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Proximate and Anti-nutrient Contents of Kersting's Groundnut (*Macrotyloma geocarpum*) Subjected to Different Fermentation Methods

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Authors' contributions

This work was carried out in collaboration between both authors. Author CA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Author VOO managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The study aimed to ascertain the effect of different fermentation methods and time on the proximate and reduced the anti-nutrient contents Kersting's groundnut (*Macrotyloma geocarpum*).
Design of the Study: A comparative effect of different fermentation methods on the proximate and anti-nutrient content was evaluated.
Place and Duration of Study: Department of Microbiology and Central Science Laboratory, Federal University of Technology, Akure, Nigeria, between March 2013 and June 2014.
Methodology: The seeds were de-husked and sorted to remove extraneous matters. Liquid and solid fermentation were carried out using standard analytical methods. The proximate and anti-nutrients (trypsin inhibitor, tannin, oxalate, phytate) of the fermenting samples were analysed every 24 h during fermentation.
Results: Proximate analysis revealed that moisture content significantly (P=0.05) decreased from 6.25±0.14% to 4.69±0.06% and 4.89±0.02% after 24 h of liquid and solid fermentation respectively. Liquid fermentation significantly (P=0.05) increased the protein content of the groundnut from

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18.11±0.50% to 21.10±0.49% and 22.47±0.48% after 24 h and 48 h respectively. No significant difference (*P*=0.05) was observed after 72 h of fermentation. While solid fermentation significantly (*P*=0.05) increased protein content from 18.11±0.50% to 19.08±0.01% after 72 h. Both fermentation methods significantly (*P*=0.05) reduced crude fibre and increased fat content respectively. Soluble carbohydrate decreased significantly (*P*=0.05) from 63.72±0.39% to 58.17±0.87% after 48 h of liquid fermentation. Anti-nutrient (trypsin inhibitor, tannin, oxalate and phytate) contents decreased significantly (*P*=0.05) more in liquid state fermentation than in solid state fermentation. Trypsin inhibitor unit significantly (*P*=0.05) reduced from 72.18±0.00 mg/g to 12.28±0.38 mg/g and 24.17±0.01 mg/g after 24 h of liquid and solid fermentation respectively. However, a significant (*P*=0.05) increase was observed in trypsin inhibitor content after 48 h of both fermentation methods.
Conclusion: The research has revealed that 24 and 72 h liquid and solid fermentation of Kersting's groundnut significantly (*P*=0.05) enhanced the proximate content. The anti-nutrient contents also reduced significantly (*P*=0.05) in liquid fermentation.

Keywords: Fermentation; Kersting's groundnut (Macrotyloma geocarpum); proximate; anti-nutrient.

1. INTRODUCTION

Kersting's groundnut also known as Geocarpa groundnut or Ground bean is the seed of *Macrotyloma geocarpum* [1]. It is the third subterranean legume after groundnut and bambara groundnut [2,3]. It is an annual leguminous crop that belongs to the family leguminosae and the subdivision Papilionoideae [4].

Kersting's groundnut is used for preparing many dishes such as fried paste (koose), and steamed paste (tubani) [1]. Mature seeds are boiled with salt and eaten with palm oil or groundnut oil, accompanied with fermented cassava flour ('gari'), yams or rice. Also the cooking water from *Macrotyloma geocarpum* seeds is used in upper west region, for the treatment of stomach pains, intestinal cramps and as an antidote to food poisoning because it induces emesis when ingested. Concoctions prepared from the leaves act as a febrifuge [5,6]. Leaf decoction is also used as vermifuge [1].

Kersting's groundnut is an indigenous crop cultivated in parts of West Africa for food [7,8]. It is a promising alternative source of high quality protein and feed for the tropics, but presently, it is an under-utilized legume [3,9]. This is as a result of the high labour demand, lack of information regarding their nutritive values, presence of anti-nutrients in the legumes, cultural beliefs, low yield, prolong cooking time [10].

Cooking is said to destroy the heat-labile anti-nutritional factors [11,12], 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 [13-15]. However, fermentation process has been reported to represent an alternative technique for improving the nutrient values of legumes, to enhance the keeping quality, eliminate anti-nutritional factors, and reduce cooking time of the product [16-19].

