycoprotein synthesis and the control of protein level in the blood. They may also bind soluble extracellular and intercellular glycoproteins. Some lectins are found on the surface of mammalian liver cells that specifically recognize galactose residues (Nathan, 2003). They play a role in the biological recognition phenomena involving cells and proteins (Brudner et al, 2013). Not much is known about the functions of lectins in the organisms they are formed.

2.5.2 FUNCTIONS IN PLANTS

Lectins play an important role in the defense mechanism of plant against the attack of microorganisms, pest and insects. The large concentration of lectins in plant seeds decrease with growth, and suggests a role in plant germination and perhaps in the seed's survival itself. Several plants have been found to recognize non-carbohydrate ligands that are primarily hydrophobic in nature, including adenine, auxins, cytonin and indole acetic acid, as well as water-soluble porphyrins. It has been suggested that these interactions may be physiologically relevant, since some of these molecules function as phytohomones (Komath et al, 2006). In legumes, the role of lectins in the recognition of nitrogen-fixing bacteria, Rhiizobium genus, which have sugar-containing substances, he received a special attention. Among the possible functions of plant lectins is their participation in binding nitrogen-fixing bacteria to legume roots. Other functions of lectins in plants may include storage of protein, defense mechanism, cell wall extension, nitogenic stimulation transport of carbohydrates and packaging and/or mobilization of storage materials (Barondes, 1981).

2.5.3 USE IN SCIENCE, MEDICINE AND TECHNOLOGY

Purified lectins are important in a clinical setting because they are used for blood typing (Sharon, 2011).

Some of the glycolipids and glycoprotein on an individual's red blood cells can be identified by lectins e.g

- A lectin from *Dolichos biflorus* is used to identify the H blood group antigen.
- A lectin from *Vicia graminea* is used to identify the N blood antigen.
- A lectin from coconut milk is used to identify Theros antigen.
- A lectin from Dorex is used to identify R antigens.

In neuroscience, the anterograde labeling method is used to trace the efferent axons with PHA-L, a lectin in the kidney bean (Carlson, 2007).

2.6 TOXICITY OF LECTINS

2.6.1 ABSORPTION

Many lectins are relatively resistant to both heating and digestion; many have a high thermal stability at temperature at 70^0C for 30 minutes, and do not completely degrade with cooking. Some are also relatively resistant to stomach acid and proteolytic enzymes (Kilpatric, 2001). Thus, while some lectins are degraded and others pass through the gut, about 1% to 5% absorb into the blood stream of animals, which is efficient to cause immune response.

Furthermore, lectin absorption can be higher if eaten raw, or eaten by individuals efficient in stomach acid, proteolytic enzymes, or secretory lgA antibodies which bind lectins in the gut (Pusztai et al, 1981).

2.6.2 HUMAN REACTIONS

In humans, lectins have been reported to cause damage, including mass food poisoning from raw or undercooked kidney bean and hemolytic anemia and jaundice from Mexican fava beans (Goldstein et al, 1986).

2.6.3 DIGESTIVE DISTRESS

Foods with high concentrations of lectins, such as beans, cereal, grains, seeds, nuts and potatoes, may be harmful when consumed in excess in uncooked or improperly cooked from diverse effects may include nutritional deficiencies, and immune reactions. A recent in vitro study has suggested that the mechanism of lectin damage may occur by interfering with repair of already- damaged epithelial cells (Miyake et al, 2007). Lectins can cause acute gastrointestinal symptoms, including nausea and vomiting. They bind to the luminal surface of absorptive enterocytes in the small intestine. This severally damages the microvilli of the enterocytes, disrupting and absorption lectins can increase intestinal weight and cell number 60-80%, creating gas, fluid and mucus (Pusztai et al, 1981). Lectins can even promote the growth of harmful bacteria in the gut.

2.6.4 PROTEIN MALABSORPTION

Lectins can disrupt protein absorption. In the gut, lectins bind to enterocytes, causing lesions and inflammation, blocking the product of enterokinase, a protein enzyme. This interferes with protein breakdown and with nitrogen absorption in the gut. And it explains why animals on high lectins diets show increased fecal and urinary nitrogen loss, resulting in a negative nitrogen balance, and retardation of long-term growth (Pusztai et al, 1981).

2.6.5 CARBOHYDRATE MALABSORPTION

Lectins can also disrupt carbohydrate absorption and metabolism. Lectins can reduce intestinal glucose uptake by 50% (Freed, 1979). Concanavalin A in jack beans, wheat germ agglutinin, and other lectins can even bind to insulin receptors on cells, disrupting glucose metabolism (Sharon et al, 1986). Finally, because of the high lectin content in grain, it is speculated that lectins cause inflammatory bowel and celiac disease in humans (Nachbar and Oppeneim, 1980). Freed, 1979, found that the gliadin toxin is an isolectin of wheat germ agglutinin.

2.6.6 IMMUNE RESPONSE

Lectins can evoke a variety of immune responses, but they primarily cause type 2 allergies (Pusztai et al, 1981).

2.6.6.1 TYPE 2 ALLERGIES

Lectins primarily evoke 1gG and 1gM antibodies causing Type 2 allergies. Pusztai results showed high titre of circulating lectin specific 1gG antibodies (bit no reaction to other foods), and a direct relationship between the severity of toxic symptoms and the antibody titre (Pusztai et al, 1981). Lectins can cause; fatigue, headache, achiness, diarrhea, nausea, vomiting, irritability, and hemolytic anemia (Breneman, 1984).

2.6.6.2 TYPE 1+ 3 ALLERGIES

Lectins can also cause Type 1 allergies involving 1gE antibodies (Pusztai et al, 1981). And in large quantities they can even induce histamine release from blood basophiles and from mast cells without 1gE intervention (Freed, 1979). Lectins can also combine with complement and neutrophils to form Type 3 immune complexes. This can precipitate in the blood vessels, causing vascular lesion, resulting in thrombosis and hemorrhage (Goldstein et al, 1986) or it can circulate through the blood to the kidney, where it lodges in the glomerular tufts causing inflammation or nephritis (Breneman, 1984).

