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Effect of Liquid and Solid Fermentation on Mineral and Amino Acid Contents of Kersting's Groundnut (*Macrotyloma geocarpum*)

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Authors' contributions

This work was carried out in collaboration between both authors. Author CA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Both authors managed the literature searches, analyses of the study performed the spectroscopy analysis and managed the experimental process and author CA identified the species of plant. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The research aimed to reveal the effect of liquid and solid fermentation on the mineral and amino acid contents of Kersting's groundnut (*Macrotyloma geocarpum*).
Design of the Study: A comparative effect of liquid and solid fermentation on the mineral and amino acid contents was evaluated.
Place and Duration of Study: Department of Microbiology and Central Science Laboratory, Federal University of Technology, Akure, Nigeria from June 2013 to April 2014.
Methodology: The seed of Kersting's groundnut were de-husked and subjected to liquid and solid fermentation using standard methods. The titratable acidity (TTA), Mineral and Amino acid contents (essential and non-essential) of the fermenting samples were accessed every 24hrs during fermentation.
Results: TTA decreased (*P*=.05) from 0.035 at 0hr to 0.025 mg lactic acid/g at 72hrs of both liquid and solid fermentation. TTA decreased significantly (*P*=.05) as the fermentation time increased. Potassium (K) content decrease significantly (*P*=.05) from 300.83±0.11 mg/100 g at 0hr to

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217.60±0.07 and 289.26±0.15 mg/100 g at 72hrs of liquid and solid fermentation respectively. Magnesium content reduced significantly (*P*=.05) from 18.65±0.03 mg/100 g at 0hr to 6.93±0.01 and 7.32±0.04 mg/100 g after 24hrs of liquid and solid fermentation respectively. The value was observed to increase significantly (*P*=.05) to 15.24±0.60 mg/100 g and 24.27±0.03 mg/100 g after 72hrs of both liquid and solid fermentation respectively. Copper was observed to be absent in the groundnut. A total of 17 amino acids were analysed in the groundnut. Leucine was found to be the most abundant essential amino acid with a value of 7.19±0.00 g/100g protein while Glutamic was the most abundant non-essential amino acid with a value of 15.84±0.01 g/100 g protein. The total essential amino acid (TEAA) ranges from 46.39% to 46.85% of the total amino acid (%TAA).

Conclusion: The study revealed variation in the mineral contents has the fermentation time increased. Amino acid content was observed to increase significantly after 24 and 48hrs of liquid and solid fermentation respectively.

Keywords: Fermentation; Kersting's groundnut (Macrotyloma geocarpum); minerals and amino acids.

1. INTRODUCTION

Food legumes represent a diverse group of plants, which are found worldwide. These are an important component of animal and human diets [1]. They are derived from Leguminosae family, also called Fabacae and classified as vegetables. The increasing demand for sources of protein in developing countries, coupled with the relatively high cost of imported proteins, has led to a search for alternatives, particularly unconventional legumes indigenous to the tropics [1]. Legumes play an important role in human nutrition since they are rich sources of carbohydrate, protein, minerals (zinc, calcium and magnesium) and vitamins (vitamin E, niacin, riboflavin and thiamine) [2]. They have been reported to contain adequate amounts of lysine, except for the sulphur-containing amino acids, which are methionine and cystine [3,4].

Fermentation process is a process which involves the conversion of large molecules to small molecules or molecular oxidation/ reduction mechanisms mediated by selected microorganisms [5]. The mechanism of food fermentation is essentially the conversion of carbohydrates to alcohols and carbon dioxide or organic acids by yeasts, bacteria or a combination thereof, under anaerobic conditions [6]. Fermented foods are defined as those foods which have been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification to the food [7,8].

Cowpea, Groundnut, Bambara nut, Soybean, Sesame, Pigeon pea, African yam bean, and Groundbean are consumed in Nigeria. However, some of these legumes are underutilized. The low consumption or underutilization of some of these legumes are likely due to hard-to-cook characteristic of legumes, lack of information regarding their nutritive values and presence of antinutrients in the legumes [9]. Kersting's groundnut also known as groundbean is one of the lesser known and under-utilized legume indigenous to West Africa. It is a member of the Fabaceae family, which produces pods in the ground. The pod contains about 1 to 3 seeds with helium and relatively thick seed coat [9].

Recent problems linked with meat consumption as source of protein have led to renewed interest in vegetation diet [10]. The consumption of a high quantity of meat increases the risk of cardiovascular diseases and some types of cancer. It is to this end that intensive efforts are being made to find alternative sources of protein from the underutilized leguminous plants in nutrition and in the formulation of new food products [11,12].