In physiological terms, fermentation is defined as the type of metabolism of a carbon source in which energy is generated by substrate level phosphorylation but to the microbiologist, the term fermentation is a form of energy-yielding microbial metabolism in which an organic substrate, usually a carbohydrate, is incompletely oxidized, and an organic carbohydrate acts as the electron acceptor [20,21]. It is therefore pertinent to examine the effect of liquid and solid fermentation methods so as to ascertain the suitable fermentation method and effective time that will best improve the proximate and reduce the anti-nutrient contents of Kersting's groundnut.

2. MATERIALS AND METHODS

2.1 Source of Kersting's Groundnut

Kersting's groundnut (*Macrotyloma geocarpum*) seeds were purchased from a seller at Isolo market, in Akure South Local Government area of Ondo State, 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. The seeds were de-husked, cleaned and sorted to remove extraneous matters.

2.2 Processing of Kersting's Groundnut

The sorted seeds were divided into three portions, coded A, B and C of 500 g each using

electronic weighing balance (Electronic balance, MT-301 Model). Portion A was subjected to solid fermentation, where the de-husked seeds were wrapped in blanched banana (*Musa acuminata*) leaves and allowed to ferment for three days at 28±2℃ in a clean plastic bowl with cover. Portion B was subjected to liquid fermentation in which the sorted seeds were soaked in water in the ratio of 1:3 w/v in a clean plastic bowl with cover and allowed to ferment for three days at 28±2℃. Portion C which serves as control was analyzed raw. Sample were taken out at 24 h interval and analysed immediately. Procedure employed for the processing is illustrated in Fig. 1.

2.3 Proximate Estimation

The moisture, crude protein, crude firbe, crude fat, ash and soluble carbohydrate (by difference) content were determined in accordance with methods described by [22]. The proximate analyses were carried out in triplicate and reported in percentage.

2.4 Anti-nutrients Determination

2.4.1 Trypsin inhibitor activity (TIA)

Trypsin inhibitor activity (TIA) was determined by the method of [23], using N-alpha-benzoyl-DL arginine-p-nitro anilide hydrochloride (BAPNA) as substrate. Crystalline porcine pancreatic trypsin 40mg was dissolved in 0.001M HCl such that standard trypsin solution contains 40µg trypsin. Each sample dilution was used with BAPNA substrate and trypsin solution at 37℃. The reaction was allowed to take place in water bath for 10 min and their absorbance read at 410 nm against each sample blank. About 1 g of finely ground and sieved sample of the seed flour was defatted for three hours using n hexane. The sample was mixed with 50 ml of 0.01 M NaOH and the pH was adjusted to 9.5 using 0.1M NaOH or 0.1 M HCl. The mixture was left for 10 min at 1,000 rpm. The extract from the sample was diluted with distilled water to obtain a dilution whereby 1ml extract produced trypsin inhibition activity of between 40-60%.

2.4.2 Tannin determination

About 0.2 g of finely ground sample was weighed into a sample bottle. Ten millimeters (10 ml) of 70% aqueous acetone was added and properly covered. The bottle were put in an ice bath shaker and shaken for 2 hours at 30℃. Each solution was then centrifuged and the supernatant stored in ice. A 0.2 ml of each solution was pipetted into the test tube and 0.8 ml of distilled water was added. Standard tannin acid solutions were prepared from a 0.5 mg/ml of the stock and the solution made up to 1 ml with distilled water. Folin Ciocaeteau reagent (0.5 ml) was added to both sample and standard followed by 2.5 ml of 20% Na2CO3 the solution were then

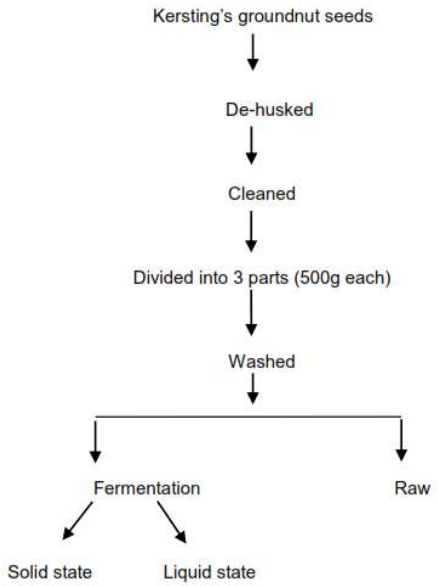

Fig. 1. Flow chart of methods used in processing Kersting's groundnut

vortexed and incubated for 40 minutes at room temperature after which its absorbance was read using AJ- IC03 spectrophotometer at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid curve that was prepared [22].

