KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

FACULTY OF BIOSCIENCES

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

CONSUMPTION PATTERNS, PROTEIN QUALITY AND HAZARDS OF "NULS" UTILIZATION

By

ISAAC WILLIAMS OFOSU

NOVEMBER, 2017

CONSUMPTION PATTERNS, PROTEIN QUALITY AND HAZARDS OF "NULS" UTILIZATION

By

ISAAC WILLIAMS OFOSU

(PG 1892507)

A thesis submitted to the Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, Kumasi, in partial fulfilment of the requirements for the award of

DOCTOR OF PHILOSOPHY IN FOOD SCIENCE AND TECHNOLOGY

NOVEMBER, 2017

DECLARATION

I write to declare that I entirely undertook this study and was duly supervised by Professors; William O. Ellis and Ibok N. Oduro. I also declare that references have been made to portions I cited during the preparation of the thesis. This dissertation is entirely the report of my research activities.

…… ……

Signature Date

Isaac W. Ofosu

…… ……

Signature Date

Prof. William O. Ellis

…… ……

Signature Date

Prof. Ibok N. Oduro

…… ……

Signature Date

Dr. F.D. Wireko-Manu

(Head of Department)

ACKNOWLEDGEMENT

I acknowledge the unimaginable gift of knowledge the Almighty, and all Gracious, Omnipresent God, bestow on mankind. His grace has been abundant for me. Indeed, from Him, all knowledge and understanding flow, and to Him, all wisdom and power go forth!

I wish to acknowledge the immense contributions of my supervisors, Prof. William O. Ellis and Prof. Ibok N. Oduro. I especially want to thank you for your patience, motivation and guidance that drove me on, in spite of all the challenges I faced. I want to also specially thank Prof. Kwabena Nsiah. I am grateful for your immense assistance in shaping manuscripts derived from this study for publication.

I wish to thank my students, especially Michelle Oppong Siaw and you, William Ofori Appaw and the entire laboratory staff. Special thanks goes to you, Pastor Randy Nii Amoo Addy. My sincerest thanks goes to you, Dr. (Mrs.) Gloria M. Ankar-Brewoo, my office mate and a close friend and also to you, Dr. Herman E. Lutterodt and Dr. Emmanuel de-Graft Johnson Owusu-Ansah. You have provided a solid support and a fountain of encouragement throughout my study. I also wish to thank all my colleagues most sincerely, in all the diverse ways you advised and prayed with me.

Last, but not the least, I would like to thank my family, especially my daughter; Christa and my wife; Vida Ofosu. You were never tired of listening to the regular briefings on the progress of my study. I also wish to express my heartfelt thanks to my elder brother, Bright Adofo Dede, and my cousin, Benneth Ofosu Ameyaw. Above all, I wish to thank my elder sister, Esther Owiredua. You continue to fill the vacuum left by our departed mother to provide the motherly love, care and prayers.

God bless you all!

DEDICATION

To the Memory of Three Great Women in My Life who I wished had lived to witness my graduation,
But passed on as I laboured in my studies;

My Grandmother:
Comfort Adwoa Ofosuah,

My Mother:
Nancy Afua Mirekuah,

And
My younger Sister:
Cynthia Abena Ntirirwaah.

May you find peace, wherever you are!

ABSTRACT

Many people consume large quantities of plant-based products, including neglected and underutilized legumes (NULs), because of their availability or affordability. This makes NULs a critical food security resource. However, NULs are known to contain toxins such as lectins (glycoproteins) which persistently resist heat inactivation, making them a potentially serious health hazard. In order to promote their continuous and sustainable utilization, consumption profile, safety and nutritional quality by way of essential amino acids need to be investigated. Furthermore, suitable ways of attenuating the possible risks of lectins in NULs need to be investigated. A survey was carried out aimed at finding the processing and consumption patterns of five selected NULs; *Vigna subterranea, Cajanus cajan, Mucuna pruriens, Phaseolus lunatus* and *Canavalia ensiformis* focusing on consumer characteristics such as: age, house hold numbers, educational levels, marital status and occupation. Also considered were, the familiarity of NULs, perception of hazards and the risks, in terms of exposure assessment and exposure frequency of dishes consumed per body weight of consumers. Time-heat inactivation of the lectins in NULs flours, as well as their NULs' model dishes were studied, from which risk was evaluated, using the hazard-based approach. Five different NULs protein extracts were profiled to ascertain the essential amino acids, and the quality evaluated by matching them against the standard FAO/WHO dietary indispensable amino acids for three age groups; infants, children and adults. γ–Radiated starches prepared using radiation doses from 3 to 42 kGy were composited at 10% composition with defatted NULs flours, with respect to quantities of native starches present in NULs. The composites were agitated in an extruder which operated within intrinsic temperature ranging from 2 to 12 ^{o}C. Residual lectins from the extrudates were quantified by ELISA analysis. The statistical analysis of the collected survey data involved the use of Palisade @Risk software to fit each measured parameter to the most adequate probabilistic distribution, based on its Akaike information criterion. Subsequently, the central tendency characteristics of the measured parameters, together with their variations, uncertainties and distribution functions were recorded. In the survey of the consumption of NULs, it was observed that, majority of the respondents who were over 40 years (67.6%) were familiar (59.4%) with NULs and consumed NULs dishes. The most popular dishes were prepared from *Vigna subterranea* (15.9%) and *Phaseolus lunatus* (14.95%). The majority of consumers (66%), perceived the presence of hazards in NULs, citing pesticide residues (58.7%), but no intrinsic hazards as threat. However, a few (16.6%)

considered the interaction between pesticide residues and food additives as dangerous. Majority (66.1%) regarded NULs dishes as safe, while at the same time complained of stomach discomfort (97.2%) after consumption. In spite of these observations, they would still recommend their use to others. Through the ELISA analysis, significantly high quantities of lectins still remained in *Vigna subterranea* flours even after cooking for 1 h. Risk analysis of the modelled foods revealed that the hazard quotient (HQ) of all the NULs dishes were above 1, meaning consumers are at risk of systemic toxicity. Assessment of essential amino acids revealed that, out of the five NULs studied, *Vigna subterranea* had adequate quantities of histidine (26.2 mg/g) that met the dietary requirements of all the three age groups. However, the levels of isoleucine (35.9 mg/g) and the aromatic amino acids phenylalanine and tyrosine (94 mg/g) were adequate for only children and adults. In the case of *Phaseolus lunatus*, lysine (53.6 mg/g) and threonine (44 mg/g) were relatively high. However, this could only meet the adults' requirement. In spite of these contributions of amino acids, protein quality of *Vigna subterranea* and *Phaseolus lunatus* proteins, based on digested indispensable amino acid scores (DIAAS) presented a different outlook. Thus, the DIAAS of *Vigna subterranea* (3.6) and *Phaseolus lunatus* (2.5), show low quality, relative to international standards. In the studies involving the attenuation of lectins in NULs, Mamdani type fuzzy logic inference system was used to model the two input variables of the NULs flour treatment to predict the optimal lectin inactivation at 100% accuracy. While lectins from *Canavalia ensiformis* recorded 83% inactivation, lectins from *Vigna subterranea* rather potentiated up to 44%. Thus, γ-radiated starches incorporation into NULs flours during low temperature extrusion treatment, inactivated NULs lectins at varying degrees and these were adequately predicted by the model.

CONTRIBUTION TO KNOWLEDGE

1. Ofosu, I.W., Ellis, W.O., Nsiah, K. and Oduro, I.N. (2017): Exposure assessment, habitual cooking and eating habits and consumers' characteristics. *Journal of Food Security, 5* (5):169-175

2. Ofosu, I.W., Ellis, W., Nsiah, K. and Oduro, I.N. (2017): Neglected and underutilized legumes (NULs) hazards and probabilistic risks associated with some selected dietary lectins. *Journal of Food Security, 5* (6):212-222.

3. Ofosu, I.W., Ellis, W.O., Nsiah, K. and Oduro, I.N. (2017): Essential amino acid quality profile in neglected and underutilized legumes (NULs). *Journal of Food Science and Quality Management, 68*:16-22

4. Ofosu, I.W. (2017): Fuzzy modelling γ-radiated starches as inactivating agents of NULs lectins, *Macromolecules: An Indian Journal, 12* (3):109-126

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: GENERAL INTRODUCTION

1.1. Background

In order to survive and live successfully, man is endowed with biodiversity made up of food, feed and other resources such as medicine in the form of herbal products (UNEP, 1995). For several decades, activities of man have scaled down this biodiversity resulting in the reduction or shrinkage of the food baskets. Consequently, consumers in developing countries including rural dwellers and the urban poor people are believed to be affected by hidden hunger (Padulosi *et al.*, 2000). There is a gradual acceptance of otherwise underutilized species for food and feed as a result of the outcomes of several research activities by institutions. While the opening up of consumers to underutilized species is gradually increasing, the processing difficulties of these species have still not been fully addressed (Padulosi *et al.*, 2011a).

Studies have shown how underutilized species including neglected and underutilized legumes (NULs) can be processed to remove the hazards in them before consumption (El-Moneim *et al.*, 2000; Fasoyiro *et al.*, 2010; Granito *et al.*, 2010). Indeed, many different diets have been prepared from NULs and positioned for industrial take-off from such research activities. Underutilized species, and in particular NULs, are known to tolerate harsh environmental conditions and are also suitably adapted as food security crop to fight poverty and hunger (Padulosi *et al.*, 2011a). Yet, important characteristic components of their foods, such as the amino acid profile have been argued not to be adequate, compared to other sources of proteins such as soybean or meat (Gebrelibanos *et al.*, 2013). While NULs use intrinsic secondary metabolites as survival adaptive chemicals to live successfully in their natural wild habitats, they tend to cause harm when human beings and farm animals consume them. Indeed, numerous hazards have been identified including compounds such as non-protein amino acids; L-3,4-

dihydroxyphenylalanine **(**L-DOPA) in *Mucuna pruriens* (Bressani, 2002) β-N-Oxalyl-l-α,β-diaminopropionic acid (β-ODAP) and β-L-glutamyl aminopropionitrile (β-BAPN) in grass pea (Fikre *et al*., 2008). Others include canaline (Rosenthal, 1992), canavanine, and canatoxin in *Canavalia ensiformis* (Sridhar and Seena, 2006). While research is currently ongoing to make the consumption of NULs safer, it should be a matter of urgency to direct some attention to risk assessment of legume consumption as a result of the impact of these hazardous substances. Thus, the risk assessment and their interventionary cooking protocols must be carried out side by side.

The main criteria for classifying some legumes as NULs stems from lack of marketing, conservation and general lack of research (Azam-Ali, 2010; Padulosi *et al*., 2000). However, some NULs are now receiving research attention and these include *Phaseolus lunatus*, *Mucuna pruriens*, *Vigna subterranea*, *Canavalia ensiformis* and *Cajanus cajan* among others. Some of their amino acids and hazard profiles compared to frequently used legumes or beans such as cowpea and soybean are not well documented. Rural dwellers and generally the urban poor continue to use NULs in a variety of foods such as in the preparation of soups where *Phaseolus lunatus*, *Mucuna pruriens* and *Canavalia ensiformis* are generally used as thickeners (Osei-Bonsu *et al*., 1995) and also in the preparation of "*Koose*" and "*Tubani*" where cowpea and/or Bambara groundnut are usually used (Plahar *et al*., 2002).

The potential exists and many researchers have indeed worked towards the positioning of these relatively cheap protein resources for industrial applications (Ghadge *et al*., 2008). A number of processing methods such as fermentation, pressure cooking, commercial canning, and legume flour production have been used to process them (Subuola *et al*., 2012). While these NULs remain a major food resource for a section of the population, their adverse impact is

worth monitoring. Apart from lectin and phytate, NULs are also known to have large quantities of goitrogens, protease inhibitors and estrogens (Subuola *et al*., 2012). Many low-income families continue to make a living in cultivating and consuming these NULs. One need not imagine how difficult it will be to restrain the low socio-economic class from using these NULs as their food source. Thus, steps must be taken to make their processing and consumption safer; since indeed the large populations who consume these may be at a potential risk.

Lectins are proteins commonly present in some NULs and they are known to bind to specific complex carbohydrates. Plants use this mechanism as adaptive feature, but when man feeds on them problems arise. Several reports exist that show that at certain concentrations, lectins withstand thermal treatment and even digestive actions. Lectins are hazards and tend to have adverse health effect when used as food or feed (Greer and Pusztai, 2009; Makkar *et al*., 2007; Marzo *et al*., 2002; Ryder *et al*., 1994). Lectins are believed to be the main cause of leaky gut (Pusztai *et al*., 1979), perhaps leading to many of the diseases afflicting humans. In spite of these problems, enough weight-of-evidence has not been built by the food systems risk community towards the hazardous nature of lectins, thus, warranting its risk assessment. The communication of risk associated with the consumption of lectins has not been effective. It appears many people including scientists, believe that once lectins are proteins they would denature completely when cooked. However, a number of studies have also shown that many lectins persist even after cooking and in some cases, they even potentiate in so many of the foods ingested (Lawley *et al*., 2008). Perhaps among the several hazards present in NULs, it is probably, lectins that have not been studied with the view to estimating consumer exposure and the adverse health impact.

By nature, lectins are able to recognize and bind to sugars. One advantage of the binding in plants is to strategically offer defense against fungal attack (Broekaert *et al.*, 1989). Thus, advantage can be taken of such a mechanism to inactivate lectins at low temperatures, using naturally occurring complex carbohydrates or some sugar derivatives. Common examples of such carbohydrates include N-Acetyl-D-glucosamine (NADG) and chitin. Some other carbohydrates are believed to have the capacity to bind to lectins but these have hardly been studied (Timoshenko *et al.*, 1995).

Apart from using simple heating methods, less research attention has gone in the direction of inactivating lectins before consumption. The continuous use of high temperatures to inactivate lectins from food can pose serious problems since high temperatures decompose amino acid into hazardous compounds such as methyl imidazolequinolines (Hughes and Arnett, 1983). Some studies have shown that temperatures of up to 170 °C is capable of inactivating lectins (Pedrosa *et al.*, 2012). Other studies, however, show that potentiation of lectins rather occur at ordinary cooking temperatures (Lawley *et al.*, 2008). These alternating views do not bring clarity in defining an efficient and precise method in the thermal processing of NULs with the goal of inactivating lectins. In order to preserve amino acids, low temperatures must be used during thermal processing of legumes. Alternatively, natural or derived carbohydrates can be employed as binders to achieve the goal of inactivating lectins in foodstuffs.

1.2. Problem statement and justification

A large number of low income earners continue to use legume grains as part of their food baskets. More particularly, rural dwellers and the urban poor use these legumes to a very large extent, while preferring to sell their livestock to raise money for their families. NULs and

legumes in general have certain advantages with respect to the provision of nutrients for the improvement of health. However, these also contain some hazardous substances such as lectins, saponins and insecticides which have been observed to have adverse effects such as; "leaky gut" (Pusztai *et al.*, 1990), cell apoptosis (Podolak *et al.*, 2010) and pancreatic cancers (Roebuck *et al.*, 1985) in test animals. Though every effort is being made by policy makers to improve productivity of some legumes such as soybean, a large number of farmers continue to make the cultivation of NULs their major livelihood (Weinhold *et al.*, 2011). The cultivation of these beans requires empirical information as to the extent of adoption and cultivation by the farmers. It is often difficult for the rural farmers to keep up with the production of these exotic beans probably due to the high cost involved, compared to other NULs. Some studies have suggested the potential role of NULs as future crops particularly in water-stressed regions (Chivenge *et al.*, 2015). Therefore, research attention must be directed to NULs, to improve the cultivation and sustainable consumption, while maintaining the livelihood of the subsistent farmer and also supporting the climate change agenda.

It is important to note that exotic legumes such as cowpea and soybean also contain hazardous anti-nutrients that are also present in NULs. However, soybeans are given more attention and its cultivation encouraged because of its quality amino acid profile. While this is a laudable idea, formulation models can also be developed that could composite various NULs to enhance the protein quality as that of soybean. Thus, dual advantage can be taken of the adaptive cultivation of NULs, to produce protein sources from NULs, promote some economic returns for farmers and also to provide further alternatives in tackling the problem of climate change (Padulosi *et al.*, 2011b). For a wider acceptability and utilization of the NULs, greater attention should be given to the study of NULs, especially with respect to processing

protocols to address potential hazards such as lectins. There could also be the added value of the impact of NULs on the farmer's house hold and the rural communities. The outcomes would help feed into the policy relating to the potential uses of NULs.

1.3. General objective

This project was designed to study the consumption and processing profile of NULs, their protein quality, hazards, the risks of lectins ingestion and their attenuation.

1.3.1. Specific objectives

1. To determine some characteristics of consumers of NULs; their demographic and anthropometric features.
2. To evaluate the familiarity, consumption and hazards of NULs and determined the risks associated with lectin ingestion.
3. To determine the essential amino acid quality of some selected NULs.
4. To determine the effect of β-starches on lectin inactivation during low temperature extrusion cooking.

CHAPTER 2: LITERATURE REVIEW

2.1. Neglected and underutilized legumes (NULs)

The population of the world keeps increasing and many people face food insecurity because food supply and access are not matching demand. There is therefore a continuous search for other complimentary food resources. Some of these include underutilized legumes. Leguminous seeds hold such great promise and has the potential to supply cheaper and more affordable alternative protein materials (Chivenge *et al.*, 2015). To ensure food sovereignty, many ethnic groups across the globe usually engage in the cultivation of specific legumes that they use to support their food resource. However, these legumes have one problem or the other associated with their consumption. Alongside their cultivation, researchers continue to find ways of making their consumptions sustainably safer.

The International Plant Genetic Resources Institute (IPGRI, 2002) defined NULs as those 'legumes that have the potential to support food or feed resource and subsequently deliver economic benefits, as well as nutritional support for the growing masses but they are hardly considered by research'. Among such legumes are *Canavalia ensiformis* (Jack bean), *Vigna subterranea* (Bambara groundnut), *Mucuna pruriens* (Velvet beans), *Phaseolus lunatus* (Lima beans) and *Cajanus cajan* (Pigeon pea)(Padulosi *et al.*, 2013; Padulosi *et al.*, 2011a; Williams and Haq, 2002).

A review on *Canavalia ensiformis* suggests that *Canavalia gladiata* originated from Asia while *Canavalia ensiformis* originated from South America and spread around the world (Sridhar and Seena, 2006). *Canavalia maritima* is widely distributed in the USA, particularly towards the Southern Florida, whereas *Canavalia cathatica*, (ancestral form of *Canavalia gladiata*) is also

distributed throughout tropical Asia and Africa (Purseglove, 1974). *Mucuna pruriens* has also been widely investigated and it is believed that many varieties are available (Lampariello *et al*., 2012; Nwankwoala and Georgewill, 2010). *Phaseolus lunatus*, which is popularly consumed is known to have toxic agents such as cyanogenic glycosides (Nartey, 1980). There are however, other popular species such as *Phaseolus vulgaris* which is known to contain almost no cyanogenic glycosides (Ballhorn *et al.*, 2005). *Vigna subterranea* is another bean used as food and feed and believed to have originated from West Africa (Nwaichi and Ayalogu, 2010). Indeed, some authors have attested to its potential cost-effectiveness and its environmentally sustainable nature (Hillocks *et al*., 2012). Even though the origin of *Cajanus cajan* is disputed it is believed to have been introduced into East Africa from India (Odeny, 2007). The production of *Cajanus cajan* is worldwide, however, on the African continent, there is vast cultivation in East and West Africa (Aiyeloja and Bello, 2006; Boehringer and Caldwell, 1989; Kamanga *et al*., 2003).

2.1.1. NULs utilization as food and feed

The use of *Mucuna pruriens* is widespread. It has been reported that some South Asian and Oceanic people consume the boiled seeds (Rajaram and Janardhanan, 1991). Its use is also very popular within Nigeria but it is concentrated in the North-Eastern zone (Onweluzo and Eilittä, 2003). However, exploratory work shows a decline in consumption in the South Eastern parts of Nigeria. The decline has been attributed to the increasing exotic use of soybeans (Akaninwor and Okechukwu, 2006). Studies have shown that majority of Nigerians who eat *Mucuna pruriens* rather preferred "*Egusi*" melon which is even more expensive, but was considered tastier than *Mucuna pruriens* (Agrawal and Ebrahim, 2013). The reasons have been particularly attributed to the taste, appearance, edibility, aroma as well as the ease of

processing. However, those who consumed the white-seeded *Mucuna pruriens* observed that the black variety was toxic.

In Ghana, *Mucuna pruriens* is usually used as a paste to thicken soup and stews (Osei-Bonsu *et al*., 1995) particularly, in the forest regions among the villages in Offinso and Goaso. According to Onweluzo and Eilittä (2003) among some of the uses of *Mucuna pruriens* in Nigeria include; flour which is used in preparing sauce called "*Akpokoji*" or "*Nkashi*" or "*Una*". Some also use it as a gel called "*Okpa*", while others use it as a roasted snack. In some areas, it is even used in the preparation of porridge and fried cake popularly called "*Akara*", while others use it as pudding, popularly called "*Moimoi*".

It has been reported (Osei-Bonsu *et al*., 1995) that *Canavalia ensiformis* beans are usually boiled with chilies and onions, usually after the seed coat and water used in boiling them have been discarded. The seeds were then ground together with other ingredients into a fine paste and subsequently, flavored with salt, heated with red palm oil and eaten with yam, plantain or cocoyam. The report further shows that both *Canavalia ensiformis* and *Phaseolus lunatus* were sometimes ground with chilies and eggplant or cocoyam leaves and used to prepare soups containing either meat or fish and eaten with "*Fufu*".

Vigna subterranea is mainly used for human consumption. The seeds are consumed either in the immature state or when fully ripe and dry (Plahar, 2000; Swanevelder, 1998). In South America, the immature seeds are consumed fresh, grilled or boiled as a complete meal while sometimes, it is mixed with groundnut or maize. In some cases, the dried seeds are ground into flour and boiled to a stiff porridge. The seeds may also be further processed into roasted beans and usually broken into pieces, boiled and eaten like porridge. In Angola and

Mozambique, the seeds are boiled, salted and served in restaurants as appetizers (Swanevelder, 1998). In Ghana, a popular dish, "*Tubani*" is prepared by milling the dry seeds into flour, mixing with water, salt and saltpetre into paste, moulded in leaves or polythene pouches and steamed or boiled in water. The resulting dish is served with a sauce of grounded chilies and onions fried in oil (Akpalu *et al.*, 2013; Plahar, 2002). A report has also been made that up to about 17 % *Vigna subterranea* flour is often used as a substitute to cowpea for the preparation of "*Koose*" (Akpalu *et al.*, 2013), another delicacy in the Northern Regions of Ghana. In the Volta region of Ghana, the dried beans are usually boiled to tenderize, sweetened with sugar and served. The servings are either with fried ripe plantain while in some areas it is rather salted and served with "*Gari*" (Doku, 1996).

The main dish called "*Ase*", is prepared from *Cajanus cajan* by simply boiling the seeds until tender, normally in seasoned palm-nut soup. It may also be served with seasoned palm oil and eaten with fried ripe plantain and/or gari (Personal communication). In Nigeria, for instance, the cooked and tender beans are mixed with cocoyam or sometimes maize and either dried cocoyam grits or freshly cooked cocoyam (Enwere, 1998). In Eastern Africa, a popular dish called "*Dhal",* is prepared by cooking *Cajanus cajan* seeds until tender, seasoned with palm oil and mixed with vegetables before consumption (Harinder *et al.*, 1999). It is also ground into flour and baked into products such as bread, cookies and "*Chapattis*".

2.1.2. Processing of NULs

Due to the underutilization of these materials, several attempts have been made to improve their food utilization due to the increasing demand of plant proteins. To this end, several industrial applications have engage in studies to achieve faster cooking times and the improvement of their textural qualities. Various protocols involving the processing of *Cajanus cajan* seeds

into flour with varying sensory characteristics have been reported (Nakitto *et al*., 2015). Among the processing methods included soaking the beans in warm water for 30 min, followed by oven drying at 50˚C for 8 h and milling through 1 mm mesh size sieve, into powder. After dehulling and winnowing, the roasted seeds were often processed into "*Akara*" and "*Moimoi*" and evaluated against cowpea flour. A similar report has been made on *Cajanus cajan* seed flour (Ghadge *et al*., 2008) but a more elaborate processing protocol was suggested with reduced cooking time of 14 min. In this protocol, the peas are pressure-cooked for 20 min, cooled and frozen to -20˚C for about 3 h and dried out in cabinet dryer (65 ˚C, 3½ h).

A two-step processing of *Mucuna pruriens* beans has also been reported (Egounlety, 2003). This involves preparation of the beans and production of the bean food using basic fermentation protocols. The preparation of the *Mucuna pruriens* beans, involved boiling of the seeds for 45 min in a 1:6 bean:water ratio. The dehulled beans were then chopped, soaked for 12 h and subsequently re-boiled for 45 min. For *Mucuna pruriens* bean "*Tempe*" and also for *Mucuna pruriens* weaning food, the seeds are treated as before, but it is soaked twice after re-boiling in 1:3 bean:water ratio, drained and recooked at 1:6 bean:water ratio for 45 min. While the condiment preparation involved fermentation with *Bacillus subtilis*. for 48 h, the preparation of "*Tempe*" however involved the inoculation of the fungus *Rhizopus oligosporus* for 48 h at 0.4 g/kg. For the weaning food, an appropriate mixture of maize and *Mucuna pruriens* bean was made. The maize is usually soaked for up to 3 days, milled, sieved using a 1mm mesh size sieve and fermented for 48 h using lactic acid bacteria. While the source of the lactic acid bacteria was from the maize itself, co-fermentation involved either maize: *Mucuna pruriens* bean (50:50) or maize: *Mucuna pruriens* bean: melon (50:40:10). *Mucuna*

pruriens bean is also known to be rich in non-protein amino acid, L-3,4-dihydroxyphenylalanine (L-DOPA). However, it is reported that such treatments reduce the levels drastically to below 0.1%, possibly as a result of L-DOPA degrading enzyme activity (Egounlety, 2003).

Processing of *Canavalia ensiformis* have been done using processes such as soaking, thermal processing and fermentation to improve the quality of the beans as feed material (Udedibie *et al.*, 1994). Pressure cooking and lactic acid fermentation of *Canavalia ensiformis* have also been reported (Carlini and Udedibie, 1997; Sandberg, 2002).

The production of milk from *Vigna subterranean* has been reported (Murevanhema and Jideani, 2013). The resulting flour of dried *Vigna subteranea* seeds have been used in composite flour for cereal-based confectioneries such as biscuits or cakes (Addo and Oyeleke, 1986). Extrusion cooking has also been reported for use in processing bean (Okafor *et al.*, 2014). Research has also shown that protein isolates from seeds of *Vigna subterranea* proteins could be used as the sole ingredient in extruded meat analogs. Commercial canning of *Vigna subterranea* seeds in gravy has been reported (Swanevelder, 1998). A technical report presented by the Food Research Institute of Ghana (Plahar, 2000) which show tremendous contributions towards the preparation of three *Vigna subterranea* seed flours using three protocols. Raw *Vigna subterranea* seed flour was simply produced through milling after cleaning and sorting. While roasted *Vigna subterranea* seed flour was prepared by roasting and milling, the improved *Vigna subterranea* seed flour had to be boiled for 25 min followed by drying between 60 and 75 ^{O}C, and winnowing. Dishes such as *"Akla"* and "*Tubani*" have been prepared from these flours. It was suggested that the method developed for the

production of the improved *Vigna subterranea* seed flour enhanced the cooking time and utilization of the product. It has been suggested (Plahar, 2000) that the use of Chile saltpetre for the pre-soaking and cooking was an effective means of reducing cooking time for most of the hard–to-cook varieties of *Vigna subterranea* seeds.

Phaseolus lunatus has also been worked on with respect to its potential industrial application. Quite recently, a protocol was developed using a solid-state bioconversion method in producing the bean functional flour (Guzmán-Uriarte *et al.*, 2013). In this method, while the bean seeds were soaked at 25 ^{o}C for 16 h in acetic acid solution of pH 3.0, the dehulled beans were rather soaked in acetic acid at 90 ^{o}C for 30 min and cooled to 25 ^{o}C for 3 h and inoculated with *Rhizopus oligosporus*. After fermentation (108 h) and drying (50 ^{o}C for 8 h) the dehulled beans and seed coats were separately milled to 0.18 mm screen and blended. Key responses, such as total phenolics, antioxidant activity, anti-hypertensive activity, water absorption index and dispersability, pointed to an enhanced product. The studies further recommended the product as a preferable alternative to nutraceutical ingredient for the control of diseases such as hypertension and oxidative stresses.

Phaseolus lunatus continues to receive research attention in the area of pasting studies for its potential for the production of noodles. For instance, the thermal properties of these beans have been studied and its applications in the manufacture of noodles tested (Wang *et al.*, 2013). Studies of the physicochemical properties of the bean starch centered on the starch granules morphology, size distribution and their relative crystallinity. It was obvious from the studies that the amylose: amylopectin ratio, as well as the molecular weight of starch had profound impact on the pasting properties, a key attribute in the manufacture of noodles. The authors indicated an increasing popularity of the product among health-conscious consumers.

Thus, there is the full market potential for some of these underutilized legumes as dry edible bean products.

A report of low glycemic index and high fiber content of the *Phaseolus lunatus* has been made (Ramírez-Jiménez *et al.*, 2014). Together with the presence of resistant starches and low oil absorption capacity, it makes the *Phaseolus lunatus* flour, an alternative for low calorie foods.

2.2. Protein content of NULs

Several NULs lack one essential amino acid or the other; thus, they can be composited to improve quality (Sparvoli *et al.*, 2016). These composites may be comparable or even sometimes surpass the quality in terms of some amino acids relative to key legumes such as cowpea or soybean. Increasingly, there is a gradual drift of consumers from the consumption of meat to a plant-based diet (Tuso *et al.*, 2013). Thus, there is increasing research attention towards the use of alternative protein resources relative to meat, to meet consumer demands. Underutilized legumes present a huge opportunity for the ready-to-eat products, as they serve as a source for a variety of proteins that can readily be made available for consumption.

Studies have indicated that *Phaseolus lunatus* could contribute substantially towards the search for alternative protein resource. This is due to the fact that the chemical score for essential amino acids was high in moderate acid medium. However, the quantities reduced considerably in a basic medium, though palatability was enhanced (Nestares *et al.*, 2001). In another study, *Phaseolus lunatus* cultivars such as "Guateian 6662" and "Rheotibag" were found to contain high crude protein, iron and zinc contents (Prolla *et al.*, 2010).

Investigation of the impact of processing techniques on the nutritional (total protein) and anti-nutritional composition of *Canavalia ensiformis* such as L-DOPA, lectin, anti-trypsin activity, polyphenol content, tannins and free radicals have been made (Doss *et al.*, 2011). The total crude protein content of *Canavalia ensiformis* bean was reported as 29.8 g/kg dry matter. Similar values of total proteins have been reported in *Mucuna pruriens*, as 24.9 g/kg dry matter and in *Canavalia gladiata* as 29.3 g/kg dry matter (Udedibie and Carlini, 1998; Siddhuraju and Becker, 2001).

In furtherance of the study on nutritional profile of *Canavalia ensiformis*, studies have been made on the impact of geographical distribution on some physical parameters, proximate protein and mineral compositions (Doss *et al.*, 2011). It was found that the physical characteristics of the bean seeds, such as seed weight, length and width of five different accessions were different. These accessions were found to present crude protein content of between 29 and 32%. Protein fractionation studies revealed that globulin formed the major fraction of between 14.9 to 16.5 g/100 g flour, whereas albumin content was between 4.0 to 5.9 g/100 g seed flour.

A study by Linnemann and de Bruijn (1987) showed that *Vigna subterranea* seeds contained 19% protein and 6.5% oil and a very high carbohydrate fraction of 63%. It has also been reported (Okafor *et al.*, 2014) that processing methods had an impact on the qualities of *Vigna subterranea* seed flours in relation to their acceptability in extruded products. In their work, they compared the germination of the bean samples prior to drying, while in the other cases, they were simply cleaned, roasted and milled. They concluded that the samples that were roasted before extrusion yielded extruded snacks with improved taste and general

acceptability, compared to the other treatments. In addition, the other treatments such as germination and roasting of beans presented fairly balanced nutritional and antinutritional qualities after extrusion.

Fermentation has been used to fairly improve the protein content of *Cajanus cajan* seeds as fermentation time increased (Adebowale and Maliki, 2011). The proximate composition data presented in the study is a clear evidence of increased total crude protein content from 21.8 to 23.9%, probably resulting from the solid state fermentation. The protein contents of *Cajanus cajan* have been reported by various studies to range between 18 to 30%, depending on the variety (Reddy *et al.*, 1979; Swaminathan and Jain, 1975). Studies have shown that some researchers developed varieties of *Cajanus cajan* seeds that had above 30% protein content with significant sulphur containing amino acids (Singh *et al.*, 1990). These varieties of *Cajanus cajan* were thus, shown as reliable source of sulphur containing amino acid. A variety of products have also been made in India in particular, making some researchers suggest that the various potential applications of *Cajanus cajan* can only be limited by human imagination (Odeny, 2007).

2.2.1. Amino acid profile of NULs

The WHO recently announced on behalf of the 'International Agency for Research on Cancer Monograph Working Group' that processed meats can cause cancer (Bouvard *et al.*, 2015). Major media houses were in total disbelief and have not been kind on the announcement either. For consumers who are conscious of their health, however, this came as no surprise. Even before this announcement, many consumers had significantly made a transition from meat to plant proteins already. For instance, in the US, the per capita consumption of meat

declined in a survey conducted (Daniel *et al.*, 2011). At the same time, sales of meat analogues increased steadily (Wallis, 2016). Thus, this announcement has the potential to cause a major transition from the consumption of meat and other refined meat products to the consumption of plant proteins and their products.

Legumes are comparatively cheaper and are readily available. In Africa and in particular the indigenous regions, there are many different types of legumes that are edible. However, exotic legumes such as soybeans and a few other legumes have been developed and highly promoted over and above other indigenous types, for various reasons. Some of the reasons border on the nutrient content, particularly, essential amino acids and the ease of cooking these legumes. Soybeans have particularly been developed to the neglect of other indigenous legumes. Undaunted, many indigenous farmers continue to cultivate these indigenous NULs and make a living out of them. The cultivation of these indigenous legumes persists because they do not require any extra agricultural inputs that have cost implications for the already poor farmer. It is for these reasons that the frontiers for protein resources should be expanded to cover these indigenous legumes which currently, have very little industrial applications.

Underutilized legumes are of different types and varieties and even within the same family, variability is known to occur (Adebowale *et al.*, 2005). In order that plant proteins can be used successfully in food applications, they should possess several characteristics so they can perform various functional properties desirable in the food industry. These functional properties are largely based on the intrinsic overall proteins structures, on one hand, and the amino acids building blocks on the other hand. It is rare to find one protein structure that is endowed with the various functional properties sought after in the food industry. Also worth

noting is that, NULs possess several antinutrients and these antinutrients have different impact on the digestibility of the storage proteins. Irrespective of these, some researchers (Adebowale *et al*., 2007) have tried to formulate food products which are natural, cost effective and adaptable functional foods based on the protein isolates obtained from NULs.

Legumes are inexpensive sources of proteins being good sources of amino acids, especially inexpensive essential amino acids for the food sector. As we look for sources of ingredients for the manufacture of food, we strive in particular, to acquire readily available and inexpensive sources of food ingredients especially those which are rich in nutrients such as essential amino acids. It is thus, no surprise that researchers have of late turned their attention to legumes that are otherwise neglected and underutilized.

2.2.2. Characterization of amino acids

Amino acids are characterized, using several methods but in the recent past, the method of choice is the use of amino acid analyzer (Alsohaimy *et al*., 2007). Due to challenges of prolonged hydrolytic procedures with some amino acids, cysteine and methionine contents have specially been determined in perfomic acid methods (Gehrke *et al*., 1987). To avoid its degradation, tryptophan content of protein has been determined using alkaline hydrolysis rather than using acid hydrolysis (Landry and Delhaye, 1992) and sometimes colorimetrically (Pinter-Szakacs and Molnar-Perl, 1990). Other methods involve the use of the High Performance Liquid Chromatography (HPLC) using derivatization techniques (Adebowale *et al*., 2007; Alsohaimy *et al*., 2007; Bartolomeo and Maisano, 2006; Vasconcelos *et al*., 2010).

In order to measure the quality of proteins for food and human nutrition, several parameters that provide some objective scores of proteins are made. Among them are chemical score (CS), biological value (BV), net protein utilization (NPU), protein efficiency ratio (PER) and protein digestibility corrected amino acid score (PDCAAS). Chemical score defines the protein quality by comparing its limiting amino acid profile to the same type of amino acids in a standard or reference protein (Schaafsma, 2000). Chemical scores are theoretical quantification parameter suggesting potential availability of test amino acids. The reality is that not all ingested proteins actually go through digestion and assimilation. WHO/FAO frequently commission experts to review the various quality evaluations and requirement of proteins and amino acids for human nutrition, among others (FAO/WHO/UNU, 1991). Lately, the WHO expert body has recognized the limitations of the PDCAAS as assuming 100% digestion of proteins (Schaafsma, 2005). This lapse has subsequently been corrected and a new and the more advanced "digestible indispensable amino acid score" (DIAAS) has been put in its place. DIAAS is considered as "the ratio of milligram of digestible dietary indispensable amino acid in one gram of the dietary protein to the milligram of the same dietary indispensable amino acid in one gram of the reference protein (FAO, 2013). Infact, DIAAS uses the individual amino acid digestibilities evaluated at the end of the small intestine in its calculation. However, it does not round up the evaluations to 1 unlike in the case of the PDCASS. For truly evaluated quality of specific proteins, the best method is to determine the ileal digestibilities of these specific proteins. The resulting essential amino acids can then be used in quality evaluation depending on the limiting amino acid. However, for ethical reasons and animal rights issues that prohibit excessive animal sacrifices, some studies are carried using bioreactors primed with required enzymes for such digestions. For instance, using exogenous enzymes, Han *et al.*

(2007) carried out a study that indicated that in-vitro protein digestibility (IVPD) of raw legumes ranged from 72% for soybeans to 83% for dry green peas.

2.3 Potential toxicity of NULs

To some extent, NULs usage is not very popular and some of the reasons could be the presence of antinutritional factors and other toxins. Some of the antinutritional factors include flatulence factors (Gebrelibanos *et al.*, 2013), goitrogens (Subuola *et al.*, 2012) and lectins (McPherson, 1990; Miyake *et al.*, 2007; Nachbar *et al.*, 1980; van Damme *et al.*, 1998; Vasconcelos and Oliveira, 2004). The presence of these compounds may be due to the fact that NULs use these substances as adaptive chemicals, but some of these turn out to be toxic for human consumption. The situation becomes even more serious during lean seasons or during the dry seasons, when humans turn to these NULs and use them either as food or animal feed. Thus, there is the potential likelihood that these toxic substances could enter the food chain directly or indirectly and present problems in the food systems.

Several toxigenic substances have been identified in *Canavalia ensiformis,* among which Concanavalin A is probably the most studied since it was isolated several decades ago (Sumner and Howell, 1936). It is a protein that is known to bind to carbohydrates. *Canavalia ensiformis* seeds have not less than 20% of its total proteins present as Concanavalin A (Dalkin and Bowles, 1983). Concanavalin A is most effective in the presence of excess metal ions such as Mn^{2+} and Ca^{2+} (Brown *et al.*, 1982). Concanavalin A binds specifically with D-mannose and D-glucose (Liener, 1974). Studies have shown that the synthesis of concanavalin A and canavalin occurs 30 days after flowering, leading to the accumulation of the former (Yamauchi and Minamikawa, 1990). While canavalin synthesis occurs between the 30^{th} and

50^{th} day after flowering, concanavalin A levels increase until over 80 days. The case of concanavalin A is serious because it affects the gastrointestinal tracts of several experimental animals by binding to the intestinal villi (Mendez *et al.*, 1998).

Canavalin is a non-protein toxic amino acid that is present in many leguminous plants including *Canavalia ensiformis* (Rodrigues and Torne, 1992). It is thermostable with a melting point of 184 ^{O}C (Budavari *et al.*, 1989). Canavalin is similar in structure to arginine having a nitrogen rather than a carbon atom in the side chain that covalently links to a guanine group. The levels of canavalin are generally higher and it accounts for 30% in *Canavalia maritima* compared to 5% arginine of the total free amino acids (Yu and Kwon, 1992). It has been reported (D'Mello, 1989) that birds can tolerate the presence of canavalin in their diets because of the presence of arginase. However, the presence of canavalin in diets of growing chicks, significantly reduced feeding and hence growth, to about 25% (Michelangeli and Vargas, 1994). Canatoxin is a neurotoxic protein isolated from *Canavalia ensiformis* that possesses zinc and nickel as part of its dimeric polypeptide (Follmer *et al.*, 2001). Canatoxin has also been shown to be unstable in acidic medium and thus, non-toxic for human consumption (Carlini and Guimaraes, 1991). *Canavalia ensiformis* contains fractions or extracts that inhibit the digestion of proteins. Isolated protease inhibitor extracted from *Canavalia ensiformis* has been found to effectively block proteolytic enzymes produced by the pancreas of rabbit (Kumari and Pattabiraman, 1990). However, germination in *Canavalia ensiformis* was found to decrease the proteolytic inhibitors (Akpapunam and Sefa-Dedeh, 1997).

Mucuna pruriens has long been suspected to contain toxic substances (Bailey, 1947) and was eventually predicted to contain neurotoxic agents (Ghosal *et al.*, 1971). The presence of

nicotine, physostigmine and serotonin (Duke, 1981) and haemagglutinating activities (Rajaram and Janardhanan, 1991) were reported in *Mucuna pruriens*.

The major toxic substance in *Mucuna pruriens* is L-DOPA (Lampariello *et al.*, 2012). The adverse clinical consequences of these toxic chemicals include; aggression, hallucinations, severe depression and dementia. The presence of L-DOPA in food is unacceptable because substances such as N, N-dimethyl tryptamine and bufotenine are regarded as narcotics and actually psychoactive substances even at very low doses (Lorenzetti *et al.*, 1998). To reduce the anticipated toxicity, elaborate preparation methods are put in place. The seeds of *Mucuna puriens* are taken through elaborate pretreatments to reduce the level of L-DOPA significantly (Siddhuraju and Becker, 2001; Vijayakumari *et al.*, 1996). Despite its toxicity, *Mucuna pruriens* is consumed in various forms particularly in Nigeria, Ghana and South Eastern regions of Africa (Flores, 2002; Infante *et al.*, 1990; Osei-Bonsu *et al.*, 1996).

Phaseolus lunatus is known to be largely cyanogenic (Baudoin *et al.*, 1991). Studies investigating plant cyanogenesis in *Phaseolus lunatus* affirmed this legume accumulates cyanogenic compounds capable of releasing hydrogen cyanide (Ballhorn *et al.*, 2005). These releases were from the leaves of the young and matured plants. The findings of this study had serious implications with respect to farm animals that are fed on fodder, since there are several reports of the use of *Phaseolus lunatus* beans for feeding farm animals (Huisman *et al.*, 1990; Magalhaes *et al.*, 2007; Paduano *et al.*, 1995).

Legume seeds are very important sources of proteins and minerals in many human populations (Beebe *et al.*, 2000). However, they contains several anti-nutritional agents such

as lectins usually in varying amounts (Dhurandhar and Chang, 1990; Lyimo *et al.*, 1992). The use of these beans have sometimes impacted adversely and there have been instances resulting from human food poisoning attributed to undercooked beans. Treatments such as heating, autoclaving and extrusion cooking are known to reduce the levels of these toxic agents. Heating foods above 100 ^{o}C can significantly lower the lectin level. However, below this temperature, potent levels of lectin toxicity have been reported (Akande *et al.*, 2016; Bollini *et al.*, 1999; Carvalho *et al.*, 1998). In particular, the undercooked and the raw beans have been reported to have toxic substances resulting in feeding inefficiency, and histopathological damages. Studies on *Phaseolus vulgaris* provided evidence to support the fact that some fractions of the bean flour act to decrease blood sugar (Pereira *et al.*, 2012). At the same time, high levels of the bean were noted to have serious consequences on the histopathology of some of the major organs involved in metabolism. However, NULs can be safely utilized after carefully selected measures to remove these hazardous compounds.

