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CHANGES IN NUTRIENT AND ANTINUTRITIONAL CONTENTS OF SESAME SEEDS DURING FERMENTATION

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ABSTRACT

Sesame seeds were fermented using the traditional method for four days and samples taken for analysis each day till the last day of fermentation to monitor the compositional changes in the seeds as fermentation progressed. The viable count obtained ranged from 8.0×10^3 after 24 h to 2.93×10^6 cfu/g on the 4th day. The crude protein and fat content increased as fermentation progressed reaching 27.84% and 51.58% respectively. Fermentation yielded positive effect on the phytic acid, phytin phosphorus and oxalate content of the flour samples when compared with the control. Phytic acid content ranged from 31.59 mg/g for raw seed to 18.13 mg/g for fermented seed flour. Sesame seed are high in minerals such as calcium, magnesium, potassium, sodium and slight increase in values were obtained at the end of processing. Sesame seeds are rich in both essential and non-essential amino acids with leucine, methionine, phenylalanine, threonine and valine values higher than the recommended daily allowance. Processing significantly increased the amino acid values. Sesame flour demonstrated ability to scavenge free radicals. Fermentation of sesame seeds resulted in reduction in the antinutrients in the seed and the seed can serve as soup condiment and seasoning with improved nutritional composition with respect to protein and amino acid.

Keywords: Sesame seeds, fermentation, Antinutritional factors, mineral, protein, amino acid

INTRODUCTION

Sesame (*Sesamum indicum*) seed is a staple food among many ethnic groups in Nigeria and it is cultivated in several countries of the world such as India, China, Ethiopia, Uganda and Nigeria (Abou-Garbia et al., 2000). Sesame seeds are contained in the pods of a tropical plant. They are tiny, flat ovals, measuring about 3 mm (1/8 in) long. Seed colour can vary, though they are usually beige or creamy white when husked. The seeds are sold dried and whole or ground to form tahini paste. Sesame seeds are highly valued for their high content of sesame oil which is very resistant to rancidity. The seeds are high in edible oil (44-50%), protein (19-25%) and Calcium (Hui, 1996). The protein of sesame seed, though deficient in lysine, is rich in sulphur amino acids such as methionine and cystine which make it an appropriate supplement to diets based on groundnut, soyabean and certain cereals, all of which tend to be deficient in the sulphur amino acids (Elkafi et al., 1991). Raw sesame seeds contain antinutrients like phytate and oxalate, usually found in the seed hulls (Akanji et al., 2003) these substances can adversely affect mineral bioavailability in human nutrition. Fermentation brings about numerous biochemical, nutritional and organoleptic changes in the raw materials, including the breakdown of certain constituents, the reduction of antinutritional factors in growing legumes and the synthesis of B vitamins (Egounlety and Aworh, 2000). Sesame seeds could be fermented and ground to make an oily paste called *ogiri*, which can serve as flavouring condiment (Uaboi et al., 2008). The paste possesses a very strong pungent smell with some ammoniacal odour. The fermented seed has a pleasant aroma in soups and sauces and can also contribute to the protein and essential fatty acid intake in consumer, to sustain and optimize the production process, it is necessary to obtain information about the fermentation process and the compositional changes. This is significant especially as protein-calorie malnutrition and essential fatty acid deficiencies are widespread.

The objective of our study was to determine the proximate, physicochemical properties, antinutritional and mineral composition of sesame seed during fermentation and determine the amino acid profile of raw and fermented sesame seed with their antioxidant properties.

MATERIAL AND METHODS

Source of materials

Sesame seeds were purchased from Oba market in Akure, Ondo State, Nigeria, the plantain leaves were obtained from the Federal University of Technology, Akure. All reagents used were of analytical grade.

