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An Investigation Into The Effects Of Boiling And Fermentation On The Anti-Nutritive Values Of Locust Beans

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ABSTRACT:*The effects of boiling and fermentation on the anti-nutritive values of locust beans were investigated. The anti-nutritional screening was carried out on the hull, raw seed, boiled seed and the fermented locust beans. Some samples were taking to the laboratory to determine the anti-nutritive values before boiling, the boiled and fermented samples were also tested. The results showed the presence of the anti-nutrients except for cyanide which was negative after fermentation. The anti-nutritional values of the hull of the locust beans has Tannin of (0.46 mg/100g), Phytate (1.98 mg/100g), Oxalate (0.98 mg/100g), Cyanide (0.12 mg/100g), Trypsin inhibitor (0.53 mg/100g), Flavonoid (36.12%), Alkaloid (21.12%). The result showed that the boiling and fermentation processes reduced the Tannin (0.31 mg/100g), Phytate (1.68 mg/100g), Oxalate (0.64 mg/100g), Cyanide (0.08 mg/100g), Trypsin inhibitor (0.37 mg/100g), Flavonoid (23.14%), Alkaloid (13.12%) of raw seeds to Tannin (0.14 mg/100g), Phytate (1.12 mg/100g), Oxalate (0.26 mg/100g), Cyanide (Nil), Trypsin inhibitor (0.26 mg/100g), Flavonoid (11.12%) and Alkaloid (8.78%) of fermented seeds (iru) respectively. The analysis of variance of the results were carried out which showed that there is no significant different in the anti-nutritive values of the raw seed, boiled and fermented locust beans. Therefore, it was recommended that boiling and fermentation reduce the anti-nutritive values of locust beans in the same proportion.*
KEYWORDS*: Locust beans, anti-nutritional parameters, anti-nutritive values, boiling, fermentations*

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I. INTRODUCTION

The African locust bean tree with the botanical name parkiabiglobosa is a perennial deciduous tree that is fairly widely distributed all over the natural grassland of Northern Nigeria (Sobande, 2013, Ojewumi et al., 2016a). The tree starts to bear fruits from five to seven years after planting. It is planted mainly for the food value of its fruit. It grows in the savannah region of West Africa. The tree has a height ranging from 7 to 20 m; in some exceptional cases, some might reach heights of up to 30 m, with a spreading umbrella-shaped crown (Teklehaimot, 2004, Ojewumi et al., 2016b). It performs an essential function ecologically in cycling of nutrients from deep soils, and in holding the soil particles with the aid of the roots to prevent soil erosion (Alabi et al., 2005). The tree also provides shade for man. The tree requires an altitude of about 300 meters with an average rainfall of 400-700 millimeters per year and an average mean annual temperature of 28^0C. It prefers well-drained, deep, cultivated soils, but can also be found on shallow, skeletal soils and thick laterites (Protabase, 2014). The African locust bean seeds are contained in branches of pods that make up the most valuable part of the plant. The pods are flat and large irregular cluster of up to 30 seeds (Omafuvbe et al., 2004). Parkiabiglobosa is beneficial to the soil situated beneath, which is made useful and valuable by the dung and urine of animals that shelter under the tree's shade. It can also be used as timber for making pestles, mortars, bows, and seats (Ntui et al., 2012). All trees of the parkia species are carefully and usually kept safe by the people in the particular area where they grow because of their valuable sources as a reliable source of food, especially the seeds which serves as source of useful ingredients for consumption.

Processing of locust bean fruits to food condiment involves different unit operations after harvesting, such unit operations include de-podding, removal of the yellowish pulp to produce locust bean seeds. Other processing operations are cleaning, washing, re-cooking, and the fermentation to produce the food condiment which is used for soup seasoning spices (flavoring agent) (Beaumont, 2002). Several constraints are identified in the production and consumption of the condiment. These include, among others, low production due to the use

of rudiment equipment, high wood consumption,and poor manufacturing practices. De-hulling and cooking of the locust bean seeds are time consuming, laborious and inefficient. Consequently, the production of this condiment has not increased substantially. Its declining popularity, especially among the growing urban population has led to rapid increase in an import of foreign soup flavors (Beaumont, 2002). The flow chart of the processing line of locust bean pods to food condiment is shown in Figure 1