2.4.3 Oxalate determination

One gram of the sample was weighed into 1000 ml conical flask. H_2SO_4 (0.75 M) was added and the solution was carefully stirred intermittently with a magnetic stirrer for 1 hour and then filtered using Whatman No. 1 filter paper. Sample filtrate (25 ml) was collected and titrated hot (80-90℃) against 0.1 M $KMnO_4$ solution to the point when pink colour appeared that persisted for at least 30 seconds [22].

2.4.4 Phytate determination

Four grams (4 g) of finely ground samples were soaked in 100 ml of 2% HCl for three hours and then filtered. 25 ml of the filtrate was placed in a 100 ml conical flask and 5 cm^3 of 0.03% of ammonium thiocynate solution (NH_4SCN) solution was then added as indicator. Distilled water (50 ml) was then added to give it the proper acidity and this was titrated against 0.00566 g per milliter of standard iron III chloride solution that contain about 0.00195 g of iron per milliliter until a brownish yellow colouration persist for 5mins. The equivalent was obtained and from this, the phytate content in mg/100 g was calculated [22].

2.5 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 16.0 version). Differences were considered significant at *P*=0.05.

3. RESULTS AND DISCUSSION

3.1 Changes in Proximate Composition during Fermentation

The moisture content of the raw sample was 6.25%. After 24 h, the moisture content reduced significantly (P=0.05) to 4.89% and 4.69% in solid and liquid fermentation respectively. After 48 h in solid fermentation, moisture content significantly (P=0.05) reduced to 3.56% in the solid fermentation, while at same hour, it increased from 4.69% to 5.83% in liquid fermentation. After 72 h, it increased significantly (P=0.05) from 3.56% to 4.28% and 5.83% to 6.54% in solid and liquid fermentation respectively (Table 1). Decrease observed in moisture content may be as a result of the utilization of water by the microorganisms involved in the fermentation, during hydrolysis of complex compounds to similar ones. This is in agreement with the findings of [24] who recorded decrease in moisture content during fermentation of soybean. It was also observed that the bean solute rose as the solvent decreased after 48h and 72 h of liquid fermentation. This phenomenon may have resulted into an increase in the moisture content due to absorption of water from the fermenting medium. This agreed with the findings of [25] during fermentation of soybean. Increased observed in the moisture content during fermentation may also be due to proteolytic activity of microorganisms that released water through hydrolysis of peptides [26,27]. Moreover, microorganisms have metabolic activities that produce carbon dioxide and water [28], which consequently can increase the moisture content of the medium. Increase in moisture content was also reported by [21] during open and control fermentation of cowpea. Decrease in moisture content observed after 48h of solid fermentation may be as a result of evaporation or metabolic activities of the organisms involved in the fermentation [29,30].

Crude protein content of the unfermented sample was 18.11%. The content increased significantly (P=0.05) from 18.11% to 21.10% and 22.47% after 24 and 48 h of liquid fermentation respectively. No significant difference (P=0.05) was observed after 24 and 48 h of solid fermentation. Crude protein increased significantly (P=0.05) to 19.08% after 72 h of solid fermentation. No significant difference (P=0.05) was observed after 48 h and 72h of liquid fermentation (Table 1).

The increase observed in the crude protein during both methods of fermentation may be as a result of enzyme hydrolysis of complex/insoluble plant protein to amino acids and short chains peptides, thereby causing an increase in total nitrogen content which in turn increased protein availability [17,31]. It may also be due to structural proteins that are in integral part of the microbial cell [17,32]. This finding agreed with the work of [24] during fermentation of soybean.