Kersting's groundnut is a promising alternative source of high quality protein and feed for the tropics, but it is an under-utilized legume [13]. This is as a result of the high labour demand, prolong cooking time and lack of information regarding their nutritive values and presence of anti-nutrients in the legumes. Although, Cooking is said to destroy the heat-labile anti-nutritional factors [2,14], but it may cause changes in the composition of numerous chemical constituents such as amino acids, vitamins and minerals depending on the temperature and time of thermal treatment used [15,16]. However, Fermentation process has been reported to represent an alternative technique for improving the nutrient values of legumes, eliminate anti-nutritional factors, reduce cooking time of the product, converts insoluble proteins to soluble components and increases the level of lysine [17-19]. It is therefore pertinent to examine the effect of liquid and solid fermentation on the mineral and amino acid contents Kersting's groundnut so as to ascertain the suitable fermentation methods and effective time that will best improve the nutrient contents of the groundnut.

2. MATERIALS AND METHODS

2.1 Source of Kersting's Groundnut

Kersting's groundnut (*Macrotyloma geocarpum*) seeds were purchased from a seller at Oja-oba market, in Akure, Nigeria. The seeds were identified and authenticated at the Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure, Ondo state.

2.2 Processing of Kersting's Groundnut

The solid and liquid fermentation were carried out by the modification of [20] and [21] respectively (flow chart shown in Fig. 1). The sorted seeds were divided into three portions, coded i, ii and iii of 500 g each using electronic weighing balance (Electronic balance, MT-301 Model). The first portion (i) was subjected to Solid fermentation, where the de-husked seeds were wrapped in blanched banana (*Musa acuminata*) leaves and allowed to ferment for 72hrs at 28±2℃ in a sterile fermentor. The second (ii) portion was subjected to Liquid fermentation in which the sorted seeds were soaked in water in the ratio of 1:3w/v in a clean fermentor, for 72hrs at 28±2℃. The third (iii) portion which serves as control was analyzed raw. Samples were taken out every 24hrs during fermentation for analysis.

2.3 Total Titratable Acidity

Total Titratable acidity (TTA) of a solution is an approximation of the solution's total acidity. TTA of a solution is measured by reacting the acids present with a base such as NaOH to a chosen end point as indicated by an acid sensitive colour indicator. Five gram (5 g) of the unfermented and fermented sample was blended with a sterile blender, homogenized in 45 ml of distilled water (1% w/v) and filtered using Whattman filter paper. 8.0 ml of filtrate was titrated with 0.1M NaOH to an end point of permanent pink colour using 1% phenolphthalein as indicator [22].

2.4 Determination of Mineral Elements

The mineral composition (Potassium, Sodium, Calcium, Magnesium, Zinc, Iron, and Copper) of the analysed sample was determined by wet ashing method followed by spectrophotometric reading of the level of mineral.

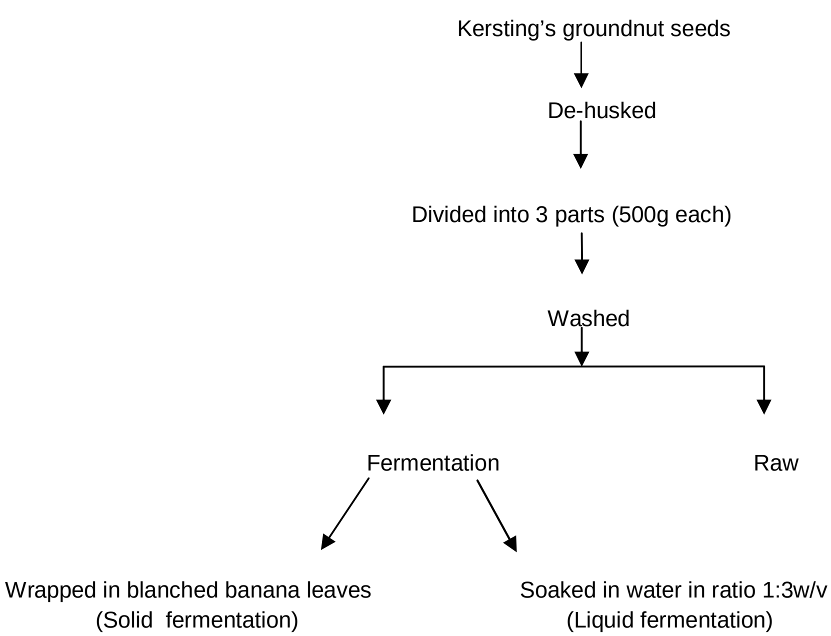

Fig. 1. Flow chart of methods used in processing Kersting's groundnut

One gram (1 g) of the samples was ashed in muffle furnace at 450℃ for 5-6 hours. The ashed samples were removed and transferred into the desiccators to cool after which the samples were dissolved with 1ml of 0.5% HNO_3, filtered into a clean small plastic bottle using Whattman filter. Distilled water was later used to dilute the solution up to 50 ml. Atomic absorption spectrophotometer (Buck 201, VGP) was used in determining the mineral content [23]. The mineral content was calculated using the formula below:

$$\text{Mineral (mg/100 g)} = \frac{R \ x \ V \ x \ D}{Wt}$$

When R = Solution concentration, V = Volume of sample digested, D = Dilution factor, and Wt = Weight of sample

