Neglected and Underutilized Legumes (NULs) Hazards and Probabilistic Risks Associated with Some Selected Dietary Lectins

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Abstract NULs are increasingly becoming food security crop but consumers complain about their safety after consumption. This is a serious matter that must be investigated so that the exposure and risks associated with their usage are evaluated and any uncertainties quantified. A structured interview schedule was used to collect data on NULs familiarity and consumption and the perception of consumers regarding their potential hazards. Also, time-temperature inactivation of the agglutinins of NULs flours, together with the NULs' model dishes were studied, from which the risk assessment of lectin's systemic toxicity was evaluated using the hazard-based approach. It was observed that, majority of the respondents who were over 40 years (67.6%) were also familiar (59.4%) with NULs and consumed NULs dishes (59.4%). The most popular dishes were obtained from the seeds of *Vigna sp*. (15.9%) and *Phaseolus sp.* (14.95%). The majority (66%) perceived the presence of hazards in NULs, citing pesticide residues (58.7%) but not intrinsic hazard as threat. A few also considered pesticide residues to be interactive with food additives (16.6 %) as dangerous. Majority (66.1%) considered NULs dishes as safe, while at the same time complained of discomfort (97.2%) after consumption. Yet, they would still recommend their use to others. Significantly high quantities of agglutinins remained in *Vigna sp*. flours even after cooking for 1 h relative to others. The hazard quotients of all the NULs dishes were above 1, meaning consumers are at risk of systemic toxicity. Respondents were somewhat confused about their perception of NULs safety, especially towards intrinsic hazards. Since extrinsic toxicity can be controlled, consumers must be made aware of the potential inherent threats that are associated with NULs consumption.

Keywords: neglected legume dishes, intrinsic hazard, agglutinins, hazard quotient, lectin risk

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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 [1]. The discovery of soy [2] and its rapid internationalization, the production and utilization of indigenous legumes, for instance, 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 led to loss of market premium [3].

Legumes contain intrinsic hazards which poses considerable risks after consumption. For instance, protease inhibitors have a role in plant defense system against insect [4], which abound in grain legumes, and consequently posing serious threat to consumers due to their impact as pancreatic carcinogens [5]. Similarly, saponins are insecticides [6], 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 [7]. Moreover, the debilitating effect of saponins become synergistic [8] in the presence of lectins. Lectins are carbohydrate binding proteins. 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 [9].

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 practises are

known to accumulate pesticides in the endosperm of the grains over a period [10].

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 [11]. 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 [12]. However, there are reports of increasing interest towards the exploitation of NULs to alleviate malnutrition, in developing countries [13]. By addressing the challenges of the presence of intrinsic and extraneous hazards, it is believed that the full benefits of grain legumes utilization could be enhanced. For instance, there are uncertainties and variabilities among the data required for risk assessment of NULs dishes.

Institutions such as the US Environmental Protection Agency[14] and the European Food Safety Authority [15] 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" [15]. A "hazard" on the other hand is defined as "a substance which has the potential to cause adverse effects upon exposure" [14]. It has been declared by Paracelsus (the father of modern toxicology) that "all substances are poisonous and there is none that is not a poison, it is the dose that determines when it becomes a poison" [16]. However, 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" [15]. 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" [14]. The NOAEL is often obtained by calculation because it is extrapolated [17,18]. 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"

[14]. Before hazards can cause any adverse effect, they 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" [14]. 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" [14]. Thus, risk assessment involves four emerged 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 [17].

A serious call has been made for a large number of people to consume legumes[19] 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 [20]. However, 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 since lectins are 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 [21]. These residual lectins are reckoned as hazards, as they pose risks. These risks and uncertainties associated with the consumption of NULs dishes have not been quantified, but this must be addressed in order to maintain confidence and sustainability. Thus, this study was designed to determine respondents' comprehension of hazards and the attendant risks associated with the consumption of NULs seeds. Secondly, the study aimed at determining the probabilistic risk assessment of the intrinsic dietary lectins in some selected NULs dishes.

2. Materials and Methods

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 [22] procedure and the results presented in Table 1.

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

NULs	Phaseolus sp.	Cajanus sp.	Vigna sp.	Mucuna sp.	Canavalia sp.
% Protein	17.2(±1.2)	21.3(±2.2)	15.2(±1.7)	25.2(±0.2)	17.4(±0.3)

2.2. Methods

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 safety interview schedule. The interview schedule 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 consumption 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 reviewed interview schedule was then administered by trained and experienced assistants.

