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The Impact of Traditional (Natural) Fermentation and Roasting on the Nutrients, Antinutritional Factors and Phytochemical Constituents of *Glycine Max* (Soybean) Seed

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A B S T R A C T

Legume seeds are rich sources of nutrients and secondary metabolites but face the problems of nutrients digestibility and utilization as a result of inherent antinutritional factors. Processing may alleviate or remove these problems for better nutrients utilization. This study appraised the impact of processing (traditional fermentation and roasting) on the nutrients, antinutrients and phytochemicals of raw *Glycine max* seed. Proximate composition indicated significant ($p<0.05$) differences in protein of all samples. No significant ($p>0.05$) difference between the ether extract of raw and fermented samples and the crude fibre of fermented and roasted samples. Carbohydrate reduced significantly ($p<0.05$) upon processing while mineral elements were significantly ($p<0.05$) increased upon fermentation. Antinutrients were reduced upon processing. Fermentation and roasting (at 150°C for 20 minutes) can enhance the nutritive values and phytochemical constituents of *G. max* (making it potentially beneficial to health) and decrease antinutrients thus increasing the potentials of nutrients bioavailability for sustainability.

Keywords: Antinutritional factors, *Glycine max*, Nutrients, Phytochemicals, Roasting, Traditional fermentation

1. Introduction

Processing foods and/or food products in ways that are beneficial to the wellbeing of humans and even animals is key to a sustainable healthy living especially in a situation of lack and dwindling economy.

Foods are beneficial only when it nourishes the body, that is, for its nutrients. It is thus essential to harness all nutrients without leaving any to wastage or unutilized. All foods are composed of very different or similar nutrients which are peculiar to them.

The qualities of nutrients depend majorly on its digestibility and utilization in the body. However, many of the nutrients in foods cannot be utilized because they may contain natural toxins.

Legumes, which are plants belonging to the family *Fabaceae* (or *Leguminosae*) are major plant food consumed throughout Nigeria where they have different application as to how they are prepared. Some plants foods can be safely eaten raw, unfortunately, legumes (especially soybean) do not belong to this category of foods. A major problem the utilization of nutrients in legume is faced with is the presence of antinutritional factors inherent in them. Antinutrients reduce the availability of one or more nutrients in food (Yacout, 2016).

Soybean is an annual legume which belongs to the family of pea (*Fabaceae*). It is the fourth most important crop that provides oil and protein (Ainsworth *et al*., 2012). It is an edible seed and the seed is of utmost economic importance because various culture adopt its usage in various capacity of preparation for several meal types. It is a major source of protein for millions of people worldwide particularly because it is of plant origin and are cheaper than the expensive animal based substitute of protein (Mukherjee *et al.*, 2016). In Nigeria, soybean is a major source of protein in homes especially where it is utilized as milk, bean cake, in making soup and as substitute for cheese from animal milk.

Soybean, if consumed in its raw form may lead to digestive problem which may be deleterious to health as a result of the antinutritional factors in them (Sharma *et al*., 2011).

These antinutritreints (such as trypsin inhibitors, saponins, phytate amongst others) make it difficult for the nutrients in soybean to be of utmost benefit unless they are gotten rid of. Ways of getting rid of these antinutritional factors include employing various processing techniques which may consequentially remove or reduce the level of these antinutrients.

Several processing methods are usually employed traditionally in Nigeria such as fermentation, germination, soaking and roasting amongst others which has been reported to improve the nutritional status of foods (Michael *et al.*, 2018; Akande and Fabiyi, 2010). The present study is therefore employing such processing techniques to ascertain their effectiveness in improving the nutritive values of soybean seeds with the specific objective of appraising the impact of traditional fermentation and roasting on the nutrients, antinutrients and phytochemical constituents of *glycine max* (soybean).

2. Materials and method

2.1 Source of sample

Raw *G. max* seed sample was purchased at a local market in Ada, Osun state in South Western part of Nigeria.

2.2 Sample preparation

Sample seeds (750 g) were sorted to remove dirt and debris. The samples were divided into three equal parts such that a part was maintained as raw (unprocessed) sample, a part was fermented and the third part was roasted.