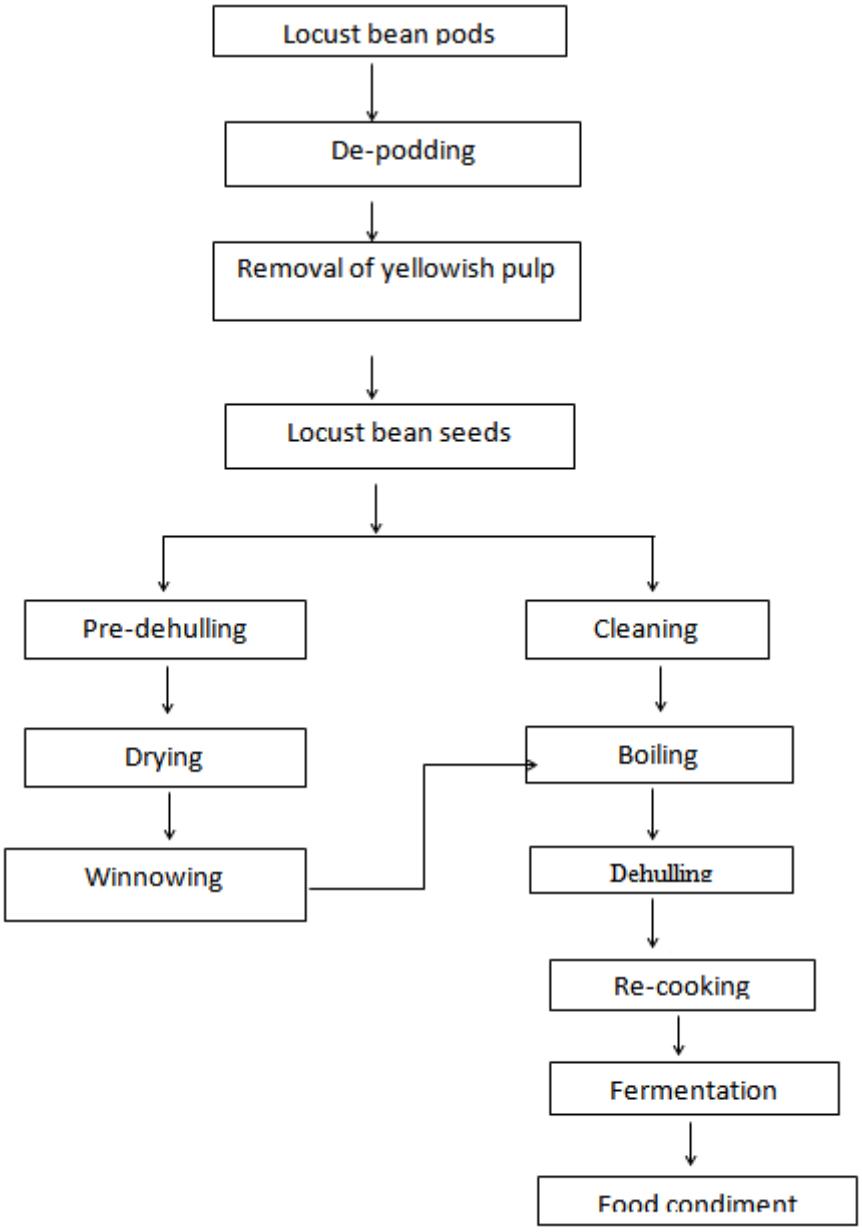

Figure1: Flow chart for traditional processing of locust bean fruits to food condiments (Aniyi, 2004).