2.6.6.3 OTHER IMMUNE REACTIONS

Lectins such as concanavalin A in jack beans can bind to T-cells and other cymphocytes triggering cell mitosis (Goldstein and Etzler, 1983). Tomato lectin agglutinates not only erythrocytes, but human lymphocytes and granulocytes (Kilpatric, 2001). Lima beans and other lectins bind to adenine and some cytokinins (Goldstein and Etzler, 1983). Lectins can alter host resistance to infection and to tumor by exhausting the immune system (Nachbar and Oppenheim, 1980).

2.7 HEMAGGLUTINATION

2.7.1 BLOOD TYPES

Blood types are themselves antigens, glycoprotein (or glycolipid) molecules on the surface of red blood cells. They are part of the immune system, and as such are known to react with foreign substance, such as: antibodies, bacteria, virus, parasites, toxins and lectins. There are some two-dozen bloods (ABO, MN, Rh, etc), comprising over 400 blood types (Issitt, 1981).

Blood types chemistry

A = N- acetyl –D- galactosamine

B = D- galactose

O = L- fucose

M = NANA or Sialic acid

N = galactose.

2.7.2 AGGLUTINATION

Agglutination is the sticking together, by serum antibodies called agglutinins, of such microscopic antigenic particles as red blood cells or bacteria so that they form visible clumps (Oxford Medical Dictionary, 2007). Lectins can agglutinate erythrocytes (RBC) and sometimes lymphocytes. Out of 119 known dietary lectins, about half are panhemagglutinins, which bind to any erythrocyte. The remainder are blood-type specific, and will bind to blood types A, B, O, AB, M or N, or subtypes A1 or A2. Later, phagocytes (killer cells, monocytes, or neutrophils) may attach, agglutinating the blood cell; or complement via alternate pathway may bind and lyses the cell (Ritt, 1989). It is then destroyed in the liver. This is classic type 2 immune response. In large numbers, this can cause hemolytic anemia and jaundice (Breneman, 1984).

Consequently, dietary lectins in excess can cause major physiological effect: gastrointestinal damage, type 2 lgG immune response, and hemagglutionn. Findings include 119 lectins: 54 are panhemagglutinins are blood-type specific (Laura Power, 1991).

2.7.3 LECTIN STRUCTURE

The major property of lectin is their specific saccharide-binding sites. Some lectins are composed of subunits with different binding sites. These include the lectin from the red kidney bean, *Phaseolus vulgaris.* It is composed of two different subunits combined into five different forms of noncovalently bound tetramers (Suddath et al, 1986). Since subunits have very different specificities for cell surface receptors, each combination is considered to have a different function. The specific of binding sites of the lectins suggest that there are endogenous saccharide receptors in the tissues from which the lectin is specialized to interact (Nachbar and Oppenhein, 1980).

2.8 IDENTIFYING LECTINS IN VITRO

Blood types specific of lectins is determine by simple in vitro testing, similar to blood typing. Common foods are purchased from several source, this is because different food sample may contain varying amounts of lectins. Foods are individually blended until homogenized, filtered and mixed with saline or NaOH to adjust pH (Nachbar & Oppenheim, 1980). They are then tested against outdated human blood by mixing 1 or 2 drops of each and centrifuging for 30 minutes (Nachbar et al, 1980).

2.9 CRITERIA FOR LECTIN SELECTION

The following are criteria for lectin selectin.

- All should be edible food lectins or medicinal herbs.
- All react with non-enzyme treated human blood.
- Where only the seeds react, foods are included only if the seeds are normally eaten (i.e bananas, group).
- Wheat germ is included under blood type M, because blood type M is sialic acid, and sialic acid is the RBC binding site for wheat germ agglutinin (Gallagher, 1981).

2.10 EFFECT OF PROCESSING ON ANTI-NUTRIENTS LECTINS

Anti-nutritional compounds are removed/destroyed by employing physical and chemical methods such as soaking, cooking, germination, fermentation, enzymatic removal and irradiation.

2.10.1 SOAKING

Soaking and then discarding the soak medium can remove/reduce some of the unwanted components such as lectins, enzyme inhibitors. Soak temperature, medium type, type of food, length of soaking and solubility of the components are the factors affecting the solution increase the permeability of cell membrane increasing the amount of anti-nutrients such as well as some loss of desirable nutrients such as soluble vitamins and proteins (Bressani et al, 1979).

2.10.2 BOILING (COOKING IN BOILING WATER)

Boiling inactivates heat sensitive trpsin and chymotrypsim inhibitors but not completely. Lectins can be deactivated by boiling beans for ten minutes; the ten minutes at boiling point 100^0C (212^0F) are sufficient to degrade the toxin, but not cook the beans. For dry legumes, the U.S Food and Drug Administration (FDA) also recommend initial soak of beans at least 5 hours in water which should then be discarded (FDA, 2009). The beans are cooked at a temperature below boiling (with a preliminary boil), as in a slow cooker, the toxic effect of hemagglutinin is increased. Beans cooked at 80^0C (176^0F) are reported to be up five times as toxic as raw beans (U.S Food and Drug administration, 2009).

2.10.3 DRY HEATING (ROASTING)

A survey of the fresh and processed foods found lectins in about 30% of the food stuffs tested, including each common food as salad, fruits, spices, dry cereals, and roasted nuts. However, dry heat may not completely effect on hemagglutinin activities of certain legumes and activity was still detectable offer 18 hours of heating.

Barber et al (1988); Bressani et al (1970), Carlin and Udebible (1988) have also reported the superiority of most heat over dry heat as a method for processing jack bean seeds. Most heating is often more effective than dry heating and the degree of inactivation depends on temperature duration of heating. Osborn and Mendel (1997) noted that dry heat was less effective than cooking (moist heat) for the improvement of growth promoting action in soybeans.

