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Nutritional Composition of Roasted and Fermented *Nigella sativa* Seeds from Minna, Nigeria

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ABSTRACT

This work studied the proximate, mineral, amino acid profile, functional properties and phytochemical content of fermented and roasted Nigella sativa (black seed) using standard analytical methods. The seeds were divided into two; one portion was roasted at 100 °C for 15 mins while the second portion was fermented for 48 hours in the presence of yeast. The proximate analysis of the roasted and fermented samples revealed the values of moisture, ash, crude lipid, Crude protein, crude fibre, carbohydrate and calorific values of the fermented sample to be 1.72±0.18 %, 4.20±0.50 %, 37.27±0.59 %, 18.16±0.31 %, 5.08±0.25 %, 33.26±0.56 % and 474.49±0.19 kcal/100g while values for the roasted sample were 1.58±0.04 %, 4.67±0.07 %, 30.28±1.30 %, 17.28±0.31 %, 24.43±0.39 %, 22.09±0.37 % and 374.46±0.19 kcal/100g respectively. Mineral elements present were K (54.87±0.15 mg/100g), Mg (9.40±0.27 mg/100g), P (18.66±0.24 mg/100g), Na (3.24±0.24 mg/100g), Ca (11.95±0.21 mg/100g) and Fe (1.63±0.19 mg/100g) for the fermented sample and values obtained for K, Mg, P, Na, Ca and Fe in the roasted sample were 56.14±0.12, 7.65±0.13, 20.12±0.37, 3.75±0.17, 12.9±0.22 and 1.82±0.31 mg/100g respectively. Results of phytochemical content showed that fermented Nigella sativa contained phenol (271.09±0.26 mg/100g), tannins (14.21±0.78 mg/100 g), saponins (27.88±1.07 mg/100 g), flavonoids (25.06±0.71 mg/100 g) and alkaloids (9.40±1.03 mg/100 g) while phytochemical content of the roasted sample were 278.84 ± 0.74, 16.79±0.74, 26.06±1.16, 21.30±0.81 and 13.74±0.78 mg/100g for phenols tannins, saponins, flavonoids and alkaloids respectively. Measured values for bulk density, water absorption capacity, oil absorption capacity were 0.36±0.03 g/cm^3, 3.00±0.04 % and 2.00±0.01 % for the fermented sample while 0.62±0.07g/cm^3, 3.50±0.02 % and 1.00±0.03 % are the bulk density, water absorption capacity and oil absorption capacity respectively of the roasted sample. The amino acid profile revealed that the fermented sample had higher concentration of the essential amino acids compared to the roasted sample. Results obtained indicate that the consumption of N. sativa can contribute to the nutritional requirements of the body.

Keywords: *Nigella sativa, amino acid, minerals, nutritional, roasted, fermented*

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INTRODUCTION

Food preservation is any method used to keep food from spoilage after harvest or slaughter. Such practices date to prehistoric times. Among the oldest methods of preservation are drying and fermentation (Singh & Desrosier, 2023). Several fermented and roasted food products are known to exist, which are very useful in meeting the large dietary needs of the rapidly growing global population (Beveridge *et al.,* 2013). Roasting and fermentation are operations which can be applied to reduce anti-nutritional factors and to increase bio-availability as well as microbiological safety (Wanink *et al.,* 1994). Most trees and shrubs present in Nigeria produce fruits; some of which have pods and seeds that can be investigated in other to promote nutrient availability and formulations of drugs (Mathew *et al*., 2021; Franzo et al., 2013). There is need for effective promotion of most underutilized crops including seeds, pods and other portions that are mostly disposed due to the lack of knowledge in terms of their nutritional compositions (Bello, 2014).