The unfermented seed is known as karwa in Hausa; Iyere in Yoruba; Soumbala in Burkina Faso, Mali, Cote d'ivoire and Guinea. They are traditionally used as food condiment and are known to be rich in protein and contain easily digestible calcium, they also contain 20% edible oil. In the third world countries where the need for protein supplementation is high for both adult and infants, 'Iru' is very important (Emujiugha, 2005). African locust bean (Parkiabiglobosa) seed grows on a common perennial leguminous tree known as African Locust bean Tree which belongs to the sub-family mimosoideae and family leguminosae (now family fabaceae), (Abdoulaye, 2012). They grow in the savannah region of West Africa up to the southern edge of the Sahel zone 13°C, (Campbell-Platt, 1980). The plant produces brownish seeds, which are arranged in pods. When processed, the seeds constitute an important condiment that adds taste and flavor to soup. The processed cake, known as

'Iru' is used widely in the south-western and middle-belt zones of Nigeria. The pods, which are slightly flattened and slender, turn from pink-brown to dark brown when matured. These pods are about 45 cm long and 2 cm. wide and may contain up to 18seeds embedded in a yellowish fleshy endocarp.
The seeds have a hard testa (averagely weighing about 0.26 g/seed) with large cotyledons forming about 70% of their weight. Locust bean fruit is normally processed into food condiment, which is popularly taken in the western part of Africa and it is used as a spice that gives an African meal a pleasant flavor. Since the last 10 years, they are considered as the most 15 important food condiments consumed everywhere by rural poor people, (Azokpota et al., 2005).

The physical, chemical and nutritional characteristics of the African Locust Bean seeds changes immediately after fermentation since the raw African locust beans are nutritionally deficient and unpalatable (Amoa-Awu et al., 2005). Nigeria has a variety of people and culture that it is difficult to pick one national dish. Each area has its own regional favorite food that depends on customs, tradition, and religion (Abdel and Dadir, 2009; Adebayo et al., 2010). The fermentation processes for these foods constitute a vital body of indigenous knowledge used for food preservation, acquired by observations and experience, and passed on from generation to `generation (Aworh, 2008; Chelule et al., 2010). The fermentation techniques are often on a small scale and household basis, characterized by the use of simple non-sterile equipment, chance or natural inoculums, unregulated conditions, sensory fluctuations, poor durability and unattractive packaging of the processed products resulting in food of unpredictable quality and variation (Olanrewaju et al., 2009). The African locust bean pulp is sweet to taste when ripe, which indicates the presence of natural sugars and thus a potential energy source. The attractive yellow colour indicates the presence of phyto-nutrients, possibly carotenoids, which are important precursors of retinol (vitamin A). It has a sour taste when unripe which indicates the presence of ascorbic acid (vitamin C) (Gernah et al., 2007a).

According to Uwaegbute (1996), the powdery fruit pulp contains more carbohydrate than the seeds, the carbohydrate being primary reducing sugars, non-reducing sugars, and other complex carbohydrate. High carbohydrate content of feed is desirable; a deficiency causes depletion of body tissue (Barker, 1996). Gernah et al. (2007) reported that the fruit pulp contains 67.30% carbohydrate that is comparable to lentils and Bambara nuts with a carbohydrate value of 65.00% (Muller, 1988). The reducing sugar content in carbohydrate sources is partly responsible for browning as a result of Mallard reaction between the reducing sugar and the protein content of the sample. Maillard reaction might not pose any problem in those samples with low level of both protein and reducing sugars (Adewusi et al., 1995). Bello et al. (2008) reported a sugar content of 4.27mg/g, a starch content of 151.88 mg/g and a protein content of 5.25 mg/g for parkia fruit pulp.

Gernah et al. (2007b) reported a crude fibre value of 11.75% for parkia pulp which is on the high side. The crude fibre is higher in the parkia fruit pulp than those of other legumes which ranged from 2.10% in groundnuts to 7.60% in kidney beans (Ihekoronye and Ngoddy, 1985).

Gernah et al. (2007) reported a mineral content value of 4.18%. This is within the range for most legumes of 2.00% in peas to 5.00% in soybeans and a vitamin C content of 191.20mg/100g for parkia fruit pulp respectively. Bello et al. (2008) reported vitamin C content of 215mg/100g for parkia fruit pulp.