2.11 INFORMATION ON LEGUMES AND CEREALS

A legume fruit is a simple dry fruit that develops from a simple carpel usually dehisces (opens along a seam) on two sides. Many legumes contain symbiotic bacteria called *Rhizobia* within roof nodules of their roof system. These bacteria have the special ability of fixing nitrogen from atmospheric, molecular nitrogen (N_2) into ammonia (Postgate, 1998).

Farmed legumes can belong to many agricultural classes, including forage, grains, and blooms, pharmaceutical/industrial, fallow/green manure and timber species. Forage legume is of two broad types. Some like alfa, clover, vetch, stylo or Avachis, are sown in pasture and grazed for their seeds, and are also called pulses. Grain legumes include beans, lentils, lupine, peas and peanuts (Kurlovich and repyer, 1995).

2.11.1 SOYBEAN (GLYCINE MAX)

The soybean (US) or soya bean (UK) (*Glycine max*) is a species of legume, native to East Asia, widely grown for its edible bean which has numerous uses the plant is classified as an oilseed rather than a pulse by the UN food and Agricultural Organization (FAO), Fat-free (defatted) soybean meal is a significant and cheap source of protein for animal feeds and many repacked meals. Soybean products such as textured vegetable protein (TVP) are ingredients in many meat and dairy analogues (Riaz, 2006).

Traditional non-fermented food uses of soybean include soymilk and from the lather tofu and tofu skin. The oil is used in many industrial applications (Singh et al, 2006).

The main producers of soy are the United States (35%), Brazil (27%), Argentina (19%) and India (4%) (Singh et al, 2006). The beans contain significant amounts of phytic acid, alpha-linolenic acid, and are flavones. The plant is sometimes referred to as greater bean or yellow bean.

The genus name *Glycine* was originally introduced by Carl Linnaeus (1737) in his first edition of genera *plantanum. Glycine* soya is the wild ancestor of *Glycine max* and grows wild in China, Japan, Korea, Taiwan, and Russia (Singh et al, 2006).

2.11.2 BLACK-EYED PEA

The black-eyed pea or black-eyed bean, a legume is a subspecies of the cowpea grown around the world for its medium-sized, edible bean. The common commercial one is called the California Black-eyed; it is pale coloured with a prominent black spot. The currently accepted botanical name is *Vignia Unuiculata subspungniculatea,* although previously it was classified in the genus *Phaseolus* (Joseph E, 2010).

The first domestication probably occurs in West Africa, but the black-eyed pea is widely grown in many countries in Asia; it was introduced into Southern United States as early as the 17th century in Virginia (Joseph E, 2010). The planting of crops of black-eyed peas was promoted by George Washington carver because, as a legume, it adds nitrogen to the soil and has high nutritional value (Melissa Johnson, 2007).

2.11.3 PEANUT

The peanut or groundnut (*Arachis hypogaea*) is a species in the legume or bean family (*fabaceae*). The peanut was probably first domesticated and cultivated in the valley, of Paraguay. Pods are 3 to 7 cm (1-2 to 2-8 in) long containing 1 to 4 seeds (Michael, 2006).

Peanuts are known by many other local names such as earthnuts, groundnuts, goober peas, monkey nuts, pygmy nuts and pig nuts. Despite its name and appearance, the nut is not a nut, but rather a legume. The domesticated peanut is an amphidiploids or allotertraploid, meaning that it has two sets. Of chromosomes from two different species, thought to be A. *duranesis* and A. *ipaensis.* These likely combined in the wild to form the tetraploid species A. *monticola*, which gave a rise to the domesticated peanut (Seijo et al, 2007).

The domestication of peanut might have taken place in Paraguay or Bolivia, where the wildest strains grow today. Many pre-colonial cultures, such as the moche, depicted peanuts in their art (Berrien et al, 1997).

Lectin in peanut is called “peanut agglutinin”

2.11.4 BEAN

Green bean, also known as string bean or snap bean, in the northern and Western United States, or ejotes in Mexico, are the unripe fruit of various cultivars of the common bean, *Phaseolus vulgaris* (Taylor, 1996). Green beans are of nearly universal distribution. They are marketed canned, frozon, and fresh, beans contain high concern fraction of lectins and may be harmful if consumed in excess in uncooked or improperly cooked form. The flavonol niquelianin can be found in green beans (plumb et al, 1999).

2.11.5 KIDNEY BEAN

The kidney bean is a variety of common beans (*Phaseolus vulgaris*). It is named for its visual resemblance in shape and colour to a kidney. Red kidney beans can be confused with other beans that are not red, such as adzuki beans (Philips and Rix, 1993). Kidney beans are classified into:

- Kidney bean (also known as commonly kidney bean)
- Light speckled kidney bean
- Red speckled kidney bean
- White kidney bean

Kidney beans are more toxic than most other bean varieties if not pre-soaked and subsequently heated to the boiling point for at least 10 minutes (Press L., 2002).

The toxic compound phytohemagglutinin, a lectin is present in many common bean varieties, but is especially concentrated in red kidney beans. White kidney beans contain about a third as musc toxin as red variety (Souci et al, 2008).

Others include;

ACHA (Fonio)

Fonio is the term for cultivated grains in the *Digitaria genus.* This is notable in the part of West Africa in addition to one species in India. The grains are very small. Fonio are one of two cultivars which are white fonio *(Digitaria exilis*)aka Asha and black fonio (*Digitaria iburua*).

Asha *(Digitaria exilis*) also known as hungry rice is the most important of diverse group of wide and domesticated Digitarian species that are cultivated in the savannahs of West Africa. Fonio have the smallest seeds of the species of millet. It has potential to improve nutrition boost food security, foster b rural development and support sustainable use of the land.