Nigella (*Nigella sativa* L.), known as black cumin, is an annual herbaceous plant of the *Ranunculaceae* family. It originated from the Mediterranean region but occurs in many countries of Europe and Asia (Saleh *et al*., 2018). Black cumin (*Nigella sativa* L.) seeds are used almost entirely for its edible and medicinal purposes, such as for seasoning many kinds of backer products and for treatment of some diseases (Yearul *et al*., 2019). *N. sativa* has many biological effects such as anti-inflammatory, antihyperlipidemic, antimicrobial, anticancer, antioxidant, antidiabetic, antihypertensive as well as effects on the reproductive, digestive, immune and central nervous system such as anticonvulsive and analgesic activity (Hafize, 2019).

MATERIALS AND METHODS

Sample Collection and Preparation

Nigella sativa seeds used for the research were obtained from Tunga market in Minna, Nigeria. The seeds were sun dried and sorted to remove dirt and bad seeds before they were divided into two (2) portions of 1000 g each for roasting and fermentation.

Fermentations

The seeds of *Nigella sativa* (1000 g) were crushed and dehulled, then ground to powder and sieved with a 1 mm mesh sieve. About 100 g of powder was weighed into 250 cm^3 conical flask, 30 $cm^3$of distilled water was added followed by the addition of 1.0 g yeast (*Saccharomyces cerevisiae*) and thoroughly mixed then covered and left for 48 hours to ferment. Then freeze dried to end fermentation and kept for analysis (Mathew *et al*., 2020).

Roasting

About 1000 g of *Nigella sativa* seeds were roasted in an open pan at a temperature of 100 ^oC for 15 minutes, then left to cool in a desiccator before it was dehulled. The dehulled seeds were milled into flour using attrition mill then oven dried at a temperature of 60^oC (Makinde *et al*., 2016). The sample was sieved to obtain fine flour and packaged in an air tight container ready for analysis.

Proximate Analysis

Moisture content was determined by drying 5 g of the powdered sample to constant weight in an oven at 105 ^oC. The difference in weight before and after drying was recorded as the moisture content of the seed (AOAC, 2006). Ash content was quantified according to the method described by Ceirwyn (1998), which involves dry ashing in muffle furnace at 600^oC until grayish white ash was obtained. Crude lipid content was determined using soxhlet apparatus with n-hexane as solvent according to the AOAC (2006) method. Crude protein of the sample was determined by multiplying the value (obtained from Kjeldahl's nitrogen analysis) by a protein factor of 6.25, (AOAC, 2006). Carbohydrate content was estimated using the method where the sum of the percentage ash, crude lipid, crude protein, and crude fiber was subtracted from 100 % (AOAC, 2006):

$$Carbohydrate(\%) = 100 - (\%\,ash + \%\,protein\ + \%\,lipid\ \%\,fibre) \quad (1)$$

Calorific value was calculated using expression described by Asibey-Berko and Taye (1999):

$$Energy(kcal/100g) = (g\ crude\ protein\ x\ 2.44) + (g\ crude\ lipid\ x\ 8.37) + (g\ available\ carbohydrate\ x\ 3.57) \quad (2)$$

Mineral Determination

Six (6) g of oven dried powdered sample was weighed into dry crucible and ignited in a muffle furnace at 600 ^oC until grayish white ash was obtained. The sample was removed and cooled in a desiccator. 5 cm^3 of 1.0 moldm^{-3} HNO_3 was added and the sample was evaporated to dryness on a steam bath and then re-heated in a muffle furnace until the formation of grayish white ash was observed. The samples were removed, cooled in a desiccator and 10 cm^3 of 1.0 moldm^3 HCl was added to each ash and filtered into a 100 cm^3 volumetric flasks. Concentrations of magnesium and calcium were determined using Atomic Absorption Spectrophotometer (AAS Model SP9) while sodium and potassium were evaluated using flame photometer. Phosphorus concentration was quantified using Jenway 6100 spectrophotometer at 420 nm (Ceirwyn, 1998).