2.3 Processing of samples

2.3.1 Raw sample

A part (250 g) of the samples tagged as raw was air dried and then milled using a grinder. It was then kept in a dry, clean and air-tight container prior to further analyses.

2.3.2 Natural Fermentation

The natural fermentation process was carried out following the method described by Adejuwon et al. (2018).

A part of the samples (250 g) was fermented by submerging with clean water in a container with a lid to cover it and then kept in a dark place for 72 h. The sample was thereafter drained and oven dried at 60 ^{o}C for 24 h, milled and kept in a dry clean plastic container prior to further analyses.

2.3.3 Roasting

Roasting of sample (250 g) was carried out at 150^{o}C for 20 minutes in a hot-air oven (Gallenkamp oven 282A, UK). The roasted sample was then allowed to cool and thereafter milled using a grinder. The pulverized sample was kept in an air-tight container until required for analyses.

2.4 Determination of proximate composition

The proximate composition of samples was determined following the standard methods described by Association of Official Analytical Chemist (AOAC, 2012).

Gross energy was calculated by multiplying the mean values of crude protein, ether extract, and carbohydrate by 5, 9 and 4 Kcal/g for respectively.

2.5 Determination of minerals

Mineral composition was determined by the method described by Denson (1953).

2.6 Determination of Antinutrients

Antinutrients were determined by the methods described by Association of Official Analytical Chemist (AOAC, 2012).

2.7 Determination of Phytochemical composition

Phytochemical constituents were determined using standard procedures as described by Harborne (1998); Trease and Evans (2002).

2.8 Statistical Analysis

Data collected were recorded as a mean ± standard deviation of triplicate determinations and subjected to analysis of variance using the model for randomized block design (Steel and Torrie, 1980). Statistical tool (SPSS 21) was used to analyse the data obtained. Significant differences between treatment means were determined at 5% confidence level using Duncan's Multiple Range Test.

3. Results

Results showing the effect of processing (traditional fermentation and roasting) on the proximate composition of *G. max* seed samples are presented in table 1. The processing techniques (fermentation and roasting) resulted in greater yield of protein. In this study, the ether extract in roasted soybean showed the highest content when compared with the raw and fermented seed samples fermented samples (table 1). The lowest value of ash was recorded in raw sample compared to the processed samples. All seed samples were found to be rich in carbohydrate. The present study revealed that seeds of *G. max* are rich sources of mineral elements including Phosphorus, Potassium, Calcium, Sodium and Iron; highest content of the minerals assessed are revealed in the fermented samples when compared with the raw and roasted samples (table 2).

Table 3 showed reduction of all the antinutrients assessed upon processing. Significant reduction was observed in the fermented sample when compared to the roasted and raw samples.

Presence of secondary metabolites was observed in the seed samples studied (table 4). The raw seed sample contain flavonoids which is greatly increased upon processing (table 4). There is reduction in the saponins, terpenoids, tannins, steroids and phlotannins content of fermented seed sample compared to the raw seed sample.

Table 1: Proximate composition (%) of raw and processed *G. max (d.w.b.)*

Parameters	Raw	Fermented	Roasted
Protein	32.70 ± 4.40^{a}	36.43 ± 5.09^{b}	34.13 ± 5.12^{c}
Ether Extract	23.60 ± 3.67^{a}	23.90 ± 3.18^{a}	27.20 ± 3.98^{b}
Ash	3.06 ± 0.23^{a}	3.80 ± 0.28^{b}	3.63 ± 0.21^{c}
Crude Fibre	4.63 ± 1.12^{a}	4.00 ± 1.30^{b}	4.00 ± 1.21^{b}
Carbohydrate	28.48 ± 5.29^{a}	23.61 ± 5.17^{b}	24.93 ± 3,34^{b}
Dry matter	92.47^{a}	91.74^{b}	93.89^{c}
Gross energy (Kcal/g)	489.82^{a}	491.69^{a}	516.17^{b}

Results are expressed as mean ± SD of triplicate determinations

Values with different superscripts across the row are significantly different

Table 2: Mineral composition of raw and processed *G. max* (mg/100g)