2.2.2. Sampling and Sample Preparation

Samples of the selected NULs, specifically; *Vigna sp., Cajanus sp., Phaseolus sp., Mucuna sp. and Canavalia sp.* were purchased from the five-different market centres; Amantin, Mampong, Ejura, Abofuor and Techiman, in the study area between the period of the interview schedule (5th to 20th 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°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.

2.2.3. Time -temperature 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. Timing commenced when the temperature of the boiling water had reached 100°C. 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°C for lectin-based ELISA analysis.

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. As explained in the outline (Figure 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 amount 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 hazard 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 1 using appropriate reference dose (R$_f$D).
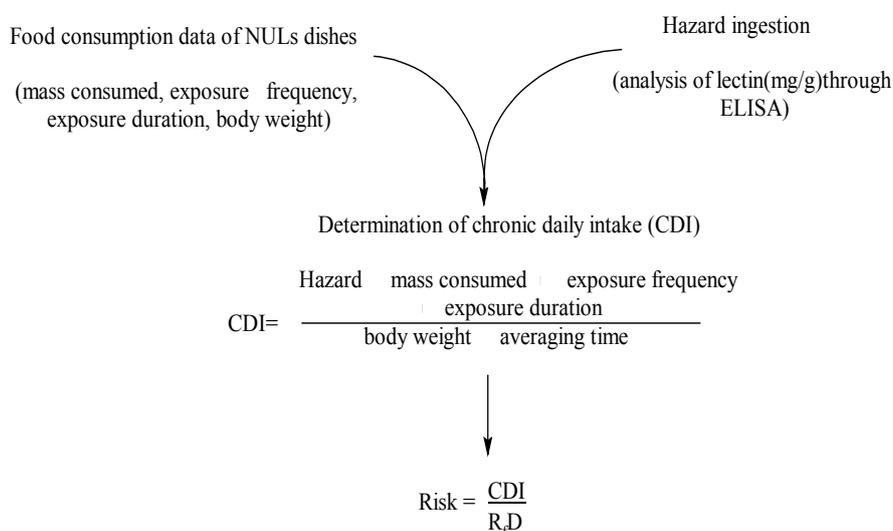

Figure 1. Outline of risk assessment of NULs dishes using their chronic daily intake (CDI) and a proposed reference dose (R$_f$D)
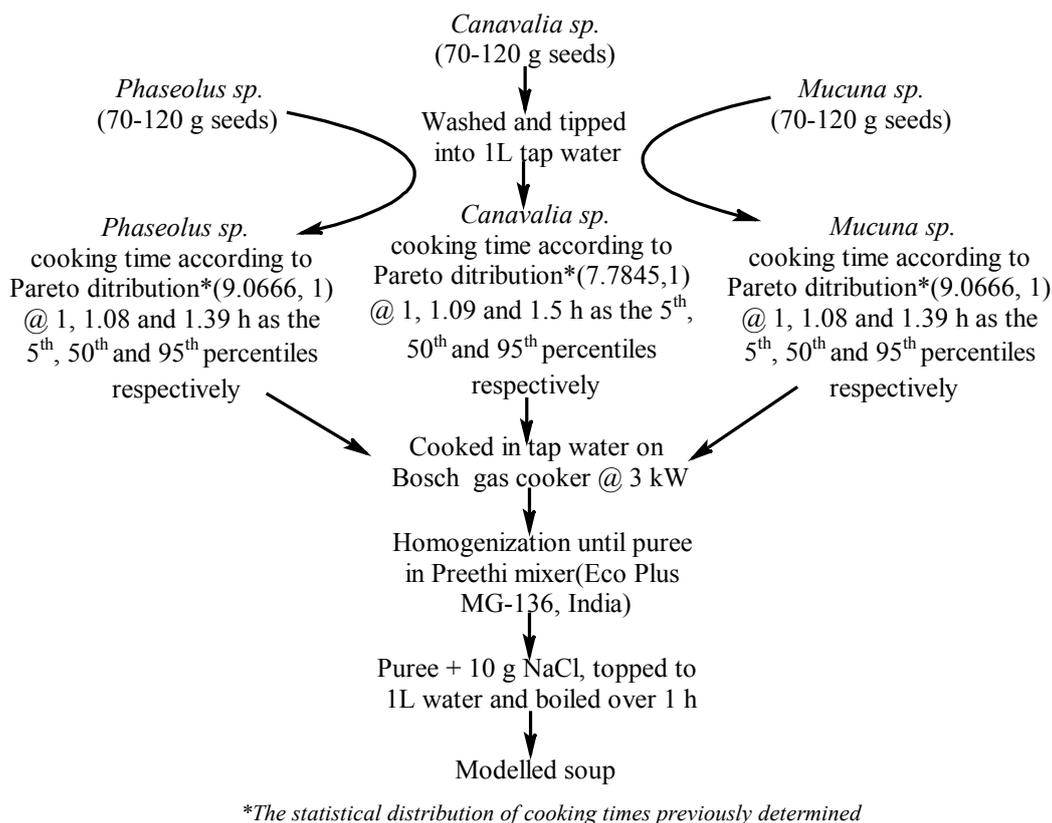
Canavalia sp.
(70-120 g seeds)