Determination of Amino Acids

Amino acid content of fermented and roasted black seeds was determined according to the method of AOAC (2006) which was also described by Barakat *et al*. (2017).

Determination of Phytochemical Content

Phenol content in the sample was quantified according method describe by Singleton *et al.* (1999). Concentration of flavonoid was determined using the method reported by Sofowora (1993). Alkaloid and saponin content were determined using the methods reported by Trease & Evans (1996) while Tannin was quantified according to AOAC (2006) method.

Functional Properties

Bulk density of the samples was determined by filling a 10 cm^3 graduated measuring cylinder gently with the sample and the bottom of the cylinder was gently tabbed until the volume of the sample in the cylinder stopped decreasing (Yoshiyuki & Yukata, 2003). Water and oil absorptions capacities were determined according to the method reported by Njintang *et al*. (2001).

RESULTS AND DISCUSSION

Proximate composition

Results of the proximate composition, mineral content, phytochemicals, amino acid profile and functional properties of roasted and fermented *Nigella sativa* seed are presented in (Tables 1-5). The mean values of the proximate composition of roasted and fermented *Nigella sativa* seed are presented in (Table 1). Moisture content of the roasted sample (1.58±0.04 %) was lower than the 1.72±0.18 % obtained for the fermented sample. These values were significantly (p>0.05) lower than the 10.70±0.21 % and 2.00±0.82 % reported for fermented and roasted *Tamarindus indica* seeds (Musah *et al*., 2022). High moisture content can reduce shelf life of the sample and cause the growth of molds. From the results, roasted sample is expected to have a higher shelf life and lower possibility of mold growth during storage than the fermented sample. The ash contents of roasted and fermented *Nigella sativa* seed were 4.67±0.07 and 4.20±0.50 % respectively. These values were slightly lower than 5.11±0.61 and 4.82±0.22 % obtained by Musah *et al*. (2020) and Anjum *et al*., (2006) for seeds of *Vigna unguiculata sub spp. Sesquipedalis* and roasted sunflower seeds respectively indicating a low-level presence of inorganic components in both fermented and roasted samples.

The crude protein content was 17.28±0.31 and 18.16±0.31 % for roasted and fermented samples respectively. These values were lower than the protein value of 20.3±0.63 % obtained by Yearul *et al*., 2019 for *N. sativa* seed however; both values were higher than 8.12±0.02 % reported for Artoarpus altilis seed (Tukura & Obliva, 2015). The higher protein content in the fermented seed could be due to the ability of fermentation to enhance the bioavailability of certain nutrient (Wanink *et al*., 1994) including proteins, by breaking them down into digestible forms while roasting causes nutrient loss including protein denaturation. A study comparing yam bean flour before and after the fermentation process showed an increase in protein from 21.33% to 25.02% on a dry basis (Chinma *et al.,* 2020). The crude protein content is an indication the samples could be good sources of protein.

The crude fiber content of the seeds is 24.43±0.39 and 5.08±0.25 for roasted and fermented seeds respectively, indicating a high difference in values between the fermented and roasted seeds. The difference could be due to the utilization of fiber as a source of energy for microorganisms, the level of this component in the final product is reduced (Espinosa-paez *et al.,* 2017). The 5.08±0.25 % obtained for the fermented seed is slightly similar to the 5.75±0.33 and 5.22±0.74 % obtained by Musah et al., (2022) for fermented and roasted *Tamarindus indica* seeds respectively. Crude fibres aid in digestion and nutrient absorption in the body.

The crude lipid content of the roasted *Nigella sativa* was 30.23±1.20 which was lower than that of the fermented sample at 37.27±0.59. This result is similar to that of Yearul *et al.,* 2019 where lipid was the major constituent of dried *Nigella sativa* seeds at 45.4±0.53%. The higher value could also be attributed to the fermentation process. Besides proteins, fermentation also affects fat content (Eliza *et al.,* 2023). Higher amount of SCFA (Short chain fatty acids produced during the fermentation of dietary fibers by beneficial bacteria) was reported in fermented soybeans than in the unfermented sample. Its concentration increased from 2.90 to 39.89 μmol/g during 0–72 h fermentation (Chen *et al*., 2022).