Minerals	Raw	Fermented	Roasted
Calcium	163.30	170.00	178.30 ±14.76^{c}
Potassium	±11.35^{a}	±13.98^{b}	431.20 ±30.02^{c}
Sodium	421.70	440.00	41.70 ±6.13^{c}
Magnesium	±33.54^{a}	±34.76^{b}	71.70 ±7.43^{b}
Iron	40.00 ±6.32^{a}	43.30 ±7.01^{b}	7.69 ±3.50^{c}
Phosphorus	65.00 ±7.16^{a}	70.00 ±7.28^{b}	255.00 ±17.90^{c}
	7.50 ±3.76^{a}	7.80 ±3.44^{b}	
	241.70	265.00	
	±16.27^{a}	±16.80^{b}	

Results are expressed as mean ± SD of triplicate determinations

Values with different superscripts across the row are significantly different

Table 3: Antinutritional factors of raw and processed *G. max* (mg/100g)

Antinutriemts	Raw	Fermented	Roasted
Phytate	58.30 ±8.15^{a}	26.70 ±4.54^{b}	40.00 ±5.80
Oxalate	12.00 ±3.40^{a}	6.70 ±2.40^{b}	c
Lectin	1.33 ±0.01^{a}	0.50 ±0.00^{b}	9.00 ±3.80^{c}
Trypsin Inhibitor	3.66 ±0,00^{a}	1.30 ±0.00^{b}	0.90 ±0.00^{c}
Tannin	53.30 ±7.09^{a}	35.00 ±4.60^{b}	2.40 ±0.00^{c}
			43.30 ±5.00
			c

Results are expressed as mean ± SD of triplicate determinations

Values with different superscripts across the row are significantly different

Table 4: Phytochemical composition of raw and processed *G. max* (mg/100g)

Phytochemiala	Raw	Fermented	Roasted
Tannin	53.30 ±8.00 ^{a}	35.00 ±5.05 ^{b}	43.30 ±7.20 ^{c}
Phlobatannins	11.70 ±3.80 ^{a}	6.72 ±2.90 ^{b}	10.00 ±3.05 ^{a}
Saponins	43.30 ±9.15 ^{a}	35.50 ±6.75 ^{b}	30.00 ±8.00 ^{c}
Flavonoids	75.00 ±7.80 ^{a}	81.30 ±7.95 ^{b}	85.30 ±7.25 ^{c}
Steroids	45.00 ±5.00 ^{a}	33.30 ±3.85 ^{b}	40.00 ±4.20 ^{c}
Terpenoids	36.70 ±4.50 ^{a}	25.00 ±3.09 ^{b}	30.23 ±3.18 ^{c}

Results are expressed as mean ± SD of trplicate determinations

Values with different superscripts across the row are significantly different

4. Discussion

Impact of traditional fermentation and roasting on the nutrients of G. max seed

The most pertinent information on the effect of processing on the nutrients of *G. max* seed is its crude protein which is high in both the raw and processed samples and even higher than the range of protein content recorded for most legumes (Apata and Ologhobo, 1994; Estefania *et al*., 2018). Legumes had earlier been reported to be good sources of nutrients as well as provide high-quality dietary protein (Perumal *et al*., 2001; Escudero *et al*., 2006). The present study confirmed that soybean is rich in protein and may contribute to the growth and repair of worn-out tissues (Ayoola*et al*., 2012) and can thereby improve the nutrition of both humans and animals.

It was reported that the process of fermentation can have consequences which are significant to the qualities of nutrients in foods (McFeeters, 1988). Increase in protein content was also corroborated by several authors upon fermentation (El-Hag *et al*., 2002; Pranoto*et al*, 2013). The increase in protein content as a result of fermentation may be due to the breakdown of complex protein by microorganism resulting in the release of peptides and amino acids (Pranoto*et al*., 2013). Microbes are reported to be useful in the transformation of food products from time immemorial till date (Redzepi, 2011). In traditional fermentation process, several non-pathogenic microorganisms which occur in nature are exploited. Fermentation makes nutrients accessible and ensures their bioavalability (Hotz and Gibson, 2007). Roasting, as earlier noted and reported are capable of enhancing the nutrient qualities of legumes (Klan and Saini, 2016; Ajatta*et al*., 2019).