Phaseolus sp.
(70-120 g seeds)

Mucuna sp.
(70-120 g seeds)

Washed and tipped
into 1L tap water

Phaseolus sp.
cooking time according to
Pareto ditribution*(9.0666, 1)
@ 1, 1.08 and 1.39 h as the
5th, 50th and 95th percentiles
respectively

Canavalia sp.
cooking time according to
Pareto ditribution*(7.7845,1)
@ 1, 1.09 and 1.5 h as the 5th,
50th and 95th percentiles
respectively

Mucuna sp.
cooking time according to
Pareto ditribution*(9.0666, 1)
@ 1, 1.08 and 1.39 h as the
5th, 50th and 95th percentiles
respectively

Cooked in tap water on
Bosch gas cooker @ 3 kW

Homogenization until puree
in Preethi mixer(Eco Plus
MG-136, India)

Puree + 10 g NaCl, topped to
1L water and boiled over 1 h

Modelled soup

*The statistical distribution of cooking times previously determined

Figure 2. Preparation of soups based on *Canavalia sp.*, *Mucuna sp.* and *Phaseolus sp.* according to respondents' cooking practices

Vigna sp.
(500 g seeds)

Cajanus sp.
(100-200 g
seeds)

Washed, milled and
homogenized in 1500 mL tap
water, 2 g Chile salt petre, 10
g NaCl

Washed and tipped into 1 L
tap water

Filled into cellophane tubes
(12 pieces) weighing
between 15 and 26 g each

Cooking times according to
Laplace distribution*
(2, 0.24595) @ 1.5, 2 and
2.4 h for 5th, 50th and 95th
percentiles respectively

Cooking times according to
Uniform distribution*
(0.99038, 2.0096) @ 1, 1.5
and 1.96 h for 5th, 50th and
95th percentiles respectively

Cooked in tap water on
Bosch gas cooker at 3 kW

Cooked in tap water on
Bosch gas cooker at 3 kW

"Ase"

"*Tubani*"

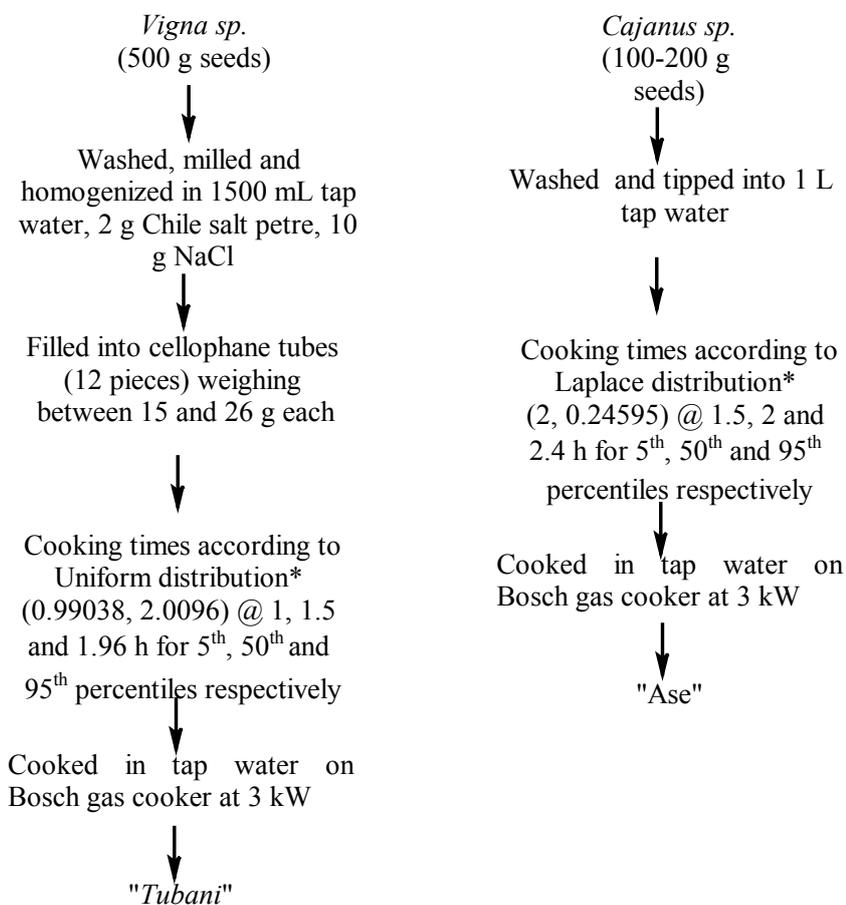** The statistical distributions of the cooking times were previously determined*