Methods

Production of sesame condiment

The method of Odunfa (1985) was slightly modified and employed as shown in the flow chart in Figure 1. Preliminary cleaning of the seed was done by winnowing and washing with clean water. Thereafter the seeds were soaked overnight with 3% sodium chloride. It was dehulled by crushing in a wooden mortar with pestle, thereafter rinsed with water and decanted to remove the hulls; the clean seeds were boiled to soften for 6 hours, drained and allowed to cool. It was wrapped in clean leaves, kept at ambient temperature for 4 days of fermentation. The resulting mash was harvested and dried in an oven at 60°C for 6 h. It was then milled into flour and store at 4 °C in preparation for further analysis.

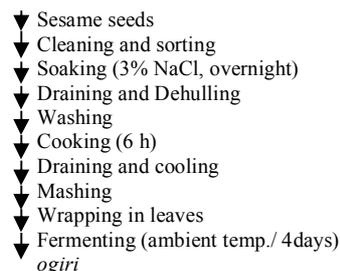


Figure1 Flow chart for the production of *ogiri* from sesame seed
Source: Odunfa, 1985.

Microbial count of sesame seed during fermentation

Microbial count was carried out as described by ICSMF (1978). The media used were Plate Count Agar and Potato Dextrose Agar. One gramme of fermented mash was added to 9 mL sterile normal saline solution, further dilutions up to 10⁻⁷ were made. Each dilutions of 10⁻⁴ and 10⁻⁵ (1 mL) was pour plated in sterile petri-dishes, incubated at 27°C/24 h for bacteria and room temperature/48 h for fungi and the colonies counted using colony counter.

Physicochemical Measurements

Determination of pH and temperature

The pH of the fermenting mass was determined by weighing 2 g of the fermenting mass homogenized in a blender with 20 mLs of distilled water and the pH was determined using a pH meter and the temperature monitored daily using a laboratory thermometer.

Determination of Total Titratable Acidity (TTA)

The amount of lactic acid in the fermenting mass was determined as described by Pearson (1976). 20 mL filtrate obtained from 2 g of fermenting sesame seed dissolved in 20 mLs distilled water was titrated against 0.1 M NaOH using phenolphthalein as indicator. The titre value was then used to calculate the titratable acidity as percentage lactic acid.

Determination of Proximate composition

The proximate composition of the samples was determined by AOAC (2000). Protein content was determined by the kjeldahl method. Ash content was determined by dry ashing in muffle furnace at 550°C. The fat content was determined using soxhlet apparatus. Crude fibre was determined by acid digestion, while carbohydrate was calculated by difference.

Determination of Antinutritional factors

The method of Young and Graves (1940) was employed for phytic acid and phytin phosphorus determination by soaking 4g of finely ground sample in 100 mL of 2% HCl for 3 h, filtered and adding 5 mL of 0.03% NH₄SCN to 25 mL filtrate with the addition of 50 mL distilled water then titrated against Ferric Chloride while the method of Day and Underwood (1986) was employed for the determination of oxalate content by adding 75 mL of 3 M H₂SO₄ to 1g of sample and stirring intermittently for 1 h, 25 mL filtrate was then titrated hot against 0.05MnO₄ solution to the point when a faint pink colour persisted for at least 30 seconds .

Determination of mineral composition

The dry ashing technique as described by Oshodi and Fagbemi (1992) was used for the determination of mineral composition. About 1 g of flour sample was weighed into a dry pre-weighed crucible and transferred into a muffle furnace with temperature maintained at 550°C for 8 h after which the sample turned grey with no black speck. The mineral elements were analysed from the solution obtained from dissolving the ash in 10% HCl, filtered and diluted to 100 mL volume. The solution was aspirated into the Atomic Absorption Spectrophotometer (AAS) and Flame Emission Spectrophotometer (FES) to determine the mineral elements. The minerals analysed include Ca, Mg, K, Na, Zn, Fe, Mn and Cu.

