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IMPACT OF ROASTING ON THE PROXIMATE AND ANTI-NUTRITIONAL FACTOR OF COMMONLY CONSUMED PLANT SEEDS IN NIGERIA

L. C. Ejidike[1*], O. A. Ikeh[1]., P. N. Izuakor[1], N. A. Okonkwo[1] and V. I. Eboh[1].

[1]Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka, Nigeria.
*Corresponding author's e-mail: lindaejidike@gmail.com.
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ABSTRACT

The health benefits associated with plant protein consumption, when adequately positioned in diet, have been of great interest. However, the presence of anti-nutrients in these plants tends to affect the optimum utilization of food nutrients by neutralizing the total content of food, thereby reducing digestion. This has led to the development of different food processing methods to deactivate the effect of these anti-nutrients. This study aims to determine the impact of roasting on proximate composition and the anti-nutritional factor of three commonly consumed seeds (cashew nut, cowpea, and groundnut) in Nigeria. The shelled seeds were separated into two portions. The first portion was unprocessed while the other was processed through roasting at 40 to 70^{0}C and time (20-40min). Standard procedures determined the samples' proximate composition and anti-nutritional factor for unroasted and roasted flours. A slight denaturing of protein was observed when compared to unroasted samples. All samples showed a significant decrease in the anti-nutrients present, with oxalate having the least significant value for both raw (0.08mg/100g - 0.11 mg/100g) and roasted (0.07mg/100g – 0.10 mg/100g) samples. Hence, the use of roasting helps to aid the reduction of these anti-nutrients in food.

Keywords: Anti-nutrient; roasting; food processing; nutritional composition; proteinous plants; proximate composition.

INTRODUCTION

Plant-based proteins have grown increasingly interesting because of their potential health benefits and positive environmental impact (Ahnen *et al.*, 2019). In the course of our lives, and even before we are born, we are exposed to food compounds made up of a complex mixture that can have a positive or negative impact on our health (Astley & Paul, 2016), so ensuring food safety and security by maintaining and enhancing the nutritional

quality of foods is of great importance (Das *et al*., 2022). In Africa, roasting has been reported as one of the most commonly used food processing methods to increase the nutritional quality present in plant seeds (Ajatta *et al*., 2019). This process involves using heat to dry food, facilitating a browning reaction, and improving leguminous seeds' nutritional composition (Tonfack *et al*., 2018). The contribution of legumes to nutrition is limited due to the presence of anti-nutritional factors in these seeds (Ramakrishna *et al*., 2006). These anti-nutrients are phytochemicals responsible for the decrease in protein quality and bioavailability of micro- and macro-nutrients, leading to low digestibility of these nutrients (Faizal *et al.*, 2023). The reduction or elimination of these compounds is important to improve the biological utilization of plant proteins, which generally have lower digestibility (75–80%) when compared to animal proteins (90–95%) (such as meat, poultry, egg, and milk), also serve as a substitute for animal protein due to their economic efficiency according to recent research (Sá *et al*., 2020). Consequently, these seeds are considered to be good sources of plant protein, and this has led to the development of different food processing methods to deactivate the effect of these anti-nutrients; in this way, plant sources of protein can be used as a high-quality protein (Sá *et al.*, 2020). This study aims to determine the effect roasting has on the protein profile of these seeds by investigating its influence on the proximate and anti-nutrients present in these seeds.

MATERIAL AND METHOD

Chemicals and reagents

Three varieties of plant seeds (groundnut, cashew nut, and cowpea) were obtained from Eke Awka market, Anambra state, Nigeria. The seeds were distinguished and authenticated at the Department of Botany in Nnamdi Azikiwe University, Awka. All

reagents used in the study were analytical grade.

Sample preparations

The three seed samples (groundnut, cashew nut and cowpea) were sorted, washed with distilled water, and air-dried at room temperature. The shelled seeds were separated into two portions. The first portion, which was unprocessed, was deshelled and dehulled, then prepared into flour. At the same time, the other portion was subjected to a controlled traditional open-air roasting at a temperature ranging from 40 - 70^0C and time (20 - 40 minutes). After roasting, the samples were cooled in a desiccator, then deshelled and dehulled, and then prepared into flour.

Determination of the chemical composition of seed samples

Proximate composition

The moisture content, ash content, crude fat, and crude protein of the seed flours were determined according to the methods described (AOAC, 2012). The proximate composition of the seeds was determined in triplicate determinations. A difference of 100% calculated the total carbohydrate content.