Fonio (*Digitaria exilis and Digitaria iburua*) is probably the oldest African cereals instead it was once their major food even though other people have ever heard of it, this crop still remains important in the areas scattered from Cape Verde to Lake Chad. In certain regions of Mali, Burkina Faso, Guinea and Nigeria for instance it is either the staple food of the diet. Each year west African farmers devoted approximately 300,000 hectares to cultivating fonio and the crop supplies food to about 3-4 million people.

Despite its ancient heritage and wide spread importance, knowledge of fonio evolution, origin, distribution and genetic diversity remains scarce even within West Africa. The crop

has a fraction of the attention accorded to sorghum, pearly, millet and a much trifle considering its importance in the rural economy and its potential in increasing food supply

MAIZE (Zea mays)

Maize known in some English-speaking countries as corn is a large grain plant domesticated by indigenous people in Mesoamerica in prehistoric times. The leafy stalk produces ears which contain the grain, which are seeds called kernels. Maize (*Zea mays*) kernels are often used in cooking as a starch.

Most historians believe that corn was domesticated in the Tehuacan valley of Mexico (Lance and Garren Benson 2002). The Omlec and Mayans cultivated it in numerous varieties throughout Meso-America, cooked, ground or processed through Mixtamalozation.

Beginning about 2500BC, the crop spread through much of the Americas (Roney Winter, John Winter, 2009). The region developed a trade network based on surplus and varieties of maize crops. After European contract with the Americas in the 15th and early 16th centuries, explorers and traders carried maize back to Europe and introduced it to other countries. Maize spread to the rest of the world because of its ability to grow in diverse climates. Sugar-rich varieties called sweet corn are usually grown for human consumption, while field corn varieties are used for animal feed and as chemical feed stocks. Maize is the most widely grown grain crop throughout the Americas.

Maize (*Zea mays*) is the most important cereal in the world after wheat and rice with regard to cultivation areas and total production (Purseglove, 1992, Osagre and Eka, 1998). The name maize was derived from the South American Indiana Arawak-Carib word maliz. It is known as Indian corn or corn in America (Purseglove, 1992). It was introduced in Nigeria probably in the 16th century by the Portuguese (Osagie and Eka, 1998).

Maize have some medical uses which include water filtered through charcoal obtained from maize stalk can be used as a treatment to cure Gonorrhea (Abdul Rahamann, 1997). An infusion obtained from stigma of maize inflorescence can be used for treatment of disease of the urinary tract or passage (Abdul Rahamann, 1997).

SORGHUM

Sorghum (*Sorghum bicolor*) is cultivated tropical cereal grass. It is generally, although not universally considered to have first been domesticated in North Africa, possibly in the Nile or Ethiopian region as recently as 1000bc (Kimber C.T., 2000).

The cultivation of sorghum played a crucial role in the Bantu (Black) group of people across Sub-Saharan Africa (Diamond et al., 1998).

Today, sorghum is cultivated across the world in the warmer climatic areas. It is quantitatively world fifth largest most important cereal grain, after wheat, maize, rice and barley. In Africa, sorghum is still largely a subsistence food crop, but it is increasingly forming a foundation of a successful food and beverage industries.

Throughout the Sub-Saharan Africa sorghum is the grain of choice to produce traditional cloudy and opaque (sorghum) beers. The key ingredients of this beer is sorghum malt, which provides hydrolytic enzymes (especially amylase to ferment sugar into ethanol and carbon dioxide), starch (the source of fermentable sugar), yeast nutrient and beer flavor and colour

substances in Southern Africa, malting sorghum for opaque beer brewing has developed into a large scale industry with some 150,000 tonnes of sorghum been commercially malted annually. This figure a small amount of sorghum malted for the production of sorghum malt breakfast cereal "Maltabela''. Sorghum is also malted commercially on a large scale in Nigeria for the production of lager beer and stout and non alcoholic malt based beverages. In the country of Africa where sorghum is malted commercially the respective agricultural department and commercial breeders breed sorghum cultivaters with good malting quality for brewing. The primary quality criterion is their potential to produce malt with high diastatic power (amylase activity) (Taylor Dewar, 2000, Taylor and Dewar 2001).

RICE

Rice is the seed of the plant Oryzae sativa (Asian rice) or Oryzae glaberimma (African rice) as a cereal grain it's the most widely consumed staple food for a large part of the human population, especially in Asia. It is the grain where the second highest worldwide production after corn according to Data for 2010 (FAOSTAT, 2006).

Rice is utilized in many forms, it is mainly used as a food been boiled or steamed and eaten with meat, fish and vegetable (Grist, 1996). Rice production may be derived from rough rice, brown rice, milled rice, cooked rice, dried milled flour, wet milled flour or rice starch (Juliano and Hicks, 1993).

The rice processor is interested in variety, moisture content and freedom from rice, foreign seeds, chalky kernels, soils and insects.

The quality of the rice is related to consumer acceptance which is dependent on the intended use, in 1990 it was estimated that 88.5% of Japanese was used as food, 5.2% for making wine, 4.9% for cakes, 12% for seed and 1.1% for sugar (Grist, 1996). Rice can be used as starch. It is employed as a raw material in European starch industry. In Indian villages, ground rice is steeped into alkaline and centrifuged to give an improved starch.

Rice flour can replace wheat flour for baking or dusting powder in packaging bakery industry, but since it contains no gluten, it is suitable for people on a low gluten free diet but unsuitable for bread making (IRRI, 1997).

The composition of rice differs with the variety, the nature of the soil, environmental condition, and the fertilizer applied (Jules, 2002).

MILLET (*Panicum millaceum*)

Millet is a group of highly variable small seeded widely grown around the world as cereal crops or grains for both human food and fodder. They do not form a taxonomic group but rather a functional or agronomic one. Millet are important crops in a semi-arid tropics of Asia and Africa (especially in India, Nigeria and Niger), 97% of millet production in developing countries (McDonough et al., 2000). Millet were found to have high nutritive value and comparable to that of major cereals such as wheat and rice (Parmeswaran and Sadasvian, 1994). It has also been reported that millet protein are good source of essential amino acid except lysine and threoine but relatively high in metheionine. Millet are also rich sources of phytochemicals and micronutrients (Mal et al., 2010 and Singh et al., 2012).