Determination of free-radical scavenging capacity

The free radical scavenging capacity was determined using the method described by Gyamfi et al. (1999). 1,1-di phenyl-2-picrylhydrazyl (DPPH) chemical was used, one mL of the extract from sesame sample was mixed with 1 mL 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30minutes and the absorbance of sample (A_{sample}) at 517 nm was determined with a UV-spectrophotometer against methanol as blank. The percentage concentration of DPPH in the reaction medium was calculated using the following formula:

$$\%DPPH = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Statistical analysis

The results obtained from the various analyses were subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 16.0. Means were separated with Duncan Multiple Range Test (DMRT) at 95% confidence level (p<0.05).

RESULTS AND DISCUSSION

Microbial count

The result of bacteria and fungi count is presented in Table 1. Raw sesame seed produced too numerous to count microbial load. However, after dehulling, the microbial load was reduced to 1.9×10⁴ cfu/g. The total viable count of microorganisms increased as fermentation period progressed from 8×10³ cfu/g after 24 h to 2.93×10⁶ cfu/g within the 96 h of fermentation which suggests multiplication of microorganisms as fermentation progressed. The steady increase in the microbial load during fermentation could have been influenced by the accumulation of compounds such as organic acids and other metabolites. Several researches have shown that fermented foods are desired and regarded as safe due to the production of inhibitory substances by the microorganisms involved in the fermentation process (Gobbetti et al., 2005; De Vuyst et al., 2006). Similar result was observed during the fermentation of African oil bean seed (Egounlety, 2003; Enujiugha et al., 2008). The fungal count on the other hand decreased progressively with dehulling and as the fermentation period progressed.

Table 1 Microbial count of sesame seed during fermentation

Characteristics	TVC (cfu/g)	Fungi (sfu/g)
Raw sesame seed	Numerous	3.63×10 ⁵
Dehulled sesame seed	1.9×10 ⁴	2.3×10 ⁴
Dehulled fermenting sesame 24hrs	8×10 ³	5.1×10 ³
Dehulled fermenting sesame 48hrs	1.3×10 ⁴	2.3×10 ³
Dehulled fermenting sesame 72hrs	2.9×10 ⁵	1.8×10 ³
Dehulled fermenting sesame 96hrs	2.93×10 ⁶	1.2×10 ³

TVC - Total Viable Count

Physicochemical changes during fermentation

Changes in physical and chemical composition of sesame seed during fermentation is shown in Table 2. The pH of the fermenting mass increased with an increase in fermentation period from 4.8 at 0 h to 8.3 at the end of the 96 h fermentation period which depicts that fermentation of sesame seed for the production of soup condiment *ogiri* is an alkaline fermentation. Most bacteria are favoured by reactions near neutrality while a few are favoured by an alkaline reaction. High pH inhibits many microorganisms and determines the types of organisms that grow in fermenting mass. In this study, the increase in pH during fermentation could have been contributed by the strong proteolysis of the sesame seed proteins into some basic products like ammonia. Owens et al. (1997) reported that a rise in pH value from 4.5 to 8.5 was observed in the fermentation of soybeans with the development of strong ammoniacal odour because of the hydrolysis of seed protein and the metabolism of the resultant amino acids. In addition, Egounlety (2003) reported increase in pH values during fermentation of mucuna tempe and condiment due to protein hydrolysis. On the other hand, the total titratable acidity (TTA) decreased with increasing pH from 0.061 to 0.025% as the fermentation progressed, while the temperature increased from 28°C at 0hr to 39°C at the end of 96 h fermentation as a result of the activities of the fermenting micro-organisms. Optimized temperature of 35°C was suggested for fermentation of dehulled soybean by *B. subtilis* (Owens et al., 1997).

