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Effect of Heat Treatment and Fermentation on Anti-Nutrients Content of Lima Bean (*Phaseolus lunatus*) During Production of *Daddawa* Analogue

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Authors' contributions

This work was carried out in collaboration between all authors. Author HAA designed the project, supervised the project and supervised the writing of this article. Author HAA supervised the M.Sc thesis on which this article is based. Author EOF carried out the analyses and prepared the article from her M.Sc thesis. Author VAO was involved in the design of the study and preparation of the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: To produce *daddawa* analogue (a fermented condiment) from Lima bean (*Phaseolus lunatus*) seeds which should have safe levels of anti-nutrients comparable to *daddawa* from locust bean (*Parkia biglobosa*) seeds.
Design of the Study: Lima bean and locust bean seeds were separately heat-processed and subjected to natural fermentation for 72 hours to produce *daddawa* analogue and *daddawa* respectively.
Place and Duration of Study: Department of Food Science and Technology and Central Science Laboratory, Obafemi Awolowo University, Ile-Ife, Nigeria between March 2010 and June 2011.
Methodology: The pH, titratable acidity, and the anti-nutrients (tannin, phytate, cyanide, trypsin inhibitor) of the fermenting samples were evaluated every 12 hours during

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fermentation.
Results: pH increased (*P* = .05) from 7.46 at 0 *h* to 8.50 after 72 *h* and from 7.50 at 0 *h* to 8.74 at 72 *h* of fermentation in lima bean and locust beans respectively. Titratable acidity decreased in the fermented lima beans from 0.216 at 0 *h* to 0.045 mg lactic acid/g at 72 *h* of fermentation. All anti-nutrients analyzed decreased with fermentation time. Tannin content decreased significantly (*P* = .05) from 19.0 ± 0.10 at 0 *h* to 2.0 ± 0.01mg/kg at 72 *h* and from 9.50 ± 0.02 at 0 *h* to 3.06 ± 0.01 mg/kg at 72 *h* of fermentation in lima beans and locust beans respectively. Fermentation significantly decreased phytate content (*P* = .05) from 22.0 ± 0.03 at 0 *h* to 7.0 ± 0.01mg/kg at 72 *h* in lima beans. Cyanide content decreased significantly (*P* = .05) from 0.97 ± 0.01 at 0 *h* to 0.25 ± 0.01mg/kg at 72 *h* and from 15.0 ± 0.05 at 0 *h* to 0.29 ± 0.10 mg/kg at 72 *h* of fermentation in lima beans and locust beans respectively. Trypsin inhibitor content also decreased significantly (*P* = .05) from 4.40 ± 0.10 at 0 *h* to 1.76 ± 0.02 mg/kg at 72 *h* and from 0.24 ± 0.0 at 0 *h* to 0.10 ± 0.1 TIU/g at 72 *h* of fermentation in lima beans and locust beans respectively.
Conclusion: The study has shown that *daddawa* analogue from lima beans was comparable to *daddawa* from locust beans in terms of anti-nutritional contents.

Keywords: Daddawa; lima bean; locust bean; anti nutrients; fermentation; heat processing.

ACRONYMS

Ppm = parts per million; Tiu = Trypsin inhibitor unit; w/u = weight per volume; TTA = Titratable acidity.

1. INTRODUCTION

Legumes are very good source of protein, calorie, minerals and vitamins. They could be fermented into condiments (*daddawa*). *Daddawa* are added to food in form of sauce, powder, spread, seed or similar thing to enhance and improve the flavor and taste of such food (1,2). Locust bean (*Parkia biglobosa*) has been the traditional raw material for processing daddawa (daddawa), but parkia trees are now getting into extinction hence the need for use of alternative raw material for processing *daddawa*. Lima bean (*Phaseolus lunatus*) could serve as an alternative raw material for producing *daddawa*. It is a nutritious food stuff which is cultivated primarily for immature vegetables or mature dry seeds (3). In Africa, about 120,000 to 200,000 hectare is devoted to lima bean cultivation in the sub-humid and humid areas indicating the need for its maximum utilization (4).