For example, pearl millet was found significantly rich in starch, soluble and insoluble dietary fibre, minerals and anti-oxidants (Regee et al., 2006). It contains about 92.5% dry matter,

2.1% ash, 2.8% crude fibre, 7.8% crude fat, 13.8% crude protein and 62.2% starch (Ali et al., 2003). Also, foxtail millet protein characterization showed that its protein concentrate is a potential functional food ingredient and the essential amino acid pattern suggests possible use as a supplementary protein source to most cereals because it is rich in lysine (Mohamed et al., 2009).

Finger millet also is known to have several potential health benefit and some of the health benefits are attributed to its Polyphenol contents (Chethan and Malleshe, 2007).

Thus, the presence of all the required nutrients in millets makes them suitable for large scale utilization in the manufacture of food products such as baby foods, snacks foods and dietary food and increasingly more millet products have entered into the daily lives of people, including millet wine, millet nutrition powder from both grain and flour form (Subramanian and Viswanathan, 2007, Liu et al., 2012).

NB: phytohemagglutinin (PHA) actually consist of two closely related protein, called leucoagglutinin (PHA-L) and PHA-E. The letters E and L indicates these proteins agglutinate Erythrocytes and Leukocytes, respectively. Phytohemagglutinin has carbohydrates-binding specificity for a complex oligosaccharide containing galactose, N-acetylglucosamine, and mannose (Dao-Thi et al, 1996).

3.0 MATERIALS AND METHODS

3.1 MATERIALS

The food materials for the work, namely black-eyed pea, soybean, are rice (Oryzea sativa), maize (Zea mays), millet (Panicum millaceum), acha (Digitaria exelis), sorghum (Sorghum bicolour), red kidney bean, peanut and green bean were purchased from Ubani Market, Umuahia, Abia State, Nigeria.

About 3mls of fresh whole blood cells from human blood groups A, B, AB and O were collected from healthy donors at Abia State University Medical centre, Umuahia Location. Abia State, Nigeria.

3.2 EQUIPMENT AND CHEMICALS

High reactive and analytical grade chemicals, reagents and laboratory equipment were obtained from Abia State University Uturu, Abia State, Nigeria.

3.3 SAMPLE PREPARATION

Preparation of legume samples:

About 100g each of the legumes (Black-eyed peas, green beans, peanuts, red kidney beans and soybeans) were coarsely milled using attrition mill to reduce their sizes and enable effective and efficient yield of extracts during soaking. About 25g each of the flour was soaked in 100mls of distilled water, respectively, in conical flask. Gentle shaking and stirring at intervals of 10 minutes were done for 1 hour. The solutions were filtered through 3 layers of muslin cloth.

The filtrate was allowed to stand for 12 hours at room temperature after which the supernatants were decanted, while the residues/sediments were discarded.

One cup each of the legume samples (except peanut) were boiled in $1^1/_2$ litre of distilled water respectively for 30 minutes. The boiled water was allowed to cool. After which it was collected and the bean discarded. Peanut was roasted for 20 minutes, soaked in water, after which the supernatant was collected.

Preparation of cereal samples:

The cereal samples purchased from the market was treated by three different processing operations after division of the samples into two different groups. First group was milled by hammer milling and then soaked in distilled water for three hours, while second group of samples were treated by boiling for 40-80mins.

3.4 PREPARATION OF LECTIN BUFFER

Lectin buffer was prepared, according to the method described by Brooks et al, (1997), as follows. About 6.057g of tris base, 8.70g of sodium chloride, 0.203g of magnesium chloride and 0.11g of calcium chloride were weighed out using Satorius weighing balance. The tris base and salts were mixed in a volumetric flask and dissolved with 100mls of distilled water. Concentrated hydrochloric acid was added to adjust the pH from 9.8 to 7.6. The volume was made up to 1000mls with distilled water.

3.5 PREPARATION OF RED BLOOD CELLS

The whole blood cells were obtained with 5ml syringe, transferred into sterile EDTA bottles. The ethylene diamine tetracetic acid in the bottle served as anti-coagulant. The bottles were gently shaken to ensure thorough mixture of blood with EDTA.

The whole bloods (3mls each) were transferred into test tubes, respectively. About 10mls of lectin buffer (pH 7.6) was added, respectively. The blood sample was centrifuged at 2000rpm for 10 minutes to enable sedimentation and collection of red blood cells at bottom.

The supernatant (plasma and buffer) layer was carefully removed. Another 10mls of lectin buffer was reintroduced into the blood samples and the washing was repeated 3 times to obtain clear supernatant. The supernatant was carefully removed, leaving the red blood cells (RBC).

About 2mls of the washed red blood cells from blood group A, B, AB and O, respectively, were placed in conical flask and diluted with 50mls of lectin buffer to obtain 4% suspension erythrocytes.

3.6 PRECIPITATION OF PROTEINS (CRUDE LECTINS)

Extraction from legumes: The method described by Brooks et al, (1997) was employed in recovery of protein (crude lectin) from supernatants of both fresh and boiled samples. The crude proteins (crude lectins) in the supernatants were precipitated by stirring ammonium sulphate into the liquid to give a 10% w/v solution (i.e 10g of ammonium sulphate was dissolved in 100mls each of the supernatants) of each of the samples (both fresh and boiled), respectively.

The solutions were allowed to stand overnight to give room for complete precipitation. After which the crude protein precipitates (crude lectins) were collected and supernatants were discarded.

The crude lectins extracts were put in test tubes, respectively and about 5ml of lectin buffer was added to each of the test tubes. After vigorous shaking, the crude lectins extracts dissolved in the lectin buffer and lectin solution was obtained for each of the unprocessed and processed samples.