The impact of fermentation on antinutrients such as cyanide and phytate in composite millet and *Cajanus cajan* seeds has been reported to be significant (Onweluzo and Nwabugwu, 2009). Different procedures have been used to remove toxic substances such as trypsin inhibitors, haemagglutinin and saponins from *Cajanus cajan* seeds to make it wholesome to feed African cat fish (Francis *et al.*, 2001; Grimaud, 1988; Ogunji *et al.*, 2005). The anti-nutritional factors in *Cajanus cajan* seeds have been removed by boiling in water and/or boiling with potash and toasting (Onu and Okongwu, 2006).

2.4 Lectins

Lectins are proteins or glycoproteins, sometimes referred to as phytohaemagglutinins; thus,

they have plant origins. They are capable of specifically recognizing and irreversibly binding to specific carbohydrates (Miyake *et al.*, 2007; Sitohy *et al.*, 2007). All foods are known to contain variable amounts of lectins. They are harmful when ingested in large quantities, but at lower levels, some health benefits have been attributed to them (Lam and Ng, 2011). Food groups such as grains, legumes, dairy and nightshades are known to have potentially hazardous lectins. Lectins are ubiquitous and thus, can be isolated from sources such as plants and even animals (Mojica and Merca, 2004). A couple of reports have shown that removal of lectins from diets makes consumers less susceptible to some disorders (Coppo *et al.*, 1992; Freed, 1979). Some of these conditions include rheumatoid and osteoarthritis. It has been reported that 50% of *Phaseolus vulgaris* seed storage proteins is known to also have lectin properties (Emani and Hall, 2008). The relationship between the phytohaemaglutinic properties of *Phaseolus vulgaris* and its allergenic properties in some individuals has been reported (Kumar *et al.*, 2013). A wide range of conditions such as hormonal disturbances leading to premenopausal symptoms, low testosterone levels in males, osteoporosis, breast cancers and high blood cholesterol have been attributed to lectins (Sullivan, 2008). Other conditions such as hyper insulinaemia, diabetes, celiac diseases, allergy and schizophrenia, drive studies into interventionary techniques to control lectins.

For instance, several attempts have been made to eliminate lectins from diets though methods like inactivation of lectins through soaking, sprouting and cooking (Cuadrado *et al.*, 1996; Sanchez *et al.*, 2006). Heating is the most commonly used method for inactivating lectin and indeed, excessive heating would cause denaturation (Lemke *et al.*, 2013) and decrease their water solubilities. Consequently, many of their surface functional properties would be affected. In addition, extensive heating can also impact negatively on the quality of protein and the

bioavailability of many essential amino acids (Lokuruka, 2011). There is the possibility of using instant controlled pressure drop to remove lectins. In this process, steam pressures of up to 8 bar and heating up to 170 ^{o}C for a very short time of up to 3 min was used (Pedrosa *et al.*, 2012). A reduction of the levels of lectins in legumes such as soybean, lupine, chick pea and lentil were achieved. In recent past, there has been the application of low dose gamma irradiation of food proteins in an attempt to eliminate lectins such as Concanavalin A: this has achieved some successes (Vaz *et al.*, 2011). This was as a result of finding the possibility of allergic responses using low dose gamma irradiation as an alternative to control lectins present in the protein portion that leads to allerginicity.

2.4.1 Lectins and carbohydrates

Lectins have been defined as ubiquitous proteins or glycoproteins of non-immune origin that bind to specific carbohydrate receptors with high affinity (Pusztai, 1991). Lectins can agglutinate cells, as well as binding to macromolecules such as carbohydrates (Miyake *et al.*, 2007). They achieve this by binding to and cross-linking their surface carbohydrates. The carbohydrate recognition domains (CRDs) of many lectins have been reviewed and shown to be closely related (Weis and Drickamer, 1996). The review also shows that the carbohydrate-lectin binding occurs through interactions such as hydrogen bonding, salt linkage and van der Waal's forces. The review further shows that on the basis of recognition, lectins may be classified as animal type, legume type or glycolipid binding type. Other groups identified are those found to be mineral (Ca^{2+})-dependent. There are also galactose binding lectins, mannose-6-phosphate binding and sialo sugars binding lectins. The activity of some types of lectins depend on divalent mineral (Ca^{2+} and Mn^{2+}). Essentially, these divalent cations stabilize

the globular lectins or the sugar ligands electrostatically (Iobst *et al*., 1994; Kolatkar and Weis, 1996; Sandberg, 2002). As in all van der Waals interactions, significant non-polar binding forces results from the interactions of hydrogen and carbon atoms close by, with the aromatic side chains of proteins, such as tryptophan and phenylalanine. It is also believed that glycerol patches existing in some liposugars also present non-polar surface to promote interactions with the hydrophobic portions of proteins such as tyrosine (Wright, 1984). Specific sugars that selectively bind to lectins are thus, known to present additional atoms structurally positioned to offer extensive hydrophobic interactions (galactose and mannose). It has also been observed that there exists sub site multivalency systems that extend the binding sites (Rini, 1995). Thus, it is these sites that increase the affinity between the carbohydrate-lectin interactions.

Two independent binding sites for sugars in lectins have been suggested though one appears to be more significant (Bourne *et al*., 1994). This observation reechoes other findings (Wright, 1984) that there exist binding sites that may still be present in lectins. It has been suggested that these secondary sites which are more common in bacterial sugars, such as sialyl lactose, have no structural similarities, compared to the primary sites (Sauter *et al*., 1992).

Though closely related lectins as in the case of galectins effectively bind β-galactose, they show selectivity for other sugars such as lactose and N-acetyl lactosamine. Thus, the CRD is flexible in their sugar-lectin binding. Many plant lectins are known to be homologous, but, other closely related lectins have variable recognitive CRD and bond with specific sets of sugars. Studies have shown that lectins from *Canavalia ensiformis* and pea basically bind to mannose type sugars (Bourne *et al*., 1994). It has however, been demonstrated that soybean agglutinin (SBA) usually bind to galactose sugars (Dessen *et al*., 1995). The binding has been

observed to be conservative and the characteristic signature binding sites are specific for very few sugars such as mannose and galactose tough oligosaccharides and even polysaccharides are recognized as well. Studies in *Phaseolus vulgaris* indicate that these carbohydrate-binding points on lectins are found mostly in the β pleats of the 7-chain sheet (Hirabayashi *et al.*, 2011; Gabius *et al.*, 2011). These authors also explained that van der Waals interactions are predominant and that the tyrosine and phenylalanine which are predominant in lectins are observed to associate with mannose and galactose. These van der Waals interactions were further re-enforced as hydrogen bonds are formed between the sugars and the amino acids; asparagine and aspartic acid, that are situated in the binding sites. Banerjee *et al.* (1996) presented the structural design of the monomers of *Phaseolus vulgaris* lectins as composed of three β-sheets in antiparallel 6-stranded back sheet, together with a concave shape 7-stranded front sheet, and a smaller 5-stranded sheet. Compared with other legume lectins, studies have indicated that short α-helices are usually found in *Phaseolus vulgaris* lectins (Nagae *et al.*, 2014).

2.5 Hazardous effects of lectins

Dietary or nutrient proteins are generally digested as they pass through the gastrointestinal tract either by digestive enzymes or by the gut flora and fauna. Lectins resist degradation by either the gut flora and fauna or by the digestive enzymes (Pusztai and Bardocz, 1996). From this observation, it means lectins are able to survive the gastrointestinal tract. For instance, *Phaseolus vulgaris* and *Canavalia ensiformis* lectins have very high binding capacity to the epithelial cells of the small intestines. A recovery rate of above 90% of lectin activity has been reported after feeding native lectins to experimental animals(Pusztai and Bardocz, 1996). There has been further evidence that apart from some lectins being resistant to bacteria and

digestive enzymes, some lectins are also heat-stable.

There has been a long history of lectin research showing its cancer fighting potential (Giacometti, 2015; Reynoso-Camacho *et al*., 2003) and other therapeutic potentials such as HIV type 1 and type 2 strains (Balzarini *et al*., 2004). There have been reports of isolated glycoproteins; γ-conglutin, that have glucose lowering properties (Lovati *et al*., 2012). The report also showed that ability of this protein to cross the intestinal mucosa, suggesting that the proteins resist proteolytic digestion apart from exhibiting insulin–like activity. The study also catalogued a number of organs such as small intestine, spleen and thymus that were severely affected. However, consumption of *Phaseolus vulgaris* and *acutifolius* continue to be the normal part of the native diet in South Americans (Jones, 1996).

The toxicity of *Phaseolus vulgaris* seeds, which are widely consumed in Northern Africa. Studies have been carried out to assess the residual activities of lectins of certain processing methods (Nader *et al*., 2015). They explained that heat processed beans presented lesser subunits of lectins relative to native beans. This is a clear suggestion that processing procedures may probably have led to the loss of some of the subunits of lectins. That lectins pose food safety problems is an understatement. It is therefore, very important to present lectins as a hazard, thus, paving the way for weight-of-evidence or serious attention to be focused on them. In hazards identification, procedures are adopted to determine whether a particular agent has the capacity to cause adverse health effects or not. It is important to note, however, that even though individuals may be exposed to the hazard, not all individuals in a population will be susceptible to diseases resulting from the hazard in question.

The action of lectins on the gut is well documented. The first line of attack as a result of ingestion is the gastrointestinal tract (Makkar *et al.*, 2007; Miyake *et al.*, 2007; Pusztai *et al.*, 1979). It has been generally agreed by these investigators that experimental animals fed with lectin led to the erosion of the mucus layer of the intestine leading to bacteria and protozoa infections. It has also been observed that lectin bind to specific carbohydrate moieties and particularly to injured cells in this process of gastrointestinal attack. It has been explained that gut injuries often resulted in hyperplasia and hypertrophy of the small intestine as well as organ weight and physiological activity in experimental rats (Marzo *et al.*, 2002). Similar findings were made by Reynoso-Camacho *et al.* (2003) who attributed tepary bean lectin as the cause of the degeneration of the thymus and spleen.

2.6 Hazard characterization

Hazard characterization in general deals with the quantification of the adverse effects observed in the hazard identification stage of the risk assessment process. Some of the main processes in hazard characterization include dose-response studies of the hazard in question, to which a test organism is exposed and the resultant adverse responses measured and quantified. For example, the dose of acute toxicity studies of tepary beans conducted earlier (Ramos *et al.*, 1998), was re-evaluated (Reynoso-Camacho *et al.*, 2003), to be between 20 and 1200 µg/g body weight of test animals. Responses such as mortality and other clinical signs were noted after 15 days of exposure. Post-mortem of the dead animals as well as the dissections of those that survived, showed damages to the internal organs such as kidney, liver, thymus, lungs, small intestine and the spleen. However, these observations were reversed when lectin was removed from their diet, suggesting that, at certain concentrations of lectin the dose might be tolerable. It is this tolerable dose of lectin that is uncertain in human consumption patterns. At this

tolerable/reference dose, human populations (including sensitive subgroups such as pregnant women and the elderly) should be able to have daily oral exposure of lectin containing foods without any risk of deleterious effects during a lifetime (Tunsaringkarn *et al.*, 2012).

Though lectins are ubiquitous, the quantities present in foods vary thus, certain foods contain more than others (Nachbar and Oppenheim, 1980). Also, depending on the type and degree of processing, the same food may contain different amounts of lectins (Nader *et al.*, 2015). It has been documented that food groups that contain lectins include grains such as wheat and wheat germ, rice, barley, maize and millet (Sullivan, 2008). Nightshades such as; potato, tomato, garden eggs and pepper are also known to contain high levels of lectin even after passing through the digestive tract. Dairy products present another food group with the potential to cause harm especially if the animals from which the dietary products were obtained, were fed on grains like beans and peanuts, instead of grass (Sullivan, 2008). Lectins acquired from feeding on such grains could transfer lectins into breast milk and consequently into dairy products. Also, pasteurized milk is potentially harmful due to the reduction of secretory immunoglobulin (sIgA). It is the natural presence of sIgA that binds lectins. These sIgA are known to offer protection in the form of mucosa against antigens. Studies show that some foods may contain as high as 30% residual lectins even after thermal processing (Nachbar and Oppenheim, 1980). It is these partially inactivated lectins that are responsible for many the adverse reactions of foods containing lectins (Gell and Coombs, 1975).

Purified lectins obtained from *Discorea bulbifera* have potent actions on internal organs such as liver and the spleen of test rats (Kuku and Nkiruka, 2009). It was realized in the study that the amino acids; cysteine, tryptophan and arginine are responsible for the haemagglutinating

activities of lectins. Though these key amino acids are known to be involved at the activities at the binding sites in lectins, what is not clear is whether greater quantities of these amino acids in proteins imply greater lectin activities or not. Lectins have varying degrees of systemic toxicities but some are deadly, as reported in ricin lectins which has an LD_{50} (oral) 20 mg/kg body weight in humans (Audi *et al.*, 2005). The intraperitoneal LD_{50} in rats has also been reported as between 1100 mg/kg and 1120 mg/kg body weights for male and female respectively (Reynoso-Camacho *et al.*, 2003).

2.7 Exposure assessment

Lectins occur widely distributed in foods such as fruits, vegetables, legumes, night shades, sea foods and farmed animals (Nachbar and Oppenheim, 1980). This suggests that consumers are exposed each time they ingest each of the food groups mentioned. A database showing the oral intake of lectins by individuals is limited. Thus, it is difficult to estimate exposure range of the consumption of lectin from a reliable data base such as can be obtained from WHO/FAO.

For this reason, in order to carry out exposure assessment, a number of factors will have to be considered. Information could be gathered from a broad variety of food groups. Data will also be gathered from tests involving oral challenge of experimental animals. Unlike other toxic substances that enter consumers through inhalation as a major route, lectins entry of consumers through inhalation will be considered as a minor route, unless a sub-group of consumers could be identified who work in legume milling industries. Information on the presence of biomarkers indicative of exposure could be considered, as well as finding the stability of lectin in processed foods.

Food consumption data enable toxicologists to estimate the consumption of toxic substances. The procedure is very simple. Once the concentration of the toxic substance is known and the amount of food consumed is also determined, exposure or risk can be quantified. European Food Safety Authority (EFSA) has one of its regulations stipulating the promotion and coordination of efforts towards the development of uniform risk assessment methods (EFSA, 2012).

As part of harmonizing food consumption data, the eating pattern in a population is evaluated based on sampling methods, sub-population groups, age and dietary preparation protocols (WHO/FAO, 1997). It is expected that the form in which food is eaten must be borne in mind in order to obtain the most effective mass of food ingested and subsequently, its exposure assessment. For instance, food consumed may be in its fabricated form such as bread, or its ingredients such as flour or better still as a raw agricultural commodity such as wheat (Møller and Ireland, 2000).

When surveys are carried out, the quality of the results are usually dependent on the validity of data collection methods (Tennant, 2012). Sometimes subjects in a survey area tend to over-impress or even become economical with the truth during data collection in the field. In some other cases, the results from the survey of products on the markets may be skewed due to brand loyalty. In their haste to bring their manufactured foods to the market, manufacturers sometimes handle foods in such a way that the levels of toxic substances in them may fall far below what is expected or over and above what is usually present. In short, manufacturers of food products may be unable to produce consistent products that will have the same amount of toxic substances throughout the period of study.

For effective data collection on food consumption, validation is necessary, using other methods to verify whether subjects provided the correct information. Such verification or validation methods are quite expensive. Most of them involve the analysis of the presence of biomarkers in the excretory product of consumers. Additionally, they could use other invasive procedures such as blood sampling. Many researchers have used probabilistic modelling of food and nutrient intake to estimate the uncertainties related to food systems risks (Gilsenan *et al*., 2003; McNamara *et al*., 2003; Rubingh *et al*., 2003). Exposure to toxic substances is integrated as the product of food intake and the toxicant concentration (Gibney and van der Voet, 2003). This is an example of the Monte Carlo probabilistic models, used to estimate dietary exposure of selected food chemicals. Simulations involve random sampling from a sample population or data set over and over again; a procedure called iteration. Modelling experts argue that when the number of iterations is large, the accuracy of the outcome of the applications of the distribution of toxic substances such as the risk estimate becomes more accurate.

2.7.1 Central tendency metrics

Traditionally, the output of data gathering for the exposure of evaluation purposes, have been characterized with the use of the measures of central tendency terms; mean, median, mode, minimum, maximum and standard deviation. These terms describe "the statistical measure that identify a single value as representative of an entire distribution" (Gravetter and Wallnau, 2000). In decision making, such as in the evaluation of nutrient quality or in risk assessment, it is proper to define food consumptions individually from experimentally determined database. However, for many years, many field experiments have hardly itemized types of legumes and consumption patterns because very often all of them are clustered and referred to as either

beans or pulses (WHO/GEM, 2012). To make matters worse, crunched or central tendency values such as the mean is often used to represent large data of clustered pulse consumption. It is therefore misleading to draw general conclusions using such central tendency values. To butress this point, an explanatory paper has shown statistical evidence of the sensitivity of the mean and especially to outliers when the sample size is small (Dawson and Trapp, 2004; Manikandan, 2011)). However, descriptive statistics does not give a complete description of the entire data gathered, because the crunched central tendency values cannot effectively represent the huge data, hence, the use of probabilistic approach is preferred. Citing heterogeneous consumption population habits as reasons, conclusions from studies (van der Voet *et al.*, 2007) have reinforced the choice of the probabilistic model as the best method to process data obtained from individuals in a population study. Thus, when dataset is obtained in a localized sub-population it could provide very useful information on the variabilities or uncertainties among legumes.

2.8 Risk assessment

For foods containing proteinaceous hazards such as food allergens and food lectins, the risk assessment is quite difficult since reference doses of these hazardous substances are difficult to determine. For food allergens, a lot of work has been done to the extent that producers are required by law to include in their label, food products containing food allergens (Muraro *et al.*, 2014). For lectins, however, very little guidelines are available let alone, regulating foods containing substantial quantities of lectins. Many studies on risk assessments on food allergens have been done on peanut agglutinin showing the probability distribution models of individual peanut thresholds (Allen *et al.*, 2014). The no-observable-adverse-effect-level (NOAEL) and the least-observable-adverse-effect-level (LOAEL) of many of the peanut allergens, have been

established. For instance, the probabilistic approach, has been used to present a model to explain the risk assessment of allergen essentially outlined as hazard identification and characterization, exposure assessment and risk modelling (Spanjersberg *et al.*, 2007).

Though lectins are ubiquitous in foods, and also pose adverse health effect, there is no reliable reference dose that serves as benchmark in their applications. Thus, estimating the risk associated with ingestion of lectins is bound to face several challenges. One way though, is to carry out clinical trials in order to establish the threshold levels for lectins in foods. However, there are scattered thresholds of lectins depending on the type of lectin, their methods of quantification and their dose-response characteristics such as their NOAEL and LD_{50}. Though many hazard indices have been established in animal studies, there are only few cases where these indices have been reported in human studies. For example, the NOAEL of *Phaseolus vulgaris* grain legumes was established as 175 g/day/70 kg body weight, through another study reported a lower safety limits of 85 mg/day/70 kg body weight (Choshi, 2007).

There are two main types of hazards that are used to characterize food systems risks; non-carcinogenic (systemic) hazard and carcinogenic hazard (Nielsen *et al.*, 2008). In either case, risk characterization is reckoned as excess of hazards. The excess hazard is usually quantified as hazard quotient (ratio of the concentration of the hazard to the toxicologically accepted hazard) (Barnes *et al.*, 1988), or as an integrated product of the hazard concentration and the potency factor of the specific hazard (LaGrega *et al.*, 2010). The risk produced by a lifetime average dose of 1 mg/kg-day, also known as potency factor or slope factor are obtained from the dose-response characterization of the hazard (Gerba, 2000). Normally, the hazard quotient gives a quick estimate of risk because when the hazard quotient is less than

one, it means, there is no need for stringent regulation because the hazard is tolerable(Tennant, 2012). However, when the ratio greater than one, it means, risk is implicated, thus, health concern must be raised (Barnes *et al.*, 1988). An integrated product of the concentration of the hazard and the potency factor is used only when the hazard is a carcinogen (LaGrega *et al.*, 2010). Though the concentration of a hazard is often determined using analytical techniques (such as HPLC, ELISA, etc.), their thresholds are set by institutional risk databases (WHO/FAO, 2009, 1997). Lectins perfectly suit the classification of a hazard, since biologically active lectins found in food, result in systemic toxicities (Pusztai and Bardocz, 1996).

Test experimented animals are often used to characterize the NOAEL, and LOAEL from which reference dose could be deduced, following well-documented protocols for the extrapolation of reference dose from NOAEL and LOAEL for lectins in humans consumption (Crump, 1984; Gerba, 2000). An uncertainty factor (UF) or safety factor of 10^2 is integrated with NOAEL, to harmonize the conversion of hazard impact in humans. The first factor- 10, is used to harmonize the hazard concentration value of NOAEL (by dividing) to human concentration value, believing that such resultant value would be small enough not to cause harm to humans. The second factor-10, then leverages the dose further in order to harmonize human variability and uncertainties to the hazard in question. In some cases, however, if the NOAEL values are not available, the LOAEL could be used only when the safety factor was modified to 10^3. It is believed that risk assessors prefer to err on the side of preserving life and property (Crump, 1984). In order to determine the risk for hazards, such as lectins, where a reliable database is very scarce to find, a simple ratio comparison is made between the chronic daily intake (CDI).or the average daily dose (ADD) to a reference dose (R_fD).

The Committee for Medicinal Products for Veterinary Use, prepared a report on the European public maximum residue level (MRL) assessment on lectin extracted from *Phaseolus vulgaris* (EPMAR, 2008). Their report emphasized the concentration of lectin in the various species of kidney bean, suggesting that the levels may vary, depending on the soil and other climatic factors. Report was also made of cause and effect of lectin ingestion, defined by symptoms such as loss of appetite, nausea and other gastrointestinal tract discomfort. These symptoms were particularly observed to be dose-dependent. Other symptoms included decrease in body weight and eventual death. In their studies, the ADI could not be established and the acute oral LD_{50} value could not be determined due to non-availability of comprehensive data. Though no repeated dose toxicity was run, the study concluded that the threshold dose of *Phaseolus vulgaris* seed extract that does not produce adverse outcome in the experimental animals with respect to the small intestines and pancreas was 65 mg/kg body weight /day. Further studies using pure recombinant PHA-E lectin, produced doses of 55 and 70 mg PHA-E lectin/kg body weight for males and female rats in a 28-day toxicity trial as LOAEL. Outcomes were based on the weight of small intestines in the test animals.

It must be emphasized that the report explained a very important point by citing susceptibility to microbial infection as a direct effect of lectin consumption. Lack of reliable database is a setback in the study of the adverse health effect of lectins from the legume family. In the face of this difficulty, simple comparison of the results of the LD_{50} of the tepary bean lectin to that of the LD_{50} of ricin 0.028 mg/kg body weight (Lin and Liu, 1986) was made. It was concluded that the tepary bean lectin had relatively low toxicity (Reynoso-Camacho *et al.*, 2003). The lack of reliable threshold parameters makes the quantification of lectin risk suffer limitations, despite

the impact of agglutinin in the foods consumed. In the absence of such a reliable database, it should be possible to estimate the risk using individual threshold values reported by researchers, while using these as principal evidence reference points.

2.9 Risk communication

Risk communication presents an interactive platform for exchange of information on risk estimates, as well as cross fertilization of principles and management procedures among all stakeholders, on the safety of food. The goal of risk communication is to make the consuming public understand the risks involved in the consumption of certain food products along the food supply chain (NRC, 1989). Compared to knowledge about food allergen, where steps have been taken to indicate the presence of allergenic materials in food (Gendel, 2012), limited information has been communicated to consumers as far as the presence of lectins in foods are concerned. There is the notion that since lectins are proteins, thermal processing of foods would lead to their denaturation. Contrary to this notion, it has been reported that total removal of lectins does not occur when *Phaseolus vulgaris* is cooked at 100 ^{o}C (McPherson, 1990). Thus, care must be taken in this type of communication since depending on the type, some lectins persist even after cooking. All through literature, there are reports of human consumption of dietary lectins being associated with adverse health implications. It is worthy to note that the average consumer is exposed to street foods which have been totally processed under the control of individual food processors (Nachbar and Oppenheim, 1980). Unfortunately, many of these street food processors have little or no knowledge about the presence of these hazards in leguminous foods and so processing is done with little or no consideration of their elimination.

2.10 Lectin inactivation and impact

One common procedure for removing lectins involves thermal treatments. Various forms of heating have been done, including wet and dry heating. These different heating procedures, often leading to the production of various types of residual active lectins in foods, have been studied (McPherson, 1990).

In all the instances of adverse health reports, lectins are known to have certain active sites that bind to specific chains of complex sugars (Weis and Drickamer, 1996). These complex sugars are known to be on the surfaces of red blood cells, intestinal villi and indeed any other sites where lectins can bind readily. Sugar derivatives such as N-Acetyl-D-galactosamine (NAG) and L-fucose and sialic acid usually bind to the active site of lectins (Fernández-Alonso *et al.*, 2015). There is little information on the exploitation of these derived sugars to inactivate lectins.

By extension therefore, processes such as irradiation (Rombo *et al.*, 2004) that can lead to the production of these derivatives of carbohydrates, can serve the same purpose in inactivating lectins. Irradiation, as a method to produce modified starches is also a likely candidate for the production of derivatives of sugars, that can be evaluated for the inactivation of lectins. For example, it has been found (Rombo *et al.*, 2004) that increasing irradiation dose caused a dose-dependent increase of β (1, 3) and β (1, 4) bonded starches both in bean and maize flours. One clear mechanism through which starch derivatives were produced was by transglucosidation. However, the performance of lectin inactivation for such starches which were produced within the range of 5 and 40 kGy are yet to be determined. Quite recently, inactivation of lectin-induced haemaglutination has been carried out using glucose and

mannose (Ahmed John *et al.*, 2013). Derivatives of sugars such as rhamnose, sialic acid and N-acetyl glucosamine were found to be good candidates, and potentially, materials such as chitin, fungi and prawns derived from crustacea (Ifuku and Saimoto, 2012) can be studied.

2.10.1 Lectins thermodenaturation and adverse impacts

Due to their protein nature, lectins are supposed to denature when treated with heat. During the cooking process, high temperatures of over 100 ^{o}C, involving boiling, frying and baking are employed. In an attempt to inactivate lectins at these temperatures, both the amino acids in the lectins and the other food proteins decompose, either through their amino or carboxylate groups. Other reactions such as alkylation or acylation may also decompose proteins. Maillard reaction processes involving advanced glycation end-products (AGEs) are very common at these temperatures (Tamanna and Mahmood, 2015). Amino acids such as serine and threonine become very reactive after the β-elimination of elements of water to produce dehydroalanine (DHA). It has been explained that reactions involving carboxylic acid groups make such amino acids as glutamates and aspartates break down to the corresponding α-amino acids (Belitz *et al.*, 2009). Reactions through the amino group render amino acids such as lysine tied up with dehydroalanine (DHA) to give the corresponding lysino-alanine. In such conditions, cysteine in particular, goes through oxidation to form cysteic acid, sulphuric acid and sulphone (Manneberg *et al.*, 1995). Such high temperatures also lead to by-products such as acrylamide, decomposed from the amino acid- asparagine. However, acrylamide is classified as a probable carcinogen (Tareke *et al.*, 2002). There are also human carcinogenic heterocyclic compounds such as methyl imidazole quinolines that are formed (Warshawsky and Landolph Jr, 2005) during high temperature processing of foods that are likely to inactivate.

Extrusion is often used in food industry to provide thermo- mechanical energy required to cause physicochemical changes of raw materials. Extrusion provides shear during mixing and generates dispersion and homogenization of the food component undergoing the cooking (Anton and Luciano, 2007). An extruder has been used as a bioreactor to produce a number of products including starch hydrolysis (Govindasamy *et al.*, 1997) and degradation of toxic principles of β-N-Oxalyl-l-α,β-diaminopropionic acid (β-ODAP) (Hailu *et al.*, 2015). Extrusion cooking temperatures running at 130 ^{O}C has been used to process *Vigna subterranea* seeds-based product (Jiddere and Filli, 2015). Similarly, extrusion cooking of maize flour-soybean mixture has also been done at 170 ^{O}C and 14% moisture (Pérez *et al.*, 2008). It is cautioned that such high temperature cooking is not recommended since amino acid degradation could also occur (Manneberg *et al.*, 1995).

Extrusion can be used as an alternative to increase the utilization of legumes and also to eliminate toxic substances in legumes. However, nutritional qualities of proteins are also reduced. Extrusion cooking has been used to eliminate haemagglutinating activity in pea in order to improve utilization (Alonso *et al.*, 2000). Their extrusion conditions which were run at 148 ^{O}C, and 25% moisture proved to be very effective. Extrusion cooking involving varying levels of navy and red bean flour (15-45%) added to corn starch has been used for the production of snacks (Anton and Luciano, 2007). This reduced phytic acid and trypsin inhibitors to safe levels at 50 and 100% respectively. In the last decade, extrusion, as a processing technology has really caught on with food research scientists. Extrusion process using different rates of water injection and assessing the quality of the ready-to-eat products has been made (Stojceska *et al.*, 2009). There is however, scanty information on the reduction of phytohaemaglutinic activity, involving the addition of derived sugars used as ligands to bind

lectins. Studies have been carried out on the impact of temperature treatment on the haemagglutinic activity, either using extrusion as a bioreactor or steam cooking at 82 ^{O}C for *Phaseolus vulgaris* lectins (Kelkar *et al*., 2012).

2.11 Utility of NULs through sustainable processing

The cultivation of NULs usually requires low investment since they are largely handled by subsistent farmers, who use them as food, thus, making NULs a potential for food security resource (Chivenge *et al*., 2015). Data on the consumption habits and thermal processing of NULs as well as the characteristics of NULs consumers is unreliable. However, it is this data that is required to ensure the sustainability of NULs food usage. There seem to be an increasing drift towards the consumption of plant products and less meat consumption (FAOSTAT, 2009). Since NULs are potential protein sources, attention must be directed to these neglected legumes to exploit the possibilities of tapping into them.

NULs, as well as many legumes lag behind other food crops because of the inherent lectins and the illnesses that are associated with it (Pusztai and Bardocz, 1996). Heat treatments, among other processing factors, seem to be key in the inactivation of lectins in legumes (Friedman and Brandon, 2001). Generally, thermal treatments appear to inactivate lectins, but what happens precisely to lectins in beans when cooked appears fuzzy. In some situations, potentiation occurs, thus, resulting in increased lectin biological activity, even after heat treatment (USFDA, 2000). In other cases, there is the breakdown of lectins during thermal treatment (Nciri *et al*., 2016). Thus, studies show that a usual cooking temperature is likely to bring about inactivation of lectins. On the other hand, high temperatures would likely bring about amino acid decomposition (Damodaran, 1996). It is therefore uncertain during the

thermal treatment of lectins, how low the "low temperatures" should be and how high the "high temperatures" must be. Overall, NULs could offer a potential supply of proteins, but in order to achieve this goal, strategies must be developed for their optimum utilization, thereby, paving the way for their exploitation as potential legume resource for sustainable utilization.

CHAPTER 3: NEGLECTED AND UNDERUTILIZED LEGUMES (NULs): EXPOSURE ASSESSMENT, HABITUAL COOKING AND EATING HABITS AND CONSUMERS' CHARACTERISTICS

3.1 Introduction

Neglected and Underutilized Legumes **(**NULs) are crops which have not received much attention by research, technology, marketing systems and conservation, although their cultivation and consumption serve as livelihood options for the poor (Azam-Ali, 2010; Padulosi *et al.*, 2000). Though a large number of people make a living on them, there is scarcity of information on the NULs, such as nutritional and toxicological profiles. It is particularly conjectured that the neglect of NULs might lead to a build-up of adaptive features, possibly, the production of secondary metabolic phytochemicals required to survive the environment in which they live (Padulosi *et al.*, 2011b).

In Africa and the rest of the world, there are evidences of the special nature of NULs as food security crops (Bhat and Karim, 2009; Chivenge *et al.*, 2015; Cullis and Kunert, 2017). However, little information on NULs consumption is available to catalogue. It is these gaps that warrant consumption studies of NULs (Chivenge *et al.*, 2015; Durst and Bayasgalanbat, 2014). NULs consumption database is important in many ways. It will serve as information resource for researchers, such as epidemiologists and dieticians and food systems professionals (Yamini *et al.*, 2006). In particular, NULs consumption data is the foundation for exposure assessment and consumers' risks towards hazards (Szűcs *et al.*, 2013). Exposure assessment of hazards, which involves the evaluation of the likelihood of intake of hazard by consumers may be determined according to WHO/FAO standardized formulae for dietary exposure (WHO/FAO, 2009);

$$\text{Dietary exposure} = \frac{\Sigma(\text{Concentration of hazard in food} \times \text{Food consumption})}{\text{Body weight (kg)}}$$

It has been explained that depending on the extent of the food consumption, two approaches are available; average daily dose of hazards and chronic daily dose of hazards (Gerba, 1999). While average daily dose is used to evaluate the ingestion of hazard in a relatively short period of time, the chronic daily dose evaluates the ingestion of dose over a life time. It is the life time ingestion that demands the input of exposure frequency of food consumed within a year, in the dietary exposure formula. In order to collect this particular information, consumers are often asked to indicate the number of times of intake of food per week (7 days), which can then be easily transformed to exposure per month (30 days) by the researcher. This is reasonable since it might be too difficult for consumers to recollect NULs dishes ingestions per month. The exposure per month may easily be subsequently transformed subsequently into exposure per year (365¼ days).

A review of available data shows that the protocols used for food consumption data, such as dietary recalls, diet histories, diet diaries and use of food frequency questionnaire, are usually not consistent across many studies (Szűcs *et al.*, 2013). For instance, studies have shown that the instructional content of the collection protocols of 23 European countries food consumption survey had 15 (65.22%) that were comparable based on the adult consumers (Verger *et al.*, 2002). This shows that there is the need for the harmonization of the collection methods. However, harmonization of the surveys is complex and demands collaborations among many stakeholders (Szűcs *et al.*, 2013). The need for cataloguing NULs consumption data is warranted and a standard or a harmonized survey protocol must be found.

While we wait for the harmonization of collection methods to be leveraged, it has been pointed out that uncertainty in food consumption data collection is alarming (Chardon and Swart, 2016). Central tendency values such as mean, median and mode are used to characterize huge food consumption data. Such approaches, that use crunched or point estimates, lead to inaccurate conclusions **(**Dawson and Trapp, 2004). It has been recommended that probabilistic analysis must be used to ensure that such large data is fitted to distributions that quantify both the variabilities and uncertainties (Jedrychowski and Wichers, 2009). Thus, while a harmonized food consumption data survey protocol is required, any protocol that is used to gather data should have the quality of data preserved so that it truly reflects the consumption characteristics of the population. The use of the probabilistic approach, comes in handy as it reflects a better representation of the outcome distribution, compared to point estimates (EFSA, 2012). The reliability of data relating to NULs consumption patterns and cooking practices has the potential of addressing not only cultural attitudes but barriers that impede the harnessing of the full potential of the NULs. Thus, the study was aimed specifically at determining NULs dishes exposure assessment, time of cooking and characteristics of its consumers in the Mid-West belt of Ghana.

3.2 Methods

3.2.1 Study area and period of study

The survey was conducted in the Mid-West belt of Ghana (Figure 3.1). The area included Amantin-Atebubu District, bordering the northern part. The Jaman-South District bordered the far west, where Drobo, a major town is situated. The Ejura-Sekyedumasi, Mampong and Techiman districts were the central zone of the study area. Mampong, Techiman and Ejura are major markets. The mid-west study area covered Offinso-North District, where Abofour, a major trading town, is located. Within

these districts, there are several towns and villages that feed into these major centers of trading especially during market days.

The survey was conducted by showing samples of the five NULs to respondent (Appendix A) between 5th and 20th May, 2014 during market days which are known to attract a cross-section of the population, involving women, men and children. It was expected that the chosen days would provide the opportunity to recruit a large number of respondents within a short period, from a study area which is sparsely populated.

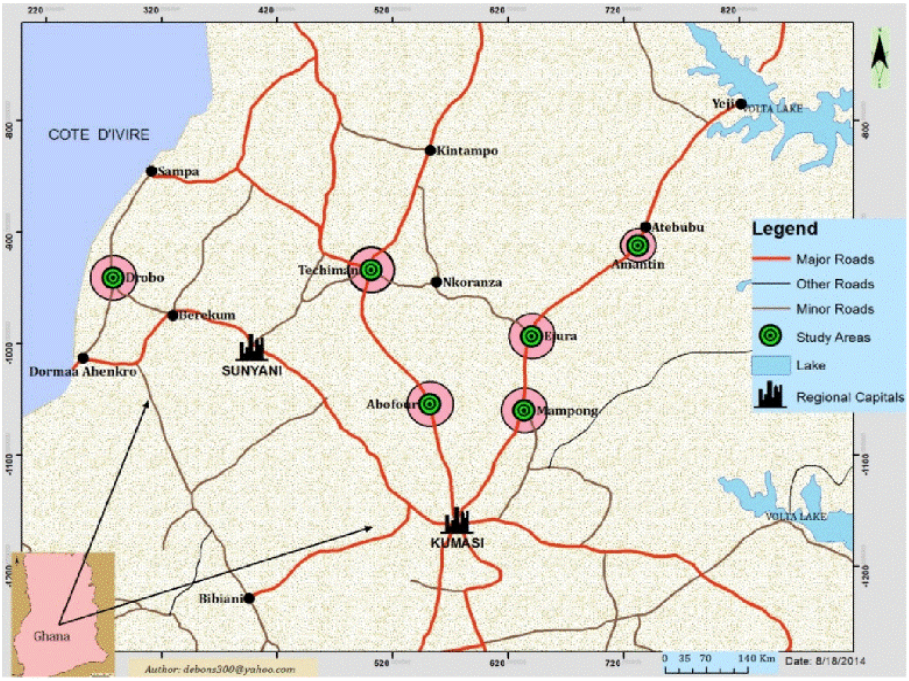


Figure 3. 1: Locations of surveyed towns in the mid-west of Ghana

3.2.2 Design of questionnaire/ Interview schedule

The structure of the study survey was specially designed purposely to facilitate the collection of a reliable database for the evaluation of risk assessment of NULs consumption, in a later study. A structured questionnaire (Appendix B), which covered factors such as NULs seeds

processing times, types of dishes and amount of dish consumed were used. Other factors on the questionnaire were gender, age, weight, level of education, occupation, marital status and house hold numbers. On thermal processing, the questionnaire was used to find out the various cooking times used by the respondents. By buying random quantities and weighing, the masses of the dishes were determined. Subsequently, the exposure frequency assessed the quantities of NULs dishes consumed by the respondents and expressed as per month.

Some of the dishes were soup, stew, *"Tubani"*, "*Koose*", bean porridge (beans composited with cereals) and boiled beans. The body weights of the respondents were determined using a scale on the field. The questionnaire was first pre-tested with about 50 respondents in the study area, and based on the feedback, the requisite modifications were made prior to the actual survey.

3.2.3 Survey

Three assistants, experienced in questionnaire administration were recruited and further trained for the data collection from respondents for the survey. During the training of the interviewers, working definitions, both in English and the local dialect were established for some key words. These included *"Tubani", "Koose",* bean porridge, boiled bean, consumption rates, exposure frequencies and the names of the NULs. The area of study had predominantly Akan speaking inhabitants. Though other migrants had settled in the communities, they also understood and spoke the Akan language fairly well. Thus, the Akan language was used to give the flexibility needed to overcome possible barriers to communications.

Prior to administration of the questionnaires, the consent of the respondents was sought. It was those who gave informed consent who were interviewed. In the consent seeking process, first, the essence of the study was carefully explained to the respondents. The data collection was random and individuals were asked questions within the precincts of the market centers, including traders of NULs inside the markets. Every market center, as indicated in Figure 3.1, was visited twice in order to increase the quality of data collected. The survey was carried out during the market days, which run from about 8:00 am to 5:00 pm. Each interview schedule took an average of 15 min. The five NULs listed on the questionnaire were; *Canavalia ensiformis*, *Cajanus cajan*, *Mucuna pruriens*, *Phaseolus lunatus* and *Vigna subterranea*. They were basically selected because these legumes are found abundantly in the area under study.

3.2.4 Data analysis

Data for each of the NULs was first captured into excel and tallies were made according to gender, age, level of education, occupation, marital status, house hold numbers, time used in cooking NULs, amount of dish consumed per day and number of times of eating dish per week. Body weights and ages were originally captured in groups of 10 kg or 10 years ranges (Appendix C). Palisade @Risk (Palisade, 2014), a Microsoft Excel add-in, was then used to fit statistical distributions for each factor. The fitted distributions were automatically ranked according to *Akaike Information Criterion* (AIC), based on the least loss of actual data and robustness during fitting (Snipes and Taylor, 2014). The best fitted distributions subsequently gave information such as the central tendencies; minimum, maximum, mean, mode, median and standard deviation. Body weights and ages were iterated (10,000) and their simulated 5^{th}, 50^{th} and 95^{th} percentiles, recorded.

3.3 Results and discussion

3.3.1 Characteristics of consumers

A total of 534 consumers of NULs aged between 10 and 100 years, and made up of 59 men and 475 women in the precincts of the commercial area on the market days, took part in the survey. The consumers were predominantly non-formally educated (373) but some respondents with basic education (119) were recorded. The remaining consumers (38) had either secondary, post-secondary or tertiary education. The consumers were predominantly traders (413), but others were non-skilled workers (51) or farmers (51). There were also artisans (8), civil servants (3) and public servants (8). Most consumers were married (398) with 108 being singles and 28 being widows.

3.3.2 Gender

It was observed that female consumers were more than the males. This is not surprising since over 80 % of the respondents in the study were females. Generally, market activities are dominated by females and since this study was carried out in predominantly market vicinities, this might have contributed to the results obtained. However, in a similar NULs consumption survey, Mexican male adolescents, were found to consume higher quantities of legumes than females (Ortiz-Hernández and Gómez-Tello, 2008). The contrast may probably be attributed to differences in cultural settings or even the study design, as well as the area of study.

3.3.3 House hold numbers of consumers

It was found that the minimum house hold numbers (Table 3.1) were between 1 to 3 but on the other hand, the maximum house hold numbers was 6 for all consumers of the NULs studied.

Table 3.1: Percentage distributions of consumer characteristics (marital status, gender, occupation and levels of education) relative to NULs consumption.

Characteristics of consumers	*Cajanus cajan*	*Canavalia ensiformis*	*Mucuna pruriens*	*Phaseolus lunatus*	*Vigna subterranea*
Marital status					
Single	17	8	20	16	25
Married	76	85	74	71	72
Widowed	7	7	6	5	3
	100	100	100	100	100
Gender					
Male	5	9	17	3	22
Female	95	91	83	97	88
	100	100	100	100	100
Occupation					
Non-skilled	7	10	5	6	16
Farmer	14	6	7	10	12
Civil servants	0	1	0	0	0
Artisans	2	0	2	1	3
Trader	77	82	81	83	69
Public servant	2	1	5	0	0
	100	100	100	100	100
Education					
JSS	14	19	29	22	26
SSS	2	2	8	4	3
PSSS	3	5	1	3	1
Tertiary	1	1	1	1	1
Non-formal	80	73	61	70	69
	100	100	100	100	100

Again, the modal house hold numbers were between 1 and 2 but the mean was 5. However, the average house hold number has been reported to be between 5 to 6, according to the recent census (Ghanadistricts, 2013). The observation suggest the use of a probability distribution system which would give the true nature of house hold numbers in the study area. The link between house hold numbers and NULs consumption stems from the fact that, NULs are relatively less expensive and easily accessible (Ghanadistricts, 2013). Therefore, the reported low per capita family income (GHS 182.5) within the Atebubu-Amantin study area suggest that many families would probably consume NULs dishes as part of the family diets (Ghanadistricts, 2013).