In Nigeria, lima bean is consumed as cooked whole beans but its long cooking time discourages potential consumers. Lima beans like other minor legumes contain some anti-nutritional factors such as trypsin and chymotrypsin inhibitors, haemagglutinins, cyanogenic glucosides, tannin, lectins, polyphenols, phytic acid and various oligosaccharides which cause flatulence (5,6). Anti-nutrients are substances which through their metabolism interfere with nutrient intake, absorption, utilization and availability in the body systems thereby affecting the health of consumers (7). Improved lima beans contain lower amounts of cyanogenic glucosides compared to the wild species. These anti-nutrients if not reduced by processing techniques could limit the utilization potential of lima beans.

Soaking, germination, dehulling, cooking and fermentation have been suggested to be capable of reducing the anti-nutritional factors in legumes (8,6). Raw lima beans have a

trypsin inhibitor activity of 32.63 TIU/mg protein while cooked lima bean has no measurable activity. Lima bean soaked in water for 2, 4 and 6 days had an inhibitor activity of 31.02, 26.87 and 21.34 TIU/mg protein, respectively. Germinated beans for 2, 4 and 6 days have inhibitor activity of 29.30, 24.02 and 18.18 TIU/mg protein respectively (9,5).

Fermentation is one of the important food processing technologies employed in solving the problems of food spoilage and food borne diseases worldwide. During the process, the actions of microorganisms or their enzymes modify the food and produce desirable attributes such as flavor and textural changes in the food (10). The palatability and nutritive value of raw seeds are also improved (11,12).

In an earlier investigation, proximate, mineral and sensory properties of a *daddawa* analogue from lima bean have been reported (2). This study evaluates the effect of heat treatment and fermentation on the anti-nutrients content of lima bean when it is processed to *daddawa*.

2. MATERIALS AND METHODS

2.1 Source of Materials

Lima bean seeds (NSWP 46) were supplied by the Grain legume Improvement Programme of Institute of Agricultural Research and Training, Ibadan, Oyo State, Nigeria while the locust bean seeds and fermenting containers (calabash) were procured from a local market in Ibadan, Oyo State, Nigeria.

2.2 Lima Bean and Locust Bean Seeds Fermentation

Lima bean fermentation was carried out by the modification of the method described by (13) (See the flow chart in Fig. 1). The lima bean seeds were roasted at 71 ∓ 0.5ºC for 5 minutes on gas powered cooker flame before further processing. The procedure employed for production of locust bean *daddawa* is shown in Fig. 2. Samples were taken out at 12 *h* interval and analysed immediately.

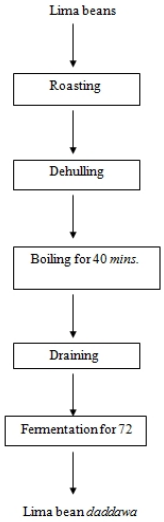

Fig. 1. Flowchart of laboratory processing of lima bean *daddawa*

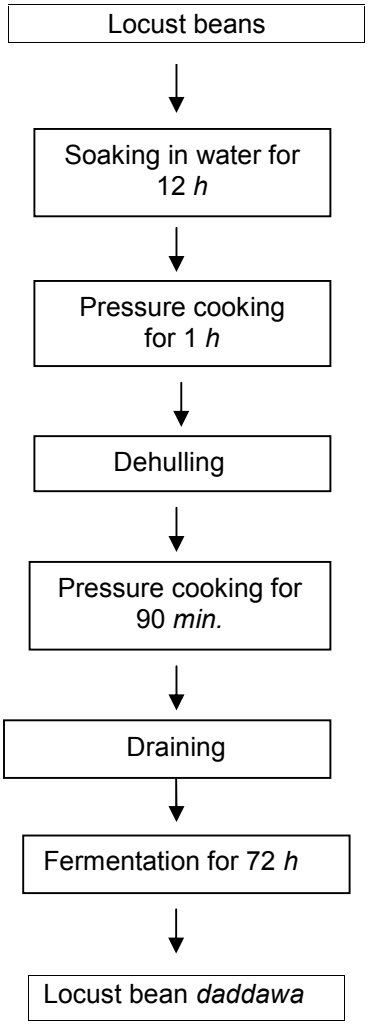

Fig. 2. Flowchart of laboratory processing of locust bean *daddawa*

2.3 Determination of pH and Titratable Acidity

Homogenate of the sample (1% w/v) was made by adding 45 ml of distilled water to 5g of sample. The mixture was blended with a sterile blender. The slurry was filtered through Whatman No1 filter paper, aliquot (10 ml) was taken and the pH read on the pH meter (ATC, Model HI-8915) standardized with buffers pH 4 and 7.