2.5 Determination of Amino Acids

The four stages involved in the determination of the amino acid content of the sample are as follows:

2.5.1 Nitrogen determination

A small quantity of 2.0 g of ground sample was weighed, wrapped in No 1 Whattman filter paper and was put in the Kjeldahl digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture containing (0.5 g) sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and silicon acids (SiO_2) in the ratio 10:5:1 was added into the flask to facilitate digestion. Few pieces of anti-bonding particles were added.

The flask was then put in Kjeldahl digestion apparatus for three hours until the liquid light green. The digested sample was cooled and diluted with distilled water to 100 ml in standard volumetric flask. 10ml of the diluted solution with 10ml of 45% sodium hydroxide was put into the distillation apparatus and distilled into 10 ml of 2% boric acid containing four drops of bromocresol green methyl red indicator until about 70% distillate was collected. The distillate was then titrated with standardized 0.01M hydrochloric acid to grey coloured end point. The percentage nitrogen in the original sample was calculated using the formular below:

$$\text{Percentage Nitrogen} = \frac{(a-b) x \ 0.01 \ x \ 14 \ x \ V}{W \ x \ C} \quad X \ 100$$

Where

a= Titre value of digested sample
b= Titre value of blast sample
V = Volume after dilution (100 ml)
C = Aliquot of the sample used (10 ml)
14 = Nitrogen concentration in mg

2.5.2 Defatting

About 2.0 g of each sample was weighed into the extraction thimble and the fat was extracted with chloroform methanol mixture using soxhlet extraction apparatus [23]. The extraction lasted for 5 hours.

2.5.3 Hydrolysis of samples

The defatted samples (30 to 35 mg) were weighed into glass ampoules. Seven 10ml of 6M HCl was added and oxygen was expelled by passing nitrogen gas into the ampoule (to avoid possible oxidation of some amino acid during hydrolysis). Each glass ampoule was then sealed with a Bunsen flame and put into an oven at 105±5℃ for 22 hrs. The ampoule was allowed to cool before breaking at the tip and the content was filtered. The filtrate was then evaporated to dryness at 40℃ under vacuum in a rotary evaporator. Each residue was dissolved with 5ml of acetate buffer and stored in a plastic specimen bottle and kept in deep freezer.

2.5.4 Loading of the hydrolysate into TSM (Technicon Sequential Multi-sample Amino Acid Analyzer)

The amount loaded was between 5 to 10 microliters. This was dispensed into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of analysis lasted for 76 minutes.

The amount of each amino acid present in the sample was calculated in g/16N or g/100 g protein using this formular:

Concentration (g/100g protein) = NH x W at NH/2 x Sstd x C

Where $C = \frac{Dilution \ x \ 16}{Sample \ weight \ (g) x \ N\% \ x \ 10 \ vol.loaded}$ divided

by NH x W (nleu)

Where

NH = Net height
W = Width at half height
Nleu = Norleucin

2.6 Statistical Analysis

All experiments were carried out in triplicates. Data obtained were analyzed by one-way analysis of variance (ANOVA) and means were compared by Duncan's New Multiple Range test (SPSS 21.0 version). Differences were considered significant at *P*=.05.

3. RESULTS AND DISCUSSION

3.1 Changes in Titratable Acidity during Fermentation

The titratable acidity of unfermented Kersting's groundnut was 0.035 mg lactic acid/g. No significant changes was observed in the TTA after 24hrs of solid fermentation, while a significant decrease (*P*=.05) from 0.035 mg lactic acid/g to 0.031 mg lactic acid/g was observed after 24hrs of liquid fermentation. The TTA reduced significantly (*P*=.05) to 0.025 after 72hrs of both liquid and solid fermentation. The decrease in TTA may be due to the alkaline condition of the fermenting medium. This alkaline condition is being attributed to proteolytic activities of the fermenting organisms with the release of ammonia following the utilization of amino acids by the microorganisms. The decrease observed in the TTA agreed with the findings of [24,25] who worked on lima bean and locust bean respectively. These researchers recorded decrease in TTA during the fermentation of those beans.