3.3.4 Educational background

A number of studies have presented NULs consumption as food for the economically poor (Naylor *et al.*, 2004; Oniang'o *et al.*, 2006; Padulosi *et al.*, 2013). However, economically poor consumers are not necessarily people who have no formal education, though there might be linkages. The results obtained from this study (Table 3.1) showed that people with no formal education ate more NULs dishes, relative to people with some level of literacy.

It is seen (Table 3.1) that over 60 to 80% of NULs dishes consumption was patronized by people who had no formal education. On the other hand, consumers with high school to tertiary education, patronized NULs dish consumption at between 14 to 26%. This finding is in agreement with the observation among Mexican adolescents, where the consumption of NULs was higher in individuals with low socioeconomic status as defined by education and economic status (Ortiz-Hernández and Gómez-Tello, 2008). This observation may be linked to the contemporary food consumption trends (Kearney, 2010) probably learnt from schools.

3.3.5 Age of consumers

With respect to age, the consumers of the NULs dishes in the study area showed a mean, mode and median ages of 49.21, 58.64 and 50.48 years, respectively. The uncertainty established for the study area was such that, the ages could be as low as 10 years and as high as 100 year. There were some variations in the ages of consumers among the specific NULs consumption (Table 3.2). The least mean age (40.81 years) was recorded for *Vigna subterranea* consumers and the highest (56.68 years) was recorded for *Cajanus cajan* consumers. The disparities between the overall mean age on one hand, and the specific mean age of consumption of

specific NULs on the other, explains why it is important to catalogue NULs consumptions along specific type of NULs.

Overall, the results shows that the 5th percentile of the respondent population was less than 20 years of age. This means the consumption of NULs was generally very low among teens. It is important to document this, since nutritional or risk assessment can be tailored to specific age groups. These findings are similar to what was observed in a study on the psychosocial factors which influence young adults' intentions to consume legumes (Folta *et al.*, 2006). Even though legumes are nutrient-dense (Drewnowski and Popkin, 1997) and inexpensive (Chivenge *et al.*, 2015) the studies show that teens consume little of it. However, there was notable decline of NULs consumption among consumers who were over 78 years old which is surprising. It may partly be explained that they are less active in the market centres and thus, were not captured in the vicinity of the commercial areas.

3.3.6 Thermal processing times

There are reports of different sensitivities of lectin toxicities in legumes and other crops to man and animals (Dolan *et al.*, 2010). However, it is reported that food proteins denature when cooked at moderate temperatures of between 60-90 ^{o}C for a couple of minutes to 1 h. Therefore, many believe that when lectin (glycoprotein) is heated at a high temperature for relatively long period of time their toxicities would be lost (Damodaran, 1996). It is in this light that the survey was carried out, to evaluate the cooking times of NULs for the evaluation of their safety in a subsequent study.

From Table 3.2, it is seen that the minimum cooking time of all the NULs was 1 h and the maximum cooking time was also 2 h. However, *Cajanus cajan* seeds were the exception because some consumers reported of cooking times of this legumes running up to 5 h. The mean values of the cooking times might explain the type of distribution (Laplace 2.0, 0.24595) of thermal coking time for this NUL. However, the mean, gave two ranges of cooking times and this grouped the NULs under study into two. One group, made up of *Mucuna pruriens*, *Canavalia ensiformis* and *Phaseolus lunatus*, had mean cooking times of between 1.11-1.18 h (Table 3.2). These NULs similarly presented similar distribution of cooking times.

Table 3. 2: Central tendencies and probability distributions of the age, house hold numbers and processing times of NUL seeds in the study area.

NULs	Variables	Probability Distributions	Central tendencies					
			Min	Max	Mean	Mode	Median	Std
Cajan cajan	Age of consumers (year)	Histogram (10,100)	10.003	100.000	55.682	50.361	55.555	20.450
	Thermal processing time(h)	Laplace (2,0.24595)	1.000	5.000	1.878	1.000	2.000	0.447
	Household numbers	Triang (1.793,6,6)	2.000	6.000	5.125	2.000	6.000	1.224
Canavalia ensiformis	Age of consumers (year)	Histogram (10,100)	10.050	100.000	50.508	49.650	51.326	16.324
	Thermal processing time(h)	Pareto (7.7845,1)	1.000	2.000	1.178	1.000	1.000	0.354
	Household numbers	Triang (1.914,6,6)	2.000	6.000	5.267	3.000	4.803	1.111
Vigna subterranea	Age of consumers (year)	Histogram (10,100)	10.001	100.000	40.809	44.795	39.998	18.213
	Thermal processing time(h)	Uniform (0.99038, 2.0096)	1.000	2.000	1.686	1.000	2.000	0.461
	Household numbers	Triang (1.9452,6,6)	2.000	6.000	5.476	3.000	6.000	0.982
Mucuna pruriens	Age of consumers (year)	Histogram (10,100)	10.033	100.000	53.727	55.503	54.670	16.356
	Thermal processing time(h)	Pareto (12.476,1)	1.000	2.000	1.109	1.000	1.000	0.279
	Household numbers	Triang (2.7383,6,6)	3.000	6.000	5.320	3.000	6.000	0.920
Phaseolus lunatus	Age of consumers (year)	Histogram (10,100)	10.006	100.000	51.867	54.190	52.998	16.579
	Thermal processing time(h)	Pareto (12.476,1)	1.000	2.000	1.154	1.000	1.000	0.342
	Household numbers	Triang (0.94735,6,6)	1.000	6.000	5.370	6.000	6.000	0.982

It is not surprising that the other group; *Vigna subterranea* and *Cajanus cajan* respectively, had different mean cooking times of between 1.69 h and 1.88 h and also different distributions of the cooking time.

In a more realistic manner, the modal (most likely) cooking time of 1 h was recorded for all the NULs. This, brings into focus the importance of probability distribution of the parameters into

the evaluations, in order to quantify uncertainties about them and also give a better outlook of cooking times among the consumers.

Other studies have reported different cooking times for some NULs far less than what consumers reported in this study. For instance, a cooking time of 25 min for soaked *Phaseolus vulgaris* seeds boiled at 97 ^{o}C eliminated lectins (Antunes and Sgarbieri, 1980).

However, cooking at 100 ^{o}C for only 10 min also eliminated completely lectins from *Phaseolus vulgaris* seeds at (Bender, 1983). In addition, observation of potentiation (unexpected increase) of lectins activities at a lower temperature (80 ^{o}C) was also made. This suggests that incomplete elimination of lectin toxicity poses risk to consumers. In this study however, longer cooking times of above 1 h (Table 3.2) were reported. Thus, it is suggested that risk of lectin toxicity could be low, provided cooking temperature was close to 100 ^{o}C (Bender, 1983). However, since the exact cooking temperatures were not investigated and reported in this study, it is not possible to conclude on the safety of NULs dishes consumed at this stage.

3.3.7 Exposure assessment metrics

One of the aims of this study was to specifically gather data that could help establish the elements for the evaluation of the exposure to the ingestion of possible hazards from NULs. In the exposure assessment, the parameters to be obtained from consumers include exposure frequency of hazard, mass of NULs consumed per day and body weight of consumers (WHO/FAO, 2009).

It was found that a minimum mass of NULs consumed per day, is as low as 160 g per day for *Phaseolus lunatus, Mucuna pruriens* and *Canavalia ensiformis* through 250 g per day for *Cajanus cajan,* to 300 g per day for *Vigna subterranea*. The maximum mass of NULs consumption ranged from 650 g per day for *Canavalia ensiformis,* to as high as 900 g per day for *Vigna subterranea.* The main central tendency, the mean consumption, ranged from about 380 g per day in *Phaseolus lunatus,* to 600 g per day in *Vigna subterranea* and the probability distributions of the true level of the masses of NULs consumptions are as shown in Table 3.3.
It has been reported by WHO Global Environmental Monitoring System (WHO/GEM, 2012), that pulse consumption rate in Ghana is 237.0 g per person, compared to what was obtained for specific NULs in this study. This value is deterministic and thus, carry uncertainties as well. For instance, the type of pulse is not known, neither the type of dishes they were used to prepare. Also, lack of the probability distribution associated with it, makes the true level of the amount of pulse consumed in Ghana difficult to assess.

Another parameter required in the evaluation of exposure assessment is exposure frequency. From Table 3.3, the weakness of the central tendency metrics are shown once again. For all the NULs consumed, the central tendencies; minimums and maximums, showed the same exposure frequencies as 1 and 24 respectively. The mode also showed a range of the least exposure frequency (3 per month) for *Mucuna pruriens* consumption, but the rest of the NULs showed a high exposure frequency (of above 4 per month) of NULs consumption. The probability distributions obtained in this study adequately describe the nature of the spread of the exposure frequency and increase the reliability of the data. However, it is difficult to discuss the exposure frequencies of the NULs any further due to paucity of data in NULs consumption or any related pulses previously studied.

Table 3. 3: Central tendencies and probability distributions of the elements for the evaluation of exposure assessment of NULs consumption in the study area

NULs	Variables	Probability Distributions	Central tendencies					
			Min	Max	Mean	Mode	Median	Std
Cajanus cajan	NULs consumed (g)	Uniform (250,800)	250.000	800.000	525.000	434.250	524.960	158.780
	Exposure Frequency (month)	Expon (5.0536,0.95488)	1.000	24.000	6.054	4.000	4.000	6.009
	Weight of consumers (kg)	Histogram (41, 100)	41.040	100.000	78.051	83.235	83.235	11.730
Canavalia ensiformis	NULs consumed (g)	Laplace (400, 114.7084)	160.000	650.000	407.780	400.000	400.000	136.590
	Exposure Frequency (month)	Pareto (0.90521,1)	1.000	24.000	4.833	3.400	3.400	5.172
	Weight of consumers (kg)	Histogram (41,100)	41.000	100.000	66.901	66.047	66.047	13.593
Vigna subterranea	NULs consumed (g)	Uniform (294.29,905.71)	300.000	900.000	600.000	600.000	600.000	196.520
	Exposure Frequency (month)	Triang (1,1,26.675)	1.000	24.000	8.849	8.000	8.000	6.554
	Weight of consumers (kg)	Histogram (31,100)	31.000	100.000	63.082	60.978	60.978	13.074
Mucuna pruriens	NULs consumed (g)	Laplace (400,120.1367)	160.000	650.000	400.910	400.000	400.000	141.020
	Exposure Frequency (month)	Pareto (0.68858,1)	1.000	24.000	7.570	3.000	3.000	7.209
	Weight of consumers (kg)	Histogram (41,100)	41.001	100.000	66.190	65.880	65.880	12.903
Phaseolus lunatus	NULs consumed (g)	Laplace (400,119.501)	160.000	650.000	382.170	400.000	400.000	138.460
	Exposure Frequency (month)	Expon (5.725, 0.95229)	1.000	24.000	6.725	4.000	4.000	6.072
	Weight of consumers (kg)	Histogram (0.016, 100)	31.000	100.000	65.095	64.982	64.832	12.397

By expressing the amount of NULs dishes eaten as food consumption per body weight, ingestion of hazards tend to be harmonized (WHO/FAO, 2009). Therefore, body weights of the consumers is another key parameter required for the evaluation of the exposure assessment. Table 3.3 shows a minimum weight of between 31 and 41 kg as the body weights of different NULs consumers, but a maximum of 100 kg was recorded for all of them. The mean weight of consumers ranged between 63 kg for those consuming *Vigna subterranea* to those 78 kg for those consuming *Cajanus cajan* with those consuming *Mucuna pruriens*, *Canavalia ensiformis* and *Phaseolus lunatus* within the limits. Thus, the average body weight of 70 kg adult (Gerba, 1999; Hathcock *et al*., 2007), usually used for nutritional and toxicological impact assessments would vary if the probabilistic distributions of body weights of consumers were used for the evaluations of nutrient or hazard ingestions in food systems and cultural diets.

3.4 Conclusion

The central tendency values required for the exposure assessment of hazards in NULs dishes have been established specifically for the five NULs *Mucuna pruriens, Phaseolus lunatus, Canavalia ensiformis, Vigna subterranea* and *Cajanus cajan*. The uncertainties associated with these central tendency values have been quantified and the statistical distribution established. Alongside the exposure assessment, the amount of specific NULs seeds/dish consumed, exposure frequencies per month and the body weights of respondents consuming them have been established. Also, the exposure assessments of NULs dishes can effectively be linked to other factors such as ages of the individuals consuming it, their characteristics such as house hold numbers, marital status, their educational background and occupation. With the establishment of the exposure assessment of the NULs, estimates of intake assessment of hazards in each NULs can be carried out.

CHAPTER 4: NULS HAZARDS, PROBABILISTIC EXPOSURE ASSESSMENT AND RISKS ASSOCIATED WITH SOME SELECTED DIETARY LECTINS

4.1 Introduction

People use legumes for many different reasons but one of the primary reason is to provide nutrients for the survival of humans and farm animals. Around the globe, many sub-populations have cultivated and consumed their indigenous legumes such as peas, beans, lentils and peanuts (Messina, 1999). The discovery of soy (Kalogeropoulos *et al.*, 2010) and its rapid internationalization as the quintessential legume, drove under, the production and utilization of indigenous legumes. For instance, it contributed to the neglect of established dietary legume culture, bequeathed over successive generations. Other factors include; limited access to market niches and low consumption. It has also been reported that lack of value addition to NULs lead to loss of market premium (FAO, 2004).

There is no denying the fact that legumes contain intrinsic hazards which poses considerable risks after consumption. For instance, protease inhibitors are insecticides (Habib and Fazili, 2007), which abound in legumes, and consequently posing serious threat to consumers due to their effect as pancreatic carcinogens (Roebuck *et al.*, 1985). Similarly, saponins are insecticides (De Geyter *et al.*, 2007), but they also bind to cholesterol in the intestinal mucosa and all other cells, posing a greater risk because the binding action causes cell injuries (Podolak *et al.*, 2010). Moreover, the debilitating effect of saponins become synergistic (Maentz *et al.*, 1999) in the presence of lectins. It has been reported that about 60% of some lectins remain biologically active, even after cooking, and these active lectins bind the intestinal mucosa leading to the "leaky gut" condition (Pusztai *et al.*, 1990).

Apart from intrinsic hazards, there is also documented evidence of pesticide residues, resulting from storage or handling practices of grain legumes. For fear of losing their produce, farmers usually use pesticides to keep insect infestations under control and in so doing, they may use approved or non-approved pesticides. These practices are known to accumulate pesticides in the endosperm of the grains over a period (Uygun *et al*., 2009).

Many traditional caterers use Chile saltpetre (known indigenously as "*Kawu*" or "*Kawe*") to process legume grains for consumption, as they believe it tenderizes the usually hard-to-cook beans. However, these salts are known to produce cancer causing nitrosamines (Song *et al*., 2015). Whether for tenderizing the hard-to-cook beans, or controlling insect infestations, or even if they occur intrinsically, the uncontrollable ingestion of pesticides, additives or phytochemicals, impact adversely on health. Despite the presence of the natural and extraneous hazards, indigenous communities across the world have been resilient in the cultivation of their traditional legumes for their sustenance (Chivenge *et al*., 2015). Indeed, there are reports of increasing interest towards the exploitation of NULs to alleviate malnutrition, in developing countries (Ade-Omowaye *et al*., 2015). By addressing the challenges of the presence of intrinsic and extraneous hazards, it is believed that the full benefits of legumes utilization could be enhanced.

Institutions such as the US Environmental Protection Agency (USEPA, 2002) and the European Food Safety Authority (EFSA, 2012) have defined basic terminologies relating to food quality and safety. These terminologies have been developed to facilitate the guiding principles of the food safety process. The safety of food is dependent on the "acceptable daily intake" of hazards in the food, defined as "an estimate of the amount of a substance in food that can be consumed

over a lifetime without presenting an appreciable risk to health" (EFSA, 2012). A "hazard" on the other hand is defined as "a substance which has the potential to cause adverse effects upon exposure" (USEPA, 2002). It has been declared by Paracelsus (the father of modern toxicology) that "all substances are poisonous and there is none that is a not a poison, it is the dose that determines when it becomes a poison" (Trautmann, 2005).

In order to study the risk analysis process, it is still important to run the dose-response test on suspected hazards, after the prior hazard identification process. The dose-response test, enables the determination of thresholds of hazards. During hazard identification, weight-of-evidence is built to support the fact that the hazard is indeed capable of causing those specific adverse health effects. The characterization of hazards involves the study of the "adverse health effects associated with the agents which may be present in food" (EFSA, 2012). Results from such studies include the identification of the "no observed adverse effect level" (NOAEL), which is defined as, "the greatest dose of a hazard at which no detectable adverse effects occur in an exposed population" (USEPA, 2002). The NOAEL is often obtained by calculation because it is extrapolated (Gerba, 1999; Tennant, 2012). On the other hand, what is easily observed during the dose-response studies is the "lowest observed adverse effect level" (LOAEL). This is "the lowest concentration that is observed to cause harm in an exposed test population" (USEPA, 2002). Before any hazard can cause adverse effect, the hazard must sufficiently accumulate in tissues when consumers are exposed to them. Thus, exposure assessment is carried out to quantify the hazard ingested. Exposure assessment, involves "the quantification of the amount of hazard ingested per body weight of an individual or population exposed to hazard" (USEPA, 2002). To complete the risk assessment, "the likelihood that a particular hazard will cause harm is

calculated in the light of the nature of the hazard, based on the extent of exposure" (USEPA, 2002). Thus, risk assessment involves four steps; hazard identification, hazard characterization, exposure assessment and risk characterization. Hazard quotient, has been used by scientists as a quick way of finding whether the exposed hazard is greater than the acceptable daily intake, also known as the reference dose. A ratio of the average daily dose to the reference dose, giving values of above one (1), has been used to indicate the presence of risk (Gerba, 1999).

A serious advocacy has been made for people to consume legumes(Foyer *et al.*, 2016) including NULs, but there is still uncertainty about the safety of NULs. For example, the cultivation, harvest and storage of NULs are not monitored or controlled since they are regarded as stop gap crops. Also regulatory bodies seem to have no guidelines restricting the ingestion of lectins (Dolan *et al*., 2010).

Legume lectins are particularly resistant to cooking; thus, improper cooking, as might occur in street vendered foods or foods cooked in the field, leave substantial amounts of active lectins in the food. It is often argued that legumes are cooked and lectins being proteins, their biological activities are supposed to be eliminated, but this is not always true because residual lectins have been isolated in many cooked foods (Nciri *et al*., 2016). These residual lectins are best described as hazards, thus, they pose risks. These risks and uncertainties associated with the consumption of NULs dishes have not been quantified, but they must be addressed in order to maintain confidence and sustainability.

This present study sought to determine the respondents' comprehension of hazards and the attendant risks associated with the consumption of NULs seeds. Secondly, the study also aimed

at conducting a probabilistic risk assessment of the intrinsic dietary lectins in some selected NULs dishes.

4.2 Materials and methods

4.2.1 Materials

Plant soybean agglutinin obtained from Gentaur Molecular Products (BVBA, Belgium) was used for the quantitative determination of NULs agglutinin. Samples of NULs were purchased from major markets in the study area. As part of the preparation of samples, protein content of the selected NULs were determined by Kjeldahl procedure (AOAC, 2000) and the results presented in Table 4.l.

Table 4. 1: Protein content of five NULs sampled from the study area.

NULs	*Phaseolus lunatus*	*Cajanus cajan*	*Vigna subterranea*	*Mucuna pruriens*	*Canavalia ensiformis*
% Crude Protein	17.2(±1.2)	21.3(±2.2)	15.2(±1.7)	25.2(±0.2)	17.4(±0.3)

4.2.2 Methods

4.2.2.1 Structured interview schedule on perception of hazard

Dataset on the perception of hazards in NULs consumed in the study area was collected, based on a survey. The survey was designed to study such factors as: the extent of familiarity of NULs, eating habits and the manifestations of associated hazards. Other factors considered included: safety of NULs dishes, perceptions of hazards, reaction to allergens and recommendations relating to the of NULs dishes, regardless of potential hazards. Pre-testing was done using 30 respondents and based on the outcomes, the requisite modifications were effected. The survey

was then administered by trained and experienced assistants within the study area in May 2015 (Appendix E). The data obtained was run in SPSS software (IBM Corp., 2016) to obtain the frequency distributions of variables that were studied.

4.2.2.2 Sampling and sample preparation

Samples of the selected NULs, specifically; *Vigna subterranea, Cajanus cajan, Phaseolus lunatus, Mucuna pruriens and Canavalia ensiformis* were purchased from the five- market centres; Amantin, Mampong, Ejura, Abofuor and Techiman in the study area, between the period of the survey (5^{th} to 20^{th} May, 2014). Each of the five market centres was visited at least twice. About 5 kg each of the beans was bought from each of the two visits made, sorted, pooled and further dried in solar tent dryer (40 ^{o}C) for two days. The dried beans were sampled by quartering to obtain about 1 kg each of representative samples. These were pulverized into flour with Schulte-Buffalo Hammer mill (LLC, W-6-H, US) to 1 µm mesh size and stored in plastic containers pending further analysis.

4.2.2.3 Degradation of NULs agglutinins

A mass of 0.5 g each of the five NULs flours was weighed into a 15 mL Eppendorf tubes after which 5 mL of distilled water was added. The mixture was agitated thoroughly to ensure homogenous mixing. In all, 20 tubes containing samples were prepared, 4 for each flour. A 200-mL saucepan, previously filled with water at half its volume, was placed on a Bosch (PCP615B80E, Germany) gas cooker and set to provide 1.7 kW heat, according to the manufacturer's specification. The cooking times which had been predetermined at 0, 10, 30 and 60 min were set, and at the end of the set times, the differently cooked pasted flours were removed and cooled immediately under running tap. Timing commenced when the temperature

of the boiling water had reached 100 ^{o}C. Using the method proposed by Roy and Das (2015), phosphate buffered saline (pH 7.5) was added to each of the pasted flour to the 10-mL mark and agitated at 250 rpm on a Pro Digital Orbital Shaker (SK-0330, US) overnight to ensure complete homogenization. Samples were initially centrifuged at low speed to sediment as much of the debris, which was discarded. Then, 1.5 mL each of the resulting supernatant samples was centrifuged at 10,000 rpm for 10 min to obtain clear solutions of soluble proteins. From the clear supernatant, 500 µl was transferred into 1.5 mL Eppendorf tubes and then kept at 4 ^{o}C for lectin-based ELISA analysis.

4.2.2.4 Dietary exposure assessment of NULs agglutinins

For hazards in foods, exposure is simply based on the amount of hazard ingested per unit body weight. The amount ingested is the product of the concentration of hazard and the amount of food consumed (Gerba, 1999).

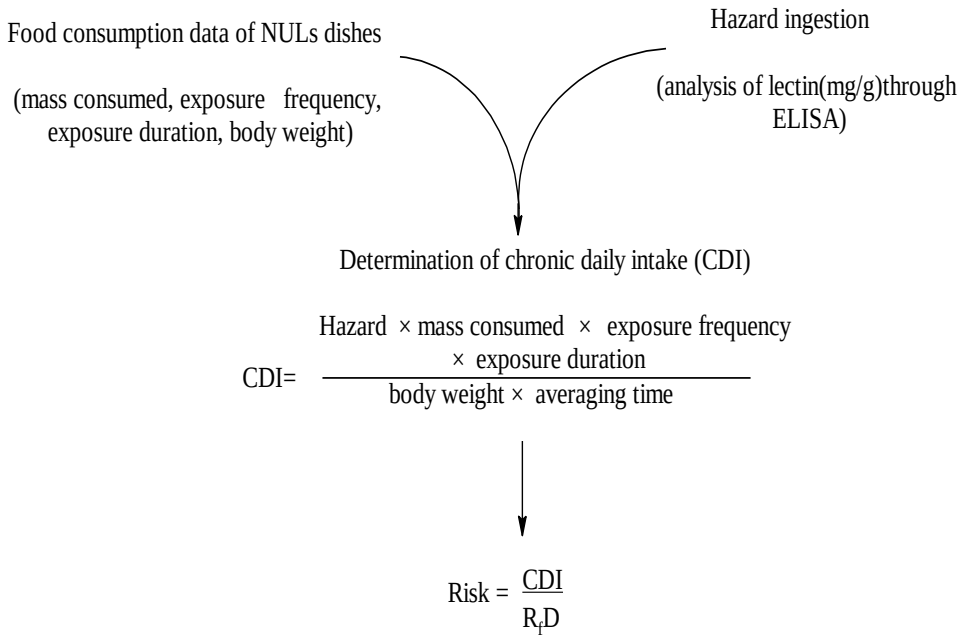
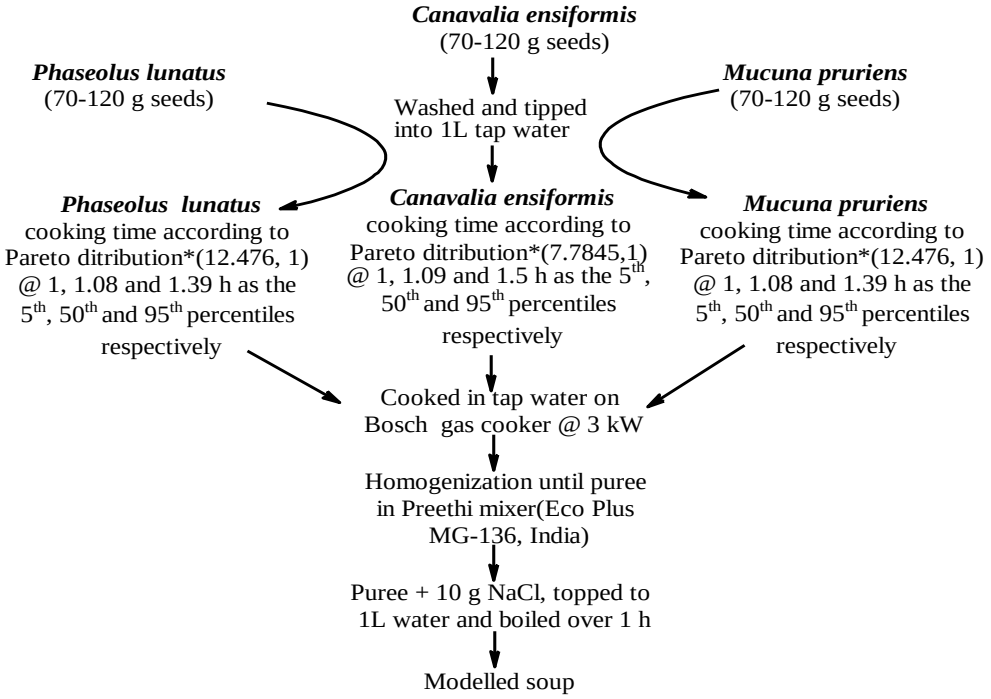


Figure 4. 1: Outline of risk assessment of NULs dishes using their chronic daily intake (CDI) and a proposed reference dose (RfD).

As explained in the outline (Figure 4.1), two separate data were required in the evaluation of the exposure assessment: the food consumption data of NULs dishes and the concentration of the hazards (quantities of agglutinins in NULs dishes) ingested. The quantities of NULs ingested were previously determined, where the central tendencies of the exposure assessment, habitual cooking and eating habits and consumers' characteristics were quantified, together with their uncertainties and statistical distribution functions. Secondly, the levels of the hazards ingested (concentration of agglutinins) was obtained from the analyses of modelled NULs dishes using ELISA quantifications. From the sections that follow, details of the ELISA quantification of agglutinins and the quantities of NULs dishes consumed are described. Subsequently, the risk (in terms of hazard quotient) was calculated as per Equation 4.1 using appropriate reference dose (R_fD).

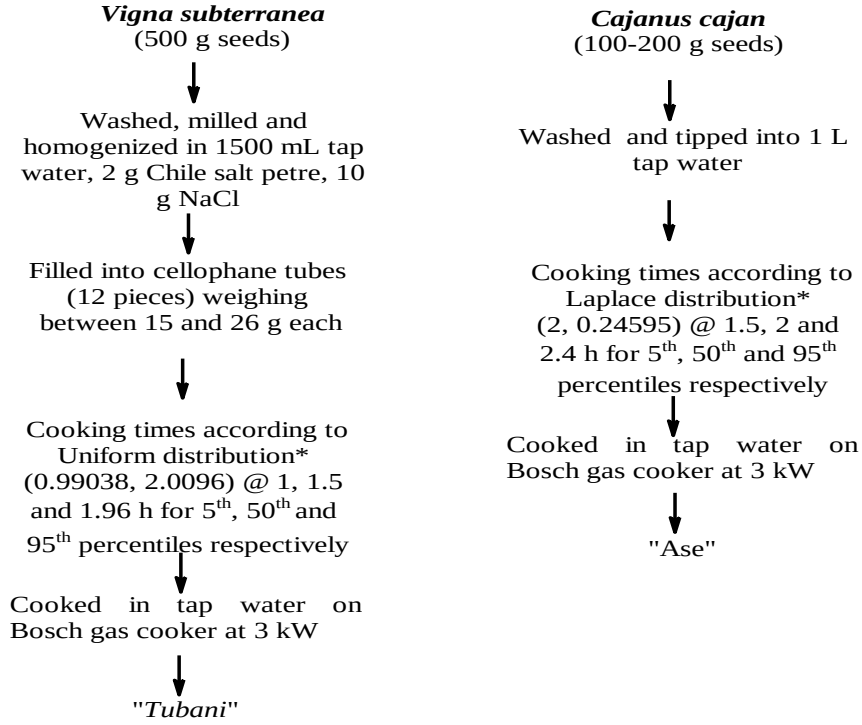
4.2.2.5 Residual agglutinins in model NULs dishes

In this study, NULs based model dishes were prepared according to what was being practised in the field. For the model soups, 18 different masses of between 70-120 g of *Canavalia ensiformis, Mucuna pruriens* and *Phaseolus lunatus* were used and each NUL gave their corresponding modelled soups (Figure 4.2). On the other hand, 23 wrapped pieces each of "*Tubani*" weighing between 15 and 26 g were prepared (Figure 4.3). Similarly, 20 batches of *Cajanus cajan* grains weighing between 100 and 200 g were used to prepare model "*Ase*"(Figure 4.3). Again, using Roy and Das's (2015) method, phosphate buffered saline (pH 7.4) was then added to 5 g sample of the cooked "*Tubani*" and "*Ase*" and homogenized into a total of 40 mL of the solution. On the other hand, 10 mL of the model soup of *Canavalia ensiformis, Mucuna pruriens* and *Phaseolus lunatus* was also homogenized into 40 mL of solution. After agitating overnight at room temperature, 2 mL of the mixture was finally centrifuged at 10,000 rpm for 10 min.



**The statistical distribution of cooking times previously determined*

Figure 4. 2: Preparation of soups based on *Canavalia ensiformis*, *Mucuna pruriens* and *Phaseolus lunatus* according to respondents' cooking practices.



** The statistical distributions of the cooking times were previously determined*

Figure 4. 3: Preparation of "Tubani" and "Ase" based on *Vigna subterranea* and *Cajanus cajan* respectively, according to respondents cooking practices.

The clear supernatant containing soluble proteins was then transferred into 1.5 mL Eppendorf tubes and kept (4 ^{o}C), until needed for the ELISA determination of lectins.

4.2.2.5 ELISA determination of lectin activity

The analysis was based on sandwich enzyme-linked immunosorbent assay procedure, similar to what was described in a study involving *Phaseolus vulgaris* (Boniglia *et al.*,2003). Purified soybean agglutinin antibody already pre-coated onto 96 well plate was used. The standard solution of lectins, test samples (from "*Tubani*", "*Ase*" and soups) and extracts of time - temperature treated NULs flours and blanks were set into the wells. All tests were done in duplicates. The plates were sealed and incubated at 37 ^{0}C for 30 min. Subsequently, the wells were washed five times with phosphate buffer containing 0.05 % Tween 20. An aliquot of 50 µl of horseradish peroxidase (HRP) conjugated anti-SBA antibody as the detection antibody, was transferred into each well, except the control well. The plates were sealed again and incubated at 37 ^{0}C for 30 min after which washing was done as before. Unbound conjugates were washed away with wash buffer. A chromogenic substrate, 3,3',5,5'-Tetramethylbenzidine (TMB) was used to visualize HRP enzymatic reaction, as the TMB was catalyzed by HRP to produce a blue color that changed to yellow after adding acidic stop solution. The optical intensity of the yellow solution was proportional to lectin amount of sample captured in the plate. The OD (optical density) absorbance read was at 450 nm in a SpectraMax Microplate Reader (Plus 384, US) within 15 min. Calculation of lectin content was done as;

$$\text{Relative OD 450 nm} = \text{OD at 450 nm of each well} - \text{OD at 450 nm of control well.}$$

A standard curve was obtained as the OD of each standard solution plotted, against the respective concentration of the standard solution which gave a regression, r^2 of 0.983. The lectin concentration of each sample was then determined from the standard curve, and appropriately multiplied by the dilution factor to obtain the true concentration.

4.2.2.6 Probabilistic modelling of the exposure of lectins in modelled dishes

Since the risk of lectin in foods is only by the oral route or pathway it was estimated based on the USEPA standard procedure for computing the Hazard Quotient (HQ), otherwise known as the non-cancer risk-equation for systemic toxicity (USEPA, 2001). Equation 4.1, integrated all the variables needed to calculate the HQ for the agglutinins. The concentration and contact rate of agglutinins is expressed, respectively as; C_L and C_R. The contact rate is actually, the total mass of NUL-based dish consumed per day. The body weight is given as B_w, whereas the reference dose is denoted as R_fD. If HQ is greater than unity, then non-carcinogenic, systemic toxicity risk is certain. Sources of each dataset of residual lectin concentrations (C_L,) in each NULs dish were obtained from ELISA determination. However, the contact rate (C_R), exposure frequency per month (EF) and the body weights (B_w) of consumers of each of the five NULs dishes were supplementary data established in a previous study (Ofosu *et al.*, 2017).

$$HQ = \frac{C_L \times C_R \times EF \times ED}{R_fD \times B_w \times AT} \quad .....4.1$$

The determination of HQ of each NULs dish for an exposure duration (ED) of one year, was done using Palisade@Risk (Palisade, 2014) Microsoft Excel (Microsoft, 2014) plug-in by integrating the distribution functions of the factors in Equation 4.1 and reference standards. Simulation was run at 100,000 iterations and the final HQs for each NUL dish were recorded. For systemic non-cancer toxic substance as lectins, 30 years was used as the averaging time (AT)

(Gerba, 1999). For this particular study, a probable threshold dose (R_fD) was assumed, based on a NOAEL of 50 mg/kg-day in animal studies recently reported (Ferriz-Martínez *et al.*, 2015). In order to use this value as a human safety factor, an uncertainty factor of 10^2, derived as conversion from animal to man, 10A, and leveraging for all humans, 10H (Barnes *et al.*, 1988) was used to harmonize the dose. The basis of the use of this reference dose stemmed from the fact that monomers of legume agglutinins are homologous and structurally well conserved (Loris *et al.*, 1998). Thus, agglutinins such as; concanavalin A, PHA-L, from pea, peanut and soybean, would probably deliver similar adverse effects in humans since, they are members of the same family.

4.3 Results and discussion

4.3.1 Familiarity with beans and frequency of consumption

A total of 118 consumers were surveyed, of which 32.4% were male and 67.6% were female. The respondents were all above 10 years of age but the majority (69.6%) were above 40 years. Majority of respondents (59.4%) were familiar with all the NULs (Figure 4.4) in the study area.

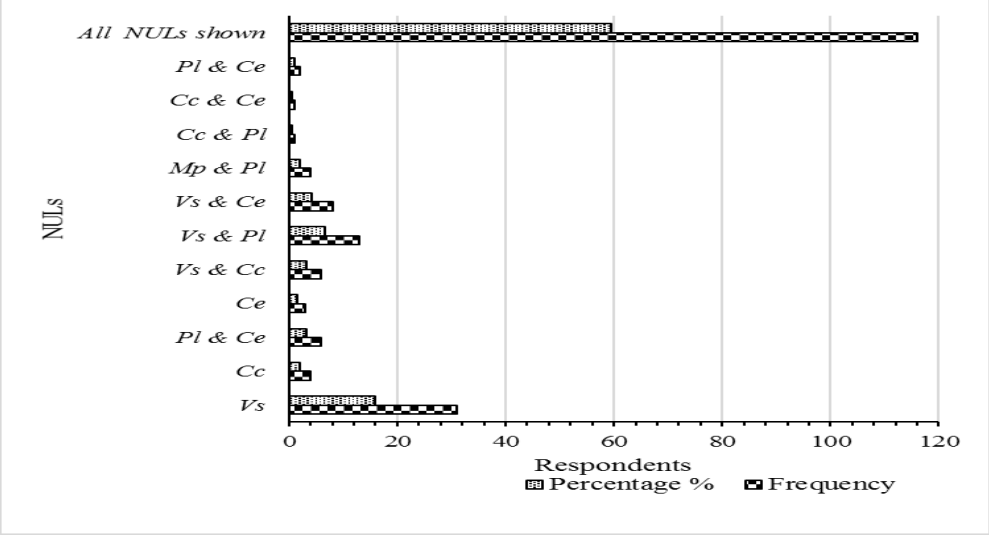


Figure 4. 4: Familiarity with NULs consumed among respondents in the study area.

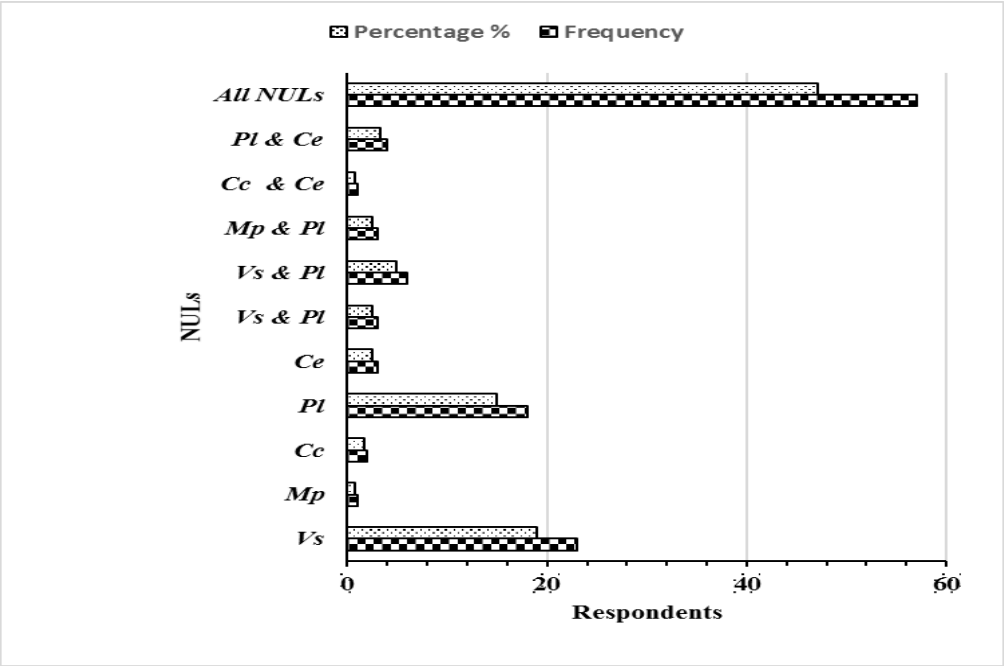


Figure 4. 5: NULs frequently consumed by respondents in the study area.
NB *Pl =Phaseolus lunatus, Ce=Canavalia ensiformis, Cc= Cajanus cajan, Vs= Vigna subterranea, Mp= Mucuna pruriens*

Figure 4.5 present respondents and the specific NULs they actually consume. Respondents who consumed a combination of two NULs (21.1%), was lower than those who consumed all the NULs (59.4%). Also, consumption of *Vigna subterranea* (15.9%) and *Phaseolus lunatus* (14.95%) were the most popular among the NULs. The results corroborate other studies which have reported that *Vigna subterranea* has been used in the preparation of dishes such as "*Koose*" (fried bean flour), and "*Tubani*" (Plahar *et al*., 2002). The popularity of *Phaseolus lunatus*., *Canavalia ensiformis* and *Mucuna pruriens* lie in their use as soup thickeners (Osei-Bonsu *et al*., 1995). However, the low levels of the utilization of boiled *Cajanus cajan* seeds (1.7%) was to be expected because "*Ase*" served with "*Gari*" compete directly with the popular cooked cowpea seeds (also served with "*Gari*"). In addition, cooked cowpea is largely served with fried plantain (Phillips and McWatters, 1991).

Table 4. 2: Perception of hazard and types of hazards in NULs consumed by respondents.

		Frequency	Percentage %
Perception of hazards in beans	Yes	115	66.1
	No	59	33.9
	Total	174	100
Type of hazards in beans		Frequency	Percentage %
Pesticides		74	58.7
Pathogenic bacteria		7	5.6
Food additives		5	4.0
Food allergens		2	1.6
Pesticide residue and pathogenic bacteria		1	0.8
Pesticide residue and environment contamination		2	1.6
Pesticides and food additives		21	16.6
All the hazard presented		14	11.1
	Total	126	100

Out of 174 respondents who were interviewed on their perception of hazards in NULs, over 66% suspected the presence of hazards in beans (Table 4.2). However, out of 126 respondents who provided information on the type of hazard in beans, 58.7% cited pesticide residues as the single most common hazard. Interactive combination of food additives and pesticides residues (16.6%) were cited as the second most common hazard. This observation was not out of place, because the legumes are likely to be consumed by insects and pests, if not controlled (Donkor *et al.*, 2015). In addition, consumers use chemicals which act as cooking aids, such as Chile saltpetre, ($NaNO_3$) ("*Kanwe*"/"*Kawu*") to hasten the process of cooking hard-to-boil beans (Plahar *et al.*, 2002). According to the respondents, the adverse impact of all the other hazards, such as contamination with pathogenic bacteria and food allergens were marginal (Table 4.2). In-depth interviews with respondents showed that the respondents were however, spilt over the adverse impact of Chile saltpetre. Some insisted that it was safe to use but others questioned its safety. It is not clearly understood why some respondents (16.6%) perceive Chile saltpetre as interactively hazardous when combined with pesticide residues. The reasons are not clear because it is

doubtful if the hazardous nature of nitrosamines (Song *et al.*, 2015) was already known to a majority of respondents who barely had knowledge on food toxicological experts. But indeed, nitrates are unsafe, since studies show that individual nitrite dietary intake of between 0.7% and 16.4% for adults and also between 10.5% to 66.2% for children are higher than the ADI (Menard *et al.*, 2008). Respondents who attributed the hazards in the beans to pathogenic bacteria (5.6%) were more than those who attributed the hazards to the presence of food allergens (1.6%, Table 4.2). This finding, supports the observation that only a small fraction of the population suffer from food allergies (Hadley, 2006). The response on perception of bean safety (66.1%, Table 4.2) was overwhelmingly in favour of those who consider NULs as unsafe. Thus, it was not surprising why 97.2% of respondents complained about discomforts after consuming NULs dishes (Figure 4.6). These gastrointestinal distresses have been reportedly linked to the presence of lectins (Pusztai and Bardocz, 1996). What this mean is that majority of the respondents show probable symptoms of lectin poisoning, because symptoms such as nausea, vomiting, or diarrhea have been reported to occur within three hours of ingestion (USFDA, 2000).