Carbohydrate values obtained for fermented and roasted sample were 33.26±0.56 and 22.09±0.37 % respectively. These values were lower than 58.94±0.23 % reported for *Tamarindus indica* seed (Musah *et al.,* 2022) but close to the 25.47±0.69 for Nigella seed obtained by Iqbal *et al.* (2011). The low carbohydrate in the samples is an indication of decreased chance of rancidity thereby increasing the shelf life of the samples (Abiodun & Umeonuorah, 2013). Fermentation of carbohydrates containing seeds results in an increased amount of resistant starch (Assensio *et al.,* 2020).

Mean calorific value of the roasted sample (374.46±0.19 kcal/100g) was lower than 474.49±0.19 kcal/100g obtained for the fermented sample. These values were higher than the 329.08±0.92 kcal/100g obtained for roasted *Tamarindus indica* seeds and 301.29±0.31 kcal/100g for fermented *Tamarindus indica* seeds (Musah *et al.,* 2022).

Table 1: Proximate Composition of Roasted and Fermented *Nigella Sativa* Seed.

Parameters	Content (%)	
	Roasted	Fermented
Moisture	1.58±0.04	1.72±0.18
Ash	4.67±0.07	4.20±0.50
Crude protein	17.28±0.31	18.16±0.31
Crude fiber	24.43±0.39	5.08±0.25
Crude lipid	30.28±1.30	37.27±0.59
Available Carbohydrate	22.09±0.37	33.26±0.56
Calorific Value(kcal/100g)	374.46±0.19	474.49±0.19

Values are expressed as mean of triplicate determination±Standard deviation

Table 2: Mineral content of Roasted and Fermented *Nigella Sativa* Seed.

Minerals	Concentration (mg/100g)	
	Roasted	Fermented
Sodium (Na)	3.75±0.17	3.24±0.24
Potassium (K)	56.14±0.12	54.87±0.15
Calcium (Ca)	12.9±0.22	11.95±0.21
Magnesium (Mg)	7.65±0.13	9.40±0.27
Iron (Fe)	1.82±0.31	1.63±0.19
Phosphorus (P)	20.12±0.37	18.66±0.24

Mineral Content

Mineral content of roasted and fermented *Nigella sativa* seed are presented in (Table 2). Results indicate high concentrations of potassium (56.14±0.12 mg/100g), phosphorus (20.12±0.37 mg/100g), sodium (3.75±0.17 mg/100g), iron (1.82±0.31 mg/100g) and calcium (12.9±0.22 mg/100g) in roasted seed sample when compared to values obtained for fermented sample. Only magnesium content (9.40±0.27 mg/100g) was higher in the fermented sample. Values obtained in this study were lower than concentrations of potassium (1498.3 ± 25.0 mg/100g), phosphorus (481.5 ± 47.4 mg/100g), magnesium (355.2 ± 2.3 mg/100g) and calcium (366.7 ± 20.1 mg/100g) for dried *Nigella sativa* seed (Yearul *et al.,* 2019), but slightly similar values 19.17±0.02 mg/100g (potassium), 12.19±0.12 mg/100g (phosphorus) and 18.17±0.02 mg/100g (magnesium) was reported for *Parkia biglobosa* fermented seed (Ndamitso *et al.,* 2020).
Values are expressed as mean of triplicate determination±Standard deviation.The result indicates that potassium was the major mineral present in the roasted and fermented black seed. Potassium helps to protect from rise in blood pressure and is essential for the synthesis of tissue protein in protein depleted animals (Dzemeku *et al.,* 2006). The high concentration of potassium in both samples indicates their consumption could help to prevent rise in blood pressure. Potassium and sodium ions co-function to maintain body electrolytic balance (Eddy *et al*., 2012). Calcium is essential for strong bone formation and for nerves to function properly (Bell *et al.,* 1996).