Determination of the Antinutritional Components of the seed samples

Alkaloid determination

Determination of alkaloids was according to the methodology by Adeniyi *et al.,* (2008). After weighing five grams (5g) of the sample into a 250 mL beaker, 200 mL of 20% acetic acid in ethanol was added, the beaker was capped, and the mixture was left to stand at 25°C for four hours. This was filtered through No. 42 filter paper, and the filtrate was concentrated to a quarter of its original volume using a MemLert water bath until the precipitation was fully formed; concentrated ammonium hydroxide was added to the extract drop by drop. After settling the mixture, the precipitate was gathered and cleaned with diluted NH_4OH (1% ammonia solution). Next, pre-weighed filter paper to filtered, then the residue was dried in an oven, and the percentage of alkaloid was expressed

mathematically as:

$$\%Weight\ of\ Alkaloid = \frac{weight\ of\ filter\ paper\ with\ residue - weight\ of\ filter\ paper}{weight\ of\ sample\ analyzed} \times 100$$

Tannin Determination

Determination of tannin was according to the methodology by (Amorim *et al.,* 2008). A mixture of 0.5g samples in 50 mL of distilled water was allowed to stand for 30 mins at 28°C and filtered using Whatman No. 42 filter paper. Followed by the pouring of about 2 mL of the extract into a 50 mL volumetric flask, two millilitres of standard tannin solution and two millilitres of distilled water were added to different volumetric flasks to function as standards. Each flask was filled with 2.5 mL of saturated Na_2CO_3 solution and Folin's reagent. After adding distilled water to fill each flask to 50 mL, the mixture was incubated for 90 minutes at 28°C. Using a spectrophotometer (ThermoFisher Scientific, Model G10 UV-VIS), their absorbance was measured at 260 nm. The device was calibrated to zero using the blank solution.

Oxalate Determination

Determination of oxalate was according to the methodology previously described by Adeniyi et al., (2008). 2g sample was weighed into a 250mL volumetric flask and suspended in 190mL of distilled water. After adding 10 mL of 6M HCl, the suspension was digested for one hour at 100ºC. After cooling, it was made up to the 250 mL mark and filtered. Four drops of methyl red indicator were added to 125 mL of the filtrate duplicate amount metered out and placed into beakers. After that, the NH_4OH solution was added (drop-wise) until the test solution's colour changed from salmon pink to light yellow (pH 4-4.5). Each sample portion was heated to 90ºC, cooled, and filtered to remove precipitate containing ferrous ions. The filtrate was further heated to 90ºC, and 10mL of a 5% CaCl solution was added and agitated. After heating, it was cooled and left

overnight at 25 ºC; the solution was centrifuged at 2500rpm for 5 minutes. The supernatant was decanted, and the precipitate completely dissolved in 10mL of 20% H_2SO_4 solution. It was made up to 300mL. Aliquots of 125mL of the filtrate were heated until near boiling and then titrated against 0.05M standard $KMnO_4$ solution to a faint pink colour, which persisted for 30 seconds.

$$Oxalate = \frac{T \times (Vme)(Df)(\frac{mg}{100})}{(ME) \times Mf}$$

Where T = titre value, Vme = volume-mass equivalent (i.e. 1mL of 0.05M $KMnO_4$ solution is equivalent to 0.00225g anhydrous oxalic acid). Df = Dilution factor (2.4) ME = Molar equivalent of KM_nO_4 Mf = Mass of sample used.

Phytate Determination

This test was carried out according to Heubner & Stadtler (1914). 0.2g of the sample was weighed into different 250mL conical flasks. Each sample was soaked in 100mL of 2% concentrated HCl for 3hr, the sample was then filtered. A 250 mL beaker was filled with 50 mL of each filtrate, and each sample was mixed with 100 mL of distilled water. A standard iron (III) chloride solution containing 0.00195g of iron per millilitre was used to titrate 10 mL of a 0.3% ammonium thiocyanate solution added as an indicator.

$$Phytic\ acid = \frac{Titre\ value \times 0.00195 \times 1.19}{weight\ of\ sample} \times 100$$

Test for terpenoids

This test was carried out according to Malik *et al*., (2023). A 5g sample (W_1) was taken and soaked in 100mL of ethanol for 24 hours. The extract, after filtration, was extracted with 10mL of petroleum ether using a funnel. The ether extract was divided into pre-weighed glass vials and waited for its complete drying. Ether was evaporated, and the yield (%) of total terpenoid contents was measured;

$$Terpenoid = \frac{weight\ loss}{weight\ of\ sample} \times 100$$

Statistical Analysis

The assay results were expressed as the average value ± standard deviation of three

replicates. The three seeds from each treatment were compared by ANOVA using IBM SPSS Statistics 26.0. Results with $p < 0.05$ were considered significantly different.

RESULT AND DISCUSSION

Effect of roasting on the proximate composition of groundnut, cashew nut and cowpea

The consumption of healthier foods increased due to the consumers' awareness of the importance of diet, its benefits and health promotion beyond nutrition (Aguilar *et al*., 2015). Legumes are the earliest food crops cultivated by mankind and are rich in nutrients, with a high content of proteins, fibers, carbohydrates (Sá *et al*., 2020). The effect of roasting on the proximate composition of groundnut, cashew nut and cowpea seed is presented in Table 1. Roasting showed a significant impact on the proximate composition of these seeds. The protein content was observed to reduce after roasting, which is an indication that denaturation of protein occurred during roasting, and it could be a result of the heat applied and polymerization of amino acid that took place in the processing operation of these seeds leading to the loss of protein content. (Santos *et al.*, 2018) and (Ajatta *et al*., 2021) also observed similar findings for walnut and marble vine flour, stating that as roasting conditions increase, their protein content decreases. The highest protein content (9.45 ± 0.04%) was obtained in groundnut, and the lowest (7.35 ± 0.05%) was in cowpea after roasting. There was a reduction in the moisture content of the seeds from 5.38 ± 0.26 - 1.83 ± 0.02% for groundnut, 3.57 ± 0.03 - 1.14 ± 0.02% for cashew nut and 5.51 ± 0.20 - 1.98 ± 0.05% for cowpea. A similar observation was reported by Lawal *et al*., (2021) for toasted sesame seed flour, which reduced from 4.06 - 1.33%. This decrease in moisture content of these seeds can be linked to the evaporation of moisture that took place in the seeds during roasting, and this could increase the seeds' shelf life and their keeping