Table 2 Changes in physical and chemical composition of sesame seed during fermentation

Measurement	Fermentation period (h)				
	0	24	48	72	96
Temp. (°C)	28±1.00 ^c	34±0.50 ^b	37±2.00 ^a	34±1.00 ^b	39±0.50 ^a
pH	4.8±0.05 ^c	6.8±0.10 ^b	7.8±0.20 ^a	8.1±0.10 ^a	8.3±0.20 ^a
TTA(%)	0.061±0.003 ^a	0.056±0.001 ^a	0.05±0.003 ^b	0.046±0.001 ^b	0.025±0.002 ^c

Values are mean± SD from triplicate determinations; different superscripts in the same row are significantly different at P<0.05

TTA - Total Titratable Acidity

Proximate composition of sesame seed as affected by fermentation period

Changes in chemical composition of sesame seed during fermentation is shown in Table 3. The moisture content ranged between 2.37% and 3.41%. Slight increase in crude protein values was observed during fermentation (20.71-27.84%) and this could be due to the action of extracellular enzymes produced by the fermenting microorganisms. The increase in protein content can be attributed to microbial synthesis of proteins from metabolic intermediates during their growth cycles (Elyas et al., 2002). In addition, observed increment in protein content after fermentation was probably due to shift in dry matter content through depletion during fermentation by action of the fermenting microorganisms

(Abdelhaleem et al., 2008). However, cells of the fermenting microorganisms could have contributed to the protein, therefore, fermentation of sesame resulted in an observable increase in crude protein content. In most human diets, protein is more limiting than other nutrients therefore, application of fermentation process that appears to increase the protein content even at the expense of other nutrients may be advantageous nutritionally (Abdelhaleem et al., 2008). *Bacillus* species implicated in oil bean seed fermentation are important producers of proteases. These extracellular proteases easily hydrolyze complex plant proteins to amino acids and short chain peptides, thereby causing an increase in total nitrogen content (Enujiugha et al., 2003). The increase of ash content from 4.41 to 5.83% in the fermented product could be attributed to the increased metabolic activities of the fermenting microorganisms. Crude fibre content decreased from 2.41% to 2.01% by the end of fermentation period, this could probably be due to the inability of the microbial agents to synthesize cellulases and hemicellulases for the hydrolysis of complex polysaccharides in the seeds. The expected decrease in fiber content during fermentation could be attributed to the partial solubilisation of cellulose and hemicellulosic type of material by microbial enzymes. A previous study has reported a significant decrease of fat, ash, and fiber contents after four days of maize fermentation (Ejigui et al., 2005).

Table 3 Proximate composition of sesame seed during fermentation

Composition (%)	Fermentation period (h)					
	Raw	0	24	48	72	96
Moisture	1.91±0.03 ^e	2.37±0.03 ^d	2.15±0.03 ^d	2.90±0.06 ^c	3.36±0.06 ^b	3.41±0.03 ^a
Protein	26.76±1.36 ^a	20.71±0.20 ^c	19.11±0.49 ^d	22.23±0.00 ^b	23.87±1.00 ^b	27.84±0.00 ^a
Fat	52.70±1.12 ^a	51.60±0.38 ^b	51.92±0.06 ^b	52.54±0.27 ^a	52.02±0.94 ^a	51.58±1.12 ^b
Ash	5.83±0.12 ^a	4.41±0.11 ^c	5.70±0.20 ^a	5.25±0.11 ^b	5.33±0.07 ^b	5.83±0.12 ^a
Crude fibre	3.56±0.13 ^a	2.41±0.09 ^b	2.33±0.15 ^b	2.28±0.10 ^b	2.07±0.01 ^c	2.01±0.13 ^c
Carbohydrate	9.77±0.63 ^c	17.81±0.83 ^a	17.81±0.83 ^a	8.51±0.26 ^c	8.13±0.61 ^c	4.26±0.18 ^d

Values are mean ± SD from triplicate determinations; different superscripts in the same row are significantly different at P≤ 0.05.

Effect of fermentation on antinutritional factors in sesame seed

The result of concentration of antinutrients in the fermented sesame seed is presented in Table 4. Raw sesame seed contains 31.59 mg/g phytic acid, 8.89 mg/g phytin phosphorus and 1.05mg/g oxalate. The phytic acid, phytin phosphorus and oxalate content of sesame seed decreased to 18.13 mg/g, 5.10 mg/g and 0.48 mg/g leading to 50%, 69% and 69% reduction respectively at the end of 96 h fermentation. The results of this study are in agreement with those reported by Abdelhaleem et al. (2008) and Makokha (2002), who stated that fermentation of sorghum, produces significant loss in phytate. Fagbemi et al. (2005) also concluded that fermentation is the most effective processing technique that reduces phytic acid. The concentrations of antinutrients in different foodstuffs may affect their nutritive values. Oxalic and phytic acids are known to precipitate or form insoluble complexes with calcium, magnesium, zinc and iron thus interfering with their utilization, fermentation as a processing technique has been shown in this study to quantitatively reduce the concentrations to permissible limit.