3.2 Changes in Mineral Contents during Fermentation

Potassium had the highest value among the entire mineral assayed for in the sample (Table 1). The potassium content of the raw sample was 300.83 mg/100 g.

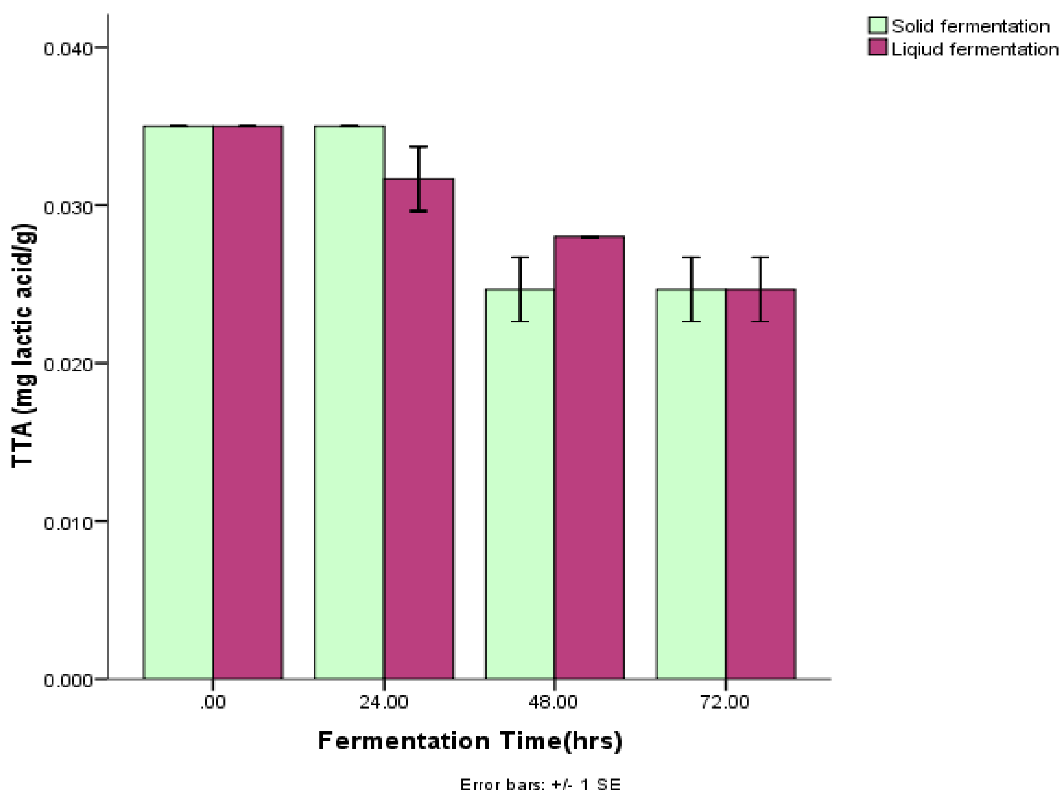

Fig. 2. Total Titratabity Acidity (mg lactic acid/g) of Kersting's groundnut during fermentation

Bars are presented as Mean ±S.E of replicates (n=3);
Key: TTA = Total Titratable Acidity

The value reduced significantly (*P*=.05) to 231.34 mg/100 g and 274.59 mg/100 g after 24hrs of liquid and solid fermentation respectively. Potassium value further reduced significantly (*P*=.05) from 231.34 mg/100 g to 218.00 mg/100 g after 72hrs liquid fermentation but increased (*P*=.05) from 274.59 mg/100 g to 289.62 mg/100 g after 72hrs solid fermentation.

Magnesium and Calcium value of the raw sample was 18.65 mg/100 g and 18.19 mg/100 g respectively. Mg value significantly decreased (*P*=.05) to 6.93 mg/100 g and 7.32 mg/100 g after 24hrs of liquid and solid fermentation respectively. The value increased back to 15.24 mg/100 g and 24.27 mg/100 g after 72hrs of liquid and solid fermentation respectively. Calcium value decreased (*P*=.05) from 18.19 mg/100 g to 9.25 mg/100g after 24hrs of liquid fermentation, but increased (*P*=.05) to 27.42 mg/100 g after 24hrs of solid fermentation.

The decrease observed in the mineral composition of Kersting's groundnut during fermentation may be as a result of leaching into the fermenting medium [26]. This is in accordance with the findings of [25] who reported decrease in mineral content during fermentation of locust bean. The decrease observed in the mineral contents also agreed with the report of [27] during fermentation of red kidney bean.