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

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 sp., Mucuna sp. and Phaseolus sp.* were used (Figure 2).

On the other hand, 23 pieces each of "*Tubani*" weighing between 15 and 26 g were prepared (Figure 3). Similarly, 20 batches of *Cajanus sp.* grains weighing between 100 and 200 g were used to prepare model "*Ase*" (Figure 3). Phosphate buffered saline (pH 7.4) was then added to 5 g sample of the cooked "*Tubani*" and "*Ase*" separately, and homogenized into a total of 40 ml of the mixture. On the other hand, 10 ml of the model soup each of *Canavalia sp., Mucuna sp.* and *Phaseolus sp.* 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 clear supernatant containing soluble proteins was then transferred into 1.5 ml Eppendorf tubes and kept (4°C), until needed for the ELISA determination of lectins.

2.2.6. 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* [23]. 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°C for 30 min. Subsequently, the wells were washed (with phosphate buffer containing 0.05 % Tween 20) five times. An aliquot of 50 µl of horseradish peroxidase (HRP) conjugated anti-SBA antibody as the detection antibody, were transferred into each well except the control well. The plates were sealed again and incubated at 37°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 reaction was catalyzed by HRP to produce a blue color that changed to yellow after adding acidic stop solution. The density of the yellow solution is proportional to lectin amount in sample captured in plate. The OD (optical density) or absorbance was at 450 nm in a SpectraMax Microplate Reader (Plus 384, US) within 15 min. Calculation of lectin content was done as;

Relative OD 450 nm

= OD at 450 of each well − OD at 450 of control well.

A standard curve was plotted as the OD of each standard solution against the respective concentrations of the standard solutions to give a standard curve with 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.

2.2.7. Probabilistic Modelling and Data Analysis of the Exposure of NULs Agglutinin in 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 [24]. Equation 1, integrated all the variables needed to calculate the HQ for the agglutinins. The concentration and contact rate of agglutinins are 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 lectins concentration (C_L,) in each NULs dish were obtained from ELISA determination of NULs dishes lectins. 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 secondary data established in a previous study [25].

$$HQ = \frac{C_L \times C_R \times EF \times ED}{R_fD \times B_w \times AT} \qquad (1)$$

The determination of HQ of each NULs dish for an exposure duration (ED) of one year, was done using Palisade@Risk [26] Microsoft Excel [27] plug-in by integrating the distribution functions of the factors in Equation 1 and reference standards. For systemic non-cancer toxic substance as lectins, 30 years was used as the averaging time (AT) [17]. Simulation was run at 100,000 iterations and the final HQs for each NUL dish were recorded. 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 [28]. 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 [29] 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 [30]. 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.

3. Results and Discussion

3.1. Familiarity with Beans and Frequency of Consumption

A total of 118 consumers were interviewed, 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) in the study area. Figure 5 present respondents who actually consume specific NULs. 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 sp.* (15.9%) and *Phaseolus sp.*(14.95%) were the most popular among the NULs. *Vigna sp.* has been used in the preparation of dishes such as "*Koose*" (fried bean flour), and "*Tubani*" [31]. This, perhaps accounts for the high levels of patronage among respondents. The popularity of *Phaseolus sp.*, *Canavalia sp.* and *Mucuna sp.* lie in their use as soup