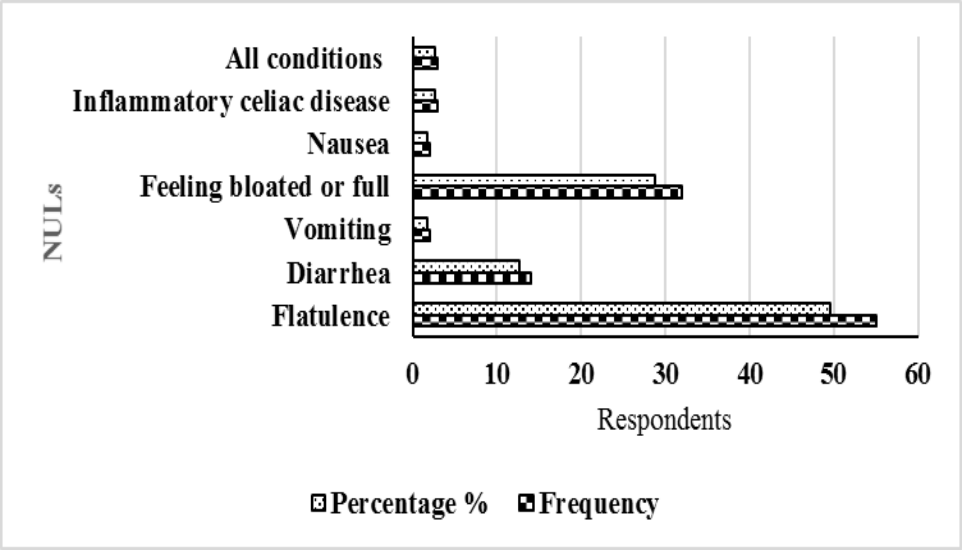


Figure 4. 6: Types of discomforts resulting from the consumption of NULs among respondents.

Table 4. 3: Responses on recommendation of NULs for consumptions and assessment of complaints.

Questions	Responses	Frequency	Percentage %
Will you recommend beans to others?	Yes	182	97.3
	No	5	2.7
	Total	**187**	**100**
Reasons for recommendation	Nutritious	179	99.4
	Discomforts	1	0.6
	Total	**180**	**100**
Why would you not recommend?	Contain hazards	10	55.6
	Stomach discomforts	8	44.4
	Total	**18**	**100**
Any complains of discomforts from others?	Yes	154	82.4
	No	33	17.6
	Total	**187**	**100**
Are the complains believable?	Yes	149	89.5
	No	12	7.5
	Total	**161**	**100**
Assessment of complaints	Heard people react	148	93.7
	People are simply exaggerating	1	0.6
	Don't know	8	5.1
	May be	1	0.6
	Total	**150**	**100**

It is also possible that many of the consumers in the study area may be showing tolerance to the levels of agglutinins present in the NULs. This observation may also indicate that consumers eat NULs as a stop gap measure.

In spite of these discomforts (97.3%), or the belief that they contain hazards (55.6%), the respondents indicated that they would recommend the consumption of NULs to others, simply because they are nutritious (99.4%, Table 4.3). This recommendation would be done even though, they have heard others (82.4%) complain about these discomforts and that they (89.5%)

know the complains were believable. It is difficult to comprehend the basis of such respondents' recommendation because of the complex and dynamic nature of population.

Table 4. 4: Reasons for continuous consumption of NULs among respondents.

Reasons	Frequency	Percentage %
It is nutritious, so it is safe	63	34.4
My parents eat, and everybody eats it the community, so it is safe	6	3.3
My parents eats it, and it is nutritious, so it is safe	26	14.2
Everybody eats it in my community, and it is nutritious, so it is safe	28	15.3
All the reasons above	60	32.8

Evidence buttressing peer pressure from the community lie in the notion that everybody in the community eat them (15.3%) and particularly, the respondents' parents eat them because they were nutritious (14.2%) (Table 4.4). The highest reported discomforts of consuming NULs dishes was largely that of flatulence (49.5%) (Figure 4.6). A small number (2.7%, Table 4.3) of respondents, however, indicated they would not recommend NUL dishes to others because they are unsafe.

4.3.2 Effect of cooking on agglutinins in NULs flour

The agglutinin content of native NULs (Figure 4.7), ranged between 64 mg/g in *Phaseolus lunatus* up to 414 mg/g in *Canavalia ensiformis*. The lectin content of legumes has been reported to vary depending on geographical location and other factors (Barampama and Simard, 1993). In this study, the geographical area covered the forest and savanna regions, providing different edaphic and ecological factors. Wild beans use these powerful agglutinins mainly for defensive purposes (Lannoo and Van Damme, 2014), probably depending on the degree of adaptation needed to fight off diseases. This might account for the variabilities in the contents of lectins.

After 10 min of cooking, NULs agglutinins had not inactivated over 200% as has been reported (Dolan *et al.*, 2010). Within this period, the agglutinins in *Phaseolus lunatus* and *Mucuna pruriens* had rather been potentiated at 20% and 9% respectively. On the other hand, agglutinins in *Cajanus cajan, Canavalia ensiformis* and *Vigna subterranea* had been inactivated at only 22%, 7% and 1% respectively. This observation is in support of studies that have also reported evidence of potentiation (USFDA, 2000). However, the boiling temperature of the flour in this study (100 ^{o}C), was well above what was used to inactivate agglutinins in other studies that was run at 80 ^{o}C (Dolan *et al.*, 2010). From Figure 4.7, after 30 min cooking, the agglutinins in *Canavalia ensiformis* had rapidly inactivated (90%) compared to *Vigna subterranea* agglutinins which had inactivated the least (6%). At the end of the 60 min of cooking, all the different of NULs were still showing varied residual lectins. Agglutinin levels of 60 mg/g and 70 mg/g of *Canavalia ensiformis* and *Vigna subterranea* were still remaining.

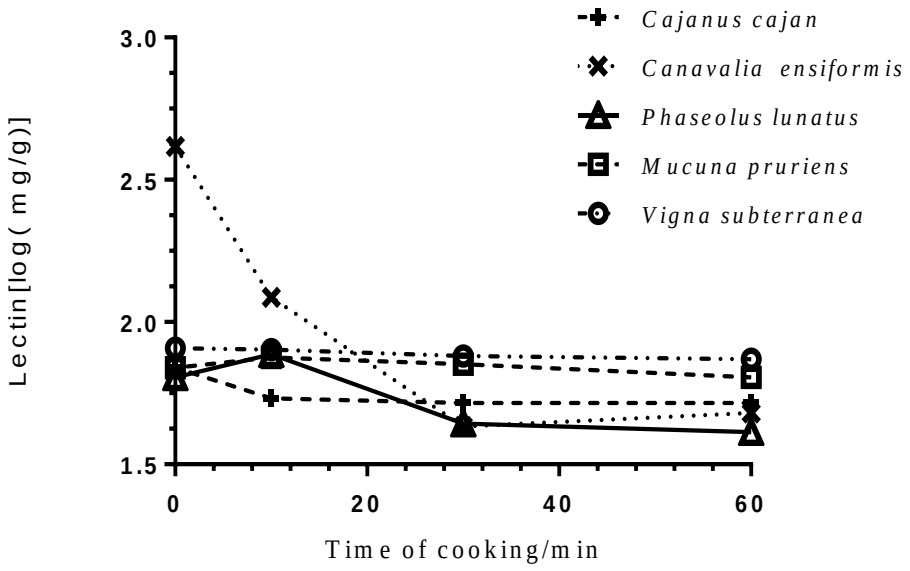


Figure 4. 7: Inactivation of lectins of five NULs flours.

Indeed, dry or moist heating of seeds at 70 ^{o}C for several hours has been reported to have little or

no effect on their lectin activity (Pusztai and Grant, 1998). The variability of the hardiness towards heat treatments, places NULs lectins in this study, into two groups; those which decomposed rapidly on one hand, and those that resist decomposition, on the other. The suggestion that soybean agglutinins (SBA) unfold by dual stage pathways, in both the monomeric and tetrameric states (Sinha and Surolia, 2005), might be applicable to the agglutinins present in other NULs.

The inactivation of *Canavalia ensiformis* and *Phaseolus lunatus* lectins were quite rapid (Figure 4.7) only in the first few minutes. This observation might probably be attributed to weak subunits stability among the multivalent binding sites (Weis and Drickamer, 1996). From Figure 4.7, the pattern of thermal degradation might suggest similar structural organizations in *Mucuna pruriens* and *Vigna subterranea* on one hand and *Cajanus cajan*, *Phaseolus lunatus* and *Canavalia ensiformis*, on the other hand, hence their similar pattern of degradation. It is important to understand the degradation patterns of these lectins since such knowledge would contribute to understanding the mechanisms of inactivating them, thus, offering means of inactivation.

4.3.3 Safety of NULs dishes

From Figure 4.8, the hazard quotients (HQ) of all the agglutinins derived from model dishes are shown. All the lectins in the modelled dishes presented hazard quotient several folds greater than the threshold of one (1) (USEPA, 2001). This means risk is implicated since dishes of these NULs were prepared according to the processing practices in the area. There were high levels of risk of lectin ingestions as reported in this study, but serious consumer-reported lectin-induced symptoms were not observed on the field probably because of tolerance or adaptation to such

diets. Reasons could be adduced from the indicated tolerance of newly weaned male Sprague-Dawley rats fed with diets of casein containing 0.2% peanuts lectins (Henne *et al.*, 1990). The argument is that it is doubtful whether such high levels of lectins ingested in the diets of consumers in the study area would still go unnoticed or only present itself as flatulence, if respondents are not well adapted to such high-lectin laden diets. In spite of tolerance to such diets, there could be serious adverse health effects among the small group of susceptible individuals in the study area.

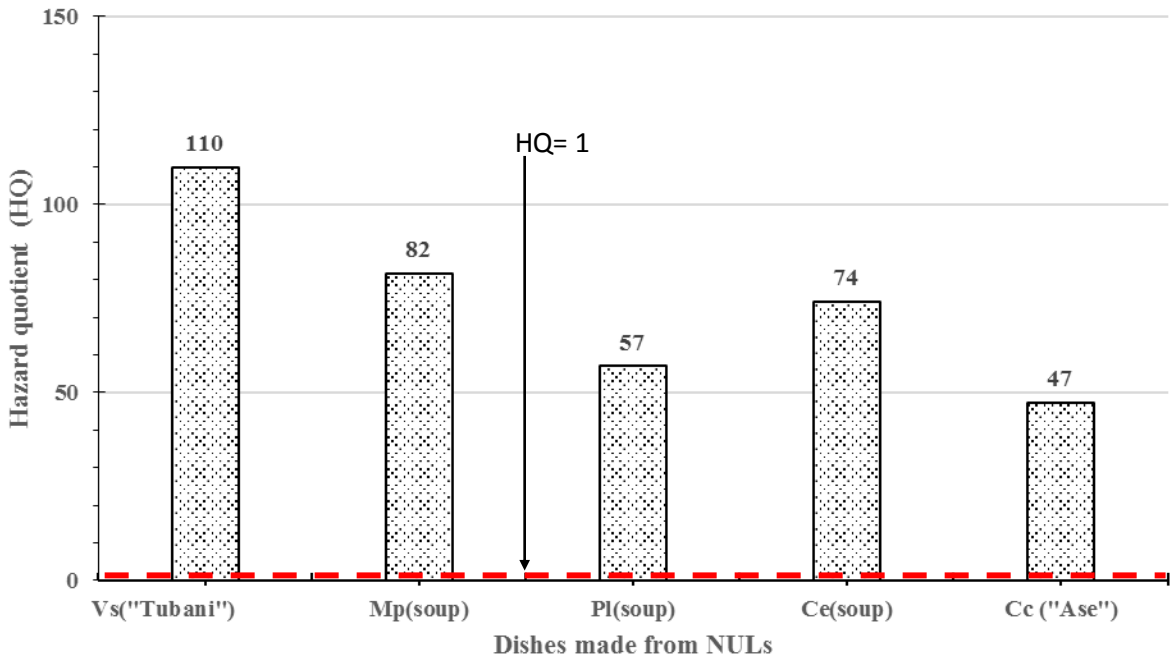


Figure 4. 8: Hazard quotients of the agglutinins of five NULs model dishes cooked according to prevailing practices of respondents in the study area.
(NB: Pl =*Phaseolus lunatus*, Ce=*Canavalia ensiformis*, Cc= *Cajanus cajan*, Vs= *Vigna subterranaea*, Mp= *Mucuna pruriens*)

4.4 Conclusion

Majority of the respondents (67.6%) were over 40 years and were familiar (59.4%) with and consumed NULs (59.4%) dishes. The most popular dishes were obtained from *Vigna subterranea* (15.9%) and *Phaseolus lunatus* (14.95%). Thus, particular attention must be given to these NULs to make dishes prepared from them safe for consumption. Very little is known about intrinsic hazards, let alone considering them as threat in NULs dishes. Since majority (66%) perceived the presence of hazards in NULs, citing pesticide residues (58.7%), it means farmer storage and handling practices are probably inadequate. Respondents also considered pesticide residues and their interactive combination with food additives (16.6%) as significantly dangerous. This is a matter of concern because little has been studied by way of their hazard identifications and potential adverse health effects. The seeming inconsistency on the part of the majority (66.1%) that considered NULs as safe, while at the same time complain of discomfort (97.2%) after consumption, shows the low level of premium consumers placed on food safety. Thus, they would recommend the use of NULs to others once they consider them as food and nutritious. Since significantly high quantities of agglutinins remained in NULs flours even after 1 h cooking, leading to an HQ greater than 1, it means consumers are at risk of systemic toxicity. Thus, a better way of processing must be found, to completely eliminate the lectins in order to make the consumption of NULs safe, if the goal of making them food security crop could be achieved.

CHAPTER 5: ESSENTIAL AMINO ACID QUALITY PROFILE OF NEGLECTED AND UNDERUTILIZED LEGUMES (NULs)

5.1 Introduction

In the past, pre-historic man lived a substantially vegetarian life much against what was previously believed (Hardy *et al.*, 2012). People still continue to maintain vegetarian lifestyles, either for religious purposes or for health reasons (Singh *et al.*, 2007a). Many people, though, continue to derive their protein sources from eating meat (Ruby *et al.*, 2016).

In the recent past however, scientific studies have provided evidence in support of the risks associated with the consumption of animal products (Brinkman *et al.*, 2014; Key *et al.*, 2004; Tuso *et al.*, 2013). It is this belief that drives consumers to search for alternative sources of protein. The search for alternative protein sources have led to a number of studies into single cell proteins (Nasseri *et al.*, 2011; Saeed *et al.*, 2016). The production of single cell proteins has its own problems such as indigestibility of some of the proteins, and high levels of nucleic acids, resulting in the production of uric acid (Adedayo *et al.*, 2011).

Other studies also suggest plant-based proteins as a possible alternative protein resource. However, plant products alone are not able to fully provide the quality and the quantities of amino acids needed to support the nutritional needs of consumers (Ghadge *et al.*, 2008). It is therefore, no surprise how the question of eating or not eating meat, has divided many communities (Ruby *et al.*, 2016).

In spite of the seemingly inadequate protein quality of plant products, in many of our communities, consumers depend on NULs as a major food resource (Adu-Dapaah and Sangwan,

2004). These indigenous NULs are cultivated and consumed in many forms, and the various end uses, are signals to the extent to which traditional legumes are deeply imbedded in the cultural diets of the people (Quasem *et al.*, 2009). The cultivation of these indigenous legumes persists because they do not require any extra agricultural inputs that have cost implications for the subsistent farmer. It is for these reasons that the frontiers for protein resources should be expanded to cover these indigenous legumes which currently have very little industrial applications.

A number of processes for improvements of protein quality, such as genetic engineering and traditional crop breeding (Falco *et al.*, 1995; Galili *et al.*, 2002; Luciani *et al.*, 2005; Roesler *et al.*, 1997; White *et al.*, 2001) have been developed. However, additional gains can be made when some resources are committed to study the factors impeding wider utilization of neglected and underutilized crops.

Generally, to ascertain the quality of protein, the essential amino acid profile, together with the digestibility and bioavailability of amino acids need to be investigated (FAO/WHO, 1990). Several techniques are available for the evaluation of the quality of proteins. These include chemical score and protein digestibility corrected amino acid score (PDCAAS)(Schaafsma, 2000). Chemical score defines the protein quality by comparing the most limiting amino acid to the same type of amino acid in a standard or reference protein. The PDCAAS is obtained when the chemical scores are multiplied by the protein digestibilities (%). These techniques are theoretical quantification methods that suggest potential availability of test amino acids. For a long time, PDCASS has been used to evaluate protein quality. However, new studies have

indicated the limitations of this method of protein evaluation. For example, in the determination of PDCASS in animal model, it is the fecal crude protein digestibility that were made, which more often, overestimate proteins that are digested or absorbed. Also, with PDCASS, values of protein quality are often truncated to a ratio of 1 or 100% as the maximum. However, a new method of protein evaluation has been recommended as digested indispensable amino acid score (DIAAS). DIAAS is usually determined on the basis of the lowest amount of the digestible dietary indispensable amino acid per unit of the dietary protein. It is defined as the "the ratio of milligram of digestible dietary indispensable amino acid in one gram of the dietary protein to the milligram of the same dietary indispensable amino acid in one gram of the reference protein (FAO, 2013)". This method uses individual amino acid digestibility analyzed at the end of the small intestine (or ileum) before it becomes fecal matter. The determinations, using this ileal matter, thus, increases the accuracy of the method.

As a food security resource, NULs are increasingly gaining acceptance because their production requires less water, land and energy, compared to farmed animals (Chivenge *et al.*, 2015). There is also the influenced of a growing consumer market demand for plant-based products (MIG, 2013) such as meat substitutes and non-diary milk from high-protein plants. It is been established that soybeans have amino acid profile comparable to that of animal protein (Hasler, 2002). However, the DIAAS of proteins of most NULs and their quality evaluated against standard thresholds of DIAAS for infants, children and adults are yet to be determined.

Studies have shown that large population of consumers are reckoned consume greater quantities of plant proteins (58%), compared to animal proteins (42%) (FAOSTAT, 2009). It is therefore

important to devote attention to the study the amino acid quality of NULs, to provide information for policy decisions with respect to the provision of quality protein for the sustenance of the different age groups that consume them. Such studies must take into account the recommended DIAAS for dietary indispensable amino acids for infants, children and adults. The objectives of the study were thus, to profile and evaluate the essential amino acids of five NULs, against DIAAS calculated on the basis of published data.

5.2 Materials and methods

5.2.1 Materials

O-phthalaldehyde (OPA), Fluorenylmethyloxycarbonyl chloride (FMOC-Cl), 3-Mercapto propionic acid and 18 standard amino acids mixture were purchased from Fluka Sigma Aldrich (St Louis MO, USA). The seeds of five types of NULs were purchased from markets in towns in Brong Ahafo and Ashanti Regions; Techiman, Ejura, Amantin, Drobo, Abofuor and Mampong.

The seeds were cleaned and solar-dried at an average temperature of 40 ^{o}C for two days. After pulverizing with Schulte-Buffalo Hammer mill (LLC, US) to 1 µm mesh size flour, they were packaged separately in plastic bags. Kjeldahl protein analyses had previously been determined (Table 4.1) on the five NULs to obtain protein contents of *Vigna subterranea* (15.2%), *Cajanus cajan* (21.3%), *Mucuna pruriens* (25.2%), *Phaseolus lunatus* (17.2%) and *Canavalia ensiformis* (17.4%).

5.2.2 Methods

5.2.2.1 Determination of amino acid composition

Accurately weighed 0.1 g quantities of each flour was separately digested in pyrex test tube at 105 ^{o}C, for 24 h in 6 mL 6 N HCl (containing 0.1 % β-mercaptoethanol + 0.1 % phenol) (Pickering and Newton, 1990). The digested samples were treated with OPA (Bartolomeo and Maisano, 2006), and subsequently separated on HPLC with phosphate (constant phase) and acetonitrile/methanol /H_2O (gradient). A gradient of 0%, 0% through 53% to 100%, 100% and ending at 0%, 0% at definite time intervals (0, 2, 12, 16, 18, 20, 24 min) was effective. Subsequently, an aliquot of 100 µL of digested samples was added to 100 uL of 0.4M borate buffer (pH=10.2) for 30 s. To this solution, 100 ul each of OPA reagent and 15 Mm FMOC-Cl (in acetonitrile) were added for derivatization to occur. Distilled water (600 uL) was added and 100 uL aliquot, run on a reverse phase C18 silica column (4.6 x 150 mm with a particle size of 5 µm) HPLC system (Cecil Adept with Shimazu 10 Axl, UV detector (λ=338 nm) at 40 ^{o}C at a flow rate of 1 mL/min (mobile phase A (40mM NaH_2PO_4/Na_2HPO_4 (1:1) buffer pH=7.8) and mobile phase B (AcN/MeOH/H_2O (45/45/10 v/v/v)).

Tryptophan was determined, after alkaline hydrolysis at 120 °C for 12 h (Çevikkalp *et al.*, 2016). The hydrolysates were filtered and the separation was performed using a mobile phase of acetonitrile and acetate buffer at pH 6.3 (1:9, v/v). The amino acids present were identified by matching their peaks (Appendix G1) with their standard retention time, and subsequently expressed as mg per g crude protein.

5.2.2.2 Statistical analysis

A one-way analysis of variance was done to determine if there were statistically significant differences among the mean mg/g amino acids of the samples of NULs under investigation

(Appendix H). Statistical significance was set at $p \leq 0.05$. Fischer pairwise comparison test was also determined (Appendix H), using the Minitab software (Ryan *et al.*, 1994). A one-sample t-test was run to test the means of the five NULs seeds against the mean thresholds of the dietary indispensable amino acids for infants, children and adults. The null hypothesis test was set to accept the mean (μ), if $\mu \geq$ hypothetical mean (reference thresholds), while the alternative hypothesis was set to reject the mean (μ), if $\mu <$ hypothetical mean (reference thresholds).

5.2.2.3 Calculating chemical and the DIAA scores

The chemical scores of the essential amino acids (EAA) of each NUL seed was calculated, using (FAO/WHO, 1990) reference amino acids as in Equation 5.2.

$$\text{EAA} = \frac{\text{mg of limiting EAA in NUL seed}}{\text{mg of limiting EAA in same standard/ reference protein}} \times 100 \quad \text{.....5.2}$$

The digestible essential amino acid (DEAA) ratio of each NUL seed was calculated, using the recommended FAO protocol (Mougham *et al.*, 2011) Equation 5.3.

$$\text{DEAA} = \frac{\text{mg of digestible essential amino acid in 1 g protein of NUL}}{\text{mg of digestible essential amino acid in 1 g protein of reference protein}} \quad \text{..... 5.3}$$

This study used digestibility ratio of each individual NUL of minimum digestibility value of 0.72 (Han *et al.*, 2007). To estimate the digestible essential amino acid of reference proteins, a reported true ileal digestibility of each of the referenced soybean meal protein's essential amino acid (Appendix G2), were used (Kong *et al.*, 2014). Also, a supplementary data of the overall mean essential amino acid content of soybean from Brazilian states, were used as reference

amino acid. They had been reported by Goldflus *et al.*(2006) as; arginine (27.06 mg/g), phenylalanine (19.26 mg/g), histidine (9.68 mg/g), isoleucine (16.06 mg/g), leucine (28.38 mg/g), lysine (23.1 mg/g), methionine (0.405 mg/g), threonine (13.15 mg/g) and valine (16.62 mg/g). Soybean reference for tryptophan was taken as 57 mg/g (Carrera *et al.*, 2011). The representative DIAAS of each of the five NULs was subsequently calculated based on recommended FAO (Moughan *et al.*, 2011) Equation 5.4.

$$\text{DIAAS (\%)} = 100 \times \text{lowest value of digested essential amino acid ratio(DEAA)} \quad \text{.....5.4}$$

5.3 Results and discussion

5.3.1 Essential amino acid composition

The results (Table 5.1) show that, of the nine essential amino acids determined, only five showed adequate quantities when matched against dietary indispensable amino acids for infants, children and adults. By definition, essential amino acid (EAA) score is based on the comparison of the concentration of the first limiting essential amino acid in the test protein, to the amino acid in a reference or standard protein (Schaafsma, 2000). Based on this premise, the essential amino acid (EAA) scores showed that, all the NULs were limiting in tryptophan (Figure 5.1). Thus, depending on the reference amino acid used, EAA score increasingly improved as the recommended threshold changed from infant through children to adult reference.

Table 5. 1: Essential amino acid composition (mg/g, mean (±S.D.), n = 3) of five NULs samples matched with dietary indispensable and recommended amino acids for infants, children and adults.

	Sources of protein					Reference amino acids		
EAA	*Cajanus cajan*	*Vigna subterranea*	*Mucuna pruriens*	*Canavalia ensiformis*	*Phaseolus lunatus*	Infants	Children	Adults
His	$8.9(\pm0.2)^{B}$	$26.2(\pm0.1)^{A*\wedge!}$	$3.9(\pm0.2)^{D}$	$4.5(\pm0.3)^{C}$	$2.9(\pm0.2)^{E}$	21.0	20.0	16.0
Ile	$3.9(\pm0.1)^{C}$	$35.9(\pm0.3)^{A\wedge!}$	$3.1(0.3)^{D}$	$6.8(0.2)^{B}$	$3.0(\pm0.1)^{D}$	55.0	32.0	30.0
Leu	$10.6(\pm1.3)^{C}$	$34.0(\pm0.1)^{A}$	$4.8(\pm0.1)^{D}$	$15.6(\pm3.5)^{B}$	$12(\pm1.2)^{C}$	96.0	66.0	61.0
Lys	$10.5(\pm0.3)^{C}$	$53.6(\pm3.3)^{B!}$	$3.7(\pm1.1)^{D}$	$13.3(\pm0.3)^{C}$	$63.1(\pm3.1)^{A\wedge!}$	69.0	57.0	48.0
Met + Cys	$3.5(\pm0.2)^{C}$	$18.7(\pm0.5)^{A}$	$2.2(\pm0.3)^{CD}$	$11.3(\pm0.3)^{B}$	$2.6(\pm0.3)^{D}$	33.0	27.0	23.0
Phe + Tyr	$3.6(\pm0.2)^{D}$	$67.6(\pm5.5)^{A\wedge!}$	$5.6(\pm0.3)^{D}$	$21.4(\pm3.3)^{B}$	$15.5(\pm1.4)^{C}$	94.0	52.0	41.0
Thr	$3.1(\pm0.3)^{C}$	$27.7(\pm0.3)^{B!}$	$2.1(\pm1.0)^{C}$	$2.1(\pm0.3)^{C}$	$56.4(\pm2.2)^{A*\wedge}$	44.0	31.0	25.0
Trp	$1.1(\pm0.2)^{C}$	$2.6(0.2)^{A}$	$1.1(\pm0.3)^{C}$	$1.2(\pm0.1)^{C}$	$1.8(\pm0.5)^{B}$	17.0	8.5	6.6
Val	$10.1(\pm2.3)^{B}$	$23.5(\pm0.3)^{A}$	$3.7(\pm0.2)^{C}$	$5.2(\pm0.3)^{C}$	$4.7(\pm0.3)^{C}$	55.0	43.0	40.0

The EAA showing means with their standard deviations. Means with some alphanumeric along the row, show no significant differences ($p>0.05$). Notations: (*) significantly ($p<0.05$) ≥ Infants (infant up to 6 months), (^) significantly ($p<0.05$) ≥ Children (children up to 3 years), (!) significantly ($p<0.05$) ≥ adults (older children, adolescents and adults).

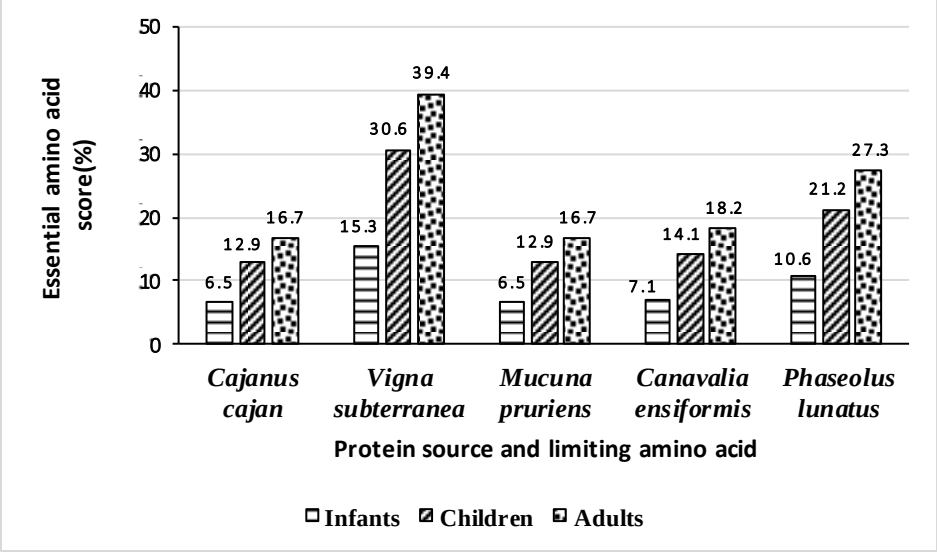


Figure 5. 1: EAA scores of NULs based on their limiting amino acid (tryptophan), compared with same limiting amino acid in the three thresholds required by infants, children and adults.

In all, the lowest EAA score, 6.5%, was found in *Cajanus cajan* and highest score of 39.4% was obtained in *Vigna subterranea* when the adult and infant references were used respectively as the threshold. Similar chemical scores of between 28% and 36%, limited to the sulphur amino acids (methionine + cysteine) in *Phaseolus lunatus* proteins has been reported (Kathirvel and Kumudha, 2011). However, the chemical score of protein extracts from *Vigna subterranea* as 0.91%, limited to tryptophan, has been reported (Yao *et al*., 2015). This lower level of chemical score of histidine compared to the levels obtained in this study for *Vigna subterranea* (15.3% (infants), 30.6% (children) or 39.4% (adult), makes the type of *Vigna subterranea* in the study area better. The variations or uncertainties in the chemical scores or concentrations of amino acids obtained in this study relative to other studies might be attributed to factors such as genetic variations of legumes (Bressani and Elías, 1980), edaphic factors (Norton *et al*., 1985) or even the procedure of the analysis of amino acids.

Histidine was found to be present in *Vigna subterranea* at 26.2 mg/g (Table 5.1) which was significantly ($p<0.05$) higher compared to those found from the other NULs. However, the least amount of histidine was found in *Phaseolus lunatus* (2.9 mg/g). The quantity of histidine determined in *Vigna subterranea* was also significantly ($p<0.05$) greater or equal to the amounts required for all the thresholds; Infants (21.mg/g), Children (20 mg/g) and Adults (16 mg/g), relative to the other NULs which were significantly lower (Table 5.1). Thus, levels of histidine determined in *Vigna subterranea* would be adequate in supplying the amount required for infants, children and adults (FAO/WHO/UNU, 2007). In contrast, the amount of histidine determined in the other NULs (Table 5.1) were significantly ($p>0.05$) lower, compared to the three reference thresholds. There are reports of values of between 29.9 mg/g and 44.4 mg/g

protein of histidine in three accessions of *Mucuna pruriens var pruriens* (Fathima *et al.*, 2010). Another study reports of histidine levels of 38.6 mg/g in *Vigna subterranea* (Yao *et al.*, 2015). It has also been reported (Ade-Omowaye *et al.*, 2015) that histidine levels range between 5.95 mg/g and 8.92 mg/g, across legumes such as *Cassia fistula, Canavalia ensiformis, Vigna subterranea, Mallotus nudiflorus, Sphenostylis stenocarpa* and *Cajanus cajan*.

The levels of isoleucine obtained in this study were significantly ($p<0.05$) different among the NULs (Table 5.1). The highest concentration of 35.9 mg/g, was found in *Vigna subterranea* and the lowest, 3.0 mg/g, was obtained in *Phaseolus lunatus* The concentration was also significantly ($p<0.05$) equal or greater than both the adult (30 mg/g) and children thresholds (32 mg/g) but not the infants' threshold (55 mg/g). For studies involving *Canavalia ensiformis*, a concentration of 53 mg/g isoleucine has been reported (Sridhar and Seena, 2006). Wide variations of isoleucine exist among NULs and this is shown in other reports of variable concentration of isoleucine. Values of isoleucine ranging between 59.4 mg/g and 69.4 mg/g among the varieties of *Mucuna pruriens var pruriens* have been reported (Fathima *et al.*, 2010). There was also a report of 54.5 mg/g isoleucine in protein extracts from *Vigna subterranea* (Yao *et al.*, 2015). In this study (Table 5.1), leucine appears to be consistently lower than what was obtained in other studies. For instance, studies involving *Canavalia ensiformis* concluded on levels of leucine ranging between 100 mg/g and 120 mg/g (Sridhar and Seena, 2006). Other studies have reported values of 102 mg/g in *Vigna subterranea* (Yao *et al.,* 2015) and levels of between 73.5 mg/g and 84.9 mg/g in *Canavalia ensiformis* and *Mucuna pruriens* (Agbede and Aletor, 2005). Therefore, the low levels of leucine obtained in this study puts the NULs in the study area as nutritionally inadequate. Interestingly, though leucine is an essential amino acid, its supplementation as a strategy to gain

muscle mass may not be significant in postprandial muscle protein synthesis (van Vliet *et al*., 2015).

There were significantly ($p<0.05$) high levels of lysine in *Phaseolus lunatus* (63.1 mg/g) and *Vigna subterranea* (53.6 mg/g) relative to the other NULs studied (Table 5.1). The quantity of lysine obtained in *Phaseolus lunatus* was significantly ($p<0.05$) equal to or greater than the threshold required for adults (48 mg/g) and children (57 mg/g), though the quantities were below what was required for infants (69 mg/g). Lysine levels in *Vigna subterranea* had been reported as 80.2 mg/g (Yao *et al*., 2015) and the lysine content of *Canavalia gladiata* cotyledon and the whole seed flour also range between 53 mg/g and 58 mg/g (Ekanayake *et al*., 1999). However, a reported lysine levels of the accessions of *Canavalia ensiformis* ranging between 13 mg/g and 150 mg/g has been made (Sridhar and Seena, 2006). Levels of lysine in *Phaseolus lunatus* ranging between 74 mg/g to 75 mg/g in wild and cultivated seeds were also reported (Kathirvel and Kumudha, 2011).

The total sulphur amino acids (methionine + cysteine) differed significantly ($p<0.05$) in the NULs studied (Table 5.1). Total sulphur amino acids ranged from a high of 18.7 mg/g in *Vigna subterranea* to a low of 2.2 mg/g in *Mucuna pruriens*. These values are inadequate, compared to all the three thresholds of the sulphur amino acids, required for proper sustenance of infants (33 mg/g), children (27 mg/g) and adults (23 mg/g) (FAO/WHO/UNU, 2007). The levels of the total sulphur amino acids are not surprising because low levels of methionine in plant-based proteins have been reported (Young and Pellet, 1994). Wide-ranging values of between 12.4 mg/g and 56.8 mg/g for methionine, and also between 5.4 mg/g and 10.1 mg/g were reported for cysteine

among the varieties of *Mucuna pruriens var pruriens* (Fathima *et al*., 2010). The peak value of 18.7 mg/g obtained in this study for *Vigna subterranea* falls within the concentrations already reported by (Fathima *et al*., 2010). Such low levels of amino acids must be complimented with cereals (Jukanti *et al*., 2012).

The aromatic amino acids were also significantly ($p<0.05$) higher in *Vigna subterranea* (67.6 mg/g) relative to *Cajanus cajan* and *Mucuna pruriens*, which gave the least of 3.6 mg/g and 5.6 mg/g respectively. This value obtained in *Vigna subterranea*, is significantly ($p<0.05$) greater than the aromatic amino acid threshold required for adults (41 mg/g) and children (52 mg/g) but not infants (94 mg/g). However, while one report (Adebowale *et al*., 2007) presented even higher quantities of the aromatic amino acids in *Mucuna pruriens* as 84.4-121.3 mg/g, another report (Sridhar and Seena, 2006) indicated a rather wider range of values in *Canavalia ensiformis* of between 19 and 116 mg/g. Thus, by comparison, the highest concentration of aromatic essential amino acids obtained in this study (67.6 mg/g) was low, though this level can support adult's and children's aromatic amino acid requirements.

Threonine level was as high as 56.4 mg/g in *Phaseolus lunatus* and this is equal to or greater than both the threonine thresholds for infants (44 mg/g) and children (31 mg/g). Threonine concentration in *Vigna subterranea* (27.7 mg/g) was significantly ($p<0.05$), lower than that determined in *Phaseolus lunatus* (Table 5.1). Generally, levels of threonine obtained in this study were comparable to the levels (37 and 42 mg/g) obtained in *Canavalia gladiata* (Eknayake *et al*., 1999) and also in *Canavalia ensiformis* (10 and 50 mg/g) (Sridhar and Seena, 2006). However, a higher level of 66.8 mg/g in *Mucuna pruriens* has been reported (Adebowale *et al*., 2007).

The quantity of tryptophan in the five NULs were all below 3 mg/g (Table 5.1). The tryptophan contents of *Vigna subterranea* (2.6 mg/g) and *Phaseolus lunatus* (1.8 mg/g) were significantly ($p<0.05$) different from each other. Similarly, the tryptophan content of the pair- *Vigna subterranea* and *Phaseolus lunatus* showed significant differences ($p<0.05$) relative to the rest of the NULs (*Mucuna pruriens*, *Cajanus cajan* and *Canavalia ensiformis*). Generally, the tryptophan content of the NULs studied, were very limiting and the values recorded were significantly ($p<0.05$) very low, compared to the recommended thresholds for infants (17 mg/g), children (8.5 mg/g) and adults (6.6 mg/g). Yao *et al.* (2015), reported levels of 6.0 mg/g in *Vigna subterranea* which was comparable to the recommended threshold of 6.6 mg/g for adults but not for infants (17 mg/g) and children (8.5 mg/g). Other studies report of high levels of tryptophan (22.3-34.6 mg/g) in *Mucuna pruriens* and in *Lupinus angustifolius* (19.56 and 26.39 mg/g) (Adebowale *et al.*, 2007; Monteiro *et al.*, 2014).

The highest level of valine was obtained in *Vigna subterranea* (23.5 mg/g) and the lowest in *Mucuna pruriens* (3.7 mg/g) (Table 5.1). Relative to the recommended thresholds for valine in the diets of infants (55 mg/g), children (43 mg/g) and adults (40 mg/g), the values obtained in this study was very limiting. Reports from other studies gave values of 8.7 mg/g in *Phaseolus vulgaris* (Nestares *et al.*, 2001) and 14.46-18.27 mg/g in *Lupinus angustifolius* (Monteiro *et al.*, 2014). Thus, the higher values of valine obtained in this study (23.5 mg/g) were relatively better than in other studies, although, highest level of valine (*Vigna subterranea*, 23.5 mg/g) obtained in this study was still inadequate to meet the requirements of infants, children and adults. Indeed, compared to valine concentration of 62.4 mg/g in *Vigna subterranea* (Yao *et al.*, 2015), or a range of valine value of between 54 mg/g and 58 mg/g in *Phaseolus lunatus* (Kathirvel and Kumudha,

2011), the levels of valine in the NULs obtained in this study are low.

5.3.2 NULs protein quality relative to digested indispensable amino acid scores (DIAAS)

The crude protein content of the individual NULs obtained in this study (Table 4.1) ranged from 15.2 g/100 g (*Vigna subterranea*) to 25.2 g /100 g (*Mucuna pruriens*). These values are even better, compared to what has been reported for rice (6.7 g/100 g), wheat flour (10.3 g/100 g) and lentils (25.8 g/100 g) (USDA, 2011). Even so, it is the amino acid composition, expressed as DIAAS, that is key to the evaluation of the quality of dietary protein. It has been theorized that, such digestibility evaluations of amino acids, closely match the actual ileal digestibility of amino acids along the ileum of humans (FAO, 2013). The DIAAS obtained and presented in Table 5.2 ranged from 1.5 for *Cajanus cajan* and *Mucuna puriens* to 3.6 in *Vigna subterranea*.

Table 5. 2: Digestible indispensable amino acid score (DIAAS) of five NULs samples evaluated using referenced mean essential amino acid content and digestibility coefficient of soybean.

Essential Amino acids	**Reference soybean protein**		**Digested essential amino acid (DEAA) ratios**				
	[*]Amino acid (mg/g)	[$]Digestibility coefficient	*Cajanus cajan*	*Vigna subterranea*	*Mucuna pruriens*	*Canavalia ensiformis*	*Phaseolus lunatus*
His	9.68	0.864	0.766	2.256	0.336	0.387	0.250
Ile	16.06	0.890	0.196	1.808	0.156	0.343	0.151
Leu	28.38	0.889	0.302	0.970	0.137	0.445	0.342
Lys	23.10	0.867	0.377	1.927	0.133	0.478	2.268
Met + Cys	40.50	0.928	0.067	0.358	0.042	0.216	0.050
Phe + Tyr	19.26	0.889	0.151	2.843	0.235	0.900	0.652
Thr	13.15	0.887	0.191	1.710	0.130	0.130	3.481
Trp	[&]57.00	0.923	0.015	0.036	0.015	0.016	0.025
Val	16.62	0.882	0.496	1.154	0.182	0.255	0.231
			Digestible indispensable amino acid score (DIAAS)				
			1.5	3.6	1.5	1.6	2.5

[$]Goldflus *et al.* (2006)
[*]Kong *et al.* (2014)
[&]Carrera *et al.* (2011)

By ranking the quality according to DIAAS, *Vigna subterranea* proteins are ranked first, followed by that of *Phaseolus lunatus*. Thus, *Cajanus cajan*, *Mucuna puriens* and *Canavalia ensiformis* proteins are poor in quality. This observation closely supports the results obtained in Table 5.1, where the amino acids from *Vigna subterranea* and *Phaseolus lunatus* proteins showed greater values. To evaluate the quality of proteins, the use of cutoffs of 100; 75-99; and below 75, has been reported to classify values of DIAAS as "Excellent", "Good" and "Low" respectively (Shaheen *et al*., 2016). Thus, all the NULs proteins evaluated in this study were of low quality in agreement with other studies (Rutherfurd *et al*., 2015). In particular, the low quality in the case of these NULs were based on the profile of tryptophan in all the NULs analyzed. The results once again reinforce the observation that these two NULs, *Vigna subterranea* and *Phaseolus lunatus* are better composited in order to impact positively, their protein quality.

5.4 Conclusion

Two of the NULs: *Vigna subterranea* and *Phaseolus lunatus* out of the five studied showed a potential to supply the recommended essential amino acids thresholds required for infants, children and adults. It was *Vigna subterranea* that had adequate quantities of histidine for infants, children and adult requirements. However, *Vigna subterranea* could provide adequate isoleucine and aromatic amino acids for children and adults. *Phaseolus lunatus,* on the other hand, could provide lysine and threonine requirements for only adults. The remaining essential amino acids; leucine, methionine + cysteine, tryptophan and valine were not adequate. The assessment of protein quality using DIAAS, however, indicated that all the NULs protein quality were low on the basis of tryptophan content. It is possible that by compositing these two NULs, together with other sources of protein a potential supplement, capable of supplying the

required essential amino acids for some consumers could be achieved.

CHAPTER 6: FUZZY MODELLING THE INACTIVATING EFFECTS OF β-STARCHES ON NULS LECTINS

6.1 Introduction

Lectins are glycoproteins that abound in plants and animals (Mojica and Merca, 2004). They are plentiful in legumes, wheat flour and nuts, and are known to resist digestion in the gastrointestinal tract (Pusztai and Bardocz, 1996). Consumption of meals containing lectins, as a result of improper cooking is known to result in conditions such as leaky gut (Makkar *et al*., 2007), bacteria and protozoa infections (Miyake *et al*., 2007), and hyperplasia and hypertrophy of the small intestine (Marzo *et al*., 2002). Such pathologies caused by lectins were avoided or minimized when diets containing lectins were removed, prior to consumption (Coppo *et al*., 1992). Many attempts have been made to inactivate lectins, using such processes as fermentation (Ma and Wang, 2010) and dialyzing out metal ions such as Ca^{2+} and Mn^{2+}, known to associate with legume lectins (Jaffe *et al*., 1997). Chemical derivatization of soybean lectins (Liener and Shohachi, 1956) and thermal processing, followed by limited proteolysis have also been used (Ma and Wang, 2010).

Carbohydrates are known to bind to lectins and the mechanism of the binding action of specific carbohydrates to lectins has been explained (Weis and Drickamer, 1996). Their explanations show that customized carbohydrate-lectin complexes underlie the inactivation process. It has been reported (Kelkar *et al*., 2012) that some level of disruption to protein structures occurs during extrusion cooking at temperatures of about 85 ^{o}C, as a result of unfolding of protein chains (Steel *et al*., 2012). Though these thermal methods do inactivate lectins to some extent, they also produce unsafe by-products such as Maillard reaction products and advanced glycated

end-products (Abate *et al.*, 2015). In addition, there is degradation of proteins and amino acids as a direct result of the high temperatures usually employed in extrusion (Cristina *et al.*, 2004).