Amino Acid Profile

The amino acid profile of roasted and fermented *Nigella sativa* seed is presented in (Table 3). The concentrations for most of the essential amino acids (EAA) in the fermented *Nigella sativa* seed were higher than the concentration of similar EAA in the roasted sample. Values obtained for the concentrations of leucine, lycine, valine, phenylalanine and arginine in the fermented sample were 6.51±0.03, 2.65±0.04, 3.80±0.00, 3.91±0.01 and 5.51±0.12 (g/100 g) respectively. These values were higher than 6.12±0.06, 2.32±0.01, 2.97±0.76, 3.42±0.06 and 4.57±0.12 (g/100 g) for roasted sample. The low value of EAA in the roasted *Nigella sativa* seed could be due to the effect of heat.

Phytochemicals Composition

Result of the phytochemical composition of roasted and fermented *Nigella sativa* seed is presented in (Table 4). The results indicate higher concentration of phenols, tannins and alkaloids in the roasted sample while flavonoids and saponins were observed in higher concentration in the fermented sample. The major phytochemicals constituent in *Nigella sativa* is phenol with a 278.84±0.74 mg/100g for the roasted sample while the fermented sample was 271.09±0.26 mg/100g. Phenols are antioxidants in human and plants (Dillard & German, 2000) and are used as a disinfectant. It is found in a number of consumer products. Skin exposure to high amounts can produce skin burns, liver damage, dark urine, irregular heartbeat, and even death. Ingestion of concentrated phenol can produce internal burns (ATSDR, 2008). The EPA has determined that exposure to phenol in drinking water at concentrations of 6 mg/L for up to 10 days is not accepted but 2 mg/L in water does not cause any adverse effect. The Occupational Safety and Health Administration (OSHA) has set a limit of 5 parts per million (ppm) of phenol in air to protect workers during 8-hour work shifts.

Table 3: Amino acid profile of roasted and fermented *Nigella Sativa* seed.

Amino acids	Concentration (g/100 g)	
	Roasted	**Fermented**
Leucine	6.12±0.06	6.51±0.03
Lysine	2.32±0.01	2.65±0.04
Isoleucine	3.94±0.02	3.25±0.05
Phenylalanine	3.42±0.06	3.91±0.01
Tryptophan	2.85±0.01	2.69±0.06
Valine	2.97±0.76	3.80±0.00
Methionine	2.84±0.64	2.66±0.06
Arginine	4.57±0.12	5.51±0.12
Histidine	2.53±0.04	2.69±0.09
Threonine	3.12±0.08	3.43±0.06
Proline	2.29±0.16	2.44±0.01
Tyrosine	2.41±0.00	2.67±0.12
Cystine	1.55±0.05	1.78±0.01
Alanine	2.70±0.05	2.37±0.03
Glutamic acid	16.98±0.42	15.37±0.22
Glycine	2.79±0.04	2.44±0.02
Serine	2.40±0.06	2.16±0.04
Aspartic acid	7.06±0.07	6.63±0.03

*= Essential amino acid (EAA)

Table 4: Phytochemical Content of Roasted and Fermented *Nigella sativa* Seeds.

Phytochemical	Concentration (mg/100 g)	
	Roasted	**Fermented**
Phenols	278.84 ± 0.74	271.09±0.26
Flavonoids	21.30±0.81	25.06±0.71
Tannins	16.79±0.74	14.21±0.78
Saponins	26.06±1.16	27.88±1.07
Alkaloids	13.74±0.78	9.40±1.03

Values are expressed as mean of triplicate determination±Standard deviation.

Table 5: Functional Properties of Roasted and Fermented Black Seed.