Titratable acidity of the homogenate expressed as mg lactic acid/g was determined using the method of (14).

2.4 Determination of Anti-Nutrients

2.4.1 Determination of tannin

Tannin content was determined using the method of (14). One gram of each sample was weighed into a beaker. Each was soaked in solvent mixture (70% acetone:glacial acetic acid, 30:20) for 5 hours to extract tannin. The extract was filtered through a double layer filter paper to obtain the filtrate. A set of standard solution of tannic acid, 10 to 50 ppm was prepared. The absorbance of the standard solution and the filtrate was read at 500nm on spectrophotometer (Spectrumlab 23A). Tannin content was expressed as mg per kilogram of the sample.

2.4.2 Determination of cyanide

Cyanide content of the samples was determined using the method described by (16). Five gram of each sample was dissolved in 50ml distilled water in a corked conical flask to extract cyanide. The cyanide extraction was allowed to stay overnight. The extract was filtered through a filter paper. Alkaline picrate solution was prepared by dissolving 1g of picric acid and 5g of sodium carbonate in warm water in a volumetric flask and making up the volume to 200ml with distilled water. To 1 ml of the sample filtrate was added 4ml alkaline picrate and this was incubated in water bath for 5min for color development. After the development of the reddish brown color, the absorbance of the solution was read at 490nm on a spectrophotometer. The absorbance of the blank containing only 1ml distilled water and 4ml alkaline picrate solution was also read. Standard cyanide solution was prepared from different concentrations of potassium cyanide solution containing 5 to 50 µg cyanide in a 500ml conical flask, 25 ml 1N HCl was added. Cyanide was expressed as mg per kilogram of the sample.

2.4.3 Determination of phytate

Phytate content was determined using the method described by (15). Sample (2 g) was extracted with 20 ml of 2% HCl for 3 hours. To 1 ml of the extract was added 1 ml of 0.3% ferric ammonium sulphate solution in a test tube and stoppered. The mixture was boiled for 30 min. in water bath. The tubes were cooled in ice for 15 min. and allowed to adjust to room temperature. It was then centrifuged at 3000 x g for 30 min. The supernatant (1 ml) was mixed with 1.5 ml of 0.02M 2,2 dipyridine solution and the absorbance was read at 519 nm against a blank (distilled water) in a spectrophotometer. Phytate was expressed as mg per kilogram of the sample.

2.4.4 Determination of trypsin inhibitor

Trypsin inhibitor was determined by the method described by (17). The samples were extracted by weighing 1 g of the sample and dissolving it in 50ml of 0.5M NaCl solution. The mixture was stirred for 30 *min.* at room temperature and then centrifuged. The supernatant was filtered through Whatman No. 1 filter paper. The filtrate (extract) was used for the determination. 20 ml of 0.1% Trypsin solution was added to 10 ml of the filterate in a test tube or beaker. A blank (10 ml) of distilled water was prepared. The content of the test tube was allowed to stand for at least 5 min. after which its absorbance was measured at a wavelength of 410 nm. Trypsin activity was expressed as number of trypsin unit inhibited (TIU) per unit weight (g) of the sample analyzed.

2.5 Statistical Analysis

Each determination was carried out in triplicate and the mean taken. Data obtained from the anti nutrient analysis were subjected to analysis of variance (ANOVA) and the mean were separated by Duncan multiple range test (SAS, 2003 Version 8). Significance was accepted at 5 % level.

3. RESULTS AND DISCUSSION

3.1 Changes in pH During the Fermentation of Lima Bean and Locust Bean to *Daddawa*

The pH of the fermenting lima beans increased significantly (P = .05) from 7.46 at 0 *h* to 8.50 at 72 *h* of fermentation while the pH of fermenting locust bean increased significantly (*P* = .05) from 7.50 at 0 *h* to 8.74 at 72 *h* of fermentation (Fig. 3). Increase in the pH as the fermentation progressed was probably due to proteolytic activities and the release of ammonia as a consequence of breakdown of proteins by the proteolytic enzymes produced by the microorganisms. The release of ammonia is responsible for the characteristic ammoniacal odour produced during the fermentation of most vegetable proteins (10,13). Increase in pH from 0 *h* to 72 *h* of fermentation in locust bean and soybean *daddawa* was also reported by (19) and (13) respectively. There was no significant difference (*P* = 0.05) in the pH of lima beans and locust beans at 0 *h* of fermentation.