Extraction from cereals: 25g of the milled samples was immersed in $1dm^3$ of distilled water contained in a conical flask. Thorough mixing was done by stirring at interval for about 1hour. The homogenates were filtered through two layers of muslin cloth and the filtrate was allowed to stand for 18 hours at room temperature, after which the supernatant was collected while the settled residue was discarded.

25g of the boiled samples was drained and the drained water was allowed to stand for 18 hours at room temperature.

3.7 ASSAYS FOR LECTIN ACTIVITY

Hemagglutinin activity of the crude lectin extracts were monitored using the method of Meimeth et al, (1982). The agglutination test was carried out by serial dilution of the lectin solutions, respectively, into other test tubes. Thus, about 0.1mg of lectin solutions of each sample was pipette into different test tube. About 0.1ml of lectin buffer was added to each in their various test tubes. This gave dilution of the lectin solutions.

About 10 micro litre of lectin solution from all samples (including diluted) were placed in the wells of the multi-well titre plate, respectively. Then 10 micro litre prepared red blood cells from blood group A were placed on top of all the solutions in multi-well titre plate. The mixture was allowed to interact for 30 minutes. The result was monitored visually.

The same was repeated with other blood groups B, AB, and O respectively. A positive agglutination test was indicated by the formation of a layer of solution over the surface of the well and thin clumps of red blood cells seen. No agglutination was indicated by unreactive resemblance of the control.

3.8 CONTROL ASSAY

The control experiment was set up according to the method described by Brooks et al, (1997).

A negative control consisting of 10 micron litre of lectin buffer placed on 10 micro litre of each of the red blood cells in multi-well titre plates were set up. They were allowed to rest for 30 minutes and then used as control.

4.0 RESULTS AND DISCUSSIONS

4.1 HEMAGGLUTININ ACTIVITY OF CRUDE LECTIN EXTRACT FROM SOYBEAN

Hemagglutinin activities of crude lectin extracts from processed and unprocessed (fresh) soybean are shown in table 4.1.

Table 4.1a: Hemagglutinin Activity of Crude Lectin Extract from Unprocessed Soybean

Blood Groups	a_0	a_1	Control
A^+	+++	++	-
B^+	+++	++	-
AB^+	+++	+++	-
O^+	-	-	-

Table 4.1b: Hemagglutinin Activity of Crude Lectin Extract from Processed (Boiled) Soybean

Blood Groups	a_0	a_1	Control
A^+	-	-	-
B^+	-	-	-
AB^+	-	-	-
O^+	-	-	-

Key:

+++	=	Strong agglutination
++	=	Moderate agglutination
+	=	Slight agglutination
-	=	No agglutination
a_0	=	Concentrated lectin extract
a_1	=	1 fold dilution of lectin extract

Results from Table 4.1a_1, shows that lectin extract from unprocessed soybean has strong hemagglutinin activity on blood group A^+, B^+ and AB^+, but has no affinity for blood group O. However, it shows that lectins in soybean can be classified as "blood type specific lectins". Individuals with blood group O may be less prone to the effect. At 1 fold dilution of the lectin solution, hemagglutinin activity was detected, though moderately with blood group A and B, and still strong with blood group AB.

Table 4.1b shows that boiling (at 100^0C) soybean for about 30 minutes may completely inhibit hemagglutinin activity of lectin extract from soybean. There was no hemagglutinin activity of lectin extract from boiled soybean.

4.2 HEMAGGLUTININ ACTIVITY OF CRUDE LECTIN EXTRACT FROM BLACK-EYED PEA

Hemagglutinin activities of crude lectin extract from black-eyed pea (both unprocessed and processed) are shown in tables 4.2.

Table 4.2a: Hemagglutinin Activity of Crude Lectin Extract from Unprocessed Black-eyed Pea.

Blood Groups	a_0	a_1	Control
A^+	-	-	-
B^+	+++	++	-
AB^+	++	+	-
O^+	-	-	-

Table 4.2b: Hemagglutinin Activity of Crude Lectin Extract from Processed Black-eyed Pea.

Blood Groups	a_0	a_1	Control
A^+	-	-	-
B^+	-	-	-
AB^+	-	-	-
O^+	-	-	-

Key:

+++	=	Strong agglutination
++	=	Moderate agglutination
+	=	Slight agglutination
-	=	No agglutination
a_0	=	Concentrated lectin extract
a_1	=	1 fold dilution of lectin extract

Result from table 4.2a shows blood-type specificity of lectin extract from black-eyed pea. The lectin extract from black-eyed pea did not agglutinate blood group A and O. Hemagglutinin activity was seen to be strong with blood group B and moderate with blood group B and moderate with blood group AB. This shows that lectin extract from black-eyed pea may have no affinity for blood group A and O.

The hemagglutinin activity of lectin extract from unprocessed black-eyed peas, with blood group B and AB shows that individuals with blood group B or AB may be potential victims. Ingestion of uncooked or improperly cooked black-eyed peas pose toxicity to people with blood group B and AB. Individuals with blood group A and O may not be affected.

Result from table 4.2b sows that boiling (at 100^0C) for 30 minutes, may completely inhibit hemagglutinating activity of lectin from black-eyed peas. There was no hemagglutinin

activity seen on all the blood groups when tested with lectin extract from boiled black-eyed peas.

4.3: HEMAGGLUTININ ACTIVITY OF CRUDE LECTIN EXTRACT FROM PEANUT

Hemagglutinin activities of lectin extracts from peanuts (both processed and unprocessed)are shown in table 4.3.