thickeners [32]. However, the low levels of the utilization of boiled *Cajanus sp*. 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 [33].
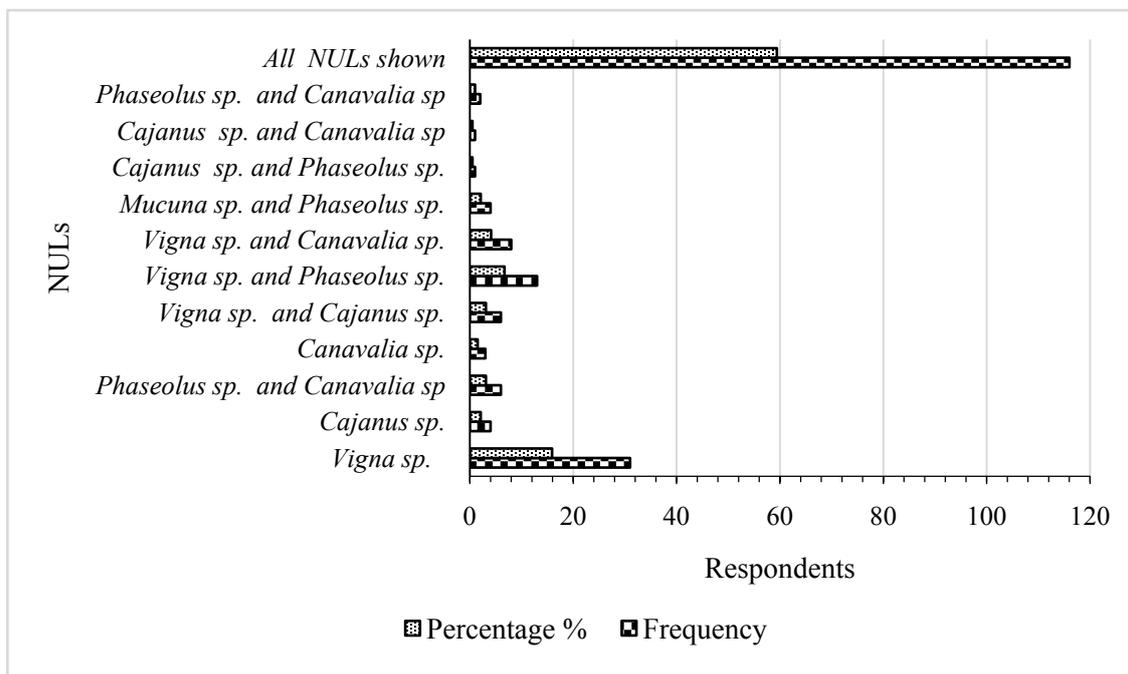

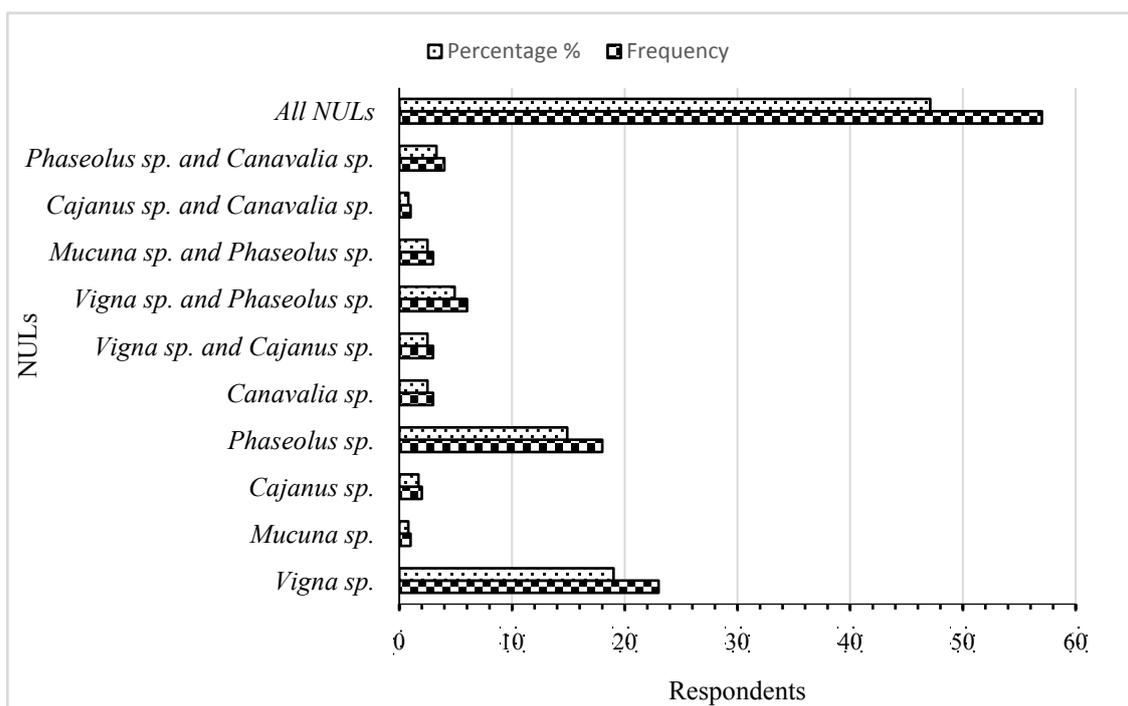**Figure 4.** Familiarity with NULs consumed among respondents in the study area.