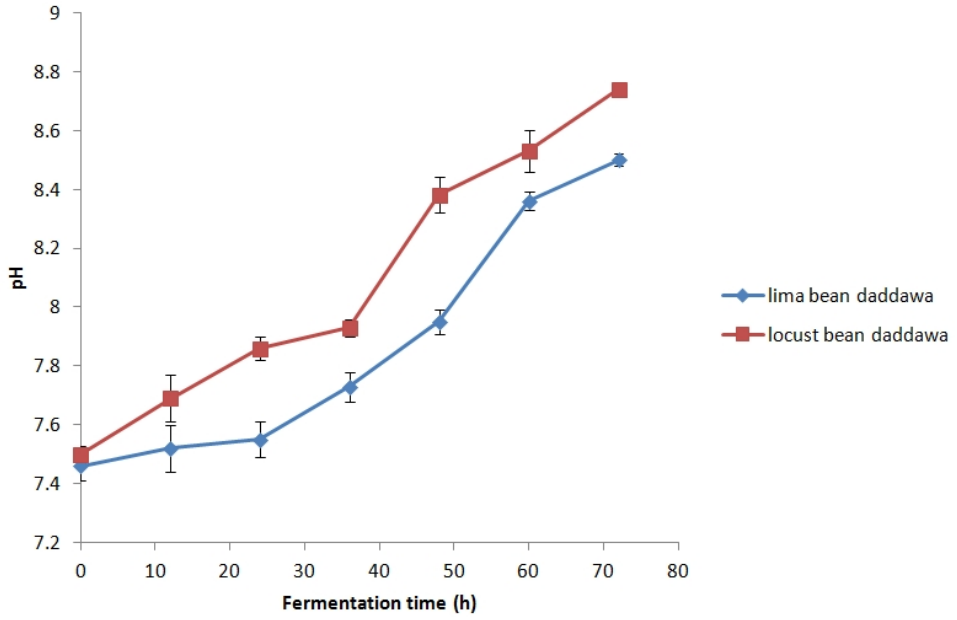

Fig. 3. Changes in pH during the fermentation process of lima bean seeds and locust bean seeds to *daddawa*

3.2 Changes in Titratable Acidity

Titratable acidity (TTA) decreased in both the fermenting lima bean and locust bean from 0.216 at 0 *h* to 0.045 at 72 *h* and from 0.198 at 0 *h* to 0.018 mg lactic acid g^{-1} at 72 *h* of fermentation respectively (Fig. 4). Decrease in TTA and increase in pH after 24 *h* of fermentation was also reported by (20) during the fermentation of castor seed to produce *Ogiri-igbo*. Progressive decrease in TTA and increase in pH in fermentation of *kpaye* was also reported by (21,22). The titratable acidity of the fermented lima bean and the fermented locust bean seeds were not significantly different at 5% significant level.

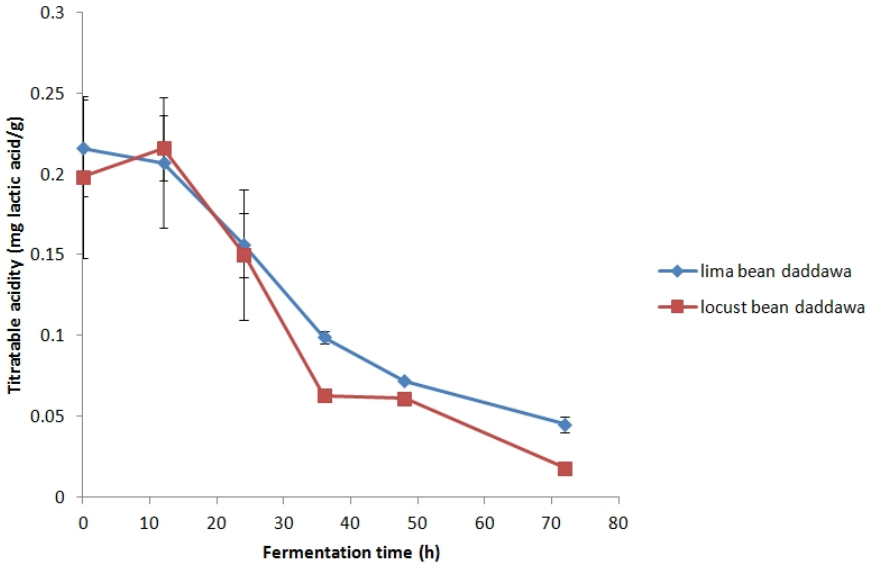

Fig. 4. Changes in titratable acidity during the fermentation of lima bean seeds and locust bean seeds to *daddawa*