Beta (1,3)-D-glucans in plants are carbohydrates that are bindable to some lectins (Wawra *et al.*, 2016). There is strong evidence showing that increased levels of β(1-3) and β(1-4)-bonded starches in bean and maize flours are produced in a dose-dependent manner, during radiation (Rombo *et al.*, 2004). The mechanism behind the production of β-starches involves the action of free radical reactions which lead to molecular depolymerization (Sokhey and Hanna, 1993). Other studies reveal the formation of dose-dependent depolymerized starch products of varying molecular weight (Lasekan and Lasekan, 2000; Sokhey and Hanna, 1993). From these studies, it has emerged that native starches irradiated from 2 to 40 kGy, produce depolymerized products of intermediate molecular weight fractions, followed by amylose-like fragments and eventually water-soluble oligosaccharides. Fragments ranging from 2 kDa to 400 kDa in maize and sorghum and also 1 kDa to 300 kDa in beans have been reported in these studies. However, it is uncertain the extent of radiation dose that would be adequate, just to produce the right molecular weight starch containing the suitable levels of β-starches to inactivate lectins.

There are uncertainties about specific temperatures employed in extrusion to achieve the desired protein inactivation, while maintaining food functional proteins or even avoiding harmful side-products. Firstly, there are different sources and levels of lectins even in the legumes that would require different operational temperatures for their inactivations. Secondly, it can be deduced from studies (Rombo *et al.*, 2004) that starch with various molecular sizes and physicochemical properties are produced when they undergo doses of radiation ranging between 2 and 40 kGy. In the use of radiations, there are also uncertainties as to whether low, moderate or high radiation

doses must be used to treat starches to produce β-starches with specific structures for the inactivation of lectins.

Situations where imprecise variables have to be studied simultaneously before a decision is taken, often times put strain on time and resources. Fuzzy logic thus, become appropriate because of its ability to arrive at a decision, in the absence of precise mathematical models. Thus, the fuzzy inference system, based on fuzzy logic is used as a decision-making tool to arrive at timely decisions (Adeyemi *et al.*, 2017). Fuzzy logic extends the principles of the classical set theory (Lababidi and Baker, 2006) and uses intersections of crisp sets, and by extension, the "if-then" rules.

In fuzzy logic, crisp sets are transformed to fuzzy sets due to the imprecise nature of the elements in the set (Perrot *et al.*, 2006). Thus, fuzzy sets contain elements that have various degrees of memberships in those sets. While it offers elements that have strong membership in one set, it shows a weak membership in another set at the same time on a scale ranging from 0-1 (Lababidi and Baker, 2006). By using the classical set operators of intersections; *and* "$\cup$" and *or* "$\cap$", fuzzy variables are mapped to outputs in an "if-then" rule relations in order to arrive at a decision. Thus, fuzzy logic is a process of presenting data and knowledge closer to human-like thinking (Zadeh, 1965).

There is ambiguity about temperature requirements in extrusion cooking (Ajitaa and Jha, 2017) but in particular, extrusion cooking at moderate to high temperatures has been reported to inactivate lectins up to 98% (Alonso *et al.*, 1998). In fact, extrusion cooking at 85 ^{o}C has been

reported (Kelkar *et al.*, 2012) to remove some lectins. However, other studies show that such high temperatures required for lectin inactivation could potentially render the extrudates unsafe (Singh *et al.*, 2007b). Thus, it is uncertain as to what temperature would be adequate, just to inactivate lectins while ensuring the safety of extrudates.

Radiation has been used on starches to produce starches of varied molecular weights (Bhatty and Macgregor, 1998) and also unique carbohydrate structures, but advantage has not been taken of these starches to inactivate lectins. Such starches can rapidly be produced by simply employing radiation technology. Though extrusion cooking alone can inactivate lectins at high temperatures, there is the attendant potential formation of unsafe extrudates.

The main drive behind this work is to evolve appropriate lectin inactivation techniques for some locally consumed NULs, involving the use of low temperature-driven extrusion treatment of β-starches produced by radiation. To be able to quickly and reliably monitor the processing conditions of this study, fuzzy logic was used as a tool to predict the outcome of imprecise input variables, required to achieve the lectins inactivation processes. The study was in two parts; the production of lectins-inactivated NULs flours by making use of β-starches, obtained from radiation and composited with the flour in extrusion treatment. Secondly, construction of a prediction model for the inactivation process of the lectins using fuzzy modeling.

6.2 Materials and methods

6.2.1. Preparation of legume flours

Five legumes (*Canavalia ensiformis, Vigna subterranea, Phaseolus lunatus, Mucuna pruriens*

and Cajanus cajan) were obtained from six locations in the mid-west districts of Ghana; specifically, *Abofuor, Techiman, Drobo, Mampong, Ejura* and *Amantin*. The legumes were dried in a solar tent dryer (36 h) with a temperature range of between 40 to 60 ^{o}C. The beans were then sorted, cleaned and subsequently milled into flour in a Tecator cyclotec hammer mill (1093, Sweden) fitted with 1 µm screen/sieve. The flours were packaged in plastic bags, sealed, labelled and stored at 4 ^{o}C, pending further use.

6.2.2. Starch irradiation

Food grade native cassava starch (labelled as tapioca starch) was obtained from *Ayensu* Starch Limited (Ghana). Irradiation was carried out using the Cobalt 60 gamma irradiation facility (RTC, GAEC, Kwabenya, Accra), with ethanol chlorobenzene (ECB) dosimetry. One kilogram each of the starch was separately weighed into plastic containers and 100 mL of distilled water was sprinkled on them and sealed. Three groups of the sealed starches weighing 1 kg each, were labeled to give a total of nine samples, and exposed to the following linguistic radiation dose variables *low* (3, 5, 8 kGy), *moderate* (13, 15, 18 kGy) and *high* (35, 38, 42 kGy). The dose rate was emitted at 2.14 kGy/h. Thus, depending on the dose required the samples were kept in the radiation chamber for different proportionate times, to achieve the required radiation doses. The range of radiation dose of between 3 and 42 kGy was selected because of their applications in dose-response production of β-starches, where starch fractions of intermediate fractions, amylose-like fractions and water-soluble oligosaccharides were resultantly obtained (Rombo *et al.*, 2004).

6.2.3. Determination of starch content of legume flour samples

The starch content of each of the five flours samples were determined, using the Megazyme

protocol, based on the original (McCleary *et al.*, 1997) total starch assay. The total starch contents were as follows; *Phaseolus lunatus* (46.1%), *Mucuna pruriens* (27.9%), *Cajanus cajan* (35.3%), *Vigna subterranea* (46.7%) and *Canavalia ensiformis* (44.3%). By determining the starch content, it was possible to condition the NUL flours (with β-starches) according to the levels of starch present in them.

6.2.4. Conditioning of the NULs flours

Conditioning of the NULs flours was achieved by adding to each of the five NULs flours *low*, *moderate* and *high* radiated starch samples equivalent to 10% starch content of each NULs flour, as reported (Section 6.2.3). The conditioning was done similar to other studies, where samples have been conditioned between 0.5-5.0% (Knorr, 1982; Yen *et al.*, 2011). The conditioning was up to 10% which is believed to be in excess to ensure that quantities of β starches were available to inactivation of all available lectins that may be present in the NULs flours.

6.2.5. Extrusion treatment

The five separate NULs flours, weighing approximately 5 kg each were cold - defatted (at 10% w/v, in three replications), using 45% n-hexane (Bitolea, Italy). The defatted flours were air - dried, packaged in plastic containers and stored at -20 ^{o}C, until further use. Extrusion treatments of NULs composites were carried out between 1 and 2 min in twin-screw Clextral extruder (Framatone BC 45, France), sketched as in Figure 6.1.

In all, the composite NULs flours containing 10% of radiated starches totalled 15 samples, excluding the native unconditioned flours. These composites were separately fed into the feed

receptacle. Treated water was injected into the composite flour (at mark 75 flow rate) by the time the feed reached the screw or shearing region. Preliminary runs were undertaken before settling on feed meal rate of 200 rpm, and a twin-screw speed at 900 rpm. The extrusion barrel which had two independent heating points (front and rear) had their extrinsic temperatures (Ex_{T1}) set at 50 ^{o}C for the incoming feed meal, and (Ex_{T2}) set at 70 ^{o}C as the extrudate exited the die of 4 mm diameter. The extrudates presented unique intrinsic temperatures (In_{T1}) as the extrinsic temperature reached 50 ^{o}C at entry point and again as they exited the die (In_{T2}) at extrinsic temperature 70 ^{o}C. As the extrudates exited, samples were taken and sealed in plastic air-tight containers for further analyses. The intrinsic temperature change was calculated (Equation 6.1), as the arithmetic difference between the initial and final intrinsic temperatures.

$$\text{Intrinsic temperature change} = In_{T2} - In_{T1} \quad \text{.....6.1}$$

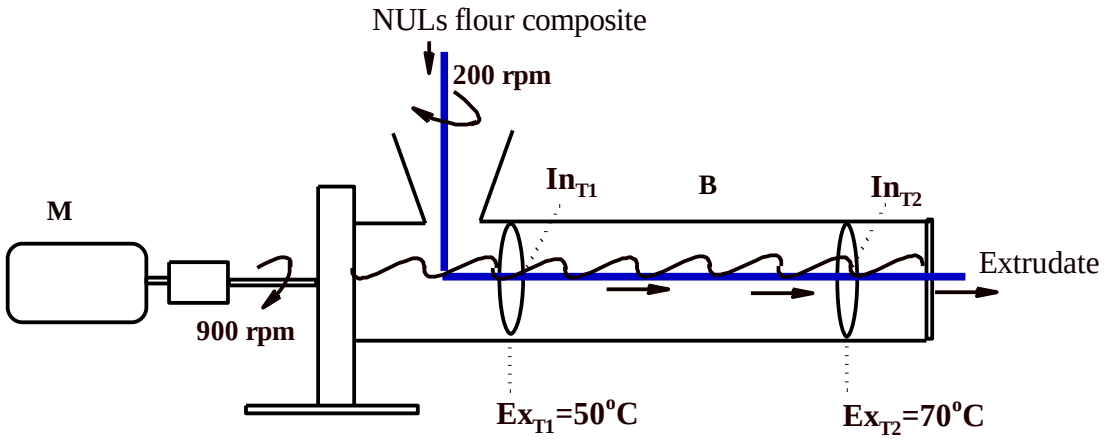


Figure 6. 1: The flow of β-starch composites of NULs flour through extruder barrel (B) as twin-screws were driven by motor (M).

6.2.6. Determination of legume agglutinins using ELISA

Five (5) grams each of the extrudate flour samples were weighed and blended into a total of 30 mL phosphate buffer saline, and quantitatively transferred into 50 mL Falcon tubes. Samples

were then agitated at 250 rpm on Pro Digital Orbital Shaker (SK-O330, US) overnight, at room temperature. After centrifugation at 10,000 rpm for 5 min, 500 µl supernatant was collected into 1.5 mL Eppendorf tube. Determinations of lectins were done, using ELISA with plant soybean agglutinin (Gentaur Molecular Products, BVBA (Belgium) as a standard, similar to other studies (Boniglia *et al.*, 2003; Rizzi *et al.*, 2003). Subsequently, % lectin change was calculated based on Equation 6.2.

$$\frac{\text{Non-extruded NULs flour Lectin content} - \text{Extruded NULs flour Lectin content}}{\text{Non-extruded NULs flour Lectin content}} \times 100\ \% \quad \text{.....6.2}$$

6.2.7. Data analysis based on fuzzy logic model

Fuzzy logic and modelling was run in Matlab (2012) fuzzy logic toolbox. Analysis is based on the classical set theory, where, the two variables (Table 6.1a) (Dose and Intrinsic temp change), were mapped to the one variable output (% Lectin change), using the "if-then" rules and the "AND" functions. The study was made in a Mamdani fuzzy inference, in which both the input and the output variables were transformed into fuzzy propositions (Lababidi and Baker, 2006) as outlined (Figure 6.2 a and b).

In the fuzzy logic tool box (Matlab, 2012), the initial step of fuzzy modelling is the fuzzification process, where the numerical data set obtained in the study (Table 6.2a) were expressed as fuzzified data (Table 6.2b). The ranges of the crisp input (Table 6.1a) and output (Table 6.1b) variables together with their membership functions Appendix I.1) had been set previously. The ranges refer to the corresponding crisp input and output values based on their universe of

discourse (universal set). Membership functions are depicted in graphical forms to characterize the degree (between 0 and 1) of the fuzziness of each of the elements in a linguistic variable in a fuzzy set (Perrot *et al.*, 2006). The option exists to choose from among several membership functions such as; triangular, gaussian and trapezoidal. For this study, triangular membership function was selected for each of the input and output variables based on its better performance relative to other membership functions (Gayathri *et al.*, 2011).

Table 6.1a : Fuzzy sets and the linguistics of inputs and their specific ranges

Inputs	Fuzzy set/Linguistics	Range of data
Radiation dose/ kGy	*Low-Moderate-High*	3-42 kGy
Intrinsic temperature change /°C	*Small-Modest-Big*	2-12 °C

Table 6.1b : Fuzzy sets linguistics of the output response range.

Output	Fuzzy set/Linguistics	Range of data
% Lectin change	*Intense decrease-Moderate decrease-Slightly low decrease-Potentiation*	-83.1 - 44.0 %

Table 6.2a : Percentage lectin inactivation obtained from treatments with two crisp variables; radiated starches and intrinsic temperature change in extruder.

	Input Variable				Output Variable		
NULs Flours	Dose(kGy)	Intrinsic Temperature (°C)			Lectin content of composited flours (mg/g dry basis)		
		Initial	Final	Change	Native	Extruded	% Change
Caj lo	3	44	48	4	16.1	12.3	23.6
Caj me	18	42	44	2	16.1	22.6	-40.4
Caj hi	35	43	45	2	14.4	18.1	-25.7
Phal lo	8	44	50	6	32.5	18.2	44.0
Phal me	20	40	44	4	15.5	14.9	3.9
Phal hi	42	42	48	6	19.2	16.9	12
Vig lo	3	51	59	8	16.8	10.6	36.9
Vig me	20	49	60	11	16.6	17.5	-5.4
Vig hi	38	47	59	12	17.8	16.7	6.2
CanEs lo	5	45	48	3	80.0	17.5	78.1
CanEs me	15	45	47	2	83.9	22.5	73.2
CanEs hi	38	45	48	3	68.3	11.6	83.0
Muc lo	5	42	44	2	12.5	6.9	44.8
Muc me	20	42	46	4	17.4	9.2	47.1
Muc hi	35	42	46	4	20.5	7.9	61.5

Having obtained the inputs and output in their fuzzified forms, mapping of the inputs to the output was done using the "if-then" rules. Consequently, a fuzzy truth reference table (Appendix I.2), representing all possible outputs for all possible inputs are obtained. Lastly, a defuzzification interface transforms the fuzzy output results into crisp numerical output results. Defuzzification is done in order to obtain crisp values. From the rule relations, it is observed that an "AND" function, also known as the algebraic product function, was used as fuzzy operator to aggregate the two inputs, in order to get the output; lectin inactivation value.

Table 6.2b .: Percentage change in lectin inactivation obtained from treatments with two crisp variables; radiated starches and intrinsic temperature change expressed as fuzzy linguistic responses.

	Input Variables		***Output Variable***
Sample Flours	*Dose/kGy*	*Intrinsic temperature change/ °C*	*% Lectin change*
Caj lo	Low	Modest	Moderate decrease
Caj me	Moderate	Small	Potentiation
Caj hi	High	Small	Potentiation
Phal lo	Low	Modest	Moderate decrease
Phal me	Moderate	Modest	Slightly low decrease
Phal hi	High	Modest	Slightly low decrease
Vig lo	Low	Big	Moderate decrease
Vig me	Moderate	Big	Potentiation
Vig hi	High	Big	Slightly low decrease
CanEs lo	Low	Small	Intense decrease
CanEs me	Moderate	Small	Intense decrease
CanEs hi	High	Small	Intense decrease
Muc lo	Low	Small	Moderate decrease
Muc me	Moderate	Modest	Moderate decrease
Muc hi	High	Modest	Intense decrease

Just as input and output crisp data were initially transformed to fuzzy data set, processed, and the output inferred, there is also the need to defuzzify the output, in order to obtain crisp value. To defuzzify is to select a single optimal point from the aggregated domain of a fuzzy membership function. In performing the defuzzification step, the mean-of-maximum (MOM) was used as the

defuzzifier among several approaches (Sugeno and Yasukawa, 1993). Establishment of the fuzzy rule relations, using the "if-then" commands, resulted in the fuzzy relational graphs (Appendix I.3). From the fuzzy relational graphs, the integrated fuzzy logic model was obtained from which the simulation of the input variables predicted the characteristics of the output variables at 100% accuracy in this study. The final fuzzy model obtained for this study, is thus, based on Matlab Simulator/Simulink (Matlab Toolbox, 2012), and presented as:

> *"A Mamdani type fuzzy model system, involving two input variables mapping to one output variable in triangular membership functions, defuzzified by aggregated mean-of-maximum".*

6.3 Results and discussion

6.3.1. Residual lectin activities of composite extrudates

Three exudates; Caj_{mo}, Caj_{hi} and Vig_{mo}, (Table 6.3) showed potentiation of residual lectin activities of up to 44% at *moderate* radiation dose and *big* intrinsic temperature change.

Table 6.3 .: Extrudates treatments conditions and residual lectin activities

Lectin activities in Extrudates	Extrudates	Treatment conditions
Intense decrease	Muc_{lo}, $CanEs_{lo}$, $CanEs_{mo}$, $CanEs_{hi}$	a) Small IT/moderate dose b) Modest IT/High dose
Moderate decrease	Caj_{lo}, $PhaL_{lo}$, Vig_{lo}, Muc_{lo}, Muc_{mo}	Modest to big IT/low to moderate dose
Slightly low decrease	$PhaL_{lo}$, $Phal_{hi}$, Vig_{hi}	Moderate to high dose/ Small or big IT
Potentiation	Caj_{mo}, Caj_{hi}, Vig_{mo}	Moderate dose/ Big IT.

NB: *IT= Intrinsic temperature change*

Potentiation is said to have occurred if after thermal treatment the activity of lectins increases several folds, compared to their native states. Studies have shown that some lectins such as

phasins (obtained from red kidney beans) are lethally toxic (Lawley *et al*., 2008), even at the level of 5 mg/kg body weight. Therefore, reports of potentiation is a serious matter of concern. The residual lectin activities obtained from this study was categorized into four residual lectins with distinctive linguistic fuzzy sets defined by lectin activities, described as *potentiation, slightly low decrease, moderate decrease* and *intense decrease*. From the studies, there is some evidence that lectins from *Cajanus cajan* were likely to potentiate. The cause of potentiation has been attributed to the well-defined domains of the tertiary and quaternary structures of lectin polypeptide subunit structures, enabling them to bind to specific sugars (Sinha and Surolia, 2005). Sometimes, disruption of the polymeric and oligomeric structures of lectins may cause unfolding and so expose extra binding sites, to offer resistance to thermal processing. Soybean lectins, for instance, is known to possess tetrameric subunits (Sinha and Surolia, 2005) which offer higher level of stability and resilience (Ghosh and Mandal, 2001). In this study, lectins potentiated up to 44% within an extrinsic temperature of between 50 ^{o}C and 70 ^{o}C, compared to phasin, which potentiated five folds at 80 ^{o}C in other reported studies (Lawley *et al*., 2008).

Moderate dose radiated starches, which probably produced amylose-like products, showed *moderate decrease* in lectin activity in *Cajanus cajan* (Table 6.3). It is to be noted that *small* to *modest* intrinsic temperatures did not favor inactivation of lectins in *Cajanus cajan* flour when *moderate* to *high* dose radiated starches (which probably produced amylose-like fragments and soluble oligosaccharides) were composited. This observation is premised on the fact that *Cajanus cajan* lectins are known to be specific for α-mannose and α-glucose (Siddiqui *et al*., 1995) but not the β-starches produced by the irradiation of starches (Rombo *et al*., 2004).

Residual lectins present in the composite extrudates from this study ranged from 14.4 mg/g in

Cajanus cajan composited flour, to 83.9 mg/g in *Canavalia ensiformis* composited flour (Table 6.2a). However, the native NULs flours had previously been determined to have lectins ranging from 64 mg/g in *Phaseolus lunatus* flour to 414 mg/g in *Canavalia ensiformis* flour, before compositing (Figure 4.7).

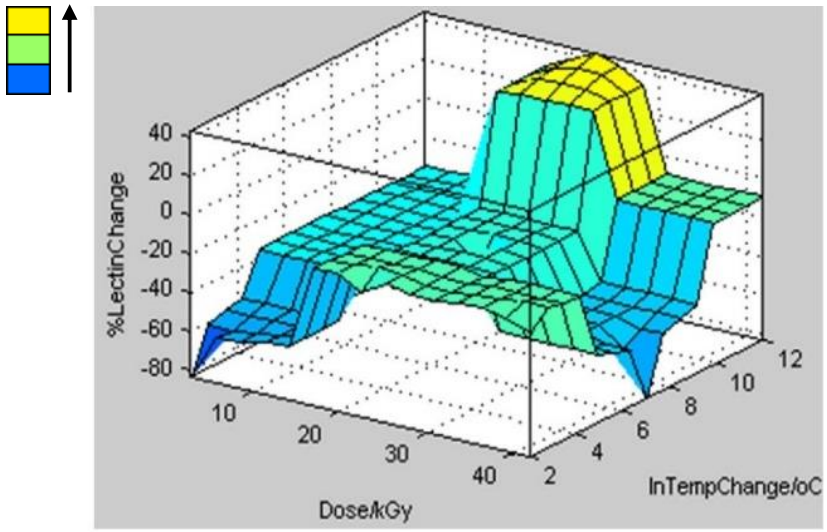


Figure 6. 2a : Surface view of the impact of two inputs (radiation dose of starch and intrinsic temperature change during extrusion) and output (% Lectin change).

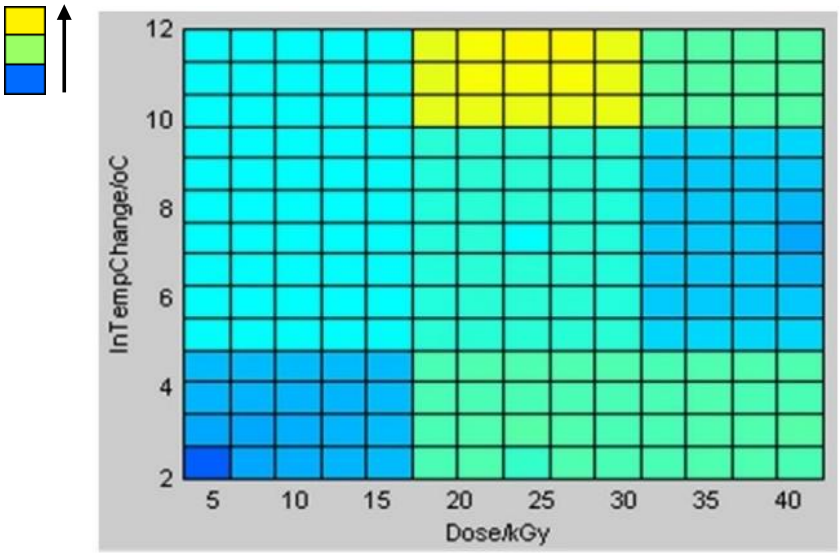


Figure 6.2b : Pseudo colour view of the impact of two inputs (radiation dose of starch and intrinsic

temperature change during extrusion) and output (% Lectin change).

The treatment of legume flours in this study recorded about 83% reduction in lectin activity in three-dimensional representation (Figure 6.2a) and contour (Figure 6.2b). These observations were made in *Canavalia ensiformis* composited with *high* dose radiated starches, extruded with modest intrinsic temperature change, and *Mucuna pruriens* combined with low radiated starches extruded with *small* intrinsic temperature range. The *high* radiation dose starch which probably contained smaller amylose-like fragments and soluble oligosaccharides (Rombo *et al.*, 2004), meant *Canavalia ensiformis* lectins had affinity for low molecular size β-starches. On the other hand, low-dose radiated starches meant *Mucuna pruriens* lectins might probably have affinity for relatively larger molecular size β-starches for inactivation. It could also mean lectins from these NULs were not oligomeric to be buried in the native state to become exposed when their subunit structures become unfolded upon intrinsic temperature agitation.

From Table 6.2a it is observed that, composited *Canavalia ensiformis* flour lectins initially between 68.3 and 83.9 mg/g, rapidly inactivated to between 11.6 and 22.5 mg/g. There seem to be certain characteristic features about the lectins in *Canavalia ensiformis* that could account for this observation. This may probably be due to relatively stronger affinity for soluble oligosaccharides that cluster the binding sites of lectin polypeptides (Mitchell *et al.*, 2001) to inactivate them subsequently. Thus, it may be inferred that the molecular size of the specific starches contributed little to inactivate the lectin but rather, it was the affinity to bind to these specific starch oligomers that had the desired impact.

Extrudates such as Caj_{lo}, $PhaL_{lo}$, Vig_{lo}, Muc_{lo}, Muc_{mo} (Table 6.3) exhibited *moderate* decrease in their legume lectin activity of between 20 and 50%. However, it appears *low* to *moderate*

radiated starches (probably containing amylose-like and soluble oligosaccharides) coupled with *modest* intrinsic temperatures effected these *moderate* decreases (20-50%.) of lectin activity, no matter the source of the legume lectin. *Vigna subterranea* lectins seem to be difficult to inactivate. The treatments for *Vigna subterranea* flour could cause a *moderate* decrease in lectin activity but could also cause potentiation (Table 6.3).

A review of protein database, specifically on the molecular structure of lectins in *Vigna subterranea* showed a paucity of information (Berman *et al.*, 2000). However, it may probably be similar to peanut agglutinin judging from the resistance of its lectin activity (Ravishankar *et al.*, 2001). Lectin resistance may also be possible if the specific starches are not suitable due to steric hindrances, or there is hydrophobicity of the complimentary amino acids (Weis and Drickamer, 1996). There could also be the potential to expose previously partially buried lectin polypeptides that would then confer carbohydrate binding activity (Hatakeyama *et al.*, 2012).

6.4 Conclusion

For extrinsic temperature range of between 50 ^{o}C and 70 ^{o}C, *Canavalia ensiformis* and *Mucuna pruriens* lectins could inactivate over 80%, at low to moderately radiated starches within *small* to *modest* intrinsic temperature. Other composited flours recorded varying extent of inactivation of lectins, at different molecular weight β-starches and different intrinsic temperatures. But lectins from *Vigna subterranea* are likely to potentiate independent of the radiation dose of starches or the intrinsic temperature applied. Thus, care must be taken in an effort to inactivate legume lectins with this procedure, since all lectins of NULs did not behave similarly. The Mamdani type fuzzy logic achieved a prediction of 100%, showing that fuzzy logic inference system, as

used in this study, is capable of being used to predict responses to lectin inactivations.

CHAPTER 7: GENERAL CONCLUSION AND RECOMMENDATION

The need for NULs utilization can better be accomplished if there is documentation of their consumption patterns, habitual cooking practices and the characterization of consumers who consume them. This study has documented the consumption patterns and quantities of the NULs dishes consumed together with their uncertainties. A modal mass of NULs dishes consumed, ranging between 400 and 600 g was determined, dependent on the types of NULs. The uncertainties is such that, quantities of dishes as low as 160 g and as high as 900 g were encountered in the study area. These quantities of NULs dishes consumed, presented different statistical distribution or spreads. While masses of "*Tubani*" and "*Ase*" consumed as main meal was of uniform distribution, masses of legume seeds used for soups, presented a more central peak (Laplace) distribution.

The uncertainty of exposure frequencies of NULs dishes ranged between a minimum of once per month to a maximum of eight times per month. However, the most likely exposure frequency of the dishes studied were between three and four times per month, with different statistical distributions. The habitual cooking times of the dishes ranged as low as 1 h and as high as 5 h. The cooking times presented different statistical distributions depending on the NULs dishes. However, the single most likely cooking time of 1 h was recorded for some NULs dishes on the field. In response to studying the impact of such cooking times on an intrinsic hazard such as lectin in NULs, modelled dishes were prepared from each of the five NULs according to the cooking practice in the study area. The risk analysis of these modelled dishes revealed that, consumers were all at risk since HQ was greater than 1. Even so, majority of consumers feed on

NULs because they believe they are nutritious. Consumers presented self-reported stomach discomforts and also believed there are extrinsic hazards such as pesticide residues, additive cooking aids, which are interactively hazardous in NULs dishes.

The consumption of NULs dishes, was reported in a house hold of between 2 to 6 members, depending on the type of dish. However, a maximum of 6 house hold members were encountered. The study also revealed that, majority of consumers are likely to fall between 44 and 56 years, though as low as 10 years, and as high as 100 years still feed on NULs dishes. Again, since majority of NULs consumer profiles were married couples, females, traders, and people with non-formal education, it was important that the quality of proteins that NULs deliver were evaluated.

Analysis of protein quality, using the digested indispensable amino acid scores (DIAAS) revealed that, out of the five NULs studied, *Vigna subterranea* and *Phaseolus lunatus* presented DIAAS of 3.6 and 2.5 respectively. Overall, this observation show that NULs proteins were of low quality relative to international standards. Even so, the majority still feed on them. Thus, measures ought to be taken to boost their protein quality by expanding into other complementary protein sources. Protein qualities are known to be linked to their digestibilities, however, intrinsic factors such as lectins abounded in the NULs studied. There were reported levels of between 64 mg/g in *Phaseolus lunatus* and up to 414 mg/g in *Canavalia ensiformis*. Apart from disrupting digestion and causing stomach and intestinal inflammation, lectins are also known to decrease the digestibilities of NULs amino acids. It is therefore important that some mitigation studies were carried out. The binding of lectin to carbohydrate is key in their mechanisms and it is this

idea that drove the inactivation studies. The inactivation studies of β-starches with lectin revealed two contrasting outcomes. While potentiation of some lectin activities occurred in *Cajanus cajan* and *Vigna subterranea* (up to 44%), inactivations of up to 80% occurred in *Mucuna puriens* and *Canavalia ensiformis* flours. Thus, the lectin mitigation studies involving β starches, might not work for all NULs lectins. This observation requires further studies with derived carbohydrates in order to control such intrinsic factors as lectins in NULs. It is believed that such studies would enhance the digestibility of NULs proteins, increase their quality and boost the utilization of NULs.

REFERENCES

Abate, G., Delbarba, A., Marziano, M., Memo, M. and Uberti, D. (2015). Advanced glycation end products (AGES) in food: focusing on Mediterranean pasta. *Journal of Nutrition and Food Science, 5*: 440-448

Addo, A. A. and Oyeleke, O. A. (1986). Nutritional evaluation and organoleptic properties of biscuits supplemented with Bambara groundnut flour. In: *A seminar paper,* (pp. 1-10).

Ade-Omowaye, B. I. O., Tucker, G. A. and Smetanska, I. (2015). Nutritional potential of nine underexploited legumes in Southwest Nigeria. *International Food Research Journal, 22* (2): 798–806.

Adebowale, O. and Maliki, K. (2011). Effect of fermentation period on the chemical composition and functional properties of Pigeon pea (*Cajanus cajan*) seed flour. *International Food Research Journal, 18* (4): 1329–1333.

Adebowale, Y. A., Adeyemi, A. and Oshodi, A. A. (2005). Variability in the physicochemical, nutritional and antinutritional attributes of six *Mucuna* species. *Food Chemistry, 89* (1): 37–48.

Adebowale, Y. A., Adeyemi, I. A., Oshodi, A. A. and Niranjan, K. (2007). Isolation, fractionation and characterisation of proteins from *Mucuna* bean. *Food Chemistry, 104* (1): 287–299.

Adedayo, M. R., Ajiboye, E. A., Akintunde, J. K. and Odaibo, A. (2011). Single cell proteins: as nutritional enhancer. *Advances in Applied Science Research, 2* (5): 396–409.

Adeyemi, O., Grove, I., Peets, S. and Norton, T. (2017). Advanced monitoring and management systems for improving sustainability in precision irrigation. *Sustainability, 9*, 353–382.

Adu-Dapaah, H. K. and Sangwan, R. S. (2004). Improving Bambara groundnut productivity using gamma irradiation and in vitro techniques. *African Journal of Biotechnology, 3* (5): 260–265.

Agbede, J. O. and Aletor, V. A. (2005). Studies of the chemical composition and protein quality evaluation of differently processed *Canavalia ensiformis* and *Mucuna pruriens* seed flours. *Journal of Food Composition and Analysis, 18* (1): 89–103.

Agrawal, S. and Ebrahim, S. (2013). Association between legume intake and self-reported diabetes among adult men and women in India. *BMC Public Health, 13* (1): 706–719.

Ahmed John, S., Sathish Claimer, C. and Prakash, M. (2013). Isolation, purification and characterization of galactose specific lectin from *Adathoda vasica*. *International Journal of Advanced Life Sciences, 6* (3): 238–245.

Aiyeloja, A. A. and Bello, O. A. (2006). Ethnobotanical potentials of common herbs in Nigeria: A case study of Enugu state. *Educational Research and Review*, *1*:16–22.

Ajitaa, T. and Jha, S. K. (2017). Extrusion cooking technology: Principal mechanism and effect on direct expanded snacks: An overview. *International Journal of Food Studies, 6*: 113–128.

Akande,T. O., Odunsi, A. A. and Akinfala, E. O. (2016). A review of nutritional and toxicological implications of castor bean (*Ricinus communis* L.) meal in animal feeding systems. *Journal of Animal Physiology and Animal Nutrition, 100* (2): 201–210.

Akaninwor, J. O. and Okechukwu, P. N. (2006). Evaluation of processed sweetpotato-crayfish – soya bean and sweet potato-crayfish-Bambara groundnut weaning mixtures. *Journal of Applied Sciences and Environmental Management, 10* (1): 55 – 61

Akpalu, M. M., Atubilla, I. A. and Oppong-Sekyere, D. (2013). Assessing the level of cultivation and utilization of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) in the Sumbrungu community of Bolgatanga, upper east region, Ghana. *International Journal of Plant, Animal and Environmental Sciences, 3* (3): 68–75.

Akpapunam, M. A. and Sefa-Dedeh, S. (1997). Some physicochemical properties and anti-nutritional factors of raw, cooked and germinated jack bean (*Canavalia ensiformis*). *Food Chemistry, 59* (1): 121–125.

Allen, K., Remington, B., Baumert, J., Crevel, R., Houben, G. and Brooks-Taylor, S. (2014). Clinical challenge data for development of allergen management thresholds for precautionary labeling of foods- VITAL 2.0. *Journal of Allergy and Clinical Immunology*. *133*, 156–164

Alonso, R., Aguirre, A. and Marzo, F. (2000). Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. *Food Chemistry, 68* (2): 159–165.

Alonso, R., Orue, E. and Marzo, F. (1998). Effect of extrusion and conventional processing methods on protein and antinutritional factor contents in pea seeds. *Journal of Food Chemistry, 63*: 505–512.

Alsohaimy, S. A., Sitohy, M. Z. and El-Masry, R. A. (2007). Isolation and partial characterization of chickpea, lupine and lentil seed proteins. *World Journal of Agricultural Sciences, 3* (1): 123–127.

Anton, A. A. and Luciano, F. B. (2007). Instrumental texture evaluation of extruded snack foods: a review evaluación instrumental de textura en alimentos extruidos: una revisión. *CYTA-Journal of Food, 5* (4): 245–251.

Antunes, P. L. and Sgarbieri, V. C. (1980). Effect of heat treatment on the toxicity and nutritive

value of dry bean (*Phaseolus vulgaris* var. Rosinha G2) Proteins. *Journal of Agricultural and Food Chemistry, 22*: 935–938.

AOAC. (2000). Official Methods of Analysis. 17th ed. Gaithersburg, Maryland.

Audi, J., Belson, M., Patel, M., Schier, J. and Osterloh, J. (2005). Ricin poisoning: A comprehensive review. *The Journal of the American Medical Association, 294* (18): 2342–2351.

Azam-Ali, S. (2010). Fitting underutilized crops within research-poor environments: Lessons and approaches. *South African Journal of Plant Soil, 27*: 293–298.

Bailey, L. H. (1947). Standard Cyclopedia of Horticulture. MacMillan, New York.

Ballhorn, D. J., Lieberei, R. and Ganzhorn, J. U. (2005). Plant cyanogenesis of *Phaseolus lunatus* and its relevance for herbivore–plant interaction: the importance of quantitative data. *Journal of Chemical Ecology, 31*: 1445–1473.

Balzarini, J., Van Laethem, K., Hatse, S., Vermeire, K., De Clercq, E., Peumans, W., Van Damme, E., Vandamme, A.-V., Böhlmstedt, A. and Schols, D. (2004). Profile of resistance of human immunodeficiency virus to mannose-specific plant lectins. *Journal of Virology, 78* (19): 10617–10627.

Barampama, Z. and Simard, R. E. (1993). Nutrient composition, protein quality and antinutritional factors of some varieties of dry beans (*Phaseolus vulgaris*) grown in Burundi. *Journal of Food Chemistry, 47*: 159–167.

Barnes, D.G., Dourson, M., Preuss, P., Bellin, J., Derosa, C., Engler, R., Erdreich, L., Farber, T., Fenner-Crisp, P., Francis, E. and Ghali, G. (1988). Reference dose (RfD): Description and use in health risk assessments. *Regulatory Toxicology and Pharmacology, 8* (4): 471–486.

Bartolomeo, M. P. and Maisano, F. (2006). Validation of a reversed-phase HPLC method for quantitative amino acid analysis. *Journal of Biomolecular Techniques, 17* (2): 131–137.

Baudoin, J. P., Barthelemy, Y. J. and Ndungo, V. (1991). Variability of cyanide contents in the primary and secondary genepools of the lima bean, *Phaseolus lunatus* L. FAO/IBPGR. *Plant Genetic Resources Newsletter, 85*: 5–9.

Beebe, S., Gonzalez, A. and Rengifo, J. (2000). Research on trace minerals in the common bean. *Food and Nutrition Bulletin, 21*: 387–391.

Belitz, H. D., Grosch, W. and Schieberle, P. (2009). Cereals and cereal products. In: *Food Chemistry* (4th). Springer-Verlag, Berlin, Heidelberg, Germany, (pp. 670-745).

Bender, A. E. (1983). Hemagglutinins (lectins) in beans. *Journal of Food Chemistry, 4*: 309–320.

Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N. and Bourne, P.E. (2000). The protein data bank. *Nucleic Acids Research, 28* (1): 235–242.

Bhat, R. and Karim, A. A. (2009). Exploring the nutritional potential of wild and underutilized legumes. *Comprehensive Reviews in Food Science and Food Safety, 8*: 305–331.

Bhatty, R. S. and Macgregor, A. W. (1998). Gamma irradiation of hulless barley: effect on grain composition, β-glucans and starch. *Cereal Chemistry, 65*: 463–470.

Boehringer, A. and Caldwell, R. (1989). *Cajanus cajan* (L.) Millsp. as a potential agroforestry component in the Eastern Province of Zambia. *Agroforestry Systems, 9*(2): 127–140.

Bollini, R., Carnovale, E. and Campion, B. (1999). Removal of antinutritional factors from beans (*Phaseolus vulgaris* L.) seeds. *Biotechnology Agronomy Society and Environment 3* (4): 217–219.

Boniglia, C., Fedele, E. and Sanzini, E. E. (2003). Measurement by ELISA of *active lectin in dietary supplements containing* Kidney bean protein. *Journal of Food Science, 68* (4): 1283–1286.

Bourne, Y., Bolgiano, B., Liao, D.I., Strecker, G., Cantau, P., Herzberg, O., Feizi, T. and Cambillau, C. (1994). Crosslinking of mammalian lectin (galectin-1) by complex biantennary saccharides. *Nature Structural Biology, 1*: 863–870.

Bouvard, V., Loomis, D., Guyton, K.Z., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Mattock, H. and Straif, K. (2015). Carcinogenicity of consumption of red and processed meat. *The Lancet Oncology, 16* (16): 1599–1600.

Bressani, R. (2002). Factors influencing nutritive value in food grain legumes: *Mucuna* Compared to other grain legumes. In: B. M. Flores, M. Eilittä, R. Myhrman, L. B. Carewand R. J. Carsky (Eds.), Food and Feed from Mucuna: Current Uses and the Way Forward (pp. 164–188). Workshop, CIDICCO, CIEPCA and World Hunger Research Center, Tegucigalpa, Honduras (April 26-29, 2000).

Bressani, R. and Elías, L. G. (1980). Nutritional value of legume crops for humans and animals. Nutritional Value of Legume Crops for Humans and Animals, (pp. 135–155).

Brinkman, B. G., Khan, A., Edner, B. and Rosén, L. A. (2014). Self-objectification, feminist activism and conformity to feminine norms among female vegetarians, semi-vegetariansand non-vegetarians. *Eating Behaviors, 15* (1): 171–174.

Broekaert, W. F., Van Parijs, J., Leyns, F., Joos, H. and Peumans, W. J. (1989). A chitin-binding lectin from stinging nettle rhizomes with antifungal properties. *Science, 245*: 1100–1102.

Brown, R. L., Griffith, R. L., Neubauer, R. H. and Rabin, H. (1982). The effect of T cell growth

factor on the cell cycle of primate T cells. *Journal of Immunology, 129* (5): 1849–1853.

Budavari, S., O'Neil, M. J., Smith, A. and Heckelman, P. E. (1989). The Merck index: An encyclopedia of chemicals, drugs and biologicals. Rahway NJ, USA: Merck and Co. *Inc*, (pp. 1384-1385).

Carlini, C. R. and Guimaraes, J. (1991). Plant and microbial toxic proteins as hemilectins: emphasis on canatoxin. *Toxicon, 29*: 791–806.

Carlini, C. R. and Udedibie, A. B. I. (1997). Comparative effects of processing methods on haemaglutinating and antitryptic activities of *Canavalia ensiformis* and *Canavalia braziliensis* seed. *Journal of Agriculture and Food Chemistry, 45*: 4372–4377.

Carrera, C.S., Reynoso, C.M., Funes, G.J., Martínez, M.J., Dardanelli, J. and Resnik, S.L.(2011). Amino acid composition of soybean seeds as affected by climatic variables. *Pesquisa Agropecuária Brasileira, 46* (12): 1579-1587.

Carvalho, D. D., Marangoni, S., Oliveira, B. and Novello, J. C. (1998). Isolation and characterization of a new lectin from the venom of the snake Bothrops jararacussu. *IUBMB Life*, *44* (5): 933–938.

Çevikkalp, S. A., Löker, G. B., Yaman, M. and Amoutzopoulos, B. (2016). A simplified HPLC method for determination of tryptophan in some cereals and legumes. *Food Chemistry, 193*: 26–29.

Chardon, J. and Swart, A. (2016). Food consumption and handling survey for quantitative microbiological consumer phase risk assessments. *Journal of Food Protection, 79* (7): 1221–1233.

Chivenge, P., Mabhaudhi, T., Modi, A. T. and Mafongoya, P. (2015). The potential role of neglected and underutilised crop species as future crops under water scarce conditions in sub-Saharan Africa. *International Journal of Environmental Research and Public Health, 12* (6): 5685–5711.

Coppo, R., Amore, A. and Roccatello, D. (1992). Dietary antigens and primary IgA nephropathy. *Journal of the American Society of Nephrology*, *2* (10): 173–180.

Cristina, M., Paes, D. and Maga, J. (2004). Effect of extrusion on essential amino acids profile and color of whole-grain flours of quality protein maize and normal maize cultivars. *Brazilian Magazine of Corn and Sorghum*, *3* (1): 10–20.

Crump, K. S. (1984). A new method for determining allowable daily intakes. *Toxicological Sciences, 4* (5): 854–871.

Cuadrado, C., Ayet, G., Robredo, L.M., Tabera, J., Villa, R., Pedrosa, M.M., Burbano, C. and

Muzquiz, M. (1996). Effect of natural fermentation on the content of inositol phosphates in lentils. *European Food Research and Technology*, *203* (3): 268–271.

