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CHEMICAL AND NUTRIENT ANALYSIS OF RAW AND FERMENTED SEEDS OF *Cassia tora*

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ABSTRACT
The quantitative analysis of nutritional values, mineral content and anti-nutritional factors of raw and fermented seeds of *Cassia tora* was determined by using standard procedures. The seed was found to be a good source of carbohydrates (33.47% and 21.80%), for both raw and fermented seeds respectively, crude protein content was found to to be 13.79% and 17.21% for raw and fermented seeds respectively, and crude fats (16.085 and 18.36%) for raw and fermented seeds respectively. It also contains substantial quantities of copper, iron, sodium, zinc, magnesium, and calcium. The results showed a significant increase of 70.36%, 24.80%, 14.68% and 51.67%, in ash, crude protein, crude fats and crude fibre respectively, in the fermented seeds. The results also indicated a decrease in the anti-nutritional factors saponins decreased by about 7.41% with the effects of boiling and fermentation could improve some nutritional values and enhance the reduction of anti-nutritional factors.

Keywords: *Cassia tora*, Food, Nutrient, Fermented seeds, Anti-nutritional

INTRODUCTION
The ultimate aim of eating food is to derive adequate nutrients, which the body needs for its normal functioning. Nutrients are the components order to grow and live a normal healthy live (Syazzie *et al.,* 1994). In Nigeria indigenous foodstuffs provide nutrients such as protein, carbohydrates, fats, water, minerals and vitamins in different proportion (Fagbemi and Oshodi 1991). Wild seeds and fruits are commonly consumed by both rural and urban dwellers, especially among low income earners. Some of these wild seeds have higher nutritional values compared with levels found in some cultivated foods. However, some of these wild seeds and fruits contain anti-nutritional factors that can affect the availability of nutrients required by body.

The preparation of foods by fermentation has good advantages such as the destruction of undesirable flavours and odours, production of a good flavour, increased digestibility, synthesis of desirable constituents and inhibitors (Odunfa 1985). Fermentation is one of the oldest methods of food preservation known to man; the art of fermentation is widespread including the processing of fruits and other carbohydrate sources to yield alcoholic and non-alcoholic beverages (Adewusi *et al*., 1991). Oil seeds, such as African locust bean, melon seed, castor oil seed, mesquite bean and soybean are also fermented to give condiments (Omafuvbe *et al.,* 2004). The characteristic ammoniacal odour and flavour of condiments enhance the taste of food in which they are used especially the various soups used as accompaniment to the starchy root and tuber diets (Simmons 1976).

Cassia tora is a common herbaceous annual occurring as a weed which belongs to the family of leguminaceae. The plant *Cassia tora* is extensively used in traditional medicine in tropical and warm subs tropical countries. The leaves *of Cassia tora,* as well as roots and seeds are used in folk medicine in Asia. It is believed to possess a laxative effect. Seeds *of Cassia tora* are roasted and foiled in water to produce tea as folk medicine (Perry 1980). Seeds of this plant yield a commercially valuable aim which is used as a food additive and as a thickener and "named for the Chinese Senna's former placement in the genus Cassia. Seeds of this plant have also been used as a substitute for coffee (Perry 1980). It has been reported that the application of paste of leaves of this plant is effective in the treatment of chronics of diseases and skin ailments. The powdered form of the plant leaves is taken internally as laxative. It tones up muscles of heart and has been reported to purify blood. It is also applied in hemorrhoids. The extract of leaves of this plant is used to treat skin rashes, and allergies. It is also used as an antidote for various poisoning. The decoction of the plant is popularly used as anti-obesity agent. Decoctions of parts of *Cassia tora* is used as an analgesic, antipyretic, antifungal, anthelmintic, diuretic, expectorant, laxative, purgative, treatment of glaucoma and hypertension, treatment of skin disease, ringworm and itch (Perry 1980).

This study provided a dietary awareness on alternatives source of protein and other nutritional values to people from low income groups has they seldom feed on meat, eggs or fish as source of protein to the required dietary standard because of socio-economic, cultural taboos and ignorance. It will also help in the optimization of the processing condition leading to a final product with improved nutritional and hygienic quality as well as improved stability. The aim of this research is to investigate the chemical and nutrient composition *of Cassia tora* seeds and to find out the effect of fermentation on the nutritional value and anti nutritional factors of the seed and the specific objectives of this research include the following:

- Proximate analysis on both raw and fermented sample of the seeds.
- Analysis of minerals composition of the seeds
- Analysis of anti nutritional factors on both raw and fermented sample of the *Cassia tora* seeds.