Gernah et al. (2007) reported phytic acid content of 60.00mg/100g for parkia pulp. Bello et al.(2008) reported a value of 0.20mg/g for parkia fruit pulp. The problem with phytic acid in foods is that it can bind some essential mineral nutrients in the digestive tract and can result in mineral deficiencies. Food that contain high amount of phytate when consumed over a long period of time can decrease bioavailability of minerals in monogastric animals (Thompson, 1993).

Gernah et al. (2007) reported a tannin content of 17.80 mg/100g for parkia pulp which could be a contributory factor to the foaming characteristics of the fruit pulp. Tannins in fruits impact an astringent taste that affects palatability, reduce food intake and consequently body growth. Tannins are known to inhibit the activities of digestive enzymes, and nutritional effects of tannins are mainly related to their interaction with protein. Tannin-protein complexes are insoluble and the protein digestibility is decreased (Carnovaleet al; 1991). Bello et al. (2008) stated that a content of 15.55TIU/g for trypsin inhibitor in parkia fruit pulp was found. Usually,parkia pulp is consumed uncooked. The presence of trypsin inhibitor in uncooked animal feed has long been known to cause diminished growth in rats, chickens and other experimental animals (Liener and Kakade, 1999). Trypsin inhibitor is heat labile and can be inactivated by heat treatment such as steaming and extrusion cooking (Liener, 2000). Oxalate is a concern because of its negative effect on mineral availability. High oxalate diet can increase the risk of renal calcium absorption and has been implicated as a source of kidney stones (Chai and Liebman, 2004). Bello et al; (2008) reported an oxalate content of 0.093g/100g for parkia fruit.

Though various locust beans processors and researchers had fermented, dried, milled and stored locust beans for preservation for future use. The effects of these processes in relation to the anti-nutritive values of locust beans still remain a challenge. Hence, the study investigated the effects of processing parameters such as; boiling and fermentation, on the anti-nutritive values of locust beans.

Plate 1: African Locust Bean Tree **(**ParkiaBiglobosa) (Igba tree). (Sobande, 2013 and Ojewumiet al., 2016)

II. METHODOLOGY

Materials: filter paper, anhydrous sodium hydroxide (NaOH), silver nitrate (AgNO3),
Equipment: Muffle furnace**,** Bunsen burner, water bath, Kjeldahl digestion flask, oven, desiccator, cooking pot, bucket, bowl, basket sieve, locust beans dehulling machine, locust beans, beaker, measuring cylinder, Dropping pipette, Conical flask, oven,

2.1 Production of Iru

7.5 kg of raw locust beanwas purchased at a local market in Osogbo, Osun state, Nigeria. Raw African locust bean was boiled for 12 hours and further soaked in the boiling water for another 12 hours (preferably overnight). Excess water was drained off and the seeds were de-hulled by using locust bean dehulling machine to remove the cooked hull from the seed. The cotyledons were again cooked for another 6 hours, the hot boil water was "drained off and the cotyledons were then spread in calabash trays, covered with wooden trays, wrapped with jute sacks and fermented for 3-4 days to produce iru.

2.2 Determination of The Anti-Nutritive Values of Locust Beans

2.2.1 Qualitative Phytochemical Analyses

Phytochemical tests were conducted on the extracts of sample using standard methods as reported elsewhere (Edoga et al., 2005; Krishnaiah et al., 2009; Egwaikhide, 2007).

Test for tannins

0.5 g of sample of sample extractwas boiled in 20 ml. of distilled water in a test tube and filtered using a conical flask and filter paper. 0.1 % ferrous chloride (FeCl3) was added to the filterate and observed for brownish green or blue black colouration which indicates the presence of tannins.

Test for saponins

2 g. of sample was boiled in 20 ml. of distilled water in a water bath and filtered. 10 ml. of the filtrate was mixed with 5 ml. of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion which indicates the presence of saponins

Test for flavonoids

A few (three) drops of 1 % ammonia solution was added to 10 ml. aqueous extract of sample extract in a test tube. A yellow colouration observed indicates the presence of flavonoid compounds.

Test for cyanide

2 ml. of acetic acid and 2 ml. of methanol was added to 0.5 g. extract of sample containing 2 ml. of H_2SO_4. The presence of cyanide was confirmed when a violet coloration that changes to blue or green was obtained.