Cullis, C. and Kunert, K. J. (2017). Unlocking the potential of orphan legumes. *Journal of Experimental Botany*, *68* (8): 1895–1903.

D'Mello, J. P. F. (1989). Toxic amino acids. In: J. P. F. DMello, C. M. Duffusand J. H. Duffus (Eds.). Antinutritional factors, potential toxic substances in plants, aspects of applied biology. WellesbourneL Warwick: Association of Applied Biologists. (pp. 29–50).

Dalkin, K. and Bowles, D. J. (1983). Analysis of inter-relationship of jackbean seed components by two-dimensional mapping of iodinated tryptic peptides. *Planta*, *157*: 536–539.

Damodaran, S. (1996) Amino Acids, Peptides and Proteins. In: Fennema, R.O., Food Chemistry, 3rd Edition, CRC Press, New York, (pp. 321-416).

Daniel, C. R., Cross, A. J., Koebnick, C. and Sinha, R. (2011). Trends in meat consumption in the USA. *Public Health Nutrition*, *14* (4): 575–583.

Dawson, B. and Trapp, R. G. (2004). Basic and Clinical Biostatistics 4th ed. New York: Mc-Graw Hill;

De Geyter, E., Lambert, E., Geelen, D. and Smagghe, G. (2007). Novel advances with plant saponins as natural insecticides to control pest insects. *Pest Technology*, *1* (2): 96–105.

Dessen, A., Gupta, D., Sabesan, S., Brewer, C. F. and Sacchettini, J. C. (1995). X-ray crystal structure of the soybean agglutinin cross-linked with a biantennary analog of the blood group I carbohydrate antigen. *Biochemistry*, *34* (15): 4933–4942.

Dhurandhar, N. V. and Chang, K. C. (1990). Effect of cooking on firmness, trypsin inhibitors, lectins and cystine/cysteine content of navy and red kidney beans (*Phaseolus vulgaris*). *Journal of Food Science*, *55* (2): 470–474.

Doku, E. (1996). Problems and prospects for the improvement of bambara groundnut. In Proceedings of the International Bambara Groundnut Symposium, July 23-25, University of Nottingham, *UK.*, (pp. 19–27).

Dolan, L. C., Matulka, R. A. and Burdock, G. A. (2010). Naturally occurring food toxins. *Toxins (Basel)*, *2* (9): 2289–2332.

Donkor, A., Osei-Fosu, P., Nyarko, S., Kingsford-Adaboh, R. and Abrefi Okyere, J.Y. (2015). Health risk assessment of pesticide residues via dietary intake of cowpea and Bambara beans among adults in Accra Metropolis, Ghana. *Research Journal of Chemical and Environmental Sciences*, *3* (1): 10–18.

Doss, A.; Pugalelenthi, M. and Vadivel, V. (2011). Nutritional evaluation of wild Jack bean (

Canavalia ensiformis DC) seeds in different locations of South India. *World Applied Sciences Journal, 13* (7): 1606–1612.

Drewnowski, A. and Popkin, B. M. (1997). The nutrition transition: New trends in the global diet. *Nutrition Reviews, 55*: 31–43.

Duke, J. A. (1981). Legume species. In: Handbook of Legumes of World Economic Importance (pp. 5–310). Springer.

Durst, P. and Bayasgalanbat, N. (2014). Promotion of Underutilized Indegenous Food Resources for Food Security and Nutrition in Asia and the Pacific. RAP Publication, 201 4/07.

EFSA. (2012). Guidance on the use of probabilistic methodology for modelling dietary exposure to pesticide residues. *EFSA Journal, 10*: 1–95.

Egounlety, M. (2003). Processing of velvet bean (*Mucuna pruriens var utilis*) by fermentation. *Tropical and Subtropical Agroecosystems, 1:* (2–3).

Eknayake, S., Jansz, E. R. and Nair, B. M. (1999). Proximate composition, mineral and amino acid content of mature *Canavalia gladiata* seeds. *Food Chemistry, 66* (1): 115–119.

El-Moneim, G., Honke, J. and Bednarska, A. (2000). Effect of frying various legumes under optimum conditions on amino acids, invitro protein digestibility, phytate and oligosaccharides. *Journal of the Science Food and Agriculture, 80*: 57–62.

Emani, C. and Hall, T. C. (2008). Phaseolin: Structure and Evolution. *The Open Evolution Journal, 2*: 66–74.

Enwere, M. J. (1998). Foods of Plant Origin Processing and Utilization with Recipes and Technology Profiles. Afro Orbis Publication Ltd. Nsukka. (pp. 224-228).

EPMAR (European Public MRL Assessment Report). (2008). Lectin extracted from red kidney beans (*Phaseolus vulgaris*) porcine species. Doc. Ref: EMEA/CVMP/491626/2007, 7 Westferry Circus, Canary Wharf, London, E14 4HB-UK. European Medicine Agency Veterinary Medicines and Inspections.

Falco, S. C., Guida, T., Locke, M., Mauvais, J., Sanders, C., Ward, R. T. and Webber, P. (1995). Transgenic canola and soybean seeds with increased lysine. *Nature Biotechnology, 13* (6): 577–582.

FAO. (2004). The state of food insecurity in the world. FAO: Rome, Italy.

FAO/WHO. (1990). Food and Agriculture Organization/World Health Organization. Protein quality evaluation,. Rome, Italy: FAO Food and Nutrition Paper 52.

FAO/WHO/UNU. (1991). Protein quality evaluation Report of Joint FAO/WHO Expert Consultation. Bethesda, Md., USA.

FAO/WHO/UNU. (2007). WHO, Report of a Joint WHO/FAO/UNU Expert Consultation. Protein and amino acid requirements in human nutrition (WHO Technical Report Series No. 935). Geneva: World Health Organization.

FAOSTAT. (2009). UN Statistics Division (FAOSTAT), Food balance sheets. Rome, Italy: WHO Press, World Health Organization.

FAO (2013). Dietary protein quality evaluation in human nutrition. Report of an FAO Expert Consultation. FAO food and nutrition paper 92, Food and Agriculture Organization of the United Nations, Rome, Italy

Fasoyiro, S. B., Akande, S. R., Arowora, K. A., Sodeko, O. O., Sulaiman, P. O., Olapade, C. O. and Odiri, C. E. (2010). Physico-chemical and sensory properties of Pigeonpea (*Cajanus cajan*) flours. *African Journal of Food Science, 4*: 120–126.

Fathima, K.R., Soris, P.T. and Mohan, V.R. (2010). Nutritional and antinutritional assessment of *Mucuna pruriens* (L.) DC var. *pruriens* an underutilized tribal pulse. *Advances in Bioresearch, 1* (2):79–89.

Fernández-Alonso, M. D., Cañada, F. J., Jiménez-Barbero, J. and Cuevas, G. (2015). Molecular recognition of saccharides by proteins. Insights on the origin of the carbohydrate-aromatic interactions. *Journal of the American Chemical Society, 127* (20): 7379–7386.

Ferriz-Martínez, R., García-García, K., Torres-Arteaga, I., Rodriguez-Mendez, A. J., Guerrero-Carrillo, M. J., Moreno-Celis, U. and Garcia-Gasca, T. (2015). Tolerability assessment of a lectin fraction from tepary bean seeds (*Phaseolus acutifolius*) orally administered to rats. *Toxicological Reports, 2*: 63–69.

Fikre, A., Korbu, L., Kuo, Y. and Lambein, F. (2008). The contents of the neuro-excitatory amino acid β-ODAP (β-N-oxalyl-L-α,β-diaminopropionic acid) and other free and protein amino acids in the seeds of different genotypes of grass pea (*Lathyrus sativus* L.). *Food Chemistry, 110* (2): 422–427.

Flores, B. (2002). Food and feed from mucuna: current uses and the way forward; proceedings. In: International Workshop on Food and Feed from Mucuna: Current Uses and the Way Forward26-29 Abr 2000 Tegucigalpa (Honduras). CIDICCO, Tegucigalpa (Honduras) IITA, Cotonou (República de Benin) World Hunger Research Center, Elgin, IL (EUA). Judson College.

Follmer, C., Barcellos, G.B., Zingali, R.B., Machado, O.L., Alves, E.W., Barja-Fidalgo, C., Guimarães, J.A. and Carlini, C.R. (2001). Canatoxin, a toxic protein from Jack beans (*Canavalia ensiformis*), is a variant form of urease (EC 3.5.1.5): Biological effects of urease independent of its ureolytic activity. *Biochemical Journal, 360*: 217–224.

Folta, S. C., Goldberg, J. P., Economos, C., Bell, R. and Meltzer, R. (2006). Food advertising targeted at school-age children: A content analysis. *Journal of Nutrition Education and Behavior, 38*: 244–248.

Foyer, C.H., Lam, H.M., Nguyen, H.T., Siddique, K.H., Varshney, R.K., Colmer, T.D., Cowling, W., Bramley, H., Mori, T.A., Hodgson, J.M. and Cooper, J.W. (2016). Neglecting legumes has compromised human health and sustainable food production. *Nature Plants, 2*: 16112.

Francis, G., Makkar, H. and Becker, K. (2001). Antinutritional factors present in plant derived alternate fish feed ingredients and their effects in fish. *Aquaculture, 199*: 197–227.

Freed, D. L. J. (1979). Dietary lectins and the anti-nutritive effects of gut allergy. In: Protein Transmission Through Living Membranes. Elsevier/North Holland Biomedical Press, (pp. 411–422).

Friedman, M. and Brandon, D. L. (2001). Nutritional and health benefits of soy proteins. *Journal of Agricultural and Food Chemistry, 49* (3): 1069–1086.

Galili, G., Galili, S., Lewinsohn, E. and Tadmor, Y. (2002). Genetic, molecular and genomic approaches to improve the value of plant foods and feeds. *Critical Reviews in Plant Sciences, 21* (3): 167–204.

Gayathri, M. J., Guna, S. N. O. and Ramash, K. K. (2011). Performance Evaluation of Membership Functions on Fuzzy Logic Controlled AC Voltage Controller for Speed Control of Induction Motor Drive. *International Journal of Computer Applications, 13* (5): 8–12.

Gebrelibanos, M., Tesfaye, D., Raghavendra, Y. and Sintayeyu, B. (2013). Nutritional and health implications of Legumes. *International Journal of Pharmaceutical Sciences and Research, 4* (4): 1269–1279.

Gehrke, C. W., Rexroad, P. R., Schisla, R. M., Absheer, J. and Zumwalt, R. W. (1987). Quantitative analysis of cystine, methionine, lysine and nine other amino acids by a single oxidation--4 hour hydrolysis method. *Journal-Association of Official Analytical Chemists, 70* (1): 171–174.

Gell, P. G. H. and Coombs, R. R. A. (1963). The classification of allergic reactions underlying disease. In: Coombs, R.R.A. and Gell, P.G.H., editors. Clinical Aspects of Immunology. London, UK: Blackwell Science;

Gendel, S. M. (2012). Comparison of international food allergen labeling regulations. *Regulatory Toxicology and Pharmacology, 63* (2): 279–285.

Gerba, C. P. (1999). Risk assessment. In: C. N. Haas, J. B. Roseand C. P. Gerba (Eds.), *Quantitative microbial risk assessment*. John Wiley and Sons, (pp. 213–234)

Ghadge, P. N., Shewalkar, S. V. and Wankhede, D. B. (2008). Effect of processing methods on qualities of instant whole legume: Pigeon pea (*Cajanus cajan* L.). *Agricultural Engineering International: The CIGR e-Journal*, 1–8.

Ghanadistricts. (2013). Ghanadistricts. http://www.ghanadistricts.com/districts/?r=6and_=93andsa=7239(Accessed: November 10, 2015)

Ghosal, S., Chaudhuri, R. K. and Dutta, S. K. (1971). Alkaloids of the flowers of Arundo donax. *Phytochemistry*, *10* (11): 2852–2853.

Ghosh, M. and Mandal, D. K. (2001). Analysis of equilibrium dissociation and unfolding in denaturants of soybean agglutinin and two of its derivatives. *International Journal of Biological Macromolecules*, *29* (4): 273–280.

Giacometti, J. (2015). Plant lectins in cancer prevention and treatment. *Medicina Fluminensis*, *51* (2): 211–229.

Gibney, M. J. and van der Voet, H. (2003). Introduction to the Monte Carlo project and the approach to the validation of probabilistic models of dietary exposure to selected food chemicals. *Food Additives and Contaminants*, 20 (Suppl 1): S1-S7

Gilsenan, M. B., Lambe, J. and Gibney, M. J. (2003). Assessment of food intake input distributions for use in probabilistic exposure assessments of food additives. *Food Additives and Contaminants*, *20*: 1023–1033.

Goldflus, F., Ceccantini, M. and Santos, W., 2006. Amino acid content of soybean samples collected in different Brazilian states: harvest 2003/2004. *Revista Brasileira de Ciência Avícola*, *8* (2): 105-111.

Govindasamy, S., Campanella, O. H. and Oates, C. G. (1997). Enzymatic hydrolysis of sago starch in a twin-screw extruder. *Journal of Food Engineering*, *32* (4): 403–426.

Granito, M., Valero, Y. and Zambrano, R. (2010). Baked product development based on fermented legumes and cereals for school children snack. *Archivos Latinoamericanos de Nutrición*, *60* (1): 85–92.

Gravetter, F. J. and Wallnau, L. B. (2000). Statistics for the Behavioral Sciences 5th ed. Belmont: Wadsworth – Thomson Learning.

Greer, F. and Pusztai, A. (2009). Toxicity of kidney bean (*Phaseolus vulgaris*) in rats: Changes in intestinal permeability. *Digestion*, *32* (1): 42–46.

Grimaud, P. (1988). The Pigeon pea (*Cajanus cajan*): A possible alternative for traditionnal pig and poultry farming in New-Caledonia. Revue d'Elevage and Veterinary Medicine of New

Caledonia (France)

Guzmán-Uriarte, M.L., Sánchez-Magaña, L.M., Angulo-Meza, G.Y., Cuevas-Rodríguez, E.O., Gutiérrez-Dorado, R., Mora-Rochín, S., Milán-Carrillo, J., Valdez-Ortiz, A. and Reyes-Moreno, C. (2013). Solid state bioconversion for producing common bean (*Phaseolus vulgaris* L.) Functional flour with high antioxidant activity and antihypertensive potential. *Food and Nutrition Sciences*, *4*: 480–490.

Habib, H. and Fazili, K. (2007). Plant protease inhibitors: A defense strategy in plants. *Biotechnology and Molecular Biology Review*, *2* (3): 68–85.

Hadley, C. (2006). Food allergies on the rise? Determining the prevalence of food allergies and how quickly it is increasing, is the first step in tackling the problem. *European Molecular Biology Organization Report*, *7* (11): 1080–1083.

Hailu, M., Workneh, T. S. and Belew, D. (2015). Review on postharvest technology of Banana fruit. *African Journal* of *Biotechnology*, *12* (*7*): 635-647

Han, I.H., Swanson, B.G. and Baik, B.K. (2007). Protein digestibility of selected legumes treated with ultrasound and high hydrostatic pressure during soaking. *Cereal chemistry*, *84* (5): 518-521.

Hardy, K., Buckley, S., Collins, M.J., Estalrrich, A., Brothwell, D., Copeland, L., García-Tabernero, A., García-Vargas, S., de la Rasilla, M., Lalueza-Fox, C. and Huguet, R. (2012). Neanderthal medics? Evidence for food, cooking and medicinal plants entrapped in dental calculus. *The Science of Nature*, *99* (8): 617–626.

Harinder, K., Kaur, B. and Sharma, S. (1999). Studies on the baking properties of wheat: Pigeonpea flour blends. *Plant Foods for Human Nutrition*, *54* (3): 217–226.

Hasler, C. M. (2002). The cardiovascular effects of soy products. *Journal of Cardiovascular Nursing*, *16*: 50–63.

Hatakeyama, T., Ryota Karino, Y., Terai, Y., Kimura, M., Unno, H. and Goda, S. (2012). An assay for carbohydrate-binding activity of lectins using polyamidoamine dendrimer conjugated with carbohydrates. *Bioscience Biotechnology Biochemistry*, *76*(10): 1999–2001.

Hathcock, J., Shao, A., Vieth, R. and Haene, R. (2007). Risk assessment for vitamin D. *American Journal of Clinical Nutrition*, *85*: 6–18.

Henne, L., Armed, E. M., George, D. E., Kao, K. J. and Harry S. Sitren. (1990). Tolerance to long-term feeding of isolated peanut lectin in the rat: Evidence for a trophic effect on the small intestines. *Journal of Nutritional Science and Vitaminology*, *36*: 599–607.

Hillocks, R. J., Bennett, C. and Mponda, O. M. (2012). Bambara nut: A review of utilisation,

market potential and crop improvement. *African Crop Science Journal, 20* (1): 1–16.

Hughes, D. L. and Arnett, E. M. (1983). Acid-base thermochemistry at elevated temperatures. *Journal of the American Chemical Society, 105* (13): 4157–4163.

Huisman, J., van der Poel, A. F. B., van Leeuwen, P. and Verstegen, M. W. A. (1990). Comparison of growth, nitrogen metabolism and organ weights in piglets and rats fed on diets containing *Phaseolus vulgaris* beans. *British Journal of Nutrition, 64* (3): 743–753.

IBM Corp. (2016). IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.

Ifuku, S. and Saimoto, H. (2012). Chitin nanofibers: preparations, modifications and applications. *Nanoscale, 4* (11): 3308–3318.

Infante, M., Perz, A., Simao, M., Manda, F., Baquete, E., Fernabdes, A. and Cliff, G. L. (1990). Outbreak of acute toxic psychosis attributed to *Mucuna pruriens. The Lancet, 336 (8723)*: doi.org/10.1016/0140-6736(90)92603-F.

Iobst, S. T., Wormald, M. R., Weis, W. I., Dwek, R. A. and Drickamer, K. (1994). Binding of sugar ligands to Ca^{2+}-dependent animal lectins: Analysis of mannose binding by site-directed mutagenesis and NMR. *Journal of Biological Chemistry, 269* (22): 15505–15511.

IPGRI. (2002). Neglected and Underutilized Plant Species Strategic Action Plan.

Jaffe, C. L., Ehrlichrogozinski, S., Lis, H. and Sharon, N. (1997). Transition-metal requirements of soybean agglutinin. *Federation of European Biochemical Societies Letters, 82*: 191–196.

Jedrychowski, L. and Wichers, H. J. (2009). Chemical and Biological Properties of Food Allergens. *CRC Press*.

Jiddere, G. and Filli, K. B. (2015). The effect of feed moisture and barrel temperature on the essential amino acids profile of sorghum malt and Bambara groundnut based extrudates. *Journal of Food Processing and Technology, 6* (5): 1-6

Jones, F. A. (1996). Herbs – useful plants. Their role in history and today. *European Journal of Gastroenterology and Hepatology, 8*: 1227–1231.

Jukanti, A. K., Gaur, P. M., Gowda, C. L. L. and Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): A review. *British Journal of Nutrition, 108* (S1): S11–S26.

Kalogeropoulos, N., Chiou, A., Ioannou, M., Karathanos, V., Hassapidou, M., Nikolaos, K. and Andrikopoulos, N. (2010). Nutritional evaluation and bioactive microconstituents (phytosterols, tocopherols, polyphenols, triterpenic acids) in cooked dry legumes usually consumed in the Mediterranean countries. *Food Chemistry, 121*: 682–690.

Kamanga, B. C. G., Shamudzarira, Z. and Vaughan, C. (2003). On-farm legume experimentation to improve soil fertility in the Zimuto Communal Area, Zimbabwe: Farmer perceptions and feedback. Risk Management Project, CIMMYT Natural Resources Group, Harare, Zimbabwe. Series 03-02

Kathirvel, P. and Kumudha, P. (2011). A comparative study on the chemical composition of wild and cultivated germplasm of *Phaseolus lunatus* L. *International Journal of Applied Biology and Pharmaceutical Technology*, *2* (4): 1–10.

Kearney, J. (2010). Food consumption trends and drivers. *Philosophical Transactions of the Royal Society B, 365*: 2793–2807.

Kelkar, S., Siddiq, M., Harte, J. B., Dolan, K. D., Nyombaire, G. and Suniaga, H. (2012). Use of low-temperature extrusion for reducing phytohemagglutinin activity (PHA) and oligosaccharides in beans (*Phaseolus vulgaris* L.) cv. Navy and Pinto. *Food Chemistry*, *133*(4): 1636–1639.

Key, T. J., Schutzian, A., Willett, W. C., Allen, N. E., Spencer, E. A. and Travis, R. C. (2004). Situating moral disengagement: Motivated reasoning in meat consumption and substitution. *Public Health Nutrition*, *7*(1A): 187–200.

Knorr, D. (1982). Functional properties of chitin and chitosan. *Journal of Food Science*, *47*: 593–595.

Kolatkar, A. R. and Weis, W. I. (1996). Structural basis of galactose recognition by C-type animal lectins. *The Journal of Biological Chemistry*, *271* (12): 6679–7785.

Kong, C., Kang, H.G., Kim, B.G. and Kim, K.H. (2014). Ileal digestibility of amino acids in meat meal and soybean meal fed to growing pigs. *Asian-Australasian journal of animal sciences*, *27* (7): 990-995.

Kuku, A. and Nkiruka, U. U. (2009). Physicochemical properties and toxicity studies of a lectin from the aerial bulb, *Dioscorea bulbifera* (Linn.). *American Journal of Research Communication*. ISSN: 2325-4076. www.usa-journals.com (Accessed: November 10 2015)

Kumar, S., Verma, A.K., Sharma, A., Kumar, D., Tripathi, A., Chaudhari, B.P., Das, M., Jain, S.K. and Dwivedi, P.D. (2013). Phytohemagglutinins augment Red Kidney bean (*Phaseolus vulgaris* L.) induced allergic manifestations. *Journal of Proteomics*, *93*: 50–64.

Kumari, N. N. and Pattabiraman, T. N. (1990). Natural plant enzyme inhibitors: isolation and properties of a trypsin inhibitor from Jack bean (*Canavalia ensiformis*). *Indian Journal of Biochemistry and Biophysics*, *27* (5): 332–338.

Lababidi, H. M. S. and Baker, C. G. J. (2006). Fuzzy Modeling. In: S. S. Sablani, M. Shafiur Rahman, A. K. Dattaand A. S. Mujumdar (Eds.), Food and Bioprocess Modelling

Techniques, CRC Press, (pp. 451–497).

LaGrega, M. D., Buckingham, P. L. and Evans, J. C. (2010). *Hazardous Waste Management*. Waveland Press.

Lam, S. K. and Ng, T. B. (2011). Apoptosis of human breast cancer cells induced by hemagglutinin from *Phaseolus vulgaris* cv. Legumi secchi. *Food Chemistry, 126* (2): 595–602.

Lampariello, L. R., Cortelazzo, A., Guerranti, R., Sticozzi, C. and Valacchi, G. (2012). The magic Velvet bean of *Mucuna pruriens*. *Journal of Traditional and Complementary Medicine, 2* (4): 331–339.

Landry, J. and Delhaye, S. (1992). Simplified procedure for the determination of tryptophan of foods and feedstuffs from barytic hydrolysis. *Journal of Agricultural and Food Chemistry, 40* (5): 776–779.

Lannoo, N. and Van Damme, E. J. M. (2014). Lectin domains at the frontiers of plant defense. *Frontiers in Plant Science, 5*: 397.

Lasekan, O. O. and Lasekan, W. O. (2000). Moisture sorption and the degree of starch polymer degradation on flours of popped and malted sorghum (*Sorghum bicolo*r). *Journal of Cereal Science, 31*: 55–61.

Lawley, R., Laurie, C. and Davis, J. (2008). The Royal Society of Chemistry. Thomas Graham House, Science Park, Milton Road, Cambridge CB4 0WF, UK.

Lemke, S., Müller, C., Lipke, E., Uhl, G. and Hildebrandt, J. (2013). May salivary gland secretory proteins from hematophagous leeches (*Hirudo verbana*) reach pharmacologically relevant concentrations in the vertebrate host? *PLoS One, 8* (9): e73809.

Liener, I. (1974). Phytohemagglutinins: Their nutritional significance. *Journal of Agricultural and Food Chemistry, 22*: 17–22.

Liener, I. E. and Shohachi, W. (1956). Chemical modification of the soy bean hemagglutinin. *Journal of Biological Chemistry, 222*: 695–704.

Lin, J. Y. and Liu, S. Y. (1986). Studies on the antitumor lectins isolated from the seeds of *Ricinus communis* (Castor bean). *Toxicon, 24*: 757– 765.

Linnemann, A. R. and de Bruijn, G. H. (1987). Traditional seed supply for food crops. *ILEIA Newsletter, 3* (2): 10–11.

Lokuruka, M. N. I. (2011). Effects of processing on soybean nutrients and potential impact on consumer health: An overview. *African Journal of Food, Agriculture, Nutrition and Development, 11* (4): 5000–5017.

Lorenzetti, E., Mac Isaac, S., Arnason, J.T., Awang, D.V.C., Buckles, D. (1998). The phytochemistry, toxicology and food potential of velvet bean (*Mucuna adans* spp. Fabaceae) In: Buckles D, Osiname O, Galiba M, Galiano G, editors. Cover crops of West Africa; contributing to sustainable agriculture. Ottawa, Canada & IITA, Ibadan, Nigeria: IDRC; p. 57.

Loris, R., Hamelryck, T., Broekaert, J. and Wyns, L. (1998). Review: Legume lectin structure. *Biochimica et Biophysica Acta, 1383* (1): 9–36.

Lovati, M.R., Manzoni, C., Castiglioni, S., Parolari, A., Magni, C. and Duranti, M. (2012). Lupin seed γ-conglutin lowers blood glucose in hyperglycaemic rats and increases glucose consumption of HepG2 cells. *British Journal of Nutrition*, *107* (1): 67–73.

Luciani, G., Altpeter, F. and Wofford, D. S. (2005). Over-expression of a soybean vegetative storage protein gene in Bahiagrass (*Paspalum notatum* var. Flugge). Poster Presented at Florida Genetics, November, 1526–1533.

Lyimo, M., Mugula, J. and Elias, T. (1992). Nutritive composition of broth from selected bean varieties cooked for various periods. *Journal of Agriculture and Food Chemistry*, *58*: 535–539.

Ma, Y. and Wang, T. (2010). Deactivation of soybean agglutinin using non-thermal treatments. *Journal of Agricultural and Food Chemistry*, *58*: 11413–11419.

Maentz, D. D., Irish, G. G. and Classen, H. L. (1999). Carbohydrate binding and agglutinating lectins in raw and processed soybean meals. *Animal Food Science and Technology*, *76*: 335–343.

Magalhaes, J.V., Liu, J., Guimaraes, C.T., Lana, U.G., Alves, V.M., Yi-Hong, W.A.N.G., Schaffert, R.E., Hoekenga, O.A., Pineros, M.A., Shaff, J.E. and Klein, P.E. (2007). A gene in the multidrug and toxic compound extrusion (MATE) family confers Aluminum tolerance in sorghum. *Nature Genetics*, *39*: 1156–1161.

Makkar, H. P. S., Francis, G. and Becker, K. (2007). Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. *Animal*, *1* (9): 1371–1391.

Manikandan, S. (2011). Measures of central tendency: The mean. *Journal of Pharmacology and Pharmacotherapeutics.*, *2* (2): 140–142.

Manneberg, M., Lahm, H.-W. and Fountoulakis, M. (1995). Quantification of cysteine residues following oxidation to cysteic acid in the presence of sodium azide. *Analytical Biochemistry*, *231* (2): 349–353.

Marzo, F., Alonso, R., Urdaneta, E., F.J. Arricibitaand F. Ibanez. (2002). Nutritional quality of

extruded kidney bean (*Phaseolus vulgaris* L. var. Pinto) and its effects on growth and skeletal muscle nitrogen fractions in rats. *Journal of Animal Science*, *80*: 875–879.

Matlab. (2012). Fuzzy Logic Toolbox Matlab Inc. US

McCleary, B. V., Gibson, T. S. and Mugford, D. C. (1997). Measurement of total starch in cereal products by amyloglucosidase-α-amylase method: Collaborative study. *Journal of Association of Official Analytical Chemists International*, *80*: 571–579.

McNamara, C., Naddy, B., Rohan, D. and Sexton, J. (2003). Design, development and validation of software for modelling dietary exposure to food chemicals and nutrients. *Food Additives and Contaminants*, *20*: 8–26.

McPherson, L. L. (1990). The effect of the consumption of Red Kidney beans *(Phaseolus vulgaris*) on the growth of rats and the implications for human populations. *The Journal of the Royal Society for the Promotion of Health*, *110* (6): 222–226.

Menard, C., Heraud, F., Volatier, J.-L. and Leblanc, J.-C. (2008). Assessment of dietary exposure of nitrate and nitrite in France. *Food Additives and Contaminants: Part A*, *25* (8): 971–988.

Mendez, A., Vargas, R. E. and Michelangeli, C. (1998). Effects of concanavalin A, fed as a constituent of Jack bean (*Canavalia ensiformis* L.) seeds, on the humoral immune response and performance of broiler chickens. *Poultry Science*, *77* (2): 282–289.

Messina, M. J. (1999). Legumes and soybeans: Overview of their nutritional profiles and health effects. *The American Journal of Clinical Nutrition*, *70(Suppl):* 439S–450S.

Michelangeli, C. and Vargas, R. (1994). L-canavanine influences feed intake, plasma basic amino acid concentrations and kidney arginase activity in chicks. *Journal of Nutrition*. *124*: 1081–1087

Microsoft. (2014). Microsoft Office Excel. US: Microsoft Corporation.

MIG. (2013). Mintel international group meat alternatives—US., www.mintel.com. (Accessed: September 1, 2016)

Mitchell, D. A., Fadden, A. J. and Drickamer, K. (2001). A novel mechanism of carbohydrate-recognition by the C-type lectins DC-SIGN and DC-SIG N-R. Subunit organization and binding to multi-valent ligands. *Journal of Biological Chemistry*, *276*: 28939–28945.

Miyake, K., Tanaka, T. and McNeil, P. L. (2007). Lectin-based food poisoning: A new mechanism of protein toxicity. *PLoS ONE*, *2* (8): e687.

Mojica, E. R. E. and Merca, F. (2004). Lectin from the body walls of black sea cucumber (*Holothuria atra Jäge*r). *Philippian Journal of Science*, *133*: 77–85.

Møller, A. and Ireland, J. (2000). LanguaL 2000: The LanguaL Thesaurus. Directorate-General for Research.

Monteiro, M. R. P., Costa, A. B. P., Campos, S. F., Silva, M. R. C., da Silva, C. O., Martino, H. S. D. and Silvestre, M. P. C. (2014). Evaluation of the chemical composition, protein quality and digestibility of lupin (*Lupinus albus* and *Lupinus angustifolius*). *The World of Health, São Paulo*, *38*: 251–259.

Moughan, P. J., Gilani, S., Rutherfurd, S. M., and Tomé, D. (2012). True ileal amino acid digestibility coefficients for application in the calculation of digestible indispensable amino acid score (DIAAS) in human nutrition. Report of a Sub-Committee of the 2011 FAO Consultation on "Protein quality evaluation in human nutrition," Food and Agriculture Organization of the United Nations, Rome, Italy.

Muraro, A., Werfel, T., Hoffmann-Sommergruber, K., Roberts, G., Beyer, K., Bindslev-Jensen, C. and Fernandez Rivas, M. (2014). EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. *Allergy*, *69* (8): 1008–1025.

Murevanhema, Y. Y. and Jideani, V. A. (2013). Potential of Bambara groundnut (*Vigna subterranea* (L.) Verdc) milk as a probiotic beverage—A review. *Critical Reviews in Food Science and Nutrition*, *53*: 954–967.

Nachbar, M. S. and Oppenheim, J. D. (1980). Lectins in the Diet: A survey of lectins in commonly consumed foods and a review of the literature. *American Journal of Clinical Nutrition*, *33*: 2338–2345.

Nachbar, M. S., Oppenheim, J. D. and Thomas, J. O. (1980). Lectins in the US diet: Isolation and characterization of a lectin from the tomato (*Lycopersicon esculentum*). *Journal of Biological Chemistry*, *255* (5): 2056–2061.

Nader, N., Hanen, B. I., Fatma, B. A.-F. and Namjun, C. (2015). In vitro assessment of *Phaseolus vulgaris* L. lectins activities against various pathogenic and beneficial microbes. *Research of Journal Biotechnology*, *10* (11): 37–47.

Nakitto, A. M., Muyonga, J. H. and Nakimbugwe, D. (2015). Effects of combined traditional processing methods on the nutritional quality of beans. *Food Science and Nutrition*, *3* (3): 233–241.

Nartey, F. (1980). Toxicological aspects of cyanogenesis in tropical foodstuffs. Toxicology in the Tropics, Taylor and Francis, London, (pp. 53–73).

Nasseri, A. T., Rasoul-Amini, S., Morowvat, M. H. and Ghasemi, Y. (2011). Single cell protein: production and process. *American Journal of Food Technology*, *6*: 103–116.

Naylor, R. L., Falcon, W. P., Goodman, R. M., Jahn, M. M., Sengooba, T., Tefera, H. and

Nelson, J. R. (2004). Biotechnology in the developing world: A case for increased investment in orphan crops. *Food Policy, 29*: 15–44.

Nciri, N., Cho, N., El Mhamdi, F., Ben Mansour, A., Haj Sassi, F. and Ben Aissa-Fennira, F. (2016). Identification and characterization of phytohemagglutinins from White Kidney beans (*Phaseolus vulgaris* L., var. *Beldia*) in the rat small intestine. *Journal of Medicinal Food, 19* (1): 85–97.

Nestares, T., Barrionuevo, M., Urbano, G. and López-Frías, M. (2001). Nutritional assessment of protein from beans (*Phaseolus vulgaris* L) processed at different pH values, in growing rats. *Journal of the Science of Food and Agriculture, 81* (15): 1522–1529.

Nielsen, E., Ostergaard, G. and Larsen, J. C. (2008). Toxicological risk assessment of chemicals: A practical guide. Informa Healthcare.(pp 216-248).

Norton, G., Bliss, F. A. and Bressani, R. (1985). Biochemical and nutritional attributes of grain legumes. Grain Legume Crops/edited by RJ Summerfield and EH Roberts.

NRC. (1989). Improving Risk Communication. Washington, D.C.: National Academies.

Nwaichi, E. O. and Ayalogu, E. O. (2010). Allelopathy as expressed by *Mucuna pruriens* and the possibility for weed management. *International Journal of Plant Physiology and Biochemistry, 2* (1): 1–5.

Nwankwoala, R. N. P. and Georgewill, O. A. (2010). The extract of *Mucuna pruriens* possesses histamine activity. *Asian Pacific Journal of Tropical Medicine, 3* (1): 29–31.

Odeny, D. A. (2007). The potential of Pigeonpea (*Cajanus cajan* (L.) Millsp.) in Africa. *Natural Resources Forum, 31*: 297–305.

Ogunji, J. O., Uwadiegwu, N., Osuigwe, D. I. and Wirth, M. (2005). Effects of different processing methods of pigeon pea (*Cajanus cajan*) on the haematology of African catfish (*Clarias gariepinus*) larvae. In: Conference on International Agriculture Research for Development, Deutscher Tropentag. Stuttgart-Hohenheim.

Okafor, J. N. C., Ani, J. C. and Okafor, G. I. (2014). Effect of processing methods on qualities of Bambara groundnut (*Voandzeia subterranea* (L.) *Thouars*) flour and their acceptability in extruded snacks. *American Journal of Food Technology, 9* (7): 350–359.

Oniang'o, R. K., Shiundu, K., Maundu, P. and Johns, T. (2006). Diversity, nutrition and food security: The case of african leafy vegetables in hunger and poverty. In: S. B. Ravi, I. Hoeschle-Zeledon, M. S. Swaminathanand E. Frison (Eds.): The role of biodiversity. report of an international consultation on the role of biodiversity in achieving the UN millennium development goal of freedom, Chennai, India: Swaminathan Research Foundation.

Onu, P. and Okongwu, S. N. (2006). Performance characteristics and nutrient utilization of

starter broilers fed raw and processed Pigeon pea *(Cajanus cajan*) Sedd Meal. *International Journal of Poultry Science, 5* (7): 693–697.

Onweluzo, J. C. and Nwabugwu, C. C. (2009). Fermentation of millet (*Pennisetum americanum*) and Pigeon pea (*Cajanus cajan*) seeds for flour production: effects on composition and selected functional properties. *Pakistan Journal of Nutrition, 8* (6): 737–744.

Onweluzo, J. and Eilittä, M. (2003). Surveying *Mucuna´* s utilization as a food in Enugu and Kogi states of Nigeria. *Tropical and Subtropical Agroecosystems, 1*: (2–3).

Ortiz-Hernández, L. and Gómez-Tello, B. L. (2008). Food consumption in Mexican adolescents. *Pan American Journal of Public Health, 24* (2): 127–35.

Osei-Bonsu, P., Buckles, D., Sozo, F. R. and Asibuo, J. Y. (1995). Traditional food uses of Mucuna pruriens and Canavalia ensiformis in Ghana. *ILEIA Newsletter, 12*: 30–31.

Paduano, D. C., Dixon, R. M., Domingo, J. A. and Holmes, J. H. G. (1995). Lupin (*Lupinus angustifolius*): Cowpea (*Vigna unguiculata*) and Navy bean (*Phaseolus vulgaris*) seeds as supplements for sheep fed low quality roughage. *Animal Feed Science and Technology, 53* (1): 55–69.

Padulosi, S., Bergamini, N. and Lawrence, T. (2011a). On-farm conservation of neglected and underutilized species : status, trendsand novel approaches to deal with climate change. proceedings of the International Conference Friedrichsdorf, Frankfurt, 14–16 June, 2011.

Padulosi, S., Heywood, V., Hunter, D. and Jarvis, A. (2011b). Underutilized species and climate change: current status and outlook. Crop Adaptation to Climate Change, (pp. 507–521).

Padulosi, S., Hodgkin, T., Williams, J. T. and Haq, N. (2000). 30 Underutilized crops: trends, challenges and opportunities in the 21st Century. In: E. J.M.M., V.R.Rao, A.H.D.Brown and M.T.Jackson (Eds.): Managing Plant Genetic Diversity. Wallingford, UK: CABI International.

Padulosi, S., Thompson, J. and Rudebjer, P. (2013). Fighting poverty, hunger and malnutrition with Neglected and Underutilized Species (NUS): Needs, challenges and the way forward. In: Bioversity International, Rome. Rome: Bioversity International

Palisade. (2014). @Risk software, version 6.3. *@Risk Software, Version 6.3*. Ithaca, New York: Palisade Corporation.

Pedrosa, M.M., Cuadrado, C., Burbano, C., Allaf, K., Haddad, J., Gelencsér, E., Takács, K., Guillamón, E. and Muzquiz, M. (2012). Effect of instant controlled pressure drop on the oligosaccharides, inositol phosphates, trypsin inhibitors and lectins contents of different legumes. *Food Chemistry, 131* (3): 862-868.

Pereira, L. L. S., Pereira, C. A., de Sousa, R. V., Santos, C. D. dos, de Moraes, C. F. and Satiro, L. C. (2012). White Bean flour (*Phaseolus vulgaris*): Therapeutic and Toxicological Research in Wistar Rats. *Journal of Applied Pharmaceutical Science, 2* (3): 1–7

Pérez, A. A., Drago, S. R., Carrara, C. R., De Greef, D. M., Torres, R. L. and González, R. J. (2008). Extrusion cooking of a maize/soybean mixture: Factors affecting expanded product characteristics and flour dispersion viscosity. *Journal of Food Engineering*, *87* (3): 333–340.

Perrot, N., Ioannou, I., Allais, I., Curt, C., Hossenlopp, J. and Trystram, G. (2006). Fuzzy concepts applied to food product quality control: A review. *Fuzzy Sets and Systems*, *157* (9): 1145–1154.

Phillips, R. D. and McWatters, K. H. (1991). Contribution of Cowpeas to nutrition and health. *Food Technology*, *45* (9): 127–130.

Pickering, M. V. and Newton, P. (1990). Amino acid hydrolysis: old problems, new solutions. *LC/GC*, *8* (10): 778–781.

Pinter-Szakacs, M. and Molnar-Perl, H. (1990). Determination of tryptophan in unhydrolyzed food and feedstuffs by the acid ninhydrin method. *Journal of Agricultural and Food Chemistry*. *38* (3): 720–726.

Plahar, W. A. (2000). Soybean protein for human nutrition and health, Accra, Ghana. In: Overview of the soybean industry in soybean industry in Ghana (pp. 1–41).

Plahar, W. A., Annan, N. T., Larweh, P. M., Golob, P., Swetman, T., Greenhaulgh, P., Coote, C. and Hodges, R. (2002). Marketing and Processing of Bambara Groundnuts (W. Africa) R7581 (ZB0232/233). Crop Post Harvest Programme. Food Research Institute (FRI): Accra, Ghana.

Podolak, I., Galanty, A. and Sobolewska, D. (2010). Saponins as cytotoxic agents: A review. *Phytochemistry Reviews*, *9*: 425–474.

Prolla, I. R. D., Barbosa, R. G., Veeck, A. P. L., Augusti, P. R., Silva, L. P. da, Ribeiro, N. D. and Emanuelli, T. (2010). Cultivar, harvest year and storage conditions affecting nutritional quality of common beans (*Phaseolus vulgaris* L. *Ciência E Tecnologia de Alimentos*, *30*: 96–102.

Purseglove, J. (1974). Tropical Crops : Dicotyledons. London : Longman.

Pusztai, A. (1991). Plant lectins. Cambridge University Press.

Pusztai, A. and Bardocz, S. (1996). Biological effects of plant lectins on the gastrointestinal tract: metabolic consequences and applications. *Trends in Glycoscience and Glycotechnology*, *8*:149–165.

Pusztai, A., Clarke, E. M. W. and King, T. P. (1979). The nutritional toxicity of *Phaseolus vulgaris* lectins. *Proceedings of the Nutrition Society*, *38*: 115–120.

Pusztai, A., Croy, R. R. D., Stewart, J. S. and Watt, W. B. (1979). Protein body membranes of Phaseolus vulgaris L. cotyledons: isolation and preliminary characterization of constituent proteins. *New Phytologist*, *83* (2): 371–378.

Pusztai, A., Ewen, S. W. B., Grant, G., Peumans, W. J., van Damme, E. J. M., Rubio, L. and Bardocz, S. (1990). Relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. *Digestion*, *46* (Suppl. 2): 308–316.

Pusztai, A. and Grant, G. (1998). Assessment of lectin inactivation by heat and digestion. *Methods in Molecular Medicine*, *9*: 505–514.

Quasem, J. M., Mazahreh, A. S. and Abu-Alruz, K. (2009). Development of vegetable based milk from decorticated sesame (*Sesamum indicum*). *American Journal of Applied Sciences*, *6* (5): *888*-896.

Rajaram, N. and Janardhanan, K. (1991). The biochemical composition and nutritional potential of the tribal pulse, *Mucuna gigantea* (Wild) DC. *Plant Foods for Human Nutrition*, *41* (1): 45–51.

Ramírez-Jiménez, A. K., Reynoso-Camacho, R., Mendoza-Díaz, S. and Loarca-Piña, G. (2014). Functional and technological potential of dehydrated *Phaseolus vulgaris* L. flours. *Food Chemistry*, *161*: 254–260.

Ramos, M. V, Mota, D. M., Teixeira, C. R., Cavada, B. S. and Moreira, R. A. (1998). Isolation and partial characterisation of highly toxic lectins from *Abrus pulchellus* seeds. *Toxicon*, *36* (3): 477–484.

Ravishankar, R., Thomas, C. J., Suguna, K., Surolia, A. and Vijayan, M. (2001). Crystal Structures of the peanut lectin-lactose complex at acidic pH: Retention of unusual quaternary structure, empty and carbohydrate bound combining sites, molecular mimicry and crystal packing directed by interactions at the combining site. *Proteins*, *43* (3): 260–270.