Table 4.3a: Hemagglutinin Activity of lectin Extract from unprocessed peanut

Blood Groups	a_0	a_1	Control
A^+	++	+	-
B^+	+++	++	-
AB^+	++	+	-
O^+	+++	++	-

Table 4.3b: Hemagglutinin Activity of Crude Lectin Extract from Roasted Peanut

Blood Groups	a_0	a_1	Control
A^+	-	-	-
B^+	+	+	-
AB^+	++	+	-
O^+	+++	++	-

Key:

+++	=	Strong agglutination
++	=	Moderate agglutination
+	=	Slight agglutination
-	=	No agglutination
a_0	=	Concentrated lectin extract
a_1	=	1 fold dilution of lectin extract

My Empirical findings are quite amazing. Peanut agglutinin was found to agglutinate all blood groups, though in different degrees. This shows that peanut agglutinin may be classified as “panhemagglutinin lectin” (i.e. lectins that agglutinate all blood types). Result from table 4.3a shows the hemagglutinin activity of lectin extract from unprocessed peanut was found to be strong on blood group B^+ and O^+ and moderate on blood group A^+ and AB^+.

At 1 fold dilution of the lectin extract, hemagglutinin activity was moderate on B and O, and slight on A and AB.

With these findings, it simply means individuals may be potential victim of hemagglutinin activity of peanut agglutinin, when unprocessed or improperly processed peanut is consumed.

Table 4.3b shows that after roasting of peanut for 20 minutes, hemagglutinin activity was still detected. The hemagglutinin activity of crude lectin extract from roasted peanut was slight on B^+, AB^+ and O^+. No agglutination was found on blood group A. At 1 fold dilution of lectin extract from roasted peanut, slight hemagglutinin activity was found on B and O. No agglutination was found on A and AB. This shows that dry heating may be very effective processing method to completely inhibit lectin's hemagglutinin activity.

4.4 HEMAGGLUTININ ACTIVITY OF CRUDE LECTIN EXTRACT FROM RED KIDNEY BEANS

Hemagglutinin activities of crude lectin extract from red kidney beans (both processed and unprocessed) are shown in table 4.4.

Table 4.4a: Hemagglutinin Activity of Lectin Extract from Unprocessed Red Kidney Bean

Blood Groups	a_0	a_1	Control
A^+	++	+	-
B^+	+++	++	-
AB^+	++	++	-
O^+	+++	++	-

Table 4.4b: Hemagglutinin Activity of Lectin Extract from Processed (Boiled) Red Kidney Bean

Blood Groups	a_0	a_1	Control
A^+	-	-	-
B^+	-	-	-
AB^+	-	-	-
O^+	-	-	-

Key:

+++	=	Strong agglutination
++	=	Moderate agglutination
+	=	Slight agglutination
-	=	No agglutination
a_0	=	Concentrated lectin extract
a_1	=	1 fold dilution of lectin extract

From table 4.4a, it can be observed that Red Kidney bean agglutinin is not blood type specific lectin. It is a type of panhemagglutinin lectin, i.e it agglutinates all blood groups. The hemagglutinin activity of red kidney bean agglutinin was found to be more severe (strong) with blood group B^+ and O. Therefore, individuals should be wary of being victimized. Similarly, it was observed that at different concentrations, crude lectin extract from red kidney gave positive reactions with all blood groups (A^+, B^+, AB^+ and O^+).
The good news is that boiling for 30 minutes was found to completely inhibit hemagglutinin activity of crude lectin extract from red kidney beans. Therefore individuals should ensure that red kidney bean is properly cooked before consumption.

Carlini and Udedible (1997) reported that it requires 45 mins of pressure cooking to completely inactivate concanavalin A in *Canavalia ensiform* and establish the fact that the protein was more resistant to heat treatment than trypsin inhibitor.

4.5 HEMAGGLUTININ ACTIVITY OF CRUDE LECTIN EXTRACT FROM GREEN BEAN

Hemagglutinin activities of crude lectin extract from green bean (processed and unprocessed) are shown in tables 4.5.

Table 4.5a: Hemagglutinin Activity of Crude Lectin Extract from Unprocessed Green Bean

Blood Groups	a_0	a_1	Control
A^+	++	+	-
B^+	++	+	-
AB^+	++	+	-
O^+	-	-	-

Table 4.5b: Hemagglutinin Activity of Crude Lectin Extract from Processed (Boiled) Green Bean

Blood Groups	a_0	a_1	Control
A^+	-	-	-
B^+	-	-	-
AB^+	-	-	-
O^+	-	-	-

Key:

+++	=	Strong agglutination
++	=	Moderate agglutination
+	=	Slight agglutination
-	=	No agglutination
a_0	=	Concentrated lectin extract
a_1	=	1 fold dilution of lectin extract

Table 4.5a shows hemagglutinin activity of crude lectin extract from unprocessed green beans. Green bean agglutinin was found to moderately agglutinate blood group A^+, B^+ and AB^+. But has no affinity for blood group O^+. At 1 fold dilution of the lectin extract from green bean, agglutination was slightly on blood group A^+, B^+ and AB^+, yet no agglutination was observed on blood group O^+. This findings shows that green bean agglutinin is an example of blood type specific lectins. This might be due to the presence of green bean agglutinin inhibitor present in blood group O^+. According to Liener et al, (1986), agglutination inhibitors weaken the force (Hydrophobic and hydrophilic forces) believed to be involved in the carbohydrate lectin interaction.

According to Roberts and Goldstein (1984); Kronis and Karver (1985), the carbohydrate-lectin complex is stabilized by intermolecular hydrogen bonds, Van der Waal forces. The destabilization of such forces by inhibitors hinders agglutination. From table 4.5b, it was observed that boiling of green bean (at 100^0C for 30 minutes) completely inactivates hemagglutinin activity of green bean agglutinin.