Figure 5. NULs frequently consumed by respondents in the study area

Out of 174 respondents who were interviewed on their perception of hazards in NULs, over 66% suspected the presence of hazards in beans (Table 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 deteriorated by insects and pests if not controlled [34]. In addition, consumers use Chile saltpetre ($NaNO_3$) as cooking aids. These salts, locally called "*kanwe*" or "*kawu*", are believed to hasten the process of cooking hard-to-boil beans [31]. According to the respondents, adverse impact of all the other hazards, such as contamination with pathogenic bacteria and food allergens were marginal (Table 2). In-depth interviews

with respondents showed that the respondents were however, split 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 respondents perceive Chile saltpetre as interactively hazardous (16.6%) when combined with pesticide residues. The reasons are not clear because it is doubtful if the hazardous nature due to the generation of nitrosamines [11] was already known to a majority of respondents who were not well informed in the toxicology of food. 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 [35].

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 2:).This finding, supports the observation that only a small fraction of the population suffer from food allergies [36]. Though the response on perception of bean safety (66.1%) was overwhelmingly in favour of those who saw NULs as safe, it was strange to observe that, 97.2% of respondents complained about discomforts after consuming NULs (Figure 6). Even though in this study, serology testing, looking for the elevation of specific antibodies for celiac disease in the blood was not done, some respondents reported the prevalence of the discomforts listed above, unusually longer than the others.

It may look like a minority of respondents (2.7%) were suffering from inflammatory celiac disease. These gastrointestinal distresses are reportedly due to the presence of lectins [37]. This means 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 [38]. It is also possible that majority of consumers in the study area may be showing tolerance to the prevalent levels of NULs agglutinins. 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 still indicated they would recommend the consumption of NULs to others, simply because they are nutritious (99.4%, Table 3). Strangely, they have heard others complain about these discomforts (82.4%) and that they know the complaints (89.5%) were believable. It is difficult to comprehend the basis of such respondents' recommendation because of the complex and dynamic nature of population. Evidence to buttress peer pressure from the community lie in the findings (Table 4) that, everybody in the community eat them (15.3%) and particularly, the respondent's parents eat them because they were nutritious (14.2%).

Irrespective of the potential risk, the reported discomforts of NULs consumption was largely that of flatulence (49.5%) (Figure 6). A small number (2.7%, Table 3) of respondents, however, indicated their decision not to recommend NUL dishes to others because they are unsafe. It was also observed that, a minority (1.6%, Table 2) of respondents were concerned about the presence of intrinsic hazards in beans. A majority (66.1%, Table 2) of consumers were very concerned about hazards and in particular; pesticides (58.7%), or in interactive combinations with pathogens (0.8%), environment (1.6%) and food additives (16.6%).