Parameter	Value	
	Roasted	**Fermented**
Bulk density (g/cm3)	0.62±0.07	0.36±0.03
Water absorption capacity (%)	3.50±0.02	3.00±0.04
Oil absorption capacity (%)	1.00±0.03	2.00±0.01

Values are expressed as mean of triplicate determination±Standard deviation.

Tannins are plant soluble polyphenols and have been reported to cause decrease in the intake of feed, rate of growth and digestibility of protein (Chuk *et al.,* 1998).

Tannins also have some health benefits which include anti-allergic, anti-oxidant, anti-inflammatory, anti-cancer and anti-microbial activities; they also enhance the uptake of glucose thereby lowering the level of blood sugar and reduce the risk of diabetes. The daily intake of tannin below the range of 1500 – 2500 mg is considered safe (Sharma *et al*., 2019). Saponin content was 27.88±1.07 mg/100g in the fermented sample which was significantly ($p>0.05$) higher than 26.06±1.16 mg/100g in the roasted sample. The difference in saponin concentration could be due to the effect of heat on the roasted sample. Onning *et al*. (1994) reported degradation of saponin during heating. Values obtained were lower than the 127.33±0.79 mg/100g and 94.69±0.29 mg/100g for fermented and roasted *Tamarindus indica* reported by Musah *et al*., (2022). Saponin is useful in the treatment of cardiovascular diseases, lowering cancer risk and decreasing blood lipid (Del-Rio *et al*., 1997). Concentrations of flavonoid in the fermented and roasted *Nigella sativa* seeds were 25.06±0.71 mg/100g and 21.30±0.81 mg/100g respectively. These values are higher than the 3.53±0.18 and 1.96±0.51mg/100g for fermented and roasted *Tamarindus indica* seeds (Musah *et al.,* 2022).

Flavonoid acts as free radical scavenger and exhibit anti-inflammatory properties (Ruiz-Cruz *et al*., 2017). Alkaloid values were 13.74±0.78 mg/100g and 9.40±1.03 mg/100g in roasted and fermented samples respectively. These concentrations were higher than to act as stimulant or depressants on the central nervous system (Victor, 2014).

Functional Properties

Result of the functional properties of roasted and fermented black seed is presented in (Table 5). Bulk density (BD) was 0.62±0.07 g/cm^3 and 0.36±0.03 g/cm^3 for fermented and roasted *Nigella sativa* seeds. These values are lower than the 0.85±0.07 g/cm^3 reported for *Vigna subterranea* (Musah *et al*., 2021). Lower bulk density is advantageous since it helps to increase the paste thickness (Mustapha *et al.,* 2015). The water absorption capacity (WAC) content was 3.50±0.02 and 3.00±0.04 for roasted and fermented *Nigella sativa* which is higher than the 1.65±0.13 % for *Vigna subterranean* by Musah *et al.* (2020). The high-water absorption capacity may be due to high polar amino acid residues of the seed proteins having strong affinity for water molecules. The oil absorption capacity (%) of the roasted and fermented seeds was 1.00±0.03 and 2.00±0.01. These values are higher than the 0.70±0.03 % obtained by Musah *et al*. (2021) for *Vigna subterranean*. The oil absorption capacity of the seed indicates that it is a poor oil absorber compared to its water absorption capacity.

Conclusion

The results obtained in this study shows that there is a decrease in the proximate constituents of the roasted sample when compared to fermented sample except the ash and crude fiber content which is higher than the later. The high lipid content of the seed makes it a good source of oil. The higher ash content of the roasted seeds resulted into higher potassium, phosphorus, sodium, iron and calcium concentrations. Assessment of the phytochemical compositions of the samples further showed the suitability of the seed for its medicinal property, especially the roasted sample. From the findings of this study, it can be deduced that the flours investigated is unique for its proximate constituents which is almost all in the right proportions like a balanced diet. There is need to further promote researches and food processing techniques targeted towards the utilization of flours from underutilized plants.

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