2.1.2 **Quantitative analysis**

Flavonoid determnation

Flavonoid was determined by using the method of Bohn and kopcipal- Abyazan (1994): 10g. of the plant sample was extracted repeatedly with 100ml. of 80% aqueous methanol at room temperature for 30 minutes. The whole solution was filtered through whatman filter paper No45 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

$$\text{Percent}(\%)\text{flavonoids} = \frac{W_2 - W_1}{W} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ i}$$

Where W= weight of sample
W_1= Weight of empty filter paper
W_2 = Weight of paper + precipitate.

Tannins determination

0.1g. of the sample was weighed and transferred to a 250 ml. conical flask. 100 ml. of distilled water was added to the sample and boiled for 1 hr. The solution obtained was diluted to 100 ml. and filtered. 5.0 ml. of the filtrate was added to 10 ml. of freshly prepared 17% sodium carbonate and 2.5 ml. of Folin Denis reagent were placed in a test tube and allowed to stand for 20 mins for colour development. Thereafter, the absorbance/optical density was read at 520 nm using the GBO Cintra 6 uv spectrophotometer. Also, the standard tannic acid curve was prepared. The blank was prepared as above without the sample (Harbone, 2000)

$$\text{Percent}(\%)\text{tannin} = \frac{\text{An}}{\text{As}} \times \text{C} \times \frac{100}{\text{W}} \times 5 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ii}$$

Where: An = Absorbance of test sample
As = Absorbance of standard solution
C= concentration of standard solution
W= weight of sample used
V_f= total volume of extract
V_a= volume of extract analyzed.

Determination of alkaloids

Alkaloids were quantitatively determined according to the method of Harborne, (2000) Two hundred milliliters of 10% acetic acid in ethanol was added to 5 g. powdered plant sample, covered and allowed to stand for 4 hrs. The filtrate was then concentrated on a water bath to one-fourth of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed and the whole solution was allowed to settle. The collected precipitates were washed with diluted ammonium hydroxide and then filtered. The residue was dried and weighed.

The alkaloid content was determined using the formula:

$$\text{Percent}(\%)\text{alkaloid} = \frac{\text{finalweightofthesample}}{\text{initialweightoftheextract}} \times \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{iii}$$

Total Cyanide Content Determination

4g. of the sample was soaked in a mixture of 40ml. distilled water and 2ml. of orthophosphoric acid. The sample was thoroughly mixed and covered and left overnight at room temperature to set free all the bounded hydrocyanic acid. The resulting mixture was transferred into distillation flask and a drop of paraffin (antifoaming) was added to the broken chip.Theflask was fitted to other distillation apparatus and distilled.about 25ml. of the distillated was collected in the receiving flask that contain 4ml. of distilled water containing 6.1g. of sodium hydroxide pallets. The distilled was then transferred into 50ml. volumetric flask and made up to mark with distilled water 20ml. of the distilled was collected and then placed in the conical flask and 1.6mwascollectediodide solution was added to the flour and titrated against 0.01M Ag(NO_3) solution. The blank was also titrated until the end point was indicated by a faint but permanent turbidity (AOC,1990) the blank (without the sample) was also titrated until the end point was indicated by a faint but permanent turbidity

$$\text{Cyanidecontent} = \frac{13.5(V_0 - V_i)}{M} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{iv}$$

Vo= titre value of the sample
Vi=titre value of the blank
M=mass of the sample

Determination of phytic acid

Phytic acid was determined using the procedure described by Lucas and Markakas (1975). A portion (2 g.) of sample was weighed into 250 ml. conical flask; 100 ml. of 2% concentrated hydrochloric acid was used to soak the sample for 3 hrs. The mixture was filtered, and 50 ml. of each filtrate was placed in 250 ml. beaker and 107 ml. of distilled water was added in each case to give proper acidity. 10 ml. of 0.3% ammonium thiocyanate

solution was added to each solution as indicator and was titrated with standard iron chloride solution which contained 0.00195 g iron per ml.