In this study, the ether extract in roasted soybean showed the highest content when compared with the raw and fermented samples (table 1). Fat is important in human diet as it may also provide an inexpensive source of high quality dietary lipid which may constitute a very good source of monounsaturated and polyunsaturated fat and consequently low in cholesterol, thereby reducing the risk of coronary heart diseases (Adegoke *et al.*, 2014).

The ash content in *G. max* is an indication that the seed has good mineral content, as a result, it can be a viable tool for evaluation of nutrients (Lienel, 2002). The lower value of ash in raw sample might be a result of the effect of antinutrients on the mineral contents of the sample as reported by Alonso et al. (2001) and Anigo et al. (2009) that anti-nutrients could interfere with the bioavailability of minerals.

As observed in the present study, the carbohydrates content of raw *G. max* was lower when compared with some legumes such as Bambara groundnut (65%), broad bean (56.9%), chicken peas (60.9%) but higher than groundnut (21.0%) as reported by Okaka (1997). All seed samples were found to be rich in carbohydrates which supply energy to cells such as brain, muscle and blood and also contribute to fat metabolism and spare protein as an energy source (Alimor and Oze, 2011). The considerable high carbohydrate content found in *G. max* is an indication of its caloric value (Enwereuzoh, 2015). Processing had a significant effect on the carbohydrates content and this agrees with the report of Alagbaoso (2015). A decrease in carbohydrates observed after fermentation was in line with the reports of Michael et al. (2018); Abang and Bough (2015); Odetokun (2000). Carbohydrates including cellulose, pepsin, lignocellulose, and starch are broken down by fermentative micro-organisms thereby reducing the fiber content of such food (Raimbault and Tewe, 2001). All samples are good sources of energy (table 1).

Just as revealed in the ash content (which is an indication of the mineral elements) the seed samples revealed to have the highest content of the mineral element assessed in the fermented samples when compared with the raw and roasted samples. The individual mineral elements assessed in this study has the highest amount in the fermented samples except calcium which was ranked to be the highest in the roasted samples (table 2). Increase in the mineral levels upon fermentation is corroborated by Pranoto et al. (2013) who in their studies discovered increase in magnesium, iron, calcium, and zinc content in some foods upon fermentation. The increase in the level of minerals might be as a result of dry matter loss during fermentation when carbohydrates and protein are degraded by microorganisms (Day and Morawicki, 2018). The mechanism through which fermentation increases the level of mineral is by reducing the phytic acid that binds minerals thereby releasing the minerals and making them available (Lopez *et al*., 1983). Fermentation also increases bioavailability of phosphorous, and iron likely due to degradation of oxalates and phytates which complex with minerals hindering their bioavailability (Sripriya*et al*., 1997).

The seeds could conveniently serve as sources of these minerals. Phosphorus has been reported to be of importance in teeth and bone formation, also contributing to energy production (Ajibade and Fagbodun, 2010).

Potassium and sodium were also revealed to be present with the most striking feature of the Na/K ratio less than one (Table 2). A diet high in potassium and low in sodium may favour lower blood pressure (Raimi *et al*., 2014).

Calcium is an essential nutrient required for critical biological functions such as nerve functions, blood coagulation, structural support for the skeleton and muscle contractions. It also aids digestion and promote good growth and regulate metabolism ((Robert *et a*l., 2003).

Iron is a major requisite of haemoglobin and myoglobin for oxygen transport and other cellular processes for growth and division (Moyo*et al*., 2011). Latunde-Dada (1991) reported that the availability of elemental iron is enhanced upon germination and fermentation of cereal. The present study also revealed the presence of magnesium which is the most required element of living cells (Andzpoana and Mombouli, 2012). Magnesium is necessary for major biological processes, including the production of cellular activities and synthesis of nucleic acid and proteins (Ajibade and Fagbodun, 2010). It also helps in regulating blood pressure, insulin release, calcium metabolism in bones and prevention of circulatory diseases (Alinnor and Oze, 2011).

Also, since anti-nutrients are heat liable, roasting could have reduced the levels of the anti-nutrients, thereby improving the bioavailability of the minerals as seen in the resultant increase in the ash content upon roasting which also agreed with the work of Audu and Aremu (2011).

However, the mineral elements composition differs upon the application of the processing methods which was corroborated in an earlier report that the chemical and nutritional compositions of soybean may vary depending on the processing techniques employed (Griesshop*et al*., 2003).