Table 1. Proximate composition of raw and fermented Kersting's groundnut

Fermentation time (H)	Moisture (%)	Protein (%)	Fat (%)	Crude fibre (%)	Ash (%)	CHO (%)
0	6.25^{e}±0.14	18.11^{ab}±0.5	5.29^{a}±0.04	3.55^{e}±0.01	3.09^{c}±0.01	63.72^{b}±0.39
24L	4.69^{c}±0.06	21.10^{c}±0.49	5.75^{b}±0.13	3.07^{d}±0.14	2.13^{a}±0.01	63.26^{b}±0.14
24S	4.89^{c}±0.02	17.15^{a}±0.00	5.57^{a}±0.06	3.22^{d}±0.05	2.16^{a}±0.01	67.02^{c}±0.13
48L	5.83^{d}±0.09	22.47^{d}±0.48	7.86^{e}±0.21	2.64^{bc}±0.11	3.04^{c}±0.01	58.17^{a}±0.87
48S	3.56^{a}±0.04	17.75^{a}±0.49	6.35^{c}±0.13	2.77^{c}±0.02	2.92^{b}±0.04	66.66^{c}±0.55
72L	6.54^{e}±0.23	22.95^{d}±0.49	8.45^{f}±0.11	2.01^{a}±0.03	2.18^{a}±0.02	57.87^{a}±0.89
72S	4.28^{b}±0.01	19.08^{b}±0.01	7.04^{d}±0.02	2.53^{b}±0.01	3.26^{d}±0.04	63.83^{b}±0.07

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: L = liquid fermentation, S = solid fermentation, CHO= soluble carbohydrate

It also agrees with the report of [31] during fermentation of an oil bean seed. These researchers recorded increase in crude protein when the beans were fermented.

The fat content of the raw sample was 5.29%. A significant increase (*P*=0.05) was observed from 5.29% to 5.75% and 7.86% liquid fermentation after 24 and 48h respectively. No significant difference was observed after 24 h of solid fermentation but it increased significantly (*P*=0.05) to 6.35% and 7.04% after 48 and 72 h of solid fermentation respectively (Table 1). Fat content increased significantly to 8.45% after 72h liquid fermentation. The increase in fat may also be as a result of fat from dead microflora, or because the fermenting microflora did not use fat from this food as source of energy [17,33]. The increase observed agreed with the findings of [34] during fermentation of starchy substrate with *A. niger*; also with the findings of [35] during fermentation of locust bean; and [17] during cocoyam fermentation.

Crude fibre content of the raw sample was 3.55%. Fibre content significantly (*P*=0.05) decreased as the fermentation time increased in both methods of fermentation (Table 1). The decrease observed was an indication of softening of fibrous tissues during fermentation [17]. The decreased observed in the fibre content in this study may be due to the activities of microorganisms which are known for the bio-conversion of carbohydrates and lignocelluloses into protein [17]. The decrease may also be due to the ability of the microorganisms involved in the fermentation to synthesis cellulase and hemicellulase for hydrolysis of the complex polysaccharide in the sample [31]. This agreed with the findings of [24] who reported decrease in fibre, carbohydrate, and other organic substances during fermentation of soybean.

The ash content of the raw sample was 3.09%. This value is close to 3.31% obtained for soybean by [24] and 3.53% reported by [36] for mung bean. Variation observed in ash content after both solid and liquid fermentation at each time interval was due to contribution by the organisms involved in the fermentation.

Soluble carbohydrate content of the raw sample was 63.72%. No significant difference (*P*=0.05) was observed after 24 h of liquid fermentation, but increased significantly (*P*=0.05) to 67.02% after 24 h of solid fermentation. The content reduced significantly (*P*=0.05) to 58.17% and 57.87% after 48 and 72 h of liquid fermentation respectively (Table 1). Increased observed in carbohydrate during solid fermentation may be due to the hydrolysis of complex oligosaccharides in the sample [31]. This hydrolysis may be due to the presence of alpha-galatosidase found in the organisms involved in the fermentation [37]. Reduction observed in the carbohydrate content may be because it had been used as source of energy during fermentation or leached out to the fermenting medium. This agreed with the findings of [24], who recorded decrease in soluble carbohydrate during fermentation of soybean; also [35] reported decrease in soluble carbohydrate during fermentation of locust bean.