3.3 Changes in the Anti-Nutrients Content of the Fermented Lima Beans and Locust Beans Samples

The raw lima beans contained 23.55 mg kg^{-1} tannin, 31.56 mg kg^{-1} phytate, 5.20 mg kg^{-1} cyanide and 28.36TIU g^{-1} trypsin inhibitor (Table 1a). The hull of the lima beans contained relatively high amount of tannin and reports have shown that the darker the color of the seed, the higher the tannin level (23). Raw locust beans contained lower level of these anti-nutrients. It contained 11.0 mg kg^{-1} tannin, 17.0 mg kg^{-1} phytate, 1.87 mg kg^{-1} cyanide and 6.32TIU g^{-1} trypsin inhibitor (Table 1b). Processing and fermentation, however, reduced the amount these anti-nutrients in both samples. Tannin content decreased with fermentation time from 19.0 at 0 *h* to 2.0 mg kg^{-1} at 72 *h* and from 9.50 at 0 *h* to 3.00 mg kg^{-1} at 72 *h* of fermentation in lima beans and locust beans respectively (Tables 2a and 2b). Drastic reduction of about 91% and 68% in tannin content were made possible after processing and fermentation of lima bean and locust bean seeds respectively. The decrease could be attributed to the hydrolysis of polyphenolic compounds of tannin complexes during fermentation. The decrease in tannin with increased fermentation time is in agreement with the findings of (24). The raw lima beans were significantly high (P = .05) in tannin compared with the fermented lima bean seeds.

Table 1a. Anti-nutritional compounds in lima beans

Sample	Anti- nutrients contents			
	Tannin (mg/kg)	Phytate (mg/kg)	Cyanide (mg/kg)	Trypsin Inhibitor (TIU/g)
Raw	23.55 ± 0.01	31.56 ± 0.01	5.20 ± 0.01	28.36 ± 0.00
Hull	16.01 ± 0.00	6.01 ± 0.00	1.90 ± 0.01	10.50 ± 0.10

Figures represent means of triplicate determination ± Standard Error

Table 1b. Anti-nutritional compounds in locust beans

Sample	Anti-nutrients content			
	Tannin (mg/kg)	Phytate (mg/kg)	Cyanide (mg/kg)	Trysin Inhibitor (TIU/g)
Raw	11.0 ± 0.02	17.0 ± 0.04	1.87 ± 0.0	6.32 ± 0.0
Hull	6.01 ± 0.03	5.52 ± 0.00	0.80 ± 0.1	1.0 ± 0.0

Figures represent means of triplicate determination ± Standard Error

Fermentation significantly decreased (p = .05) phytate content from 22.0 at 0 *h* to 7.0 mg kg^{-1} at 72 *h* in lima bean seeds (Table 2a). Reduction in phytate content of 77.82 and 73.53% were recorded for lima bean and locust bean seeds respectively after fermentation. Similar reduction in phytate level has been reported by (26) for fluted pumpkin seeds respectively during fermentation. Reduction in phytic acid during fermentation could be due to the enzymatic action of the fermenting microorganisms which hydrolyze phytate into inositol and orthophosphate. Decrease in phytate with increase in fermentation time was also reported by (27,28) for fermented black gram and fermented soybean respectively. It has also been reported that phytase hydrolyses phytate to inositol and phosphoric acid and this process releases some elements such as phosphorus thus increasing the mineral availability. Phytate has been found to decrease calcium bioavailability and forms complexes thus inhibiting the absorption of minerals particularly iron.