Reddy, L. J., Green, J. M., Singh, U., Bisen, S. S. and Jambunathan, R. (1979). Seed protein studies on *Cajanus cajan*, *Atylosia spp*. and some hybrid derivatives (Pigeon peas). International Symposium on Seed Protein Improvement in Cereals and Grain Legumes. Neuherberg (Germany, FR). 4 Sep 1978.

Reynoso-Camacho, R., de Mejıa, E. G. and Loarca-Pina, G. (2003). Purification and acute toxicity of a lectin extracted from tepary bean (*Phaseolus acutifolius*). *Food and Chemical Toxicology*, *41* (1): 21–27.

Rini, J. M. (1995). X-ray crystal structures of animal lectins. *Current Opinion Structure Biology*, *5*: 617–621.

Rizzi, C., Galeoto, Zoccatelli, G., Vincenzi, S., Chignola, R. and Peruffo, A. D. B. (2003). Active soybean lectin in foods: quantitative determination by ELISA using immobilized asialofetuin. *Food Research International*, *36*: 815–821.

Rodrigues, B. F. and Torne, S. G. (1992). Estimation of canavanine in the seeds of three *Canavalia* species. *Tropical Science, 32*: 319–320.

Roebuck, B. D., Kaplita, P. V. and MacMillan, D. L. (1985). Interaction of dietary fat and soybean isolate (SBI) on azaserine induced pancreatic carcinogenesis. *Plant Foods for Human Nutrition*, *35*: 323–329.

Roesler, K., Shintani, D., Savage, L., Boddupalli, S. and Ohlrogge, J. (1997). Targeting of the Arabidopsis homomeric acetyl-coenzyme A carboxylase to plastids of rapeseeds. *Plant Physiology*, *113* (1): 75–81.

Rombo, G. O., Taylor, J. R. N. and Minnaar, A. (2004). Irradiation of maize and bean flours: Effects on starch physicochemical properties. *Journal of the Science of Food and Agriculture, 84* (4): 350–356.

Rosenthal, G. A. (1992). Purification and Characterization of the higher plant enzyme L-Canaline Reductase. *Proceedings of the National Academy of Sciences, USA*, *89* (5): 1780–784.

Roy, A. and Das, S. (2015). Molecular mechanism underlying the Entomotoxic effect of *Colocasia esculenta* Tuber agglutinin against *Dysdercus cingulatus*. *Insects, 6* (4): 827–846.

Rubingh, C. M., Kruizinga, A. G., Hulshof, K. F. A. M. and Brussaard, J. H. (2003). Validation and sensitivity analysis of probabilistic models of dietary exposure to micronutrients: An example based on vitamin B-6. *Food Additives and Contaminants*, *20*: S50–S60.

Ruby, M. B., Alvarenga, M. S., Rozin, P., Kirby, T. A., Richer, E. and Rutsztein, G. (2016). Attitudes toward beef and vegetarians in Argentina, Brazil, Franceand the USA. *Appetite*, *96*: 546–554.

Rutherfurd, S. M., Fanning, A. C., Miller, B. J. and Moughan, P. J. (2015). Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats. *The Journal of Nutrition*, *145* (2):372-379.

Ryan, T. A., Joiner, B. L. and Ryan, B. F. (1994). Minitab^{TM}. Wiley Online Library.

Ryder, S. D., Parker, N., Parker, N., Ecclestone, D., Haqqani, M. T. and Rhodes, J. M. (1994).

Peanut lectin stimulates proliferation in colonic explants from patients with inflammatory bowel disease and colon polyps. *Gastroenterology, 106*: 117–124.

Saeed, M., Yasmin, I., Murtaza, M. A., Fatima, I. and Saeed, S. (2016). Single cell proteins: A novel value-added food product. *Pakistan Journal of Food Sciences, 26* (4): 211–217.

Sanchez, J. F., Lescar, J., Chazalet, V., Audfray, A., Gagnon, J., Alvarez, R., Breton, C., Imberty, A. and Mitchell, E.P. (2006). Biochemical and structural analysis of Helix pomatia agglutinin (HPA): A hexameric lectin with a novel fold. *Journal of Biological Chemistry, 281:* 20171–20180.

Sandberg, A. S. (2002). In vitro and in vivo degradation of phytate. In: Food Phytates (Editors, Reddy N R and Sathe S K), CRC Press, Boca Raton, Florida, (pp. 139–155).

Sauter, N.K., Hanson, J.E., Glick, G.D., Brown, J.H., Crowther, R.L., Park, S.J., Skehel, J.J. and Wiley, D.C. (1992). Binding of influenza virus hemagglutinin to analogs of its cell-surface receptor, sialic acid: analysis by proton nuclear magnetic resonance spectroscopy and X-ray crystallography. *Biochemistry, 31* (40): 9609–9621.

Schaafsma, G. (2005). The protein digestibility-corrected amino acid score (PDCAAS) - A concept for describing protein quality in foods and food ingredients: A critical review. *Journal of AOAC International, 88* (3): 988–994.

Schaafsma, G. (2000). The protein digestibility – corrected amino acid score. *Journal of Nutrition, 130*: 1865S–1867S.

Shaheen, N., Islam, S., Munmun, S., Mohiduzzaman, M. and Longvah, T. (2016). Amino acid profiles and digestible indispensable amino acid scores of proteins from the prioritized key foods in Bangladesh. *Food chemistry, 213*: 83-89.

Siddhuraju, P. and Becker, K. (2001). Rapid reversed-phase high performance liquid chromatographic method for the quantification of L-DOPA (L-3, 4-dihydroxyphenylalanine), non-methylated and methylated tetrahydroisoquinoline compounds from *Mucuna* beans. *Food Chemistry, 72* (3): 389–394.

Siddiqui, S., Hasan, S. and Salahuddin, A. (1995). Isolation and characterization of *Cajanus cajan* lectin. *Archives of Biochemistry and Biophysics, 319* (2): 426–431.

Singh, A., Singh, R. K. and Sureja, A. K. (2007a). Cultural significance and diversities of ethnic foods of Northeast India. *Indian Journal of Traditional Knowledge, 6* (1): 79–94.

Singh, S., Gamlath, S. and Wakeling, L. (2007b). Nutritional aspects of food extrusion: A review. *International Journal of Food Science and Technology, 42*: 916–929.

Singh, U., Jambunathan, R., Saxena, K. and Subrahmanyam, N. (1990). Nutrition quality evaluation of newly developed high-protein genotypes of Pigeonpea (*Cajanus cajan*). *Journal of the Science of Food and Agriculture, 50*: 201–209.

Sinha, S. and Surolia, A. (2005). Oligomerization endows enormous stability to soybean agglutinin: a comparison of the stability of monomer and tetramer of soybean agglutinin. *Biophysical Journal, 88* (6): 4243–4251.

Sitohy, M., Doheim, M. and Badr, H. (2007). Isolation and characterization of a lectin with antifungal activity from Egyptian Pisum sativum seeds. *Food Chemistry, 104* (3): 971–979.

Snipes, M. and Taylor, D. C. (2014). Model selection and Akaike Information Criteria: An example from wine ratings and prices. *Wine Economics and Policy, 3* (1): 3–9.

Sokhey, A. S. and Hanna, M. A. (1993). Properties of irradiated starch. *Journal of Food Structure*, *12*: 397–410.

Song, P., Lei Wu, L. and Guan, W. (2015). Dietary nitrates, nitrites and nitrosamines intake and the risk of gastric cancer: A meta-analysis. *Nutrients*, *12*: 9872–9895.

Spanjersberg, M. Q., Kruizinga, A. G., Rennen, M. A. and Houben, G. F. (2007). Risk assessment and food allergy: the probabilistic model applied to allergens. *Food and Chemical Toxicology, 45* (1): 49–54.

Sparvoli, F., Laureati, M., Pilu, R., Pagliarini, E., Toschi, I., Giuberti, G., Fortunati, P., Daminati, M.G., Cominelli, E. and Bollini, R. (2016). Exploitation of common bean flours with low antinutrient content for making nutritionally enhanced biscuits. *Frontiers in Plant Science, 7:* 928-942

Sridhar, K. R. and Seena, S. (2006). Nutritional and antinutritional significance of four unconventional legumes of the genus *Canavalia* – A comparative study. *Food Chemistry, 99* (2): 267–288.

Steel, C. J., Leoro, M. G. V., Schmiele, M., Ferreira, R. E. and Chang, Y. K. (2012). Thermoplastic Extrusion in Food Processing. (InTech) El-Sonbati (Ed.), In: Thermoplastic Elastomers. http://www.intechopen.com/books/ thermoplasticelastomers /thermoplastic-extrusion-in-food-processing (Accessed: 10 February, 2016)

Stojceska, V., Ainsworth, P., Plunkett, A. and İbanoğlu, Ş. (2009). The effect of extrusion cooking using different water feed rates on the quality of ready-to-eat snacks made from food by-products. *Food Chemistry, 114* (1): 226–232.

Subuola, F., Widodo, Y. and Kehinde, T. (2012). Processing and Utilization of Legumes in the Tropics. Trends in Vital Food and Control Engineering, (pp. 71–85).

Sugeno, M. and Yasukawa, T. (1993). A fuzzy-logic-based approach to qualitative modeling. *IEEE Transactions on Fuzzy Systems, 1*: 7–31.

Sullivan, K. (2008). The lectin report. http://www.krispin.com/lectin.html (Accessed: 20

December, 2015)

Sumner, J. B. and Howell, S. F. (1936). Identification of hemagglutinin of Jack bean with concanavalin A. *Journal of Bacteriology*, *32* (2): 227–237.

Swaminathan, M. S. and Jain, H. K. (1975). Food legumes in Indian agriculture. Nutritional Improvement of Food Legumes by Breeding. In: Milner. M (*ed*.): UN New York, (pp. 69-82).

Swanevelder, C. J. (1998). Bambara–food for Africa. National Department of Agriculture, Government Printer, Republic of South Africa.

Szűcs, V., Szabó, E. and Bánáti, D. (2013). Short overview of food consumption databases. *Czech Journal of Food Science*, *31*: 541–546.

Tamanna, N. and Mahmood, N. (2015). Food processing and Maillard reaction products: Effect on human health and nutrition. *International Journal of Food Science*. Article ID 526762

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S. and Törnqvist, M. (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *Journal of Agricultural and Food Chemistry*, *50* (17): 4998–5006.

Tennant, D. (2012). Food Chemical Risk Analysis. Springer Science and Business Media, (pp. 372–373).

Timoshenko, A. V., Kayser, K., Drings, P. andré, S., Dong, X., Kaltner, H., Schneller, M. and Gabius, H. J. (1995). Carbohydrate-binding proteins (plant/human lectins and autoantibodies from human serum) as mediators of release of lysozyme, elastase and myeloperoxidase from human neutrophils. *Research in Experimental Medicine*, *195* (3): 153–162.

Trautmann, N. (2005). The dose makes the poison--or does it? http://www.actionbioscience.org/environment/trautmann.html. (Accessed: 16 March, 2017)

Tunsaringkarn, T., Siriwong, W., Rungsiyothin, A. and Nopparatbundit, S. (2012). Occupational exposure of gasoline station workers to BTEX compounds in Bangkok, Thailand. *The International Journal of Occupational and Environmental Medicine*, *3*:117-125

Tuso, P. J., Ismail, M. H., Ha, B. P. and Bartolotto, C. (2013). Nutritional update for physicians: plant-based diets. *The Permanente Journal*, *17* (2): 61-66.

Udedibie, A. B. I. and Carlini, C. R. (1998). Brazilian Mucuna pruriens seeds (velvet bean) lack hemagglutinating activity. *Journal of Agricultural and Food Chemistry*, *46* (4): 1450–1452.

Udedibie, A. B. I., Esonu, B. O., Obaji, C. N. and Durunna, C. S. (1994). Dry urea treatment

prior to toasting as a method of improving the nutritive value of jackbeans (*Canavalia ensiformis*) for broilers. *Animal Feed Science and Technology*, *48* (3–4): 335–345.

UNEP. (1995). Heywood, V.H. and Watson, R.T. (eds.) Global Biodiversity Assessment. Cambridge University Press, Cambridge.

USEPA. (United States Environmental Protection Agency). (2001). Risk assessment guidance for Superfund: Volume III–Part A, Process for conducting probabilistic risk assessment; EPA 540-R-02-002; U.S. Environmental Protection Agency: Washington, DC, USA.

USFDA. (United States Food and Drug Administration). (2000). Bad bug book. Foodborne pathogenic microorganism and natural toxins handbook. Phytohaemagglutinin, Compiled by Agriculture-Led Export Businesses, 12 Dokki Street, 6th Floor, Giza, Egypt, USAID Project No. 263-0264.

USDA. (2011). NDL/FNIC food composition database home page. Retrieved March 13, 2016, from https://ndb.nal.usda.gov/

Uygun, U., Senoz, B., Öztürk, S. and Koksel, H. (2009). Degradation of organophosphorus pesticides in wheat during cookie processing. *Food Chemistry*, *117* (2): 261–264.

van Damme, E. J. M., Peumans, W. J., Pusztai, A. and Bardocz, S. (1998). The Handbook of Plant Lectins: Properties and Biomedical Applications. In: John Wiley and Sons, New York, (pp. 31–50).

van der Voet, H., de Mul, A. and van Klaveren, J. D. (2007). A probabilistic model for simultaneous exposure to multiple compounds from food and its use for risk–benefit assessment. *Food and Chemical Toxicology*, *45* (8): 1496–1506.

van Vliet, S., Burd, N. A. and van Loon, L. J. C. (2015). The skeletal muscle anabolic response to plant- versus animal-based protein consumption. *The Journal of Nutrition*, *145*: 1981–1991.

Vasconcelos, I. M., Maia, F. M. M., Farias, D. F., Campello, C. C., Carvalho, A. F. U., de Azevedo Moreira, R. and de Oliveira, J. T. A. (2010). Protein fractions, amino acid composition and antinutritional constituents of high-yielding Cowpea cultivars. *Journal of Food Composition and Analysis*, *23* (1): 54–60.

Vasconcelos, I. M. and Oliveira, J. T. A. (2004). Antinutritional properties of plant lectins. *Toxicon*, *44* (4): 385–403.

Vaz, A. F. M., Costa, R. M. P. B., Coelho, L. C. B. B., Oliva, M. L. V, Santana, L. A., Melo, A. M. M. A. and Correia, M. T. S. (2011). Gamma irradiation as an alternative treatment to abolish allergenicity of lectins in food. *Food Chemistry*, *124* (4): 1289–1295.

Verger, P., Ireland, J., Møller, A., Abravicius, J. A., De Henauw, S. and Naska, A. (2002). Improvement of comparability of dietary intake assessment using currently available

individual food consumption surveys. *European Journal of Clinical Nutrition*, *2*: 18–24.

Vijayakumari, K., Siddhuraju, P. and Janardhanan, K. (1996). Effect of different post-harvest treatments on antinutritional factors in seeds of the tribal pulse, *Mucuna pruriens* (L.) C. *International Journal of Food Sciences and Nutrition*, *47*: 263–272.

Wallis, I. (2016). Semi-vegetarianism – good for animals, good for the environment and good for humans. *Australian Zoologist*, *39* (1): 127-145

Wang, X., Hansen, C. and Allen, K. (2013). Identification of anthocyanins isolated from black bean canning wastewater by macroporous resin using optimized conditions. *Food and Nutrition Sciences*, *4*: 174–181.

Warshawsky, D. and Landolph Jr, J. R. (2005). Molecular Carcinogenesis and the Molecular Biology of Human Cancer. CRC Press.

Wawra, S., Fesel, P., Widmer, H., Timm, M., Seibel, J., Leson, L., Kesseler, L., Nostadt, R., Hilbert, M., Langen, G. and Zuccaro, A. (2016). The fungal-specific β-glucan-binding lectin FGB1 alters cell-wall composition and suppresses glucan-triggered immunity in plants. *Nature Communications*, *7*: 13188.

Weinhold, D., Killick, E. and Reis, E.J. (2013). Soybeans, poverty and inequality in the Brazilian Amazon. *World Development*, *52*: 132-143.

Weis, W. I. and Drickamer, K. (1996). Structural basis of lectin-carbohydrate recognition. *Annual Review of Biochemistry*, *65* (1): 441–473.

White, C.L., Tabe, L.M., Dove, H., Hamblin, J., Young, P., Phillips, N., Taylor, R., Gulati, S., Ashes, J. and Higgins, T.J.V. (2001). Increased efficiency of wool growth and live weight gain in Merino sheep fed transgenic lupin seed containing sunflower albumin. *Journal of the Science of Food and Agriculture*, *81*: 147–154.

WHO/FAO. (1997). Food consumption and exposure assessment of food chemicals; Food Safety Unit, WHO/FSF/FOS/97.5. http://apps.who.int/iris/bitstream/10665/63988/1/ WHO_FSF_FOS_97.5.pdf (Accessed: November 10, 2015)

WHO/FAO. (2009). Principles and methods for the risk assessment of chemicals in food. Geneva: WHO Press, World Health Organization.

WHO/GEM. (2012). Global Environment Monitoring System (GEMS/Food), data, consumption. www.who.int/nutrition /landscape_analysis/nlis_gem-food/en/ (Accessed: January 1, 2016)

Williams, J. T. and Haq, N. (2002). Global research on underutilised crops. An assessment of current activities and proposals for enhanced cooperation. International Centre for Underutilised Crops, University of Southampton, United Kingdom.

Wright, C. S. (1984). Structural comparison of the two distinct sugar binding sites in wheat germ agglutinin isolectin II. *Journal of Molecular Biology*, *178* (1): 91–104.

Yamauchi, D. and Minamikawa, T. (1990). Structure of the gene encoding concanavalin A from *Canavalia gladiata* and its expression in Escherichia coli cells. FEBS Letters, *260* (1):127-130

Yamini, S., Juan, W. Y., Marcoe, K. and Britten, P. (2006). Impact of using updated food consumption and composition data on selected my pyramid food group nutrient profiles. *Journal of Nutrition Education and Behavior, 38*: 136–142.

Yao, D. N., Kouassi, K. N., Erba, D., Scazzina, F., Pellegrini, N. and Casiraghi, M. C. (2015). Nutritive evaluation of the Bambara groundnut Ci12 landrace [*Vigna subterranea* (L.) Verdc. (Fabaceae)] produced in Cote d'Ivoire. *International Journal of Molecular Sciences, 16* (9): 21428–21441.

Yen, M. T., Yang, J. H., Tseng, Y. H., Li, R. C. and Mau, J. L. (2011). Quality of fungal chitin bread. *Journal of Food Processing and Preservation*, *35*: 708–713.

Young, V. R. and Pellet, P. L. (1994). Plant proteins in relation to human protein and amino acid nutrition. *American Journal of Clinical Nutrition, 59S*: 1203S–1212S.

Yu, G. H. and Kwon, Y. M. (1992). Alteration of arginase activity in leaf protoplasts of *Canavalia lineata*. *Korean Biochemical Journal, 25*: 196–202.

Zadeh, L. A. (1965). Fuzzy Sets. *Information and Control*, *8*(3): 338–353.

APPENDICES

A: Pictures of neglected and underutilized legumes

Vigna subterranea ***Canavalia ensiformis*** ***Cajanus cajan*** ***Phaseolus lunatus*** ***Mucuna pruriens***

B: NULs: Characteristics of consumers, cooking times and consumption patterns

Demography

Religion	None	Traditional	Muslim	Christian	Others	
	○	○	○	○	○	
Education	Non-formal	JSS	SSS	Post SSS	Tertiary	
	○	○	○	○	○	
Work	Non-Skilled	Farmer	Trader	Artisan	Civil Servant	Public Servant
	○	○	○	○	○	○
Marital status	In relationship	Married	Widowed	Single		
	○	○	○	○		
House hold number	1	2	3	4	5	≥6
	○	○	○	○	○	○

Gender

Male	○
Female	○

Legumes (Select only one)

Canavalia ensiformis	○
Cajanus cajan	○
Mucuna pruriens	○
Phaseolus lunatus	○
Vigna subterranea	○

Anthropometry

Age (years)	<10	11-20	21-30	31-40	41-50	51-60	61-70	>71
	○	○	○	○	○	○	○	○
Weight (kg)	<20	21-30	31-40	41-50	51-60	61-70	71-80	81-90
	○	○	○	○	○	○	○	○

Cooking practises — Processing time (h)

	< 1	1	1.5	2	>2
Steaming	○	○	○	○	○
Roasting	○	○	○	○	○
Frying	○	○	○	○	○
Boiling	○	○	○	○	○
Other Methods	○	○	○	○	○

Exposure Frequency (EF)

	NULs dishes (Select only one)				
EF	Sauce	Stew	Tubani	Koose	Bean porridge
1 pm	○	○	○	○	○
1-3pm	○	○	○	○	○
1 pw	○	○	○	○	○
2 pw	○	○	○	○	○
3 pw	○	○	○	○	○
4 pw	○	○	○	○	○
5 pw	○	○	○	○	○
6 pw	○	○	○	○	○

pm= per month
pw = per week

Mass consumed

NULs dishes (Select only one)	Amount purchased (GHS)						
	0.2	0.50	1.00	1.50	2.00	2.50	3.00
Sauce	○	○	○	○	○	○	○
Stew	○	○	○	○	○	○	○
Tubani	○	○	○	○	○	○	○
Koose	○	○	○	○	○	○	○
Bean porridge	○	○	○	○	○	○	○

C: Raw data of consumption survey and characteristics of consumers

Canavalia ensiformis

Gender	Religion	Education	Work	Relationships	House hold numbers	Cooking time (h)	Mass of Soup (g)	Exposure Frequency(month)
F	Chr	JSS	CS	IR	6	1	400	3
F	Chr	JSS	F	IR	5	1		1
F	Chr	JSS	F	M	6	1	400	1
F	Chr	JSS	F	M	6	2	160	1
F	Chr	JSS	F	M	5	1.5	400	3
F	Chr	JSS	F	M	6	2	400	1
F	Chr	JSS	F	M	6	1.5	650	3
F	Chr	JSS	F	M	6	1	400	3
F	Chr	JSS	F	M	5	2	400	3
F	Chr	JSS	F	M	6	1.5	400	3
F	Chr	JSS	NSW	M	6	2	650	3
F	Chr	JSS	NSW	M	6	2	300	8
F	Chr	JSS	NSW	M	6	2	400	3
F	Chr	JSS	NSW	M	6	1	400	1
F	Chr	JSS	NSW	M	6	1	650	4
F	Chr	JSS	NSW	M	6	2	650	8
F	Chr	JSS	NSW	M	4	1.5	400	16
F	Chr	JSS	NSW	M	6	1.5	300	20
F	Chr	NF	NSW	M	6	2	400	8
F	Chr	NF	PS	M	6	2	650	12
F	Chr	NF	T	M	6	1	650	4
F	Chr	NF	T	M	6	1	400	4
F	Chr	NF	T	M	6	1	650	12
F	Chr	NF	T	M	3	1	400	12
F	Chr	NF	T	M	6	1	650	4
F	Chr	NF	T	M	4	1	400	8
F	Chr	NF	T	M	4	1	400	8
F	Chr	NF	T	M	3	1	400	3
F	Chr	NF	T	M		1	400	1
F	Chr	NF	T	M	6	1	400	1
F	Chr	NF	T	M	2	1	400	3
F	Chr	NF	T	M	6	1	400	1
F	Chr	NF	T	M	3	1	400	1
F	Chr	NF	T	M	5	1	400	3
F	Chr	NF	T	M	6	1	650	1
F	Chr	NF	T	M	6	1	400	3

F	Chr	NF	T	M	3	1	400	1
F	Chr	NF	T	M	6	1	400	3
F	Chr	NF	T	M	5	1	400	4
F	Chr	NF	T	M	6	1	400	4
F	Chr	NF	T	M	5	1	650	1
F	Chr	NF	T	M	6	1	650	1
F	Chr	NF	T	M	5	1	400	4
F	Chr	NF	T	M	4	1		16
F	Chr	NF	T	M	6	1	400	12
F	Chr	NF	T	M	6	1	160	2
F	Chr	NF	T	M	3	1	400	4
F	Chr	NF	T	M	6	1	400	1
F	Chr	NF	T	M	6	1	650	4
F	Chr	NF	T	M	5	1	400	8
F	Chr	NF	T	M	5	1	400	1
F	Chr	NF	T	M	5	1	400	12
F	Chr	NF	T	M	6	1	650	12
F	Chr	NF	T	M		1	300	1
F	Mus	NF	T	M	6	1	400	4
F	Mus	NF	T	M	6	1	400	1
F	Mus	NF	T	M	6	1	650	1
F	Mus	NF	T	M	6	1	650	3
F	Mus	NF	T	M	3	1	400	1
F	Mus	NF	T	M	4	1	300	3
F	Mus	NF	T	M	4	1	400	3
F	Mus	NF	T	M	4	1	650	12
F	Mus	NF	T	M	3	1	650	3
F	Mus	NF	T	M	6	1	400	24
F	Mus	NF	T	M			650	4
F	Mus	NF	T	M	5	1	400	1
F	Mus	NF	T	M	4	2	650	12
F	Mus	NF	T	M	6	2	400	3
F	Mus	NF	T	M	6	1	400	1
F	Mus	NF	T	M	6	1	400	3
F	Mus	NF	T	M	6	1	400	3
F	Mus	NF	T	M	5	1	400	1
F	Mus	NF	T	M	3	1.5	400	1
F	Mus	NF	T	M	6		400	1
F	Mus	NF	T	M	5	1	400	1

F	Mus	NF	T	S	6	1	400	12
F	Mus	NF	T	S	6	1	650	20
F	Mus	NF	T	S		1	160	4
F	Mus	NF	T	S	6	1	400	3
F	Mus	NF	T	S	6	1.5	300	8
F	Mus	NF	T	W	3	1.5	400	
M	Mus	NF	T	W		1	650	1
M	Mus	PSSS	T	W	6	1	400	3
M	Mus	PSSS	T	W	3	1	650	3
M	Mus	PSSS	T	W	6	2	160	3
M	Mus	PSSS	T	W	6	1	300	1
M	Mus	SSS	T		6	1	400	3
M	Mus	SSS	T		6	1	160	1
M	Mus	T	T		6	1	400	1
M	Mus		T		6	1	160	
	Mus				6	1	160	1
	Non					1	300	20

Mucuna pruriens

Gender	Religion	Education	Work	Relationships	House hold numbers	Cooking time(h)	Mass of Soup (g)	Exposure Frequency(month)
F	Mus	NF	A	IR	4	1	400	16
F	Chr	JSS	A	IR	5	1	400	16
F	Mus	NF	CS	IR	6	1	400	24
F	Mus	JSS	F	IR	4	1	400	1
F	Mus	NF	F	IR	4	1	400	16
F	Mus	NF	f	M	6	1.5	650	1
F	Mus	NF	F	M	6	1	650	12
F	Mus	JSS	F	M	4	1	400	1
M	Chr	SSS	F	M	5	1		12
M	Chr	SSS	F	M	5	1	400	12
M	Chr	SSS	NSW	M	5	1	160	12
F	Mus	NF	NSW	M	6	1	400	4
F	Chr	NF	NSW	M	4	1	400	1
F	Mus	NF	NSW	M	6	1.5	650	1
F	Mus	NF	NSW	M	6	1	400	1
F	Chr	JSS	PS	M	4	1	400	1
F	Chr	NF	PS	M	6	1	400	1
F	Chr	NF	PS	M	6	1	650	1

F	Chr	NF	PS	M	6	1	300	1
F	Chr	PSSS	PS	M	6	2	400	20
F	Mus	NF	T	M	6	1	400	4
F	Mus	NF	T	M	6	1.5	650	20
F	Chr	JSS	T	M	6	1	650	24
F	Chr	NF	T	M	6	1	400	3
F	Chr	JSS	T	M	6	1	300	3
M	Chr	SSS	T	M	5	1	400	3
M	Chr	NF	T	M	6	1	650	1
F	Chr	JSS	T	M	4	1	650	3
F	Chr	NF	T	M	6	1	400	3
M	Chr	SSS	T	M	4	1.5	650	24
F	Mus	NF	T	M	6	1	400	1
M	Chr	T	T	M	4	1	650	16
F	Chr	NF	T	M	5	1	400	24
F	Chr	JSS	T	M	5	1	400	12
F	Chr	NF	T	M	6	1	400	20
F	Chr	NF	T	M	6	1	400	20
F	Chr	JSS	T	M	5	1	400	16
F	Mus	NF	T	M	6	1.5	400	12
F	Mus	NF	T	M	6	1	400	24
F	Chr	JSS	T	M	3	1	400	1
M	Chr	NF	T	M	4	1	400	3
F	Chr	NF	T	M	4	1	650	1
F	Chr	NF	T	M	5	1	160	3
F	Mus	NF	T	M	6	1	400	12
F	Chr	NF	T	M	6	1	300	12
F	Chr	JSS	T	M	3	1.5	400	1
F	Chr	JSS	T	M	5	1	650	12
F	Chr	JSS	T	M	4	1	400	1
F	Mus	NF	T	M	6	1	650	1
F	Chr	NF	T	M	5	1	160	1
F	Chr	NF	T	M	6	1	300	12
F	Chr	NF	T	M	6	1	400	12
F	Chr	NF	T	M	6	1	160	12
F	Chr	NF	T	M	6	1	400	12
F	Chr	JSS	T	M	6	1	160	12
F	Chr	JSS	T	M	5	1	160	1
F	Chr	JSS	T	M	5	1	300	12

F	Chr	NF	T	M	3	1	160	12
F	Chr	NF	T	M	3	1	300	12
F	Chr	NF	T	M	4	1	400	12
F	Mus	NF	T	M	6	2	400	12
M	Mus	NF	T	M	6	1	400	1
F	Mus	NF	T	M	6	1	160	1
M	Chr	SSS	T	M	6	1		3
M	Chr	JSS	T	M	6	1.5	400	8
M	Chr	SSS	T	M	6	1	400	1
M	Chr	JSS	T	M	5	1	400	1
M	Chr	JSS	T	M	3	1	400	3
M	Chr	JSS	T	M	6	1	400	1
F	Chr	NF	T	M	5	1	160	3
F	Chr	NF	T	M	5	1	160	3
F	Chr	NF	T	M	5	1	400	3
M	Chr	JSS	T	M	6	1	400	1
F	Chr	NF	T	M	6	1	400	1
F	Chr	NF	T	M	6	1	400	1
F	Chr	NF	T	M	5	1	400	3
F	Chr	NF	T	M	6	1	400	3
F	Chr	JSS	T	M		1	300	8
F	Chr	JSS	T	M	3	1	160	3
F	Chr	JSS	T	M	5	1	160	4
F	Chr	NF	T	S	6	1	650	3
F	Mus	NF	T	S	6	1	160	16
F	Chr	NF	T	S	5	1	400	4
F	Mus	NF	T	S	6	1	400	1
F	Chr	JSS	T	S	6	1	400	1
F	Chr	NF	T	S	6	1	650	3
F	Chr	JSS	T	S	5	1	400	3
F	Chr	JSS	T	S	5	1	400	
F	Chr	JSS	T	S	6	1	160	3
F	Mus	NF	T	S	6	1	400	8
F	Mus	NF	T	S	6	1	400	4
F	Chr	SSS	T	S	6	2	400	8
F	Chr	JSS	T	S	6	2	400	1
F	Mus	NF	T	S	5	1.5	300	3
F	Mus	NF	T	S	6	1	400	3
F	Mus	NF	T	W	6	1	400	4

M	Chr	NF	T	W	6	1	650	16
F	Mus	NF	T	W	5	2	400	8
F	No	NF	T	W	6	1	400	20
F	Chr	NF	T	W	6	2	400	16
F	Mus	NF		W	6	2	650	24

Phaseolus lunatus

Gender	Religion	Education	Work	Relationships	House hold numbers	Cooking time (h)	Mass of Soup(g)	Exposure Frequency(month)
F	Chr	NF	T	M	6	1	650	3
F	Mus	JSS	T	M	5	1	400	24
F	Mus	NF	T	M	6	1	400	16
F	Chr	NF	T	M	3	1.5	400	8
F	Chr	JSS	T	M	6	1	400	8
F	Chr	NF	T	W	6	1	650	12
F	Chr	JSS	T	M	6	2	400	16
F	Mus	JSS	T	M	5	1	400	24
F	Mus	NF	T	M	6	1	400	8
F	Chr	JSS	T	M	6	1	400	8
F	Chr	NF	T	M	6	1	300	1
F	Chr	PSSS	T	M	5	2	400	1
F	Chr	PSSS	T	M	6	1.5	400	1
F	Chr	PSSS	T	M	6	1	400	1
F	Chr	NF	T	M	6	1	300	4
F	Chr	NF	T	S	6	2	400	4
F	Chr	NF	T	S	6	1	300	4
F	Chr	NF	T	IR	6	1	300	4
F	Chr	JSS	T	M	4	1	400	4
F	Chr	NF	F	M	6	1	400	3
F	Chr	NF	T	S	1	1	400	4
F	Chr	NF	F	M	6	1	650	3
F	Mus	NF	T	M	6	1	650	16
F	Non	NF	T	M	6	1	400	4
F	Chr	SSS	T	M	6	1.5	400	8
F	Chr	NF	T	W	4	1.5	400	20
M	Chr	JSS	T	M	6	2	400	20
F	Mus	NF	T	M	6	1	400	1
F	Mus	NF	NSW	IR	6	2	400	4
F	Chr	SSS	T	M	6	2	400	8

F	Chr	NF	T	M	6	2	400	1
F	Chr	NF	T	M	6	2	400	1
F	Chr	PSSS	T	M	4	2	400	20
F	Chr	JSS	T	M	6	1	400	3
F	Chr	NF	T	M	6	2	400	24
F	Chr	JSS	T	M	6	2	400	4
F	Mus	NF	T	M	6	1	400	8
F	Mus	NF	T	M	6	1	400	4
F	Mus	NF	T	M	6	1	400	1
F	Chr	NF	T	M	6	1	160	12
F	Mus	NF	T	S	6	1	300	3
F	Chr	JSS	T	M	6	1	400	1
F	Chr	JSS	T	M	4	1	400	12
F	Chr	NF	T	M	5	1	400	16
F	Chr	NF	T	M	4	1	400	16
F	Mus	NF	T	M	6	1	650	16
F	Chr	NF	F	M	6	1	400	12
F	Chr	JSS	T	M	5	1	160	8
F	Chr	NF	T	M	6	1	160	16
F	Chr	NF	T	M	4	1	160	1
F	Chr	NF	NSW	M	6	1	400	12
F	Chr	JSS	T	M	5	1	400	4
F	Mus	NF	T	M	6	1	650	12
F	Chr	NF	T	M	5	1	400	24
F	Chr	NF	T	M	6	1	400	16
F	Mus	NF	T	M	6	1	400	3
F	Chr	NF	T	M	5	1	650	3
F	Chr	NF	T	W	4	1	400	3
F	Chr	JSS	T	IR	4	1	400	16
F	Chr	JSS	T	M	3	1	650	1
F	Chr	JSS	T	IR	5	1	650	8
F	Chr	JSS	T	M	5	1	400	1
F	Chr	JSS	T	M	3	1	650	3
F	Chr	NF	T	S	3	1	160	3
F	Chr	NF	T	M	6	1	400	12
F	Chr	NF	T	M	5	1	160	1
F	Chr	SSS	-	-	6	1.5	400	16
M	Chr	SSS	-	-	6	1.5	400	8
F	Chr	NF	T	M	5	1	160	3

F	Chr	JSS	T	M	4	1	160	3
F	Mus	NF	T	M	5	1	160	4
F	Chr	NF	T	M	6	1	160	3
F	Chr	NF	T	M	5	1	160	12
F	Chr	NF	T	M	6	1	400	12
F	Chr	NF	F	M	5	1	160	8
F	Chr	NF	T	M		2	400	1
F	Mus	NF	T		6	1	400	12
F	Chr	NF	T	M	6	1	400	4
F	Chr	NF	T	M	6	1	400	16
F	Chr	NF	T	M	6	1	400	3
F	Chr	NF	T	M	6	1	400	4
F	Chr	NF	T	M	6	1	400	3
F	Chr	NF	T	M	5	1	300	3
F	Chr	NF	T		5	1	400	1
F	Chr	NF	T	M	6	1	650	4
F	Chr	JSS	A	M	6	1	400	3
F	Mus	NF	NSW	M	6	1	300	3
F	Mus	NF	NSW	IR	6	1	650	1
F	Chr	NF	T	M	4	1	400	3
F	Mus	NF	T	M	5	1	400	1
F	Chr	NF	T	M	6	1	400	4
F	Chr	NF	T	M	6	1	300	8
F	Mus	NF	F	M	6	1	300	3
F	Chr	NF	T	M	6	1	160	4
F	Chr	NF	T	M	6	2	400	1
F	Chr	JSS	T	S	6	1.5	650	8
F	Chr	NF	T	W	6	1	160	1
F	Mus	NF	T	M	6	1	160	3
F	Mus	JSS	T	S	6	1	400	1
F	Mus	NF	T	M	6	1	400	3
F	Mus	NF	T	W	6	1	400	3
F	Mus	NF	T	M	6	1	160	3
F	Mus	NF	T	IR	6	1	400	8
M	Mus	SSS	NSW	S	6	1	400	3
F	Mus	NF	T	M	3	2	650	8
F	Mus	JSS	T	IR	6	1	400	3
F	Mus	NF	T		5	1	160	8
M	Mus	NF	T	S	4	1	160	16

F	Chr	JSS	NSW	S	4	1	160	3
F	Mus	NF	T	M	3	1	650	3
F	Chr	JSS	T	S	4	1	160	3
F	Mus	JSS	T	M	6	1	160	3
F	Chr	NF	T	M	6	1	300	1
F	Chr	NF	T	M	6	2	400	8
F	Chr	NF	T	M	4	1	650	1
F	Mus	NF	F	M	5	1	300	3
F	Chr	NF	T	M	5	1	400	8
F	Chr	JSS	NSW	S	4	1	400	8
F	Chr	NF	T	S	5	1	400	1
F	Mus	NF	F	M	6	1	400	3

Vigna subterranea

Gender	Religion	Education	Work	Relationships	House hold numbers	Cooking time (h)	Mass of Food	Exposure Frequency(month)
M	Chr	JSS	A	IR	6	1	600	12
F	Chr	JSS	A	IR	5	2	600	4
F	Chr	JSS	A	IR	6	2	600	12
F	Chr	JSS	F	IR	6	1	600	20
M	Chr	JSS	F	IR	6	2	900	4
M	Chr	JSS	F	IR	6	2	900	12
F	Chr	JSS	F	IR	3	2	300	1
M	Chr	JSS	F	IR	3	2	450	12
M	Chr	JSS	F	IR	6	2	900	24
M	Chr	JSS	F	M	6	2	600	4
M	Chr	JSS	F	M	6	2	900	3
F	Chr	JSS	F	M	6	1	600	4
F	Chr	JSS	F	M	6	1	600	3
F	Chr	JSS	F	M	6	1	600	8
M	Chr	JSS	F	M	6	1	900	3
f	Chr	JSS	F	M	6	1	300	4
F	Chr	JSS	F	M	6	1	900	4
F	Chr	JSS	NSW	M	6	1	450	20
F	Chr	JSS	NSW	M	4	1	300	4
F	Chr	JSS	NSW	M	6	1	600	3
F	Chr	JSS	NSW	M	6	1	600	4
F	Chr	JSS	NSW	M	3	1	600	4
F	Chr	JSS	NSW	M	6	2	300	16

F	Chr	JSS	NSW	M	6	2	600	4
F	Chr	JSS	NSW	M	5	1.5	300	2
M	Chr	JSS	NSW	M	3	1	600	1
M	Mus	JSS	NSW	M	6	1	900	24
M	Mus	NF	NSW	M	6	2	900	8
M	Mus	NF	NSW	M	6	1	600	4
F	Mus	NF	NSW	M	6	2	600	2
F	Mus	NF	NSW	M	6	2	900	3
F	Mus	NF	NSW	M	6	2	300	20
F	Mus	NF	T	M	6	1	300	1
F	Mus	NF	T	M	6	1	450	4
F	Mus	NF	T	M	6	2	600	20
F	Mus	NF	T	M	6	1	600	4
F	Mus	NF	T	M	6	1	450	24
F	MUS	NF	T	M	6	2	600	4
M	MUS	NF	T	M	5	1	900	8
M	MUS	NF	T	M	6	1	600	4
M	MUS	NF	T	M	6	2	600	20
M	MUS	NF	T	M		2	600	8
F	MUS	NF	T	M	4	2	600	4
F	MUS	NF	T	M	6	2	900	1
F	MUS	NF	T	M	6	2	900	4
F	Mus	NF	T	M	6	2	600	4
F	MUS	NF	T	M	6	2	900	1
F	MUS	NF	T	M	4	2	600	8
M	MUS	NF	T	M	6	2	600	20
F	MUS	NF	T	M	4	2	450	1
F	Mus	NF	T	M	6	2	450	3
F	Mus	NF	T	M	6	2	900	5
F	MUS	NF	T	M	6	2	900	8
F	MUS	NF	T	M	4	1	450	4
F	MUS	NF	T	M	6	2	600	4
F	MUS	NF	T	M	6	2	900	4
F	MUS	NF	T	M	4	2	600	8
M	Mus	NF	T	M	6	2	600	20
F	Mus	NF	T	M	4	2	450	4
F	Mus	NF	T	M	6	2	450	12
F	MUS	NF	T	M	6	2	900	20
F	Mus	NF	T	M	6	2	900	8

F	Mus	NF	T	M	4	1	300	12
F	MUS	NF	T	M	6	2	450	4
F	Mus	NF	T	M	6	2	900	4
F	Mus	NF	T	M	6	2	900	4
F	Mus	NF	T	M	6	2	600	24
F	Mus	NF	T	M	3	2	600	12
M	MUS	NF	T	M	6		900	20
F	Mus	NF	T	M	6	2	600	4
F	Mus	NF	T	M	6	2	900	12
F	Mus	NF	T	M	6	2	900	8
F	Mus	NF	T	M	2	1	450	24
F	Mus	NF	T	M	6	2	450	13
F	Mus	NF	T	M	6	2	450	4
F	Mus	NF	T	M	6	2	900	12
M	Mus	NF	T	M	6	2	900	12
F	Mus	NF	T	M	6	2	300	4
F	Mus	NF	T	M	6	2	600	8
M	Mus	NF	T	M	5	2	600	12
F	Mus	NF	T	M	6	2	600	12
F	Mus	NF	T	M	6	2	600	20
F	Mus	NF	T	M	6	2	600	20
F	Mus	NF	T	M	5	1.5	900	8
F	Mus	NF	T	S	6	1	900	1
F	Mus	NF	T	S	6	2	600	1
F	Mus	NF	T	S	6	2	900	8
F	Mus	NF	T	S	6	2	600	16
F	Mus	NF	T	S	6	2	600	4
F	Mus	NF	T	S	3	1	600	12
F	Mus	NF	T	S	6	1	600	12
F	Mus	NF	T	S	4	2	450	8
F	Mus	NF	T	S	4	1	300	12
F	Mus	NF	T	S	6	2	600	12
F	Mus	NF	T	S	5	2	600	4
F	Mus	NF	T	S	5	2	450	12
F	Mus	NF	T	S	6	2	600	12
F	Mus	NF	T	S	6	2	600	1
F	Mus	NF	T	S	6	2	600	12
F	Mus	NF	T	S	6	2	600	12
F	Mus	SSS	T	S	6	1	300	12

M	Mus	SSS	T	W	6	1	300	12
F	Mus	SSS	T	W	4	1	900	8
F	Mus	SSS		W	6	2	900	8
F	Mus	SSS			4	2	450	1
F	Mus				4	2	600	12

C2: Histogram of ages of consumers of individual NULs

Phaseolus lunatus

Group	Freq	Rel Frequency
10-20	6	0.050420168
21-30	13	0.109243697
31-40	25	0.210084034
41-50	45	0.378151261
51-60	22	0.18487395
61-70	6	0.050420168
71-100	2	0.016806723

Mucuna pruriens

Group	Freq	Rel Frequency
10-20	2	0.01980198
21-30	14	0.138613861
31-40	16	0.158415842
41-50	39	0.386138614
51-60	23	0.227722772
61-70	4	0.03960396
71-100	3	0.02970297

Vigna subterranea

Group	Freq	Rel Frequency
10-20	20	0.188679245
21-30	24	0.226415094
31-40	27	0.254716981
41-50	22	0.20754717
51-60	9	0.08490566
61-70	3	0.028301887

71-100	1	0.009433962

Canavalia ensiformis

Group	Freq	Rel Frequency
10-20	1	0.012048193
21-30	16	0.192771084
31-40	17	0.204819277
41-50	35	0.421686747
51-60	8	0.096385542
61-70	3	0.036144578
71-100	3	0.036144578

Cajanus cajan

Group	Freq	Rel Frequency
10-20	6	0.054545455
21-30	21	0.190909091
31-40	27	0.245454545
41-50	27	0.245454545
51-60	21	0.190909091
61-70	8	0.072727273

All NULs

Group	Freq	ALL
10-20	35	0.067307692
21-30	88	0.169230769
31-40	112	0.215384615
41-50	168	0.323076923
51-60	84	0.161538462
61-70	24	0.046153846
71-100	9	0.017307692

C3: Histogram of Body weight of consumers of individual NULs

Phaseolus lunatus

Group	Freq	Rel. Frequency
31-40	2	0.016393443
41-50	11	0.090163934
51-60	32	0.262295082
61-70	37	0.303278689
71-80	27	0.221311475
81-90	11	0.090163934

Mucuna pruriens

Group	Freq	Rel. Frequency
41-50	11	0.135802469
51-60	12	0.148148148
61-70	33	0.407407407
71-80	16	0.197530864
81-90	4	0.049382716
91-100	5	0.061728395

91-100	2	0.016393443

Canavalia ensiformis

Group	Freq	Rel. Frequency
41-50	10	0.103092784
51-60	27	0.278350515
61-70	21	0.216494845
71-80	23	0.237113402
81-90	11	0.113402062
91-100	5	0.051546392

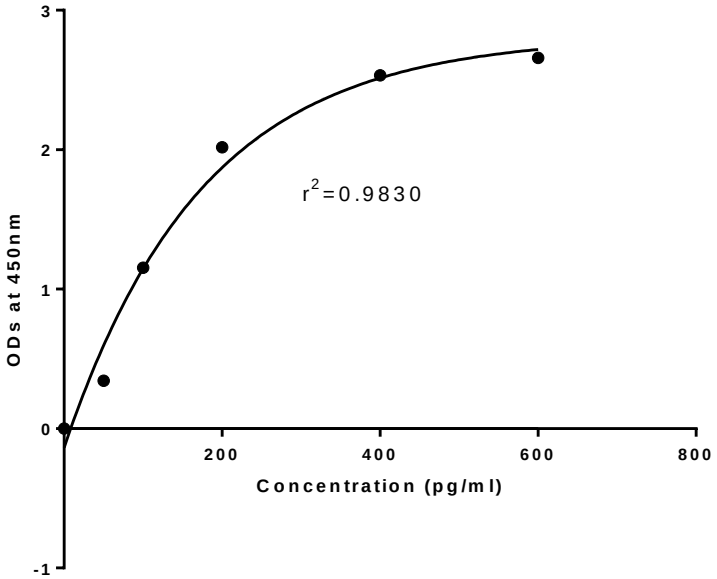
Vigna subterranea

Group	Freq	Rel. Frequency
31-40	3	0.028301887
41-50	11	0.103773585
51-60	38	0.358490566
61-70	24	0.226415094
71-80	20	0.188679245
81-90	7	0.066037736
91-100	3	0.028301887

Cajanus cajan

Group	FREQ	Rel. Frequency
31-40	1	0.005208333
41-50	6	0.03125
51-60	36	0.1875
61-70	29	0.151041667
71-80	23	0.119791667
81-90	91	0.473958333
91-100	6	0.03125

D: **Standard curve for ELISA Lectin determination**



E: Questionnaire for the Perception of safety of NULs dishes

1. Which type of beans do you normally eat? (Tick as many as applicable): Soybean☐ Cowpea ☐
   Others ☐ Specify……..

2. Are you familiar with any of the following beans? (Tick as many as applicable):
   Bambara groundnut ☐ Mucuna (adua apia) ☐ Cajanus sp☐
   Phaseolus sp ☐ Canavalia sp ☐ Others☐ Specify ……

3. Which of the beans (in 1 and 2 above) do you eat frequently?
   Soybean☐ Cowpea ☐ Bambara groundnut ☐ Mucuna sp (adua apia) ☐
   Cajanus sp☐ Phaseolus sp ☐ Canavalia sp ☐
   Others ☐ Specify……

4. Where do you get these beans for consumption? (Tick as many as applicable):
   I cultivate ☐ Purchase ☐ Others ☐ Specify……

5. If you cultivate the beans then when do you harvest for consumption? (Tick as many as applicable):
   While it is immature ☐ When it is fully mature☐ When it is dried ☐

6. Do you perceive beans to be safe for consumption? Safe☐ Not safe ☐ Probably safe ☐
   Don't know☐

7. Do you experience discomfort after consumption of beans? Yes ☐ (If yes, go to Q8) No☐

8. How long does it take before experiencing discomfort? (Tick as many as applicable):
   Immediately after consumption☐ During the night☐ The next day☐ Don't know☐
   Others ☐ Specify ……..