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

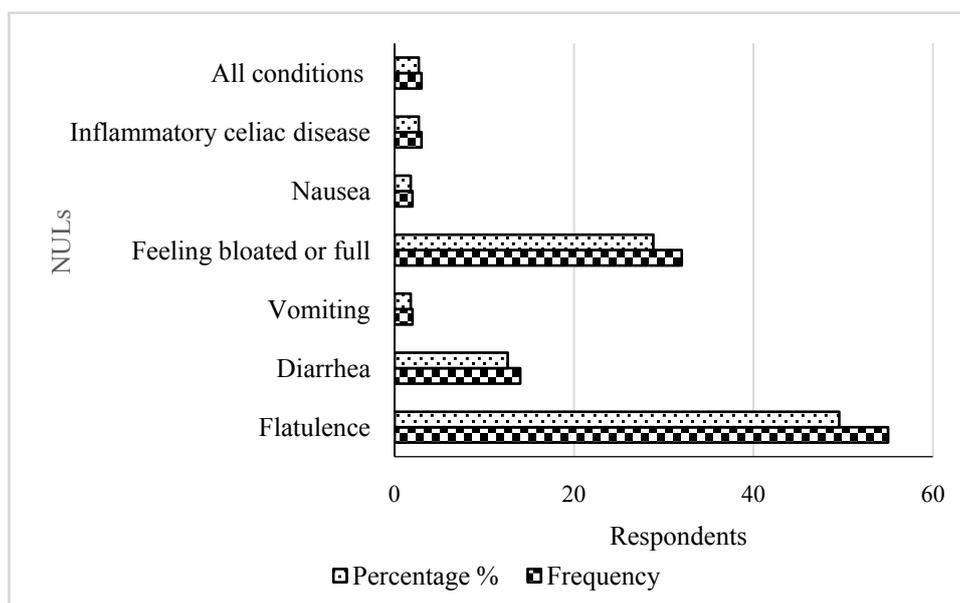|		Frequency	Percentage%
Perception of hazards in beans	Yes	115	66.1
	No	59	33.9
	Total	174	100
Type of hazards in beans		Frequency	Percentage%
Pesticides		74	58.7
Pathogenic bacteria		7	5.6
Food additives		5	4.0
Food allergens		2	1.6
Pesticide residue and pathogenic bacteria		1	0.8
Pesticide residue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

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

Table 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

Table 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

3.2. Response of Agglutinins in NULs Flour During Cooking

The agglutinin content of unprocessed NULs (Figure 7), ranged between 64 mg/g in *Phaseolus sp.* up to 414 mg/g in *Canavalia sp.* The lectin content of legumes has been reported to vary depending on geographical location and other factors [39]. In this study, the study area covered the forest and savanna regions, providing different edaphic and ecological factors. Wild beans use these powerful agglutinins mainly for defensive purposes [40], probably depending on the degree of adaptation needed to fight off diseases. These might account for the variabilities in the contents of lectins.

But within 10 min of cooking however, NULs agglutinins had not inactivated over 200% as has been reported [20]. Within this period, the agglutinins in *Phaseolus sp.* and *Mucuna sp.* had rather potentiated at 20% and 9% respectively. However, agglutinins in *Cajanus sp., Canavalia sp.* and *Vigna sp.* had inactivated at only 22%, 7% and 1% respectively. This observation is supported by studies that have also reported evidence of potentiation [38]. However, the boiling temperature of the flour in this study (100°C), was well above what was required to inactivate agglutinins in others studies that was run at 80°C [20]. However, after 30 min cooking, the agglutinins in *Canavalia sp.* had rapidly inactivated (90%) compared to especially *Vigna sp.* agglutinins which had inactivated the least (6%). At the end of the 60 min of cooking, all the different types of lectins were still showing varied residual lectins. Agglutinin levels of 60 mg/g and 70 mg/g of *Canavalia sp.* and *Vigna sp.* were still remaining. Indeed, dry or moist heating of seeds at 70°C for several hours has been reported to have little or no effect on their lectin activity [41].

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 [42], might be applicable to the agglutinins present in other NULs. The inactivation of *Canavalia sp.* and *Phaseolus sp.* lectins were quite rapid (Figure 7) only in the first few minutes. This observation might probably be attributable to weak subunits stability among the multivalent binding sites [43].
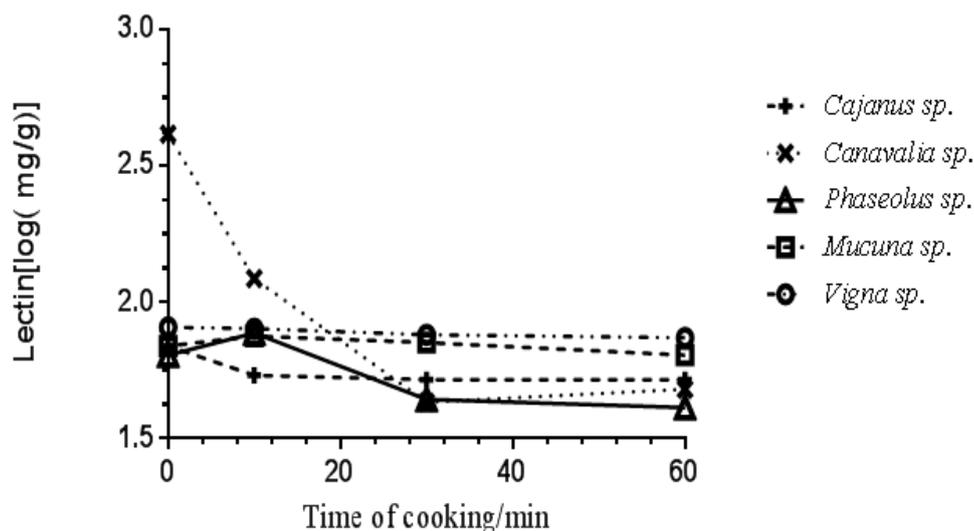
Figure 7. Time -temperature inactivation of lectins of five NULs flours