Table 2a. Influence of fermentation time on the anti-nutritional compounds of lima bean seeds

Fermentation time (h)	Anti-nutrients content			
	Tannin (mg/kg)	Phytate (mg/kg)	Cyanide (mg/kg)	Trypsin Inhibitor (TIU/g)
0	19.00 ± 0.10^{b}	22.00 ± 0.03^{b}	0.97 ± 0.01^{c}	4.40 ± 0.10^{bc}
12	15.53 ± 0.10^{c}	17.55 ± 0.03^{c}	0.81 ± 0.01^{cd}	4.63 ± 0.10^{cd}
24	13.51 ± 0.03^{d}	11.57 ± 0.03^{d}	0.80 ± 0.00^{cd}	3.42 ± 0.00^{c}
36	6.51 ± 0.04^{c}	11.01 ± 0.01^{e}	0. 80 ± 0.10^{cd}	3.42 ± 0.01^{c}
48	4.50 ± 0.00^{f}	11.10 ± 0.00^{e}	0.75 ± 0.00^{d}	3.2 2 ± 0.01^{d}
60	2.50 ± 0.01^{c}	9.02 ± 0.10^{a}	0.33 ± 0.01^{b}	2.97 ± 0.02^{b}
72	2.00 ± 0.01^{a}	7.00 ± 0.00^{f}	0.25 ± 0.01^{a}	1.76 ± 0.02^{a}

Means in the same column followed by the same superscript are not significantly different at 5% significant level

Cyanide content also decreased with processing and fermentation time. Cyanide content decreased significantly (p = .05) from 5.20 mg kg^{-1} in the raw lima beans to 0.97 mg kg^{-1}

after dehulling and cooking. Fermentation further decreased significantly (p = .05) the cyanide content from 0.97 mg kg^{-1} at 0 *h* to 0.25 mg kg^{-1} at 72 *h* of fermentation (Table 2a). Decrease in cyanide with fermentation could be due to enzymatic activities of the fermenting microorganisms (29). The value of the cyanide is within the safe level of 30.0 mg HCN equivalent kg^{-1} recommended by (30). Small amount of cyanide may act as a break on cellular oxidative process and its effect can consequently result unto death or associated with chronic neurological effect (29).

Processing and fermentation reduced the trypsin inhibitor in the fermenting lima beans. Trypsin inhibitor was highest in raw lima beans (28.36 TIU g^{-1}), but it decreased significantly (p = .05) to 1.76 TIU mg^{-1} at 72 *h* of fermentation (Tables 1a and 2a). Fermentation has been reported to reduce trypsin inhibitor activity of bambara groundnut (31).

Table 2b. Influence of fermentation time on the anti-nutritional compounds of locust bean seeds

Fermentation time (h)	Anti-nutrients content			
	Tannin (mg/kg)	Phytate (mg/kg)	Cyanide (mg/kg)	Trypsin Inhibitor (TIU/g)
0	9.50 ± 0.02^{a}	15.0 ± 0.05^{b}	15.0 ± 0.05^{ab}	0.24 ± 0.0^{a}
12	7.55 ± 0.02^{c}	14.0 ± 0.02^{c}	0.36 ± 0.1^{c}	0.21 ± 0.0^{b}
24	7.54 ± 0.01^{c}	12.5 ± 0.02^{c}	0.27 ± 0.1^{bc}	0.20 ± 0.0^{c}
36	4.08 ± 0.02^{e}	10.0 ± 0.00^{d}	0.27 ± 0.1^{bc}	0.20 ± 0.0^{c}
48	3.51 ± 0.02^{b}	9.00 ± 0.00^{de}	0.27 ± 0.1^{b}	0.20 ± 0.0^{c}
60	3.00 ± 0.00^{b}	9.00 ± 0.01^{e}	0.29 ± 0.1^{a}	0.10 ± 0.0^{c}
72	3.00 ± 0.01^{d}	4.50 ± 0.00^{a}	0.29 ± 0.1^{bc}	0.10 ± 0.1^{c}

Means in the same column followed by the same superscript are not significantly different at 5% significant level

4. CONCLUSION

Daddawa analogue could be produced from lima beans. Fermentation increased the pH and reduced the TTA of lima bean and locust bean seeds during production of *daddawa.* Product obtained from fermented lima bean seeds was comparable to locust bean seeds daddawa in terms of anti- nutritional components content.

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Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

CONSENT

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

ETHICAL APPROVAL

Not applicable.

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