Calcium is an important mineral found mainly in bone and teeth. It has central role in much physiological functional integrity of the skeletal system. Calcium is also implicated in cell membrane integrity and permeability, blood clotting, transmission of nerve signal/impulse, regulation of enzymes, and hormones. [27-29]. Dietary deficiency of calcium is one of the predisposing factors to development of ricket in children [29]. Magnesium is required for growth, maintenance of bones and proper formation of nerves and muscles. Magnesium is an activator of many enzyme systems and maintains the electrical potential in nerves [27,28,30]. Potassium regulates water balance, heart rhythm, muscles contraction and nerve-signal conduction. Potassium also influence glucose and lipid metabolism. Increase intake of potassium can lower blood pressure and may help prevent strokes Ijeomah et al. [29]. However, excessive potassium intake may lead to heart failure [29].

Zinc is involved in RNA and DNA synthesis, which influences cell division, repair and growth. Zinc may help to prevent growth of abnormal cells associated with cancer. Zinc helps in the formation of protein in the body thus helping in wound healing, taste, smell and night vision [28,29]. Lack of zinc in the body causes rapid egesting on the surface of wound which may delay quick healing [29]. Iron is reported to be very important in normal functioning of central nervous system, formation of healthy blood cells, proper growth and in the oxidation of carbohydrate, protein and fats [29]. It is a vital component of heamoglobin and part of myoglobin and many enzymes in the body. Iron deficiency leads to anemia, mal-absorption of foods, irreversible behavioral abnormalities and abnormal functioning of the brain [28]. Sodium is required for regulation of water balance in the body, maintenance of proper heartbeat, contraction of muscles and conduction of nerves impulses [28,29,31].

3.3 Changes in Amino Acid Contents during Fermentation

A total of 17 amino acids were analyzed in the Kersting's groundnut (Table 2). Glutamic acid and aspartic acid were the major amino acids in Kersting seed with a value of 15.84±0.01 g/100g protein and 11.03±0.01 g/100 g protein respectively. This observation was in agreement with the observation of (20,32,33] that glutamic acid is the most abundant amino acid in legumes followed by aspartic acid. Leucine, lysine and arginine were the most concentrated essential amino acids, with values of 7.19±0.00, 6.71±0.01, and 6.45±0.01 g/100 g protein respectively. While histidine and methionine had the lowest content which are 2.88±0.01 and 1.21±0.01 g/100 g protein respectively (Table 3). This is in accordance with the findings of [3] who reported that legumes are deficient in sulphur-containing amino acids. In unfermented Kersting groundnut, the decreasing order of essential amino acids after leucine was lysine > arginine > phenylalanine > valine > isoleucine > threonine > histidine > methionine. Cystine was revealed to be the lowest non-essential amino acid with value of 1.31±0.00 g/100 g protein while glutamic acid had the highest value 15.84±0.01 g/100 g protein (Table 4).

Table 1. Mineral composition (mg/100 g) of unfermented and fermented Kersting's groundnut

Fermentation time (hrs)	Minerals (mg/100g)						
	Fe	Mg	Zn	Na	Ca	K	Cu
0	0.36±0.02[e]	18.65±0.03[f]	0.82±0.03[d]	2.73±0.01[d]	18.19±0.01[b]	300.83±0.11[g]	0.00±0.00
24L	0.46±0.02[f]	6.93±0.01[a]	0.83±0.01[e]	7.40±0.04[f]	9.25±0.07[a]	231.33±0.09[b]	0.00±0.00
24S	0.18±0.01[b]	7.32±0.04[b]	0.73±0.01[b]	3.65±0.01[e]	27.42±0.05[d]	274.59±0.09[e]	0.00±0.00
48L	0.19±0.01[c]	11.27±0.18[c]	0.94±0.00[f]	10.36±0.24[g]	28.17±0.28[e]	234.74±0.22[c]	0.00±0.00
48S	0.08±0.00[a]	14.76±0.31[d]	0.66±0.00[a]	1.65±0.03[b]	32.87±0.03[f]	263.07±3.24[d]	0.00±0.00
72L	0.19±0.00[c]	15.24±0.60[e]	0.95±0.00[g]	1.89±0.02[c]	37.85±0.04[g]	217.60±0.07[a]	0.00±0.00
72S	0.28±0.01[d]	24.27±0.03[g]	0.75±0.00[c]	0.00±0.00[a]	18.67±0.03[c]	289.26±0.15[f]	0.00±0.00

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)
Key: S= solid fermentation, L= liquid fermentation, Fe= iron, Mg= magnesium, Zn= zinc, Na= sodium, Ca= Calcium, K= potassium, Cu= copper