4.6 CRUDE LECTIN EXTRACT FROM RICE

Table 4.6: Hemagglutinin Activity of Crude Lectin Extract from Rice

(a) Unprocessed

Blood Groups	a_0	a_1	Control
A^+	++	++	-
B^+	+++	++	-
AB^+	++	+	-
O^+	-	-	-

(b) Processed

Blood Groups	a_0	a_1	Control
A^+	-	-	-
B^+	-	-	-
AB^+	-	-	-
O^+	-	-	-

Key:

+++	=	Strong agglutination
++	=	Moderate agglutination
+	=	Slight agglutination
-	=	No agglutination
a_0	=	Concentrated lectin extract
a_1	=	1 fold dilution of lectin extract

The hemagglutanin activity of the crude lectin extract from rice grain is shown in table 4.6 (a). Rice was found to contain strong hemagglutinin activity. Crude lectin extract from the unprocessed rice was specific towards human blood group A^+, B^+, and AB^+ at various levels. But the lectin extract from processed rice showed no agglutination activity with any blood group.

Table 4.7: Hemagglutinin Activity of Crude Lectin Extract from Rice at Various Dilutions and Treatment

(a) Unprocessed

Blood Groups	a_0	a_1	Control
A^+	+	-	-
B^+	+++	++	-
AB^+	++	+	-
O^+	-	-	-

(b) Processed

Blood Groups	a_0	a_1	Control
A^+	+	-	-
B^+	++	+	-
AB^+	+	+	-
O^+	-	-	-

Key:

+++ = Strong agglutination
++ = Moderate agglutination
+ = Slight agglutination
- = No agglutination
a_0 = Concentrated lectin extract
a_1 = 1 fold dilution of lectin extract

Table 4.7 shows the behaviour of lectin extracts from millet grain and human erythrocyte. Negative agglutination was observed with blood group A^+, B^+, AB^+ in all dilutions involving extracts from unprocessed millet.

It was observed according to Kuku et al, (2009) that throughout the process of purification, the lectin activity decreased and the time for blood agglutination got longer, however, as soon as ammonium sulphate is removed via dialysis.

The lectin activity decreased, hence, it can be inferred that ammonium sulphate may inhibit the activity of hemagglutinin activity of lectin.

4.8: CRUDE LECTIN EXTRACT FROM ACHA GRAIN

Table 4.8: Heamagglutinin Activity of Crude Lectin Extract from Acha Grain at Various Dilution and Treatments.

(a) Unprocessed

Blood Groups	a_0	a_1	Control
A^+	++	+	-
B^+	-	-	-
AB^+	+	+	-
O^+	++	++	-

(b) Processed

Blood Groups	a_0	a_1	Control
A^+	++	+	-
B^+	-	-	-
AB^+	+	-	-
O^+	++	+	-

Key:

+++ = Strong agglutination
++ = Moderate agglutination
+ = Slight agglutination
- = No agglutination
a_0 = Concentrated lectin extract
a_1 = 1 fold dilution of lectin extract

Table 4.8 shows the behaviour of lectin extract from Acha grain with human erythrocytes. Agglutination was observed in blood group A^+ and O^+ at moderate levels. Also blood group AB^+ was observed to show agglutination activity but loses activity when diluted with ammonium sulphate. Hence, this also shows that ammonium sulphate can be agglutination inhibitor.

Other method such as dialysis have been used by Maria et al, (2010) to maximize extraction and enhance hemagglutinin activity while trysinization of erythrocyte has been used to modify erythrocytes and enhance lectin activity as well as inhibition, Murray et al (1990), Lis and Sharon (1986), Maricel et al, (2004).

4.9 CRUDE LECTIN EXTRACT FROM SORGHUM GRAIN

Table 4.9 Heamagglutinin Activity of Crude Lectin Extract from Sorghum at Various Dilutions and Treatments

	DILUTION AND TREATMENT				
Blood Group	a_0	a_1	a_2	a_3	control
A^+	+	++	-	+	-
B^+	-	+	+	+	-
AB^+	-	+	-	+	-
O^+	+	-	+	-	-

Key:

+++ = High agglutination
++ = Moderate agglutination
+ = Slight agglutination
- = Negative agglutination
a_0 = Lectin extract from milled and soaked rice grain
a_1 = Lectin extract from boiled rice grain
a_2 = 1 fold dilution of a_0
a_3 = 1 fold dilution of a_1
Control = without erythrocyte

Result on table 4.9 indicates that agglutination was observed from crude extract from sorghum in the blood group A^+, B^+, and AB^+ on dilution a_1 and a_3. Blood group A^+ and O^+ has agglutination activity on dilution a_0 while blood group B^+ and AB^+ losses agglutination activity on dilution a_0. Blood group B^+ has agglutination activity on all dilution but losses activity on dilution a_0, the result shows that then lectin extract in sorghum is affected by temperature not by dilution alone, hence on blood group AB^+ at unprocessed dilution a_1, and a_2. The activity agglutination was present in the blood group AB^+ but losses agglutination at its processed or boiled dilution a_1 and a_2.

5.0 CONCLUSIONS AND RECOMMENDATIONS

The study indicated that crude lectins extracts from red kidney bean, black-eyed pea, soybean, green bean and peanut have varying rate of hemagglutinin activity; green bean, black-eyed pea and soybean agglutinins were found to be blood type specific agglutinins; kidney bean and peanut agglutinins can be described as panhemagglutinins, i.e, they agglutinate all blood groups.

Also, this study indicated that hemagglutinating activity of lectin from cereal towards human erythrocytes was found to be selective to types of blood groups. Cereal consumption also may pose threat to patients with challenge in metabolizing glucose, since its main carbohydrate content is glucose.

Boiling (at 100^0C) for 30 minutes may completely inhibit hemagglutinin activity of lectins from green bean, black-eyed pea, soybean and red kidney bean. However, roasting (dry heating) may not be effective processing technique for inhibiting hemagglutinin activity of lectin extract from roasted peanut. Hence, moist heating is effective than dry heating.

Subsequent researches should be done using erythrocytes from other animals as well as evaluating the effects of other heat treatments, processing methods, storage conditions, level of maturity and other factors on hemagglutinin activity of tropical legumes.

This report will be useful tool in making food choices as well as selecting proper temperature-time regimen for food processing in general, legume processing in particular.

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