Vasanthi *et al.,* 2009 utilized mouldy sorghum and *Cassia tora* through fermentation for animal feeds purposes. An attempt was made to assess the potential addition *of Cassia tora* seeds to enhance the nutritive value of mouldy sorghum and also serve as a support base for fermentation. A trend was observed in enhancement of nutritive value particular with respect to protein, ash, fat, fibre and minerals owing to their high levels in *Cassia tora* seeds. On addition of *Cassia tora* seeds, crude fibre level increased by almost 3 times while carbohydrate and energy values did not show much increase. The analysis also revealed that fermentation resulted in 37% increase in calcium levels with addition of *Cassia tora* seeds.

MATERIALS AND METHODS

Collection and Treatment of the Sample

Matured dried pods of *Cassia tora* obtained from Yelwan Tudu of Bauchi State, Nigeria and were dehulled to release the seeds. Parts of the seeds were ground into fine powder and another part was fermented and was also ground into powder and ready for the analysis.

Fermentation

1.5kg of the seeds were boiled for 3hrs and allowed to cool overnight. These were dehulled by pounding with wooden pestle and mortar and washed with water to remove the seed coats. They were boiled again for 6hrs, water filtered out, allowed to cool and divided into three parts of 5g and packed in baskets lined and covered with aluminum foil. Yeast was inoculated onto cooked seeds. These were then kept in the dark for 3 days to ferment.

Determination **of Ash**

10g of the raw seeds and the fermented seeds were weighed and transferred into a crucible of known weight. This was then ash-burned in a muffle furnace at 500°C for 3hrs. The difference in weight was calculated in percentage.

$$\%\,Ash = \frac{Weight\ of\ Ash}{Weight\ of\ Sample} \times 100$$

Moisture content

6g of the ground sample were dried in the air oven at 105^{O}C for 24hrs. The sample was then toboggan in desiccators. Further drying was done until constant weight was obtained. The moisture content was calculated as percentage moisture according to the methods described by Owoso & Ogunmoyela (2001).

$$\%\,Moisture\,Content = \frac{M1 - M2}{M2 - M0} \times 100$$

Where; M0 = Mass of crucible in grams.
Ml = Mass of crucible and wet sample in grams.
M2 = Mass of crucible and dry sample in grams.

Crude Fats

2g of the sample were wrapped in a defatted filter paper in the soxhlet extractor containing 200ml of petroleum ether. The extraction was done for 6hrs after which the solvent was evaporated and cooled in the desiccator and weighed. The percentage of the ether extract was calculated by multiplying the increase in weight of the extraction flask by 100.

$$\%\,Crude = \frac{Weight\,of\ Fat}{Weight\,of\ Sample} \times 100$$

Determination of Crude Fibre

2g of the sample were grounded and diluted in 100ml distilled water in a conical flask. 20ml of 10% sulphuric acid were added and boiled gently for 30mins. The sample was then cooled and filtered. The filtrate was subjected to treatment using 10% sodium hydroxide. The residue was passed through 20ml of ethanol and petroleum ether and then dried at 105°C. The sample was weighed and ashed at 600°C for 90mins, cooled and reweighed and the percentage of crude fibre calculated Owoso *et al.,* 2000.

$$\%Crude\ Fibre = \frac{(wt\ of\ crude + wt\ of\ residue) - (wt\ of\ crucible + wt\ of\ sample)}{Total\ weight\ of\ sample} \times 100$$

Crude Protein and Nitrogen Determination (Micro-Kjeldahl)

The commonest method used to measure nitrogen is the Kjeldahl method, after the Danish chemist who developed it in the nineteenth century (Pearson 1983). The crude oil content of food was determined by measuring its amino-nitrogen content. In most proteineous food there is 16% elemental nitrogen, and therefore the factor to adjust the nitrogen protein is 6.25. This method used the Kjeldahl and Markham method, 0.2g of the sample was digested with 15ml concentrated sulphuric acid (98%) for 2-3 hours. The temperature of the boiling mixture was adjusted to 16°C and left for I hour before been adjusted to 370° C and this temperature was achieved by adding 40.0g sodium sulphate. The ratio of the acid to the sodium sulphate was 1:1:1.0 g of copper sulphate and a tiny speck of selenium oxide to catalyze the reaction.