quality. Also, the lower the moisture content, the less water activity and moisture, which in turn helps prevent the inhibition of microbial development.

Table 1: Proximate composition of groundnut, cashew nut and cowpea (%)

Parameters	Groundnut		Cashewnut		Cowpea	
	Raw	Roasted	Raw	Roasted	Raw	Roasted
Moisture	5.38	1.83	3.57	1.14	5.51	1.98
	±0.26	±0.02	±0.03	±0.02	±0.20	±0.05
Ash	4.79	3.83	3.98	3.83	2.45	2.39
	±0.05	±0.24	±0.53	±0.25	±0.03	±0.05
Fat	6.81	5.56	4.68	2.34	4.13	3.93
	±0.17	±0.35	±0.06	±0.05	±0.10	±0.03
Fibre	1.44	1.10	3.30	1.15	4.41	2.02
	±0.06	±0.04	±0.03	±0.02	±0.05	±0.02
Protein	10.15	9.45	9.80	9.10	13.65	7.35
	±0.03	±0.04	±0.15	±0.04	±0.11	±0.05
Carbohydrate	72.39	77.27	74.67	82.44	69.85	82.33
	±0.33	±1.05	±0.35	±0.24	±0.02	±0.14

Data were expressed as mean ± SD analyzed using one-way ANOVA. Data with the same superscripts in the row are not significantly different at $p<0.05$.

Table 2: Quantitative analysis of anti-nutrient

Parameter	Groundnut		Cashewnut		Cowpea	
	Raw	Roasted	Raw	Roasted	Raw	Roasted
Alkaloid (%)	1.46	1.14	3.84	2.44	2.16	1.24

	±0.03	±0.02	±0.12	±0.07	±0.04	±0.01
Terpenoid (%)	8.00	4.00	28.00	24.00	16.00	12.00
	±1.12	±0.06	±2.26	±1.05	±0.04	±0.03
Tannin (%)	2.84	1.34	2.34	0.89	0.84	0.11
	±0.02	±0.01	±0.70	±0.03	±0.01	±0.03
Oxalate (mg/100g)	0.10	0.09	0.08	0.07	0.11	0.10
	±0.01	±0.02	±0.01	±0.03	±0.01	±0.03
Phytate (mg/100g)	0.56	0.53	0.77	0.65	0.60	0.56
	±0.06	±0.02	±0.08	±0.03	±0.04	±0.01

Data were expressed as mean ± SD analyzed using one-way ANOVA. Data with the same superscripts in the row are not significantly different at $p<0.05$.

Effect of roasting on Anti-nutritional factors of samples

The effect of roasting on the anti-nutrients in the different seed varieties is presented in Table 2. Phytate and oxalate show no slight decrease in value after roasting. These two anti-nutrients undergo similar mechanisms, in which they can bind with minerals such as calcium, zinc, magnesium, and potassium, leading to the formation of an insoluble protein-mineral complex (Gupta *et al*., 2015; Borquaye *et al*., 2017); these insoluble complexes formed render the micronutrients present in seeds unavailable for solubilization in the small intestine, thus preventing absorption (Faizal *et al*., 2023). Tannin levels were reduced significantly after roasting, from 2.84 - 1.34% in groundnut, 2.34 - 0.89% in cashew nuts, and 0.84 - 0.11% in cowpea. These tannins are non-absorbable compounds that generate local health benefits in the gastrointestinal tract through radical scavenging and anti-viral, anti-mutagenic, and anti-nutritional effects during their colonic fermentation, which leads to health implications in several organs (Samaei *et al.*,

2020). Phytate, tannin and oxalate are responsible for decreasing the bioavailability of certain minerals or nutrients for absorption, and their overconsumption may lead to more severe disease in humans (Faizal *et al.*, 2023). Roasting, which is heat treatment, was observed to have a significant effect on the anti-nutritional factor of all the seed varieties, with oxalate having the least significant value for both raw (0.08mg/100g - 0.11 mg/100g) and roasted (0.07mg/100g – 0.10 mg/100g) samples., and terpenoid having the highest significant value for both raw (8.00 - 28.00%) and roasted (4.00 – 24.00%).

CONCLUSION

This study showed that roasting significantly impacts these seeds' anti-nutrients and proximate composition. However, further research on the impact of roasting at different temperatures is recommended.

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