Table 4 Composition of antinutritional factors of fermented sesame seed

Sample description	Antinutrients(mg/g)		
	Phytic acid	Phytin phosphorus	Oxalate
Raw sesame seed	31.59 ± 0.95 ^a	8.89±0.90 ^a	1.05 ± 0.10 ^a
Fermented sesame 0 h	30.18 ± 0.63 ^a	8.50±0.65 ^a	0.98 ± 0.03 ^a
Fermented sesame 24 h	24.17 ± 0.95 ^b	6.80±0.96 ^b	0.72 ± 0.03 ^b
Fermented sesame 48 h	20.87 ± 0.47 ^c	5.88±0.45 ^c	0.69 ± 0.052 ^b
Fermented sesame 72 h	19.23 ± 0.48 ^d	5.41±0.48 ^d	0.63 ± 0.02 ^b
Fermented sesame 96 h	18.13 ± 0.00 ^c	5.10±0.01 ^c	0.48 ± 0.052 ^c

Values are mean ± SD from triplicate determinations; different superscripts in the same column are significantly different P≤ 0.05.

Mineral composition

The result of the mineral contents analysis is presented in Table 5. This indicates that the most abundant minerals were calcium, magnesium, potassium and sodium which have physiological and therapeutic significance. It has been

reported that magnesium is an activator of many enzyme systems and maintains the electrical potentials in nerves (Ferrao et al., 1987). Calcium in conjunction with phosphorus, magnesium, manganese, vitamins A, C and D, chlorine and protein are all involved in bone formation (Aremu et al., 2005). Calcium is also important in blood clotting, muscle contraction and in certain enzymes in metabolic processes. The Na/K ratio of raw and processed sesame seed is less than one. This, on the basis of the recommendation of Nieman et al. (1992) could suggest that sesame would be suitable for reducing high blood pressure. The amount for each mineral is extremely higher than those reported for melon seeds by Ojeh et al. (2008). In addition, the values obtained for the minerals in most of the samples meet the recommended daily requirements for both children and adults (Food and Nutrition Board, 2004).

Table 5 Mineral composition of sesame seed during fermentation

Mineral (mg/100 g)	Fermenting period (h)					
	Raw	0	24	48	72	96
Ca	429±0.82	410±0.38	408±0.71	316±0.31	328±0.36	416±0.79
Mg	392±0.39	389±0.41	375±0.28	276±0.3	297±0.3	381±0.36
K	441±0.51	380±0.11	210±0.28	312±0.3	340±0.3	360±0.41
Na	371±0.38	342±0.35	301±0.18	122±0.19	144±0.17	350±0.24
Fe	109±0	99.63±0.4	90.12±0.03	58.25±0.06	64.28±0.04	99.35±0.03
Zn	91.1±0.19	82.4±0.07	76.5±0.04	56.2±0.07	58.3±0.09	88.8±0.09
Mn	26.2±0.16	23.2±0.09	19.5±0.09	10.1±0.06	13.0±0.05	23.8±0.0
Cu	22.2±0.14	20.5±0.06	19.1±0.1	12.1±0.03	14.9±0.5	21.1±0.1

Values are mean ± SD from triplicate determinations

DPPH Free Radical Scavenging Capacity

Table 6 shows the percentage free radical scavenging capacity of raw sesame seed, dehulled and fermented. The DPPH radical is a stable organic free radical with an adsorption peak at 517 nm. It loses this adsorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow (Sanchez- Moreno, 2002). Radical scavenging activity was expressed as the inhibition percentage. Raw sesame seed possessed a high inhibition percentage of about 78.3%, dehulling reduced the scavenging capacity by about 50% from 78.3% to 37.5%. It has been reported by Benguo et al. (2011) that the scavenging capacity is most predominant in the seed hulls while removal of the hulls therefore reduces the inhibition percentage.