From Figure 7, the pattern of thermal degradation might suggest similar structural organizations in *Mucuna sp.* and *Vigna sp.* on one hand and *Cajanus sp.*, *Phaseolus sp.* and *Canavalia sp.*, on the other hand. The homologous nature of lectins found in the seeds of legumes probably suggest that they might have correspondingly greater numbers of subunits that can actively bind to carbohydrates when their tertiary structures are disrupted by heating. It is important to understand the degradation patterns of these lectins. Such knowledge would contribute to understanding the mechanisms of inactivating the lectins and thus, offer an effective means of reducing them to safer levels.

3.3. Safety of NULs Dishes

From Figure 8, the hazard quotients (HQ) of all the agglutinins derived from model dishes are shown. All the agglutinins presented hazard quotient probably several folds greater than the threshold of one (1) [24]. This means risk is probably implicated since dishes of these NULs were prepared according to the processing practices in the area. The high level of risk (HQ >1) of lectin ingestion, determined in this study, appears to show less serious responses in the consumers probably because of tolerance or adaptation to such diets. The reasons are that, long-term studies on newly weaned male Sprague-Dawley rats diets of casein containing 0.2% peanuts lectins reported tolerance of agglutinins [44]. 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.
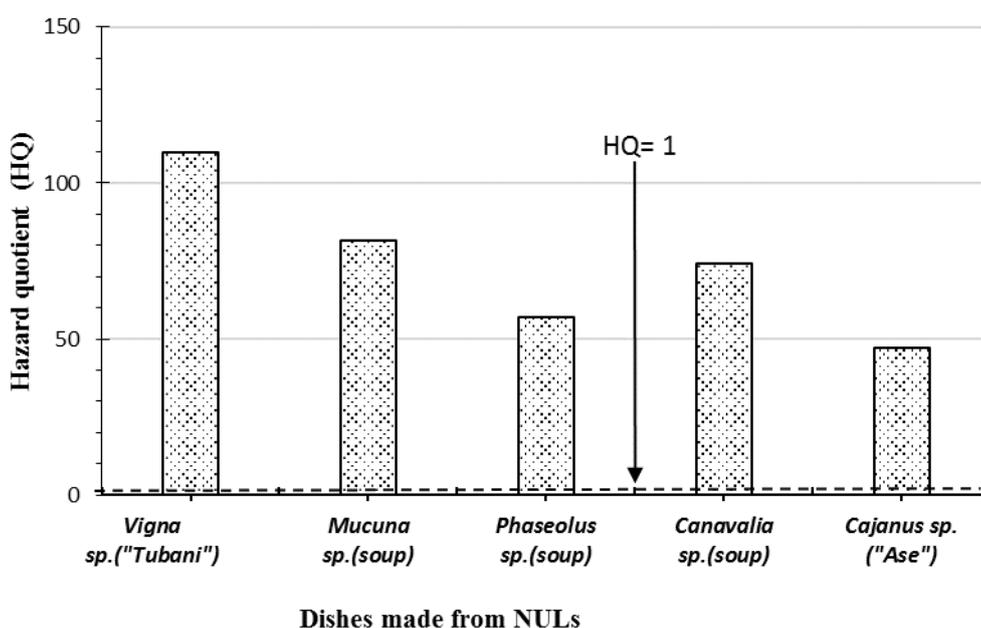

Figure 8. Hazard quotients of the agglutinins of five NULs model dishes cooked according to prevailing practices of respondents in the study area.

3.4. Conclusion

Majority of the respondents (67.6%) were over 40 years and were familiar with (59.4%) and consumed NULs (59.4%) dishes. The most popular dishes were obtained from *Vigna sp.* (15.9%) and *Phaseolus sp.* (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 potential 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 still inadequate. They also consider pesticides residues and its 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 their use 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 in order to make NULs safe for consumption if the goal of making them food security crop could be achieved.

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