Table 2. Amino acids composition (g/100 g protein) of unfermented and fermented Kersting's groundnut

Amino acid (g/100 g protein)	Fermentation time (hrs)						
	0	24L	24S	48L	48S	72L	72S
Lysine	6.71±0.01[e]	7.22±0.01[g]	6.49±0.01[b]	6.62±0.02[d]	6.90±0.01[f]	5.62±0.01[a]	6.52±0.01[c]
Histidine	2.88±0.01[d]	3.02±0.02[f]	2.93±0.01[e]	2.71±0.01[c]	2.91±0.01[e]	2.21±0.01[a]	2.40±0.01[b]
Arginine	6.45±0.01[d]	6.71±0.01[g]	6.54±0.01[e]	6.38±0.01[c]	6.62±0.01[f]	5.86±0.01[a]	6.13±0.00[b]
Aspartic acid	11.03±0.01[e]	11.66±0.01[g]	10.91±0.01[d]	10.77±0.01[c]	11.11±0.01[f]	9.63±0.01[a]	10.51±0.00[b]
Threonine	3.43±0.01[d]	4.14±0.01[g]	3.53±0.01[e]	3.28±0.01[c]	3.81±0.01[f]	3.03±0.01[a]	3.13±0.01[b]
Serine	4.95±0.01[f]	5.01±0.01[g]	4.72±0.01[c]	4.81±0.01[d]	4.91±0.00[e]	3.43±0.01[a]	4.61±0.00[b]
Glutamic acid	15.84±0.01[d]	17.33±0.01[g]	17.04±0.01[f]	15.21±0.00[c]	16.92±0.01[e]	13.21±0.01[a]	14.22±0.01[b]
Proline	3.93±0.01[e]	4.16±0.01[g]	3.72±0.01[d]	3.61±0.01[c]	4.06±0.01[f]	2.78±0.01[a]	3.37±0.00[b]
Glycine	3.69±0.00[e]	3.93±0.01[g]	3.77±0.01[f]	3.57±0.01[c]	3.65±0.01[d]	3.02±0.00[a]	3.28±0.00[b]
Alanine	4.02±0.00[d]	4.65±0.01[e]	4.01±0.01[d]	3.88±0.00[c]	3.88±0.00[c]	2.49±0.00[a]	3.61±0.01[b]
Cystine	1.31±0.00[d]	1.65±0.01[g]	1.37±0.01[e]	1.24±0.00[c]	1.45±0.00[f]	0.76±0.00[a]	1.18±0.00[b]
Valine	4.38±0.00[d]	5.20±0.00[g]	4.56±0.00[e]	4.22±0.01[c]	4.91±0.00[f]	3.10±0.00[a]	4.02±0.00[b]
Methionine	1.21±0.01[d]	1.55±0.00[g]	1.26±0.01[e]	0.98±0.01[c]	1.42±0.00[f]	0.62±0.00[a]	0.91±0.00[b]
Isoleucine	3.68±0.01[e]	4.12±0.00[g]	3.62±0.00[d]	3.56±0.00[c]	3.97±0.01[f]	2.91±0.00[a]	3.36±0.00[b]
Leucine	7.19±0.00[d]	8.10±0.00[g]	7.39±0.00[e]	6.97±0.01[c]	7.49±0.01[f]	5.50±0.01[a]	6.78±0.00[b]
Tyrosine	3.14±0.00[c]	3.65±0.01[f]	3.31±0.00[d]	3.13±0.01[c]	3.48±0.01[e]	2.31±0.01[a]	2.98±0.00[b]
Phenylalanine	5.55±0.01[e]	5.81±0.00[g]	5.11±0.01[c]	5.38±0.01[d]	5.62±0.01[f]	3.71±0.01[a]	4.83±0.01[b]

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05)
Key: S= solid fermentation; L= liquid fermentation

Table 3. Essential amino acids (g/100 g protein) in raw and fermented Kersting's groundnut