Table 6 DPPH Free Radical Scavenging Capacity

Sample description	% DPPH
Raw sesame seed	78.30 ± 0.40 ^a
Dehulled sesame seed	37.50 ± 0.11 ^d
Dehulled fermented sesame seed	47.84 ± 0.91 ^c
Whole fermented sesame seed	61.27 ± 0.32 ^b

Values are mean ± SD from triplicate determinations; different superscripts in the same column are significantly different P≤ 0.05.

Amino acid composition

Table 7 shows the amino acid composition of raw and fermented sesame flours in comparison with FAO/WHO (1991) standard. Sesame seed is a rich source of both essential and non-essential amino acids. It is majorly rich in glycine, leucine, lysine, methionine, phenylalanine, arginine, aspartic acid and glutamic acid. Sesame seed is comparably richer in some essential and non-essential amino acids than melon seed such as lysine, methionine, serine, leucine, glycine and valine (Ojeh, et al., 2008). The result showed that raw and fermented sesame seed flours contained an appreciable amount of amino acids. In addition, aspartic acid (9.35 and 10.00 g/100g) and glutamic acid (15.59 and 17.04 g/100g) were found to be the most abundant of all the amino acids in the raw and fermented seed flours. The results were similar to the report of Adeyeye (2004) that aspartic acid and glutamic acid were the most abundant amino acids in legumes and nuts. Comparing the essential amino acids of the processed sesame seed flour with the recommended daily allowances (RDA) it was observed that arginine, histidine, leucine, methionine, phenylalanine and threonine in the processed sesame seed flours were higher than the recommended daily allowance (RDA). The proportions of arginine, histidine and other essential amino acids that are present in these processed sesame seeds indicated that the

seeds may be useful in formulating weaning food. The total amino acid in the raw and fermented sesame seed flours are 75.10 and 81.70 g/100g respectively.

Table 7 Amino Acid composition of raw and fermented sesame flours

Amino Acid (g/100g)	Raw sesame flour	Fermented sesame flour	FAO/WHO (1991)
Valine	3.84	4.01	4.2
Lysine	3.38	3.89	4.2
Histidine	2.01	2.35	2.4
Methionine	2.25	2.35	2.2
Threonine	3.11	3.36	2.6
Leucine	5.99	6.04	4.8
Isoleucine	3.66	3.83	4.2
Phenylalanine	4.14	4.40	2.8
Glycine	4.11	5.03	NA
Alanine	2.93	3.09	NA
Serine	3.43	4.21	NA
Proline	3.08	3.50	NA
Arginine	5.79	5.96	2.0
Tyrosine	2.26	2.25	NA
Cystine	1.18	1.32	NA
Aspartic acid	9.35	10.00	NA
Glutamic acid	15.59	17.04	NA
Tryptophan	ND	ND	1.4

ND: Not Determined; NA: Not Available; RDA Source: FAO/WHO (1991)

RDA: Recommended Daily Allowance

CONCLUSION

Fermentation generally improved the protein value of sesame seed flour and this suggests possible use of the seed as a potential source to improve the nutritional qualities of local staples like cereals, roots and tuber flours. Most of the staples have lower values of protein and when incorporated may likely be a remedy to solving the menace of protein energy malnutrition in the developing countries. Sesame seed can be equally used for the production of soup seasoning and condiment *ogiri* as other reported fermented vegetable protein and oil seeds having exploited melon seed for this purpose for many years since there is a nutritional balance provided by this seed. In addition, fermentation used in this study is a suitable processing technique to reduce antinutrients thereby ensuring availability and absorption of minerals and protein.

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