$\text{Percent}(\%)\text{phyticacid} = y \times 1.19 \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots v$

Where y = titre value x 0.00195

III. RESULT AND DISCUSSION

Results of the proximate analysis carried out on the hull and raw locust beans before fermentation to screen for the presence of anti-nutritive is as shown in Table 1.

Table 1: Screening of raw locust beans hull and seed for anti-nutritive values.

Parameters	Hull	Raw locust beans seed
Tannin (mg/100g)		+ve +ve
Phytate (mg/100g)		+ve +ve
Oxalate (mg/100g)	+ve	+ve
Cyanide (mg/100g)	+ve	+ve
Trypsin inhibitor (mg/100g)		+ve +ve
Flavonoid(%)		+ve +ve
Alkaloid(%)		+ve +ve

The results of the anti-nutritional values of hull of raw locust beans is as shown in Table 2 below;

Table2: Anti-nutritional values of hull of raw locust beans

Parameters	Hull
Tannin (mg/100g)	0.460
Phytate (mg/100g)	1.980
Oxalate (mg/100g)	0.980
Cyanide (mg/100g)	0.120
Trypsin inhibitor (mg/100g)	0.530
Flavonoid(%)	36.120
Alkaloid(%)	21.120

The values of anti-nutrients constituents in raw seed of locust beans were found to be slightly higher when it has been boiled, the values were lower than that of raw seeds. This result have been as a result of heat treatment which dissolved the constituents at high temperature and leached it out into the water which was used to boil and soaked it. For the fermented locust beans, tannin content is 0.14 mg/100g, which is lower than some everyday consumed legumes like lima beans (140.00 mg/100g), and pigeon pea (100.00 mg/100g) as reported by osagie (1998). This is therefore considered to be acceptable and safe for consumption. Phytate has 1.12 mg/100g. this is in conformity with 1.00 mg/100g as presented by osagie (1998) for locust beans seed. Though the smallest toxic dose for phytate in man is not known, it appears that high doses are required for any applicable effects in man. Aremu (1989).

Cyanide content was found to be nil. The amount that could be in human body for 50-60 mg/kg body weight/day is 17.30 mg/100g as represented by Balagopalan et al., (1988). Which is far beyond the lethal value. The decrease in trypsin inhibitor of locust bean by fermentation may be attributed to the leaching during soaking, heat treatment during boiling and also by the action of microorganisms during fermentation. Similar result was reported by other workers on African locust beans (El- Adawy,2002.).

Table 3: Anti- nutritional values of the raw, boiled and fermented locust beans

Parameters	Raw seed	Boiled locust beans	Fermented locust beans
Tannin (mg/100g)	0.310	0.360	0.140
Phytate (mg/100g)	1.68	1.390	1.120
Oxalate(mg/100g)	0.640	0.460	0.260
Cyanide (mg/100g)	0.080	0.030	nil
Trypsin inhibitor (mg/100g)	0.370	0.310	0.260
Flavonoid(%)	23.140	19.540	11.120
Alkaloid(%)	13.120	10.640	8.780

Table 4: ANOVA table on the anti-nutritional value of the raw, boiled and fermented locust bean

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	928.969[a]	8	116.121	22.079	.000
Intercept	418.527	1	418.527	79.577	.000
Parameters	906.223	6	151.037	28.717	.000
Treatment	22.746	2	11.373	2.162	.158
Error	63.113	12	5.259		
Total	1410.609	21			
Corrected Total	992.082	20			

a. R Squared = .936 (Adjusted R Squared = .894)

Table 4 showed that there is significant different in the parameters that is; the anti-nutritional value of the locust beans. The treatment used which are raw seed, boiled locust beans,and fermented locust beans. According to the analysis, there is no significant different in the anti-nutritional values of the raw seed, boiled and fermented locust beans since the p-value (0.158) is greater than α-value (0.05) at 95% confidence interval. Thus, boiling and fermentation of locust beans have the same proportion of anti-nutritive values.

IV. CONCLUSION

The boiling and fermentation process of locust beans reduce anti-nutritive values of locust in the same proportion. The research showed that the anti-nutritive values of locust beans can be controlled by boiling and fermentation.

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