Amino acid (g/100 g protein)	Fermentation time (hrs)						
	0	24L	24S	48L	48S	72L	72S
Lysine	6.71 ± 0.01^{e}	7.22 ± 0.01^{g}	6.49 ± 0.01^{b}	6.62 ± 0.02^{d}	6.90 ± 0.01^{f}	5.62 ± 0.01^{a}	6.52 ± 0.01^{c}
Threonine	3.43 ± 0.01^{d}	4.14 ± 0.01^{g}	3.53 ± 0.01^{e}	3.28 ± 0.01^{c}	3.81 ± 0.01^{f}	3.03 ± 0.01^{a}	3.13 ± 0.01^{b}
Histidine	2.88 ± 0.01^{d}	3.02 ± 0.02^{f}	2.93 ± 0.01^{e}	2.71 ± 0.01^{c}	2.91 ± 0.01^{e}	2.21 ± 0.01^{a}	2.40 ± 0.01^{b}
Arginine	6.45 ± 0.01^{d}	6.71 ± 0.01^{g}	6.54 ± 0.01^{e}	6.38 ± 0.01^{c}	6.62 ± 0.01^{f}	5.86 ± 0.01^{a}	6.13 ± 0.00^{b}
Valine	4.38 ± 0.00^{d}	5.20 ± 0.00^{g}	4.56 ± 0.00^{e}	4.22 ± 0.01^{c}	4.91 ± 0.00^{f}	3.10 ± 0.00^{a}	4.02 ± 0.00^{b}
Methionine	1.21 ± 0.01^{d}	1.55 ± 0.00^{g}	1.26 ± 0.01^{e}	0.98 ± 0.01^{c}	1.42 ± 0.00^{f}	0.62 ± 0.00^{a}	0.91 ± 0.00^{b}
Isoleucine	3.68 ± 0.01^{e}	4.12 ± 0.00^{g}	3.62 ± 0.00^{d}	3.56 ± 0.00^{c}	3.97 ± 0.01^{f}	2.91 ± 0.00^{a}	3.36 ± 0.00^{b}
Leucine	7.19 ± 0.00^{d}	8.10 ± 0.00^{g}	7.39 ± 0.00^{e}	6.97 ± 0.01^{c}	7.49 ± 0.01^{f}	5.50 ± 0.01^{a}	6.78 ± 0.00^{b}
Phenylalanine	5.55 ± 0.01^{e}	5.81 ± 0.00^{g}	5.11 ± 0.01^{c}	5.38 ± 0.01^{d}	5.62 ± 0.01^{f}	3.71 ± 0.01^{a}	4.83 ± 0.01^{b}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05)

Table 4. Non-essential amino acids (g/100 g protein) in raw and fermented Kersting's groundnut

Amino acid (g/100 g protein)	Fermentation time (hrs)						
	0	24L	24S	48L	48S	72L	72S
Cystine	1.31 ± 0.00^{d}	1.65 ± 0.01^{g}	1.37 ± 0.01^{e}	1.24 ± 0.00^{c}	1.45 ± 0.00^{f}	0.76 ± 0.00^{a}	1.18 ± 0.00^{b}
Tyrosine	3.14 ± 0.00^{c}	3.65 ± 0.01^{f}	3.31 ± 0.00^{d}	3.13 ± 0.01^{c}	3.48 ± 0.01^{e}	2.31 ± 0.01^{a}	2.98 ± 0.00^{b}
Aspartic acid	11.03 ± 0.01^{e}	11.66 ± 0.01^{g}	10.91 ± 0.01^{d}	10.77 ± 0.01^{c}	11.11 ± 0.01^{f}	9.63 ± 0.01^{a}	10.51 ± 0.00^{b}
Serine	4.95 ± 0.01^{f}	5.01 ± 0.01^{g}	4.72 ± 0.01^{c}	4.81 ± 0.01^{d}	4.91 ± 0.00^{e}	3.43 ± 0.01^{a}	4.61 ± 0.00^{b}
Glutamic acid	15.84 ± 0.01^{d}	17.33 ± 0.01^{g}	17.04 ± 0.01^{f}	15.21 ± 0.00^{c}	16.92 ± 0.01^{e}	13.21 ± 0.01^{a}	14.22 ± 0.01^{b}
Proline	3.93 ± 0.01^{e}	4.16 ± 0.01^{g}	3.72 ± 0.01^{d}	3.61 ± 0.01^{c}	4.06 ± 0.01^{f}	2.78 ± 0.01^{a}	3.37 ± 0.00^{b}
Glycine	3.69 ± 0.00^{e}	3.93 ± 0.01^{g}	3.77 ± 0.01^{f}	3.57 ± 0.01^{c}	3.65 ± 0.01^{d}	3.02 ± 0.00^{a}	3.28 ± 0.00^{b}
Alanine	4.02 ± 0.00^{d}	4.65 ± 0.01^{e}	4.01 ± 0.01^{d}	3.88 ± 0.00^{c}	3.88 ± 0.00^{c}	2.49 ± 0.00^{a}	3.61 ± 0.01^{b}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05);
Key: S= solid fermentation; L= liquid fermentation

Table 5. Essential and non-essential amino acids (g/100 g protein) of raw and fermented Kersting groundnut