Impact of traditional fermentation and roasting on the antinutritional factors of G. max seed

Although raw soybean is a rich source of dietary nutrients yet these nutrients may become impossible to be completely utilized with the presence of antinutritional factors inherent in them. Just as processing techniques may enhance the nutritive value of food samples, so also do they possess the advantage of reducing antinutitional factors as appraised in this study (Table 3). The reduced antinutitional factors in the processed samples is in agreement with the submission of Oloyede and Abiola (2004) in their studies on the biochemical assessment of the protein status of processed *Prosopis africana* seed. Antinutritional factors are a major constraint of nutrient utilization especially in legumes (Enwere, 1998). In this study, there is high level of tannin and phytate in the raw samples.

Phytate bind minerals such as magnesium, zinc, calcium, phosphorus and make them unavailable for utilization (Nelson *et al*.,1968). Tannin belong to a class of astringent polyphenolic biomolecules which can bind and precipitate proteins and/or other organic compounds making digestibility and absorption difficult (Takuya, 2006).

Oxalate inhibits the absorption of calcium and as a result can increase the chances of developing kidney stones (Holman *et al*., 2001).

Soybean contain trypsin inhibitors which prevent the absorption of soy protein when eaten. Trypsin inhibitor act by reducing the biological activity of trypsin which is involved in the breakdown of proteins (Roy *et al*., 2010). This was reduced upon roasting and fermentation. Trypsin inhibitor is heat labile and could subsequently be remove or reduced upon application of heat (Kadam and Smithard, 1987). The reduction of trypsin inhibitors upon fermentation agrees with the submission of Vidal-Valverde et al. (1993) and Osman (2010).

Impact of traditional fermentation and roasting on the phytochemicals of G. max seed

Interestingly, the presence of secondary metabolites was observed in the seed samples studied (Table 4). Phytochemicals, also called secondary metabolites though non nutrients are chemicals produced by plants with several desirable health benefits including antioxidant properties amongst others. The raw seed sample contain flavonoids (an antioxidant) which is greatly increased upon fermentation and roasting (table 4). There is reduction in the saponins, terpenoids, tannins, steroids and phlotannins content of fermented seed sample compared to the raw seed sample. These could be as a result of the inherent fermenting microorganisms utilizing these phytochemicals thus leading to their reduction (El-Hag *et al*., 2002; Hubert *et al*., 2008). However, the flavonoids content was enhanced upon natural fermentation. this is in consonance with the submission of Wang et al. (2014) who investigated the effect of fermentation on antioxidant profiles of cereals which resulted in significant increase in the levels of flavonoids. Microorganisms break down the matrices of cereal grain matrices during fermentation leading to the release of bound phytochemicals (Dordevic*et al*., 2010).

Flavonoids may also help to prevent the body against cancer and other degenerative diseases ((Uyoh*et al*., 2013).

Saponins are naturally occurring foam producing steroidal glycosides which reduce the uptake of glucose and cholesterol. Saponins are reported to possess hemolytic activity and cholesterol binding properties (Sodipo*et al*., 2000) with the potential to lower blood cholesterol.

Tannins though reported to hinder protein digestibility amongst others as a result of decreased efficiency to convert the nutrient, are also advantageous as anticarcinogenic by possessing antioxidative property, antimicrobial and also to aid in accelerating blood clot, reduce blood pressure and blood lipid levels (Chen *et al*., 1998). Tannin was significantly reduced upon processing. This is not in agreement with the submission of Osman (2010) who reported significant increase upon fermentation.

Terpenes are organic compounds reported to be potent in the treatment of varieties of ailments (Tijani *et al*., 2013). The presence of terpenes was also shown in both the raw and processed samples with the lowest amount in the fermented samples.

5. Conclusion

Natural fermentation and roasting can increase the nutritional qualities of soybean seed. Upon roasting, mineral elements are increased particularly potassium. The antinutitional factors are reduced upon natural fermentation and roasting, an indication that nutrients in soybean will be more bioavailable upon these processing techniques. However, the antinutitional factors are better reduced upon fermentation than roasting, Flavonoids which are significant antioxidant is also improved upon the roasting process, an indication that roasting enhances antioxidant properties of soybean.

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