3.2 Changes in Anti-nutrient Content During Fermentation

The trypsin inhibitor content of the raw sample was 72.18 mg/g. The content reduced significantly (*P*=0.05) to 12.28 mg/g and 24.17 mg/g after 24 h of liquid and solid fermentation respectively. It increased significantly (*P*=0.05) after 48 and 72h of solid fermentation, but increased only after 48 h of liquid fermentation (Fig. 2). The decrease observed may be as a

result of high protease activity. This agreed with the work of [38,39] during fermentation of cowpeas and grass pea respectively.

Increase observed after 48h of both solid and liquid fermentation agreed with the report of [40] during fermentation of soybean with *Rhizopus oligosporus*. Increase in trypsin inhibitor content of leguminous grains during fermentation might be due to release of heat resistant bound trypsin inhibitor by protease enzymes produced by *Rhizopus*. spp [41,42].

The tannin content of raw sample was 0.68 mg/g. Tannin content significantly (*P*=0.05) decreased as the fermentation time increased in both methods of fermentation. More decrease was observed in liquid fermentation than solid fermentation (Fig. 3). The oxalate content of raw sample was 2.34 mg/g. It significantly (*P*=0.05) reduced to 2.16 mg/g and 1.58 mg/g after 24 h of liquid and solid fermentation respectively (Fig. 4). The content reduced significantly (*P*=0.05) in liquid fermentation as the fermentation time increased.

The decrease observed in both anti-nutrient contents may be due to the action of proteolytic enzyme produced by the microorganisms involved in the fermentation. More decrease was observed in the liquid fermentation than the solid fermentation, this may be due to the presence of more proteolytic microorganism in the liquid medium. The decrease observed in tannin content could also be attributed to the hydrolysis of polyphenolic compounds of tannin complexes during fermentation. The result is in agreement with the work of [43] who reported a decrease in tannin content during the fermentation of lima bean.

The phytate content of raw sample was 13.59 mg/g. It reduced significantly (*P*=0.05) to 9.47mg/g after 24 h of liquid fermentation but gave no significant difference (*P*=0.05) after 24 h of solid fermentation (Fig. 5). The content reduced significantly (*P*=0.05) in liquid fermentation as the fermentation time increased. Phytate content reduced significantly (*P*=0.05) after 48 and 72 h of solid fermentation.

The decrease may be due to the action of phytase that catalyses phytate degradation into inorganic orthophosphate, a series of myoinositols and lower phosphoric esters of phosphate [43]. This is in agreement with the work of [37] who worked on antinutrients in legumes and their removal. It also agreed with the findings of [43] who work on the effect of fermentation on Lima bean. These researchers observed decrease in phytate content after fermentation of Lima bean.

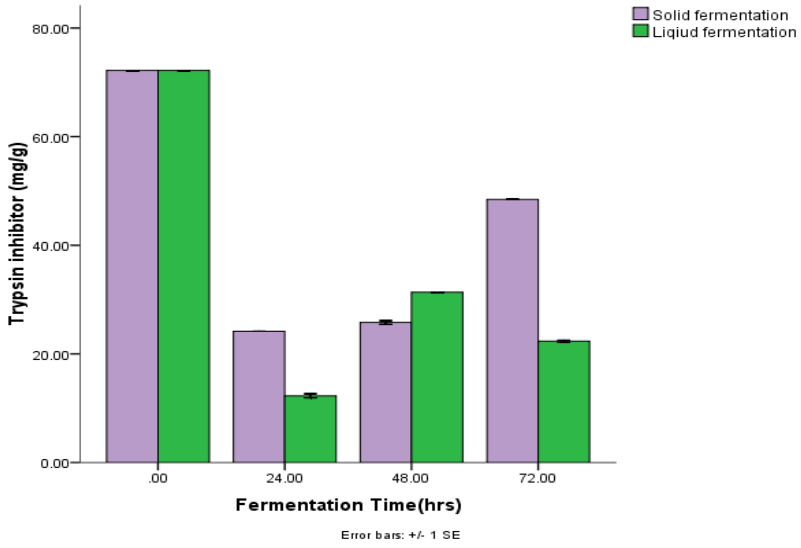

Fig. 2. Trypsin inhibitor content (mg/g) of Kersting's groundnut during fermentation
Bars are presented as Mean ±S.E of replicates (n=3)