9. If you experience discomfort, what type of discomfort do you actually have? (Tick as many as applicable):
   Flatulence ☐ Diarrhea ☐ Vomiting ☐ Feeling bloated/full ☐ Nausea ☐
   Anemic ☐ Dental carries☐ Inflammatory bowel and celiac disease☐
   Urinary tract infection ☐ Others ☐ Specify ……..

10. Would you recommend the consumption of beans to everyone? Yes ☐ (If yes, go to Q11) No☐

11. Why would you recommend the consumption of beans? It is nutritious ☐ Others☐ Specify……..

12. Why would you not recommend the consumption of beans?
   It is believed to contain hazards☐ Others☐ Specify……

1

13. Have you heard people complaining after eating any of these beans? Yes ☐ (If yes, go to Q14)
No☐

14. Do you think the complaint is legitimate? Yes ☐ (If yes, go to Q15) No☐(If no, go to Q15)

15. Which of the following best explains your position with respect to Q14?
I have heard people react when they eat these beans ☐ People are simply exaggerating☐
Don't know☐ Others☐ Specify……..

16.If your answer to Q13 is yes, is there anything you do to make consumption of these beans safer?
Yes ☐ (If yes, go to Q17) No☐

17. If yes, what can be done? (Tick as many as applicable) Adding kawu ☐ Boiling overnight ☐
Others☐ Specify……..

18. What is your age range? 10 -14☐ 15-19☐ 20 -29☐ 30 -39☐ 40 -50☐ >50☐

19. What is your gender? Male ☐ Female☐

20. Do you think there are hazards in beans? Yes ☐ (If yes, go to Q21) No ☐

21. What type of hazards do you perceive there are? (Tick as many as applicable):
Pesticide residues ☐ Pathogenic bacteria ☐ Environmental contaminants☐
Food additives☐ Food allergens ☐ Others☐ Specify…….

22. What do you perceive allergens to be? Physical reactions to food☐ Don't know ☐

23. What kind of reactions do you perceive to be allergy? (Tick as many as applicable):
Skin reactions ☐ Swelling of face ☐ Itchy lips/mouth/throat ☐
Wheezing/Trouble breathing☐ Dizziness ☐ Others☐ Specify…….

24. Do you have any of these reactions when you eat these beans? / Are you allergic to any of these beans?
Yes ☐ (If yes, go to Q25) No ☐

25. How do you know? Doctor diagnosed☐ I was told☐ Others☐ Specify…….

26. When did you last experience allergic reactions? More than 1 year ago☐ More than 1 month ago☐
Recently☐ Don't know☐

28. Do you remember how many times in the past 5 years you have reacted to food this way?
A couple of times☐ All the time☐ Others☐ Specify…….

29. Has someone died in your family as a result of allergic reactions?
Yes ☐ No ☐ Don't know ☐

30. Whose responsibility is it to make the foods we eat safe? (Tick as many as applicable)
Self ☐ Family members ☐ Sellers and farmers ☐
Government/Town council/ Sanitary inspectors ☐ Others ☐ Specify……

31. Do you buy bean products (*tubani, boiled beans, bean thickened soups)* from sellers?
Yes ☐ (If yes, go to Q32 No ☐

32. If yes, how best do you perceive them to be safe? I believe they are properly cooked ☐
I cook again before eating ☐ I take the word of the hawkers ☐
Others ☐ Specify……

33. If yes, what reasons would you offer for the safety of the beans you eat? (Tick as many as applicable):
My parents eat it so it is safe ☐ Everybody eats it in my community so it is safe ☐
It is nutritious so it is safe ☐ Government/Agric extension officers/media say it is safe ☐

34. Do you think the presence of hazards in legumes, such as allergens, is a:
serious food safety problem ☐, somewhat of a problem ☐, or not a food safety problem ☐ at all?

35. How likely it is that legumes have substances that could make you sick?
Not likely ☐ Probably ☐ Very likely ☐ Most Definitely ☐ Don't know ☐

36. Do you believe each statement is "True" or "False."
T ☐ F ☐ I know how to cook grain legumes safely.
T ☐ F ☐ I am confident that the cooked beans I buy is safe.
T ☐ F ☐ If I only eat food prepared in by professionals, I can avoid food poisoning.
T ☐ F ☐ If I eat cooked beans and I am lucky I am likely not to get food poisoning.
T ☐ F ☐ Some recalled food can be made safe to eat.
T ☐ F ☐ I think that the government is doing enough to prevent food contamination.
T ☐ F ☐ I think that food manufacturers are doing enough to prevent food contamination.
T ☐ F ☐ A food is recalled only after people have gotten sick.
T ☐ F ☐ Organic foods are less likely to be contaminated than non-organic foods.
T ☐ F ☐ Locally grown foods are less likely to be contaminated than non-locally grown foods.

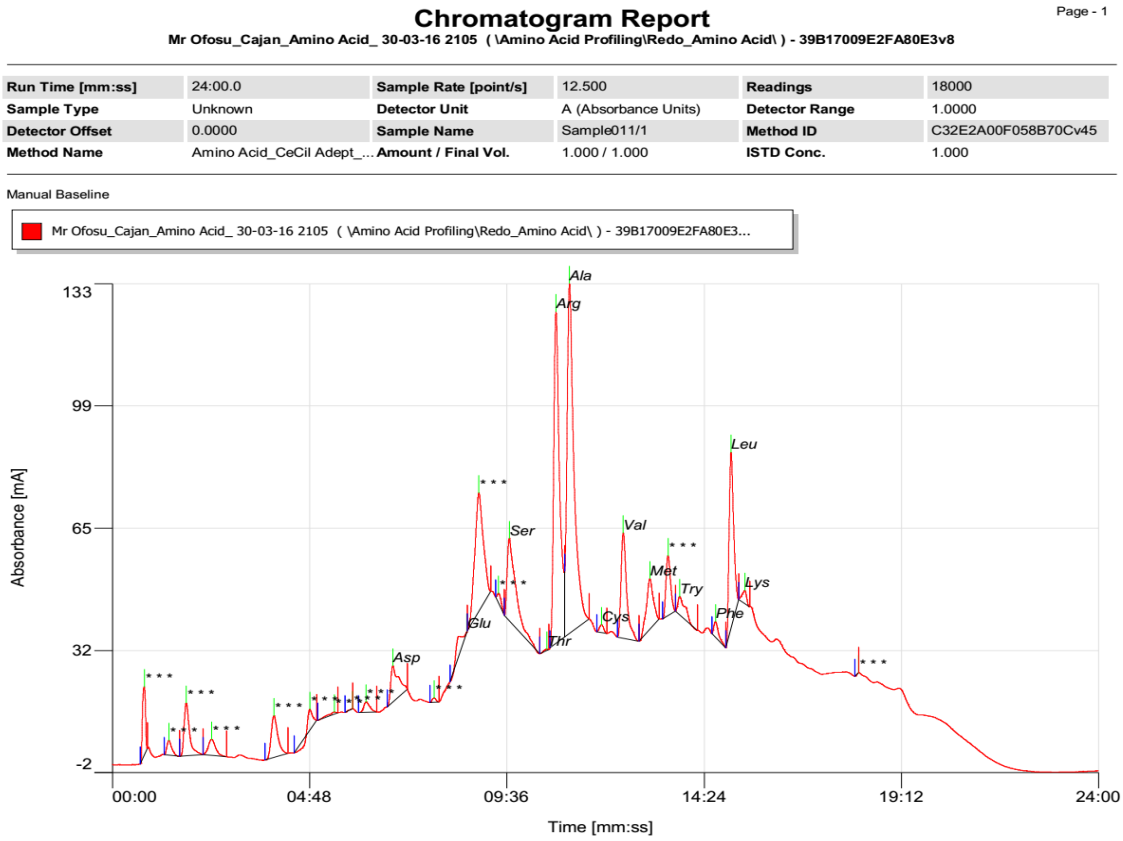
Thank You so much for taking part of this survey

F: Concentrations of lectins in model NULs dishes extracts in mg/g

Vigna subterranea (Tubani)	*Mucuna pruriens (Soup)*	*Phaseolus lunatus (Soup)*	*Canavalia ensiformis (Soup)*	*Cajanus cajan ("Ase")*
78.3	78.2	55.8	96.3	58.3
68.4	65.2	50.3	89.5	50.2
69.9	63.8	42.1	86.4	54.2
70.5	68.1	48.2	92.2	56.2
69.8	66.2	43.7	103.7	53.1
78.2	62.5	44.7	85.2	58.2
72.5	70.1	44.3	85.3	56.4
71.7	72.3	48.2	84.8	56.5
68.8	70.5	50.4	88.5	54.6
68.7	75.3	57.3	97.7	52.4
66.5	72.1	54.7	80.2	56.7
66.4	72	55.2	76.4	54.5
72.8	68.5	52.1	98.3	51.1
75.5	70.1	54.6	87.3	58.8
71.5	69.2	55.8	105.1	56.6
72.4	68.1	54.3	88.3	51.4
78.2	79.1			55.5
65.7	64.2			58.2
68.8	65.2			54.5
69.9	66.1			58.6
70.5	68.3			52.7
	68.3			51.2
	64.5			
	62.2			
	62.1			

G1: Chromatograms of amino acid determinations

Cajanus cajan



Canavalia ensiformis

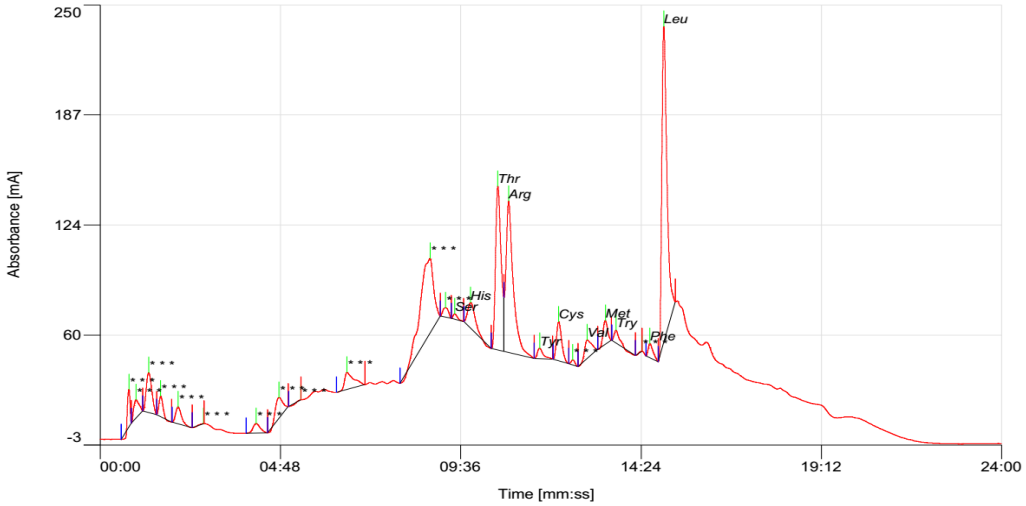
Chromatogram Report

r Ofosu_Canava_Amino Acid_30-03-16 1745 (\Amino Acid Profiling\Redo_Amino Acid\ ) - 437E5AAB0A3B672Av8

Run Time [mm:ss]	24:00.0	**Sample Rate [point/s]**	12.500	**Readings**	18000
Sample Type	Unknown	**Detector Unit**	A (Absorbance Units)	**Detector Range**	1.0000
Detector Offset	0.0000	**Sample Name**	Sample006/1	**Method ID**	C32E2A00F058B70Cv49
Method Name	Amino Acid_CeCil Adept_...	**Amount / Final Vol.**	1.000 / 1.000	**ISTD Conc.**	1.000

Manual Baseline

r Ofosu_Canava_Amino Acid_30-03-16 1745 (\Amino Acid Profiling\Redo_Amino Acid\ ) - 437E5AAB0A3B672A...



Mucuna pruriens

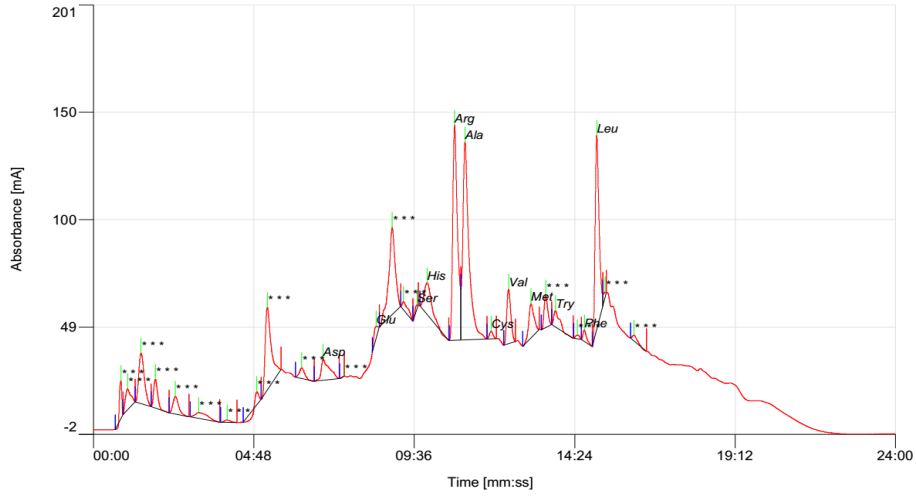
Chromatogram Report

r Ofosu_Mucuna_Amino Acid_30-03-16 1919 (\Amino Acid Profiling\Redo_Amino Acid\ ) - 2544A34FD8103903v7

Run Time [mm:ss]	24:00.0	**Sample Rate [point/s]**	12.500	**Readings**	18000
Sample Type	Unknown	**Detector Unit**	A (Absorbance Units)	**Detector Range**	1.0000
Detector Offset	0.0000	**Sample Name**	Sample008/1	**Method ID**	C32E2A00F058B70Cv50
Method Name	Amino Acid_CeCil Adept_...	**Amount / Final Vol.**	1.000 / 1.000	**ISTD Conc.**	1.000

Manual Baseline

r Ofosu_Mucuna_Amino Acid_30-03-16 1919 (\Amino Acid Profiling\Redo_Amino Acid\ ) - 2544A34FD8103903...



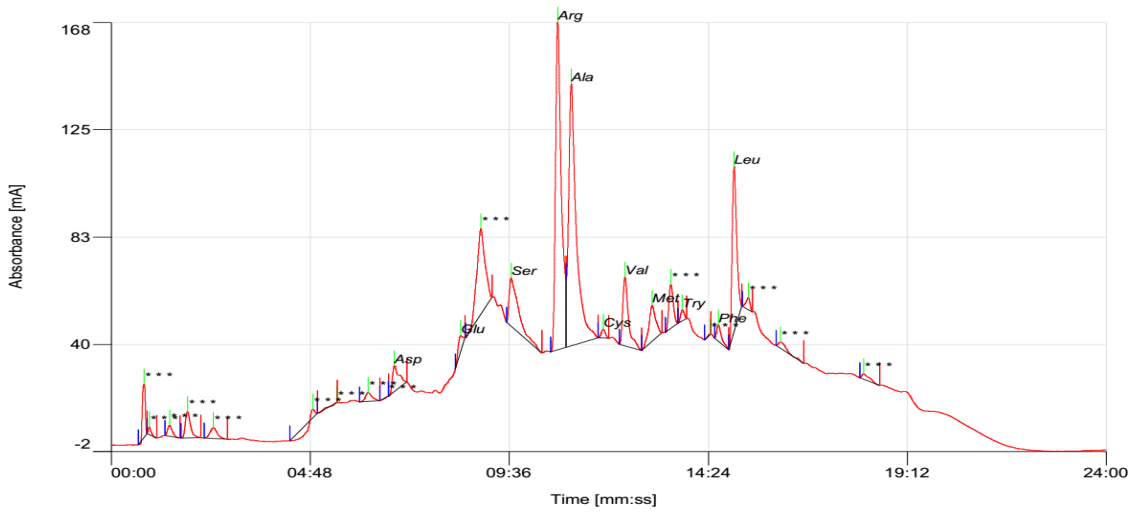
Phaseolus lunatus

Chromatogram Report

Mr Ofosu_Phaseo_Amino Acid_ 30-03-16 2149 (\Amino Acid Profiling\Redo_Amino Acid\ ) - ED5882D19085B18Cv6

Run Time [mm:ss]	24:00.0	**Sample Rate [point/s]**	12.500	**Readings**	18000
Sample Type	Unknown	**Detector Unit**	A (Absorbance Units)	**Detector Range**	1.0000
Detector Offset	0.0000	**Sample Name**	Sample012/1	**Method ID**	C32E2A00F058B70Cv46
Method Name	Amino Acid_CeCil Adept_...	**Amount / Final Vol.**	1.000 / 1.000	**ISTD Conc.**	1.000

Manual Baseline



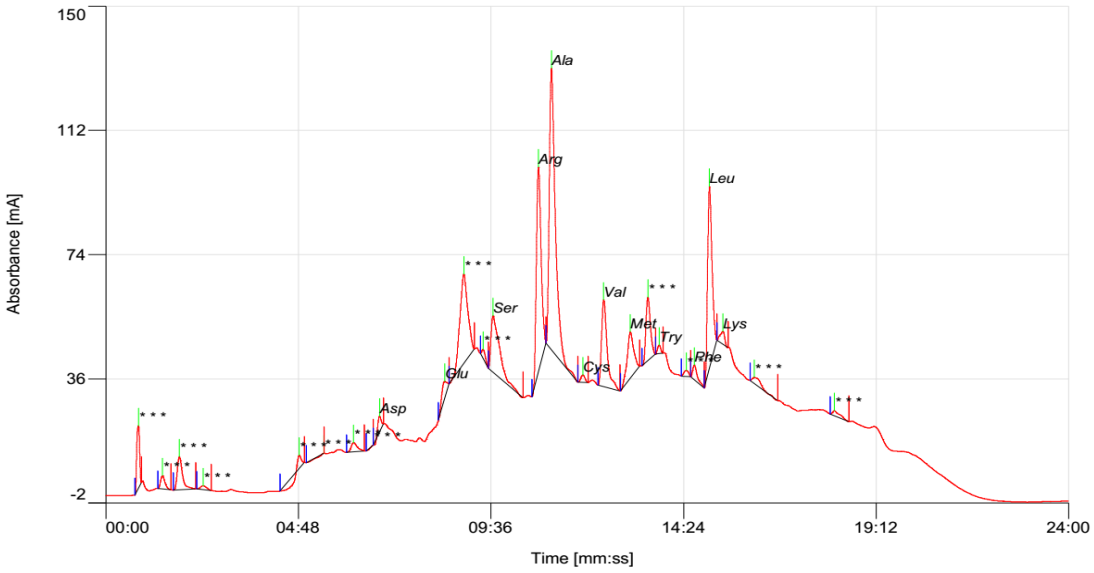
Vigna subterranea

Chromatogram Report

Mr Ofosu_Vig_Amino Acid_30-03-16 (\Amino Acid Profiling\Redo_Amino Acid\ ) - D2920E8A09A3ED97v7

Run Time [mm:ss]	24:00.0	**Sample Rate [point/s]**	12.500	**Readings**	18000
Sample Type	Unknown	**Detector Unit**	A (Absorbance Units)	**Detector Range**	1.0000
Detector Offset	0.0000	**Sample Name**	Sample010/1	**Method ID**	C32E2A00F058B70Cv48
Method Name	Amino Acid_CeCil Adept_...	**Amount / Final Vol.**	1.000 / 1.000	**ISTD Conc.**	1.000

Manual Baseline



G2. Standardized ileal digestibility of crude protein and amino acids in soybean meal (Kong *et al*., 2014)

Indispensable amino acid	Soybean meal(%)
Arg	94.7
His	86.4
Ile	89.0
Leu	88.9
Lys	86.7
Met	92.8
Phe	88.9
Thr	88.7
Trp	92.3
Val	88.2

H: ANOVA and Fischer pairwise comparison

HISTIDINE

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	1136.78	284.196	6459.00	0.000
Error	10	0.44	0.044		
Total	14	1137.22			

Fisher pairwise comparisons

Factor	N	Mean	Grouping
VG	3	26.2000	A
CC	3	8.900	B
CE	3	4.500	C
MP	3	3.900	D
PL	3	2.900	E

Test of μ = 21 vs < 21

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	8.900	0.200	0.115	9.237	-104.79	0.000
VG	3	26.2000	0.1000	0.0577	26.3686	90.07	1.000
MP	3	3.900	0.200	0.115	4.237	-148.09	0.000
CE	3	4.500	0.300	0.173	5.006	-95.26	0.000
PL	3	2.900	0.200	0.115	3.237	-156.75	0.000

Test of μ = 20 vs < 20

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	8.900	0.200	0.115	9.237	-96.13	0.000
VG	3	26.2000	0.1000	0.0577	26.3686	107.39	1.000
MP	3	3.900	0.200	0.115	4.237	-139.43	0.000
CE	3	4.500	0.300	0.173	5.006	-89.49	0.000
PL	3	2.900	0.200	0.115	3.237	-148.09	0.000

Test of μ = 16 vs < 16

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	8.900	0.200	0.115	9.237	-61.49	0.000
VG	3	26.2000	0.1000	0.0577	26.3686	176.67	1.000
MP	3	3.900	0.200	0.115	4.237	-104.79	0.000
CE	3	4.500	0.300	0.173	5.006	-66.40	0.000
PL	3	2.900	0.200	0.115	3.237	-113.45	0.000

ISOLEUCINE

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	2440.24	610.059	12709.56	0.000
Error	10	0.48	0.048		
Total	14	2440.72			

Fisher pairwise comparisons

Factor	N	Mean	Grouping
VG	3	35.900	A
CE	3	6.800	B
CC	3	3.9000	C
MP	3	3.100	D
PL	3	3.0000	D

Test of μ = 55 vs < 55

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	3.9000	0.1000	0.0577	4.0686	-885.08	0.000
VG	3	35.900	0.300	0.173	36.406	-110.27	0.000
MP	3	3.100	0.300	0.173	3.606	-299.64	0.000
CE	3	6.800	0.200	0.115	7.137	-417.42	0.000
PL	3	3.0000	0.1000	0.0577	3.1686	-900.67	0.000

Test of μ = 32 vs < 32

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	3.9000	0.1000	0.0577	4.0686	-486.71	0.000
VG	3	35.900	0.300	0.173	36.406	22.52	0.999
MP	3	3.100	0.300	0.173	3.606	-166.85	0.000
CE	3	6.800	0.200	0.115	7.137	-218.24	0.000
PL	3	3.0000	0.1000	0.0577	3.1686	-502.29	0.000

Test of μ = 30 vs < 30

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	3.9000	0.1000	0.0577	4.0686	-452.07	0.000
VG	3	35.900	0.300	0.173	36.406	34.06	1.000
MP	3	3.100	0.300	0.173	3.606	-155.31	0.000
CE	3	6.800	0.200	0.115	7.137	-200.92	0.000
PL	3	3.0000	0.1000	0.0577	3.1686	-467.65	0.000

LEUCINE

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	1478.88	369.720	120.04	0.000
Error	10	30.80	3.080		
Total	14	1509.68			

Fisher pairwise comparisons

Factor	N	Mean	Grouping
VG	3	34.0000	A
CE	3	15.60	B
PL	3	12.000	C
CC	3	10.600	C
MP	3	4.8000	D

Test of μ = 96 vs < 96

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	10.600	1.300	0.751	12.792	-113.78	0.000
VG	3	34.0000	0.1000	0.0577	34.1686	-1073.87	0.000
MP	3	4.8000	0.1000	0.0577	4.9686	-1579.63	0.000
CE	3	15.60	3.50	2.02	21.50	-39.79	0.000
PL	3	12.000	1.200	0.693	14.023	-121.24	0.000

Test of μ = 66 vs < 66

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	10.600	1.300	0.751	12.792	-73.81	0.000
VG	3	34.0000	0.1000	0.0577	34.1686	-554.26	0.000
MP	3	4.8000	0.1000	0.0577	4.9686	-1060.02	0.000
CE	3	15.60	3.50	2.02	21.50	-24.94	0.001
PL	3	12.000	1.200	0.693	14.023	-77.94	0.000

Test of μ = 61 vs < 61

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	10.600	1.300	0.751	12.792	-67.15	0.000
VG	3	34.0000	0.1000	0.0577	34.1686	-467.65	0.000
MP	3	4.8000	0.1000	0.0577	4.9686	-973.41	0.000
CE	3	15.60	3.50	2.02	21.50	-22.47	0.001
PL	3	12.000	1.200	0.693	14.023	-70.73	0.000

LYSINE

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	8990.02	2247.50	543.14	0.000
Error	10	41.38	4.14		
Total	14	9031.40			

Fisher pairwise comparisons

Factor	N	Mean	Grouping
PL	3	63.10	A
VG	3	53.60	B
CE	3	13.300	C
CC	3	10.500	C
MP	3	3.7000	D

Test of μ = 69 vs < 69

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	10.500	0.300	0.173	11.006	-337.75	0.000
VG	3	53.60	3.30	1.91	59.16	-8.08	0.007
MP	3	3.7000	0.1000	0.0577	3.8686	-1131.03	0.000
CE	3	13.300	0.300	0.173	13.806	-321.58	0.000
PL	3	63.10	3.10	1.79	68.33	-3.30	0.040

Test of μ = 57 vs < 57

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	10.500	0.300	0.173	11.006	-268.47	0.000
VG	3	53.60	3.30	1.91	59.16	-1.78	0.108
MP	3	3.7000	0.1000	0.0577	3.8686	-923.18	0.000
CE	3	13.300	0.300	0.173	13.806	-252.30	0.000
PL	3	63.10	3.10	1.79	68.33	3.41	0.962

Test of μ = 48 vs < 48

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	10.500	0.300	0.173	11.006	-216.51	0.000
VG	3	53.60	3.30	1.91	59.16	2.94	0.951
MP	3	3.7000	0.1000	0.0577	3.8686	-767.30	0.000
CE	3	13.300	0.300	0.173	13.806	-200.34	0.000
PL	3	63.10	3.10	1.79	68.33	8.44	0.993

METHIONINE + CYSTEINE

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	618.403	154.601	828.22	0.000
Error	10	1.867	0.187		
Total	14	620.269			

Fisher pairwise comparisons

Factor	N	Mean	Grouping
VG	3	18.700	A
CE	3	11.700	B
CC	3	3.4333	C
MP	3	2.700	C D
PL	3	2.600	D

Test of μ = 33 vs < 33

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	3.4333	0.1155	0.0667	3.6280	-443.50	0.000
VG	3	18.700	0.500	0.289	19.543	-49.54	0.000
MP	3	2.700	0.300	0.173	3.206	-174.94	0.000
CE	3	11.700	0.700	0.404	12.880	-52.70	0.000
PL	3	2.600	0.300	0.173	3.106	-175.51	0.000

Test of μ = 27 vs < 27

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	3.4333	0.1155	0.0667	3.6280	-353.50	0.000
VG	3	18.700	0.500	0.289	19.543	-28.75	0.001
MP	3	2.700	0.300	0.173	3.206	-140.30	0.000
CE	3	11.700	0.700	0.404	12.880	-37.86	0.000
PL	3	2.600	0.300	0.173	3.106	-140.87	0.000

Test of μ = 23 vs < 23

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	3.4333	0.1155	0.0667	3.6280	-293.50	0.000
VG	3	18.700	0.500	0.289	19.543	-14.90	0.002
MP	3	2.700	0.300	0.173	3.206	-117.20	0.000
CE	3	11.700	0.700	0.404	12.880	-27.96	0.001
PL	3	2.600	0.300	0.173	3.106	-117.78	0.000

PHENYLALANINE + TYROSINE

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	8190.13	2047.53	240.16	0.000
Error	10	85.26	8.53		
Total	14	8275.39			

Fisher pairwise comparisons

Factor	N	Mean	Grouping
VG	3	67.64	A
CE	3	21.40	B
PL	3	15.500	C
MP	3	5.600	D
CC	3	3.600	D

Test of μ = 94 vs < 94

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	3.600	0.200	0.115	3.937	-782.89	0.000
VG	3	67.64	5.45	3.14	76.82	-8.39	0.007
MP	3	5.600	0.300	0.173	6.106	-510.38	0.000
CE	3	21.40	3.30	1.91	26.96	-38.11	0.000
PL	3	15.500	1.400	0.808	17.860	-97.12	0.000

Test of μ = 52 vs < 52

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	3.600	0.200	0.115	3.937	-419.16	0.000
VG	3	67.64	5.45	3.14	76.82	4.97	0.981
MP	3	5.600	0.300	0.173	6.106	-267.89	0.000
CE	3	21.40	3.30	1.91	26.96	-16.06	0.002
PL	3	15.500	1.400	0.808	17.860	-45.16	0.000

Test of μ = 41 vs < 41

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	3.600	0.200	0.115	3.937	-323.89	0.000
VG	3	67.64	5.45	3.14	76.82	8.47	0.993
MP	3	5.600	0.300	0.173	6.106	-204.38	0.000
CE	3	21.40	3.30	1.91	26.96	-10.29	0.005
PL	3	15.500	1.400	0.808	17.860	-31.55	0.001

THREONINE

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	6887.66	1721.92	1409.10	0.000
Error	10	12.22	1.22		
Total	14	6899.88			

Fisher pairwise comparisons

Factor	N	Mean	Grouping
PL	3	56.40	A
VG	3	27.700	B
CC	3	3.100	C
CE	3	2.100	C
MP	3	2.100	C

Test of μ = 44 vs < 44

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	3.100	0.300	0.173	3.606	-236.14	0.000
VG	3	27.700	0.300	0.173	28.206	-94.11	0.000
MP	3	2.100	1.000	0.577	3.786	-72.57	0.000
CE	3	2.100	0.300	0.173	2.606	-241.91	0.000
PL	3	56.40	2.20	1.27	60.11	9.76	0.995

Test of μ = 31 vs < 31

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	3.100	0.300	0.173	3.606	-161.08	0.000
VG	3	27.700	0.300	0.173	28.206	-19.05	0.001
MP	3	2.100	1.000	0.577	3.786	-50.06	0.000
CE	3	2.100	0.300	0.173	2.606	-166.85	0.000
PL	3	56.40	2.20	1.27	60.11	20.00	0.999

Test of μ = 25 vs < 25

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	3.100	0.300	0.173	3.606	-126.44	0.000
VG	3	27.700	0.300	0.173	28.206	15.59	0.998
MP	3	2.100	1.000	0.577	3.786	-39.66	0.000
CE	3	2.100	0.300	0.173	2.606	-132.21	0.000
PL	3	56.40	2.20	1.27	60.11	24.72	0.999

TRYPTOPHAN

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	5.0760	1.26900	14.76	0.000
Error	10	0.8600	0.08600		
Total	14	5.9360			

Fisher pairwise comparisons

Factor	N	Mean	Grouping
VG	3	2.600	A
PL	3	1.800	B
CE	3	1.2000	C
MP	3	1.100	C
CC	3	1.100	C

Test of μ = 17 vs < 17

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	1.100	0.200	0.115	1.437	-137.70	0.000
VG	3	2.600	0.200	0.115	2.937	-124.71	0.000
MP	3	1.100	0.300	0.173	1.606	-91.80	0.000
CE	3	1.2000	0.1000	0.0577	1.3686	-273.66	0.000
PL	3	1.800	0.500	0.289	2.643	-52.65	0.000

Test of μ = 8.5 vs < 8.5

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	1.100	0.200	0.115	1.437	-64.09	0.000
VG	3	2.600	0.200	0.115	2.937	-51.10	0.000
MP	3	1.100	0.300	0.173	1.606	-42.72	0.000
CE	3	1.2000	0.1000	0.0577	1.3686	-126.44	0.000
PL	3	1.800	0.500	0.289	2.643	-23.21	0.001

Test of μ = 6.6 vs < 6.6

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	1.100	0.200	0.115	1.437	-47.63	0.000
VG	3	2.600	0.200	0.115	2.937	-34.64	0.000
MP	3	1.100	0.300	0.173	1.606	-31.75	0.000
CE	3	1.2000	0.1000	0.0577	1.3686	-93.53	0.000
PL	3	1.800	0.500	0.289	2.643	-16.63	0.002

VALINE

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	814.54	203.634	181.82	0.000
Error	10	11.20	1.120		
Total	14	825.74			

Fisher pairwise comparisons

Factor	N	Mean	Grouping
VG	3	23.500	A
CC	3	10.10	B
CE	3	5.200	C
PL	3	4.700	C
MP	3	3.700	C

Test of μ = 55 vs < 55

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	10.10	2.30	1.33	13.98	-33.81	0.000
VG	3	23.500	0.300	0.173	24.006	-181.87	0.000
MP	3	3.700	0.200	0.115	4.037	-444.27	0.000
CE	3	5.200	0.300	0.173	5.706	-287.52	0.000
PL	3	4.700	0.300	0.173	5.206	-290.41	0.000

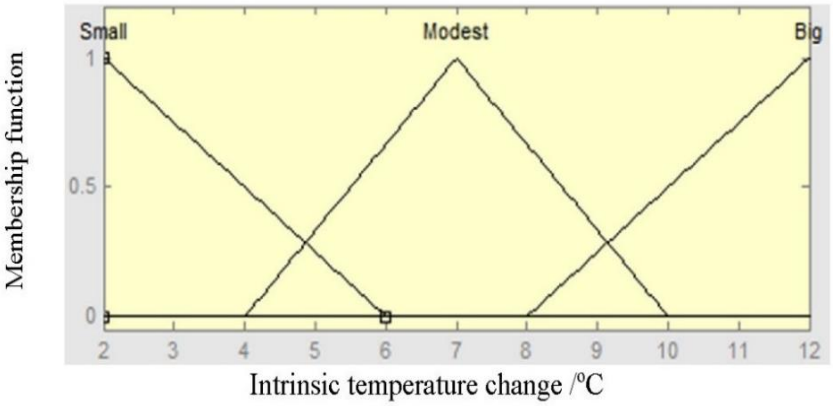
Test of μ = 43 vs < 43

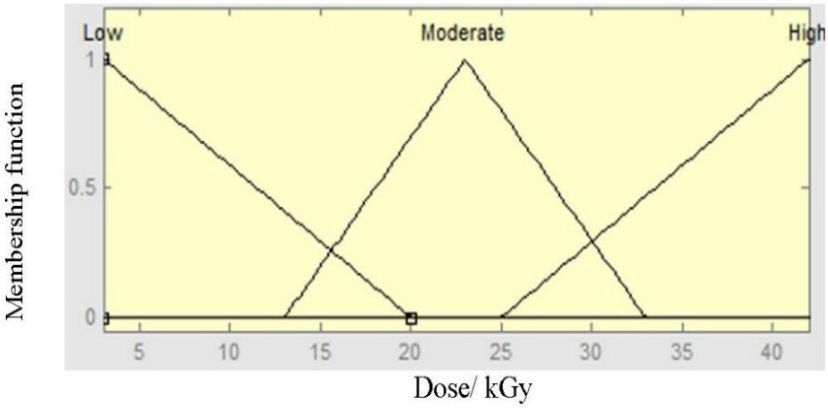
Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	10.10	2.30	1.33	13.98	-24.78	0.001
VG	3	23.500	0.300	0.173	24.006	-112.58	0.000
MP	3	3.700	0.200	0.115	4.037	-340.35	0.000
CE	3	5.200	0.300	0.173	5.706	-218.24	0.000
PL	3	4.700	0.300	0.173	5.206	-221.13	0.000

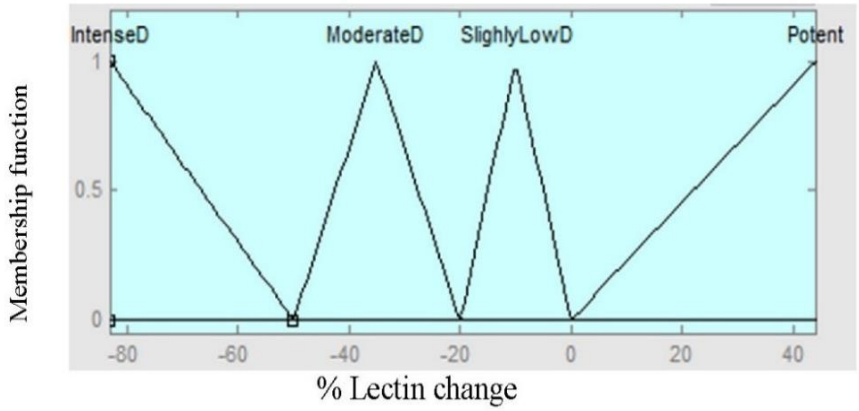
Test of μ = 40 vs < 40

Variable	N	Mean	StDev	SE Mean	95% Upper Bound	T	P
CC	3	10.10	2.30	1.33	13.98	-22.52	0.001
VG	3	23.500	0.300	0.173	24.006	-95.26	0.000
MP	3	3.700	0.200	0.115	4.037	-314.37	0.000
CE	3	5.200	0.300	0.173	5.706	-200.92	0.000
PL	3	4.700	0.300	0.173	5.206	-203.80	0.000

I 1. Membership functions of input and output variables their ranges







2. "If-then" rules showing 15 rules truth reference table

1. *If (Dose is Low) and (Intrinsic Temperature change is Modest) Then (% Lectin Change is Moderate Decrease)*
2. *If (Dose is Medium) and (Intrinsic Temperature change is Small) Then (% Lectin Change is Potentiated)*
3. *If (Dose is High) and (Intrinsic Temperature change is Small) Then (% Lectin Change is Potentiated)*
4. *If (Dose is Low) and (Intrinsic Temperature change is Modest) Then (% Lectin Change is Moderate Decrease)*
5. *If (Dose is Medium) and (Intrinsic Temperature change is Modest) Then (% Lectin Change is Slightly Low Decrease)*
6. *If (Dose is High) and (Intrinsic Temperature change is Modest) Then (% Lectin Change is Slightly Low Decrease)*
7. *If (Dose is Low) and (Intrinsic Temperature change is Big) Then (% Lectin Change is Moderate Decrease)*
8. *If (Dose is Medium) and (Intrinsic Temperature change is Big) Then (% Lectin Change is Potentiated)*
9. *If (Dose is High) and (Intrinsic Temperature change is Big) Then (% Lectin Change is Slightly Low Decrease)*
10. *If (Dose is Low) and (Intrinsic Temperature change is Small) Then (% Lectin Change is Intense Decrease)*
11. *If (Dose is Medium) and (Intrinsic Temperature change is Small) Then (% Lectin Change is Intense Decrease)*
12. *If (Dose is High) and (Intrinsic Temperature change is Small) Then (% Lectin Change is Intense Decrease)*
13. *If (Dose is Low) and (Intrinsic Temperature change is Small) Then (% Lectin Change is Moderate Decrease)*
14. *If (Dose is Medium) and (Intrinsic Temperature change is Modest) Then (% Lectin Change is Moderate Decrease)*
15. *If (Dose is High) and (Intrinsic Temperature change is Modest) Then (% Lectin Change is Intense Decrease)*

3. Integrated fuzzy relational model from which simulations is able to predict output