Amino acid	Fermentation time (hrs)						
	0	**24L**	**24S**	**48L**	**48S**	**72L**	**72S**
TAA	89.39	97.91	90.28	86.32	93.11	70.19	81.84
TEAA	41.48	45.87	41.43	40.10	43.65	32.56	38.08
TNEAA	47.91	52.04	48.85	46.22	49.46	37.63	43.76
%TEAA	46.40	46.85	45.89	46.46	46.88	46.39	46.53
%TNEAA	53.60	53.15	54.11	53.54	53.12	53.61	53.47

Key: TAA= Total Amino Acids; TEAA= Total Essential Amino Acids; TNEAA= Total Non-Essential Amino Acids
S= Solid fermentation; L= Liquid fermentation

Table 6. Essential amino acids in raw and fermented Kersting's groundnut (g/100 g protein) compared with FAO standard

Amino acid (g/100 g protein)	Fermentation time (hrs)							
	0	**FAO ref value**	**24L**	**24S**	**48L**	**48S**	**72L**	**72S**
Lysine	6.71±0.01	4.20	7.22±0.01	6.49±0.01	6.62±0.02	6.90±0.01	5.62±0.01	6.52±0.01
Threonine	3.43±0.01	2.60	4.14±0.01	3.53±0.01	3.28±0.01	3.81±0.01	3.03±0.01	3.13±0.01
Histidine	2.88±0.01	2.40	3.02±0.02	2.93±0.01	2.71±0.01	2.91±0.01	2.21±0.01	2.40±0.01
Arginine	6.45±0.01	2.00	1.65±0.01	1.37±0.01	1.24±0.00	1.45±0.00	0.76±0.00	1.18±0.00
Valine	4.38±0.00	4.20	5.20±0.00	4.56±0.00	4.22±0.01	4.91±0.00	3.10±0.00	4.02±0.00
Methionine	1.21±0.01	2.20	1.55±0.00	1.26±0.01	0.98±0.01	1.42±0.00	0.62±0.00	0.91±0.00
Isoleucine	3.68±0.01	4.20	4.12±0.00	3.62±0.00	3.56±0.00	3.97±0.01	2.91±0.00	3.36±0.00
Leucine	7.19±0.00	4.20	8.10±0.00	7.39±0.00	6.97±0.01	7.49±0.01	5.50±0.01	6.78±0.00
Phenylalanine	5.55±0.01	2.80	5.81±0.00	5.11±0.01	5.38±0.01	5.62±0.01	3.71±0.01	4.83±0.01

FAO Ref value Aremu et al. [36].
Key: S= solid fermentation, L= liquid fermentation

Amino acid content was observed to increase significantly (*P*=.05) after 24hrs of liquid fermentation and 48hrs of solid fermentation. This may be as a result of high proteolytic activities in the liquid medium by the proteolytic microorganisms. This is in agreement with the report of (2) during fermentation of soybean, garbanzo bean and groundnut. However, prolong liquid fermentation more than 24hrs and solid fermentation more than 48hrs reduced the level of these amino acids in Kersting seeds. The decreased observed in the amino acid content is due to its utilization by the microorganisms involved in the fermentation process with the release of ammonia. The ammonia released is responsible for the pungent smell perceived during the fermentation of the sample. This is in agreement with the findings of (2) that prolong fermentation up to 30hrs in liquid medium decreased the amino acid contents of Soybean, Garbanzo Bean and Groundnut.

Raw and fermented Kersting contain total essential amino acids (TEAA) ranging from 46.39% to 46.85% of the total amino acids (%TAA) (Table 5). The high percentage of TEAA found in Kersting indicates that it is a rich source of TEAA and will significantly supply essential amino acids in diet. [34] reported that food rich in TEAA will contribute to the supply of essential amino acids in diet.

When comparing the essential amino acids in the groundnut with the recommended FAO standard [35] provisional pattern, Kersting was observed to be superior in leucine, lysine, arginine and phenylalanine; adequate in threonine, valine, and histidine. It was only methionine and isoleucine that needed to be supplemented. This is in agreement with the findings of [36] who studied the chemical and amino acid composition of Bambara, Kersting's groundnut, Cowpea and Cranberry bean.

4. CONCLUSION

Kersting groundnuts are good protein source containing the essential amino acids in appreciable quantities which is capable of enriching human diets and reducing the incidence of protein-energy malnutrition. Both fermentation methods were observed to enhance the amino acids quality of the groundnut. However, liquid fermentation for 24hrs and solid fermentation for 48hrs are discovered to be the suitable fermentation methods and time that enhanced the mineral and amino acids quality of Kersting's groundnut.

CONSENT

The authors have consented that the article be published in this journal.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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