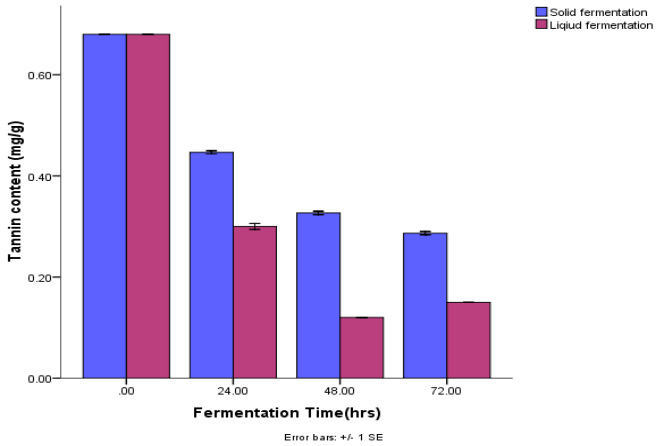

Fig. 3. Tannin content (mg/g) of Kersting's groundnut during fermentation
Bars are presented as Mean ±S.E of replicates (n=3)

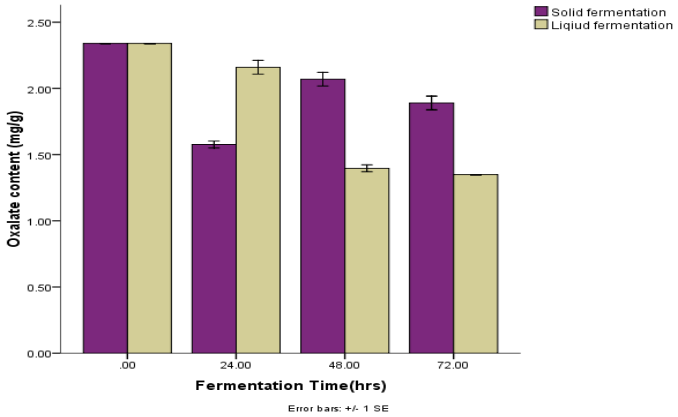

Fig. 4. Oxalate content (mg/g) of Kersting's groundnut during fermentation
Bars are presented as Mean ±S.E of replicates (n=3)

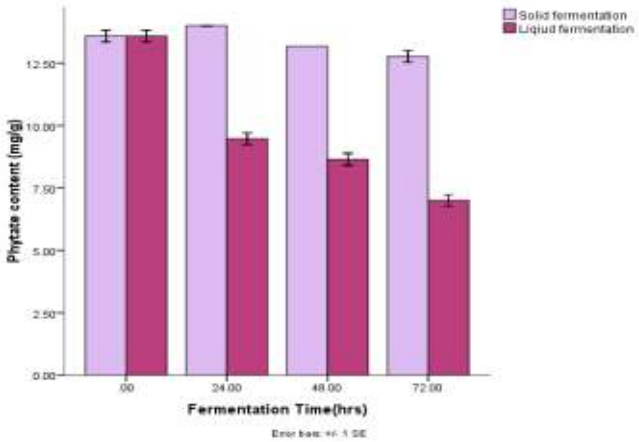

Fig. 5. Phytate content (mg/g) of Kersting's groundnut during fermentation
Bars are presented as Mean ±S.E of replicates (n=3)

4. CONCLUSION

Kersting's groundnut has the potential of providing adequate food rich in protein for the increasing population of poor people in West Africa and in the continent. Both fermentation methods significantly increase the proximate and decrease the anti-nutrient content of Kesting nut. Liquid fermentation was however found to enhance the proximate content and reduced the anti-nutrient content of the bean better than solid fermentation.

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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