Glass beads were added as anti-bubbling granule to prevent frothing which is normally caused by carbohydrate interference in the sample. A few drops of hydrogen peroxide was added to hasten digestion. The digest was allowed to cool before diluting to 250cm^3. In the digestion stage, the amino nitrogen in the sample was converted into ammonium sulphate. The ammonium sulphate was distilled by connecting to the flask containing the digest to the Markham distillation apparatus, and the digest made strongly alkaline by addition of 40% sodium hydroxide solution. The content of the then heated to boiling. The ammonia which was distilled in this way condensed and collected in Boric acid indicator. The distillate was titrated with standard 0.025 N H_2SO_4 to a pink end point.

The percentage N was calculated using the formula below:-To get the percentage crude protein= % N x 6.25

$$\%N = 0.014 \times \frac{Titre \times vol.of\ Digest \times Normality\ of\ acid}{Wt\ of\ sample \times vol.of\ aliquot\ used} \times 100$$

Determination of Carbohydrate

The carbohydrate was determined using Lane and Eynon's Method (Pearson 1983).

Determination of Invert Sugar (Reducing Sugar Glucose and Fructose)

20g of the sample was placed in a 200ml volumetric flask. 100ml distilled water was added followed by 5ml of zinc acetate and 5ml potassium ferrocynite was added and the solution was mixed together. The precipitate formed was filtered and a clear solution was obtained. The solution was filled into a sugar burette and 15ml of the solution from the burette was filled into a conical flask containing 25ml of mixture of equal volume of Fehling solution A and B. The solution was boiled and the sugar solution from the burette was added 1ml at a time of about 15 seconds interval to the boiling liquid, until the blue colour was nearly discharged.

4 drops of methyl thymol blue indictor was added and the titration continued until the blue colour was completely discharged and the supernatant liquid becomes orange- red in colour. The titration was repeated and the titre value was recorded. Using sugar table, the mg of a particular sugar corresponding to 10ml / 25ml of Fehling solution was obtained (that is the mg of sugar

present in the titrated volume of sugar solution when 10ml / 25ml of Fehling solution was used). The percentage invert sugar was calculated taking into consideration the dilution factor. Using the equation below: *percentage*

$$invert\ sugar = factor \times \frac{100}{Titre}$$

Determination of total sugar: Sucrose (non reducing sugar)
50ml of the clarified neutral sugar solution was pipetted from the determination of invert sugar into a 200ml volumetric flask. The inversion of sugar solution was done by the addition of 20ml of concentrated hydrochloric acid and it was left overnight. The inverted sugar solution was neutralized with 50% sodium hydroxide using few drops of phenolphthalein indicator. Then it was made up to mark using distilled water.

The Lane and Eynon titration was performed with the inverted sugar solution using 25ml equal volume of Fehling solution A and B. The titre value was recorded and the percentage total invert sugar was calculated using the equation:

$$Pecentage\ \ total\ \ invert\ sugar = factor \times \frac{100}{Titre}$$

The percentage sucrose was obtained by multiplying the total invert sugar by a factor of 0.95
% sucrose = % invert sugar x 0.95.
Sucrose = RS after inversion _ RS before inversion x0.95
Where RS = reducing sugar
% TOTAL SUGAR = % SUCROSE + % TOTAL INVERT SUGAR

Determination of Minerals Ion (AAS)
The buck scientific Atomic Absorption Spectrophometre VGP (variable giant pulse) system module 210 was used. The VGA uses a time specific modulation of the Hollow Cathode lamp (HC1) to produce an energy 'pulse' that contain information on both sample (Analyte) absorbance and the background (matrix) absorbance. Atomic Absorption Spectroscopy is based on the ability of an 'excited' atom of an element to absorb energy from wavelengths of thesame frequency as the element. This creates a decrease in the initial signal energy and this difference is proportional to concentration. Each has its own series of specific resonance wavelengths will have specific characteristics for sensitivity, noise and linearity. Sensitivity and noise will determine the limit of detection for that element. 20% of HNOs and 20% HC1 were prepared. After the preparation, 1g of ash sample was dissolved in the solution of HNO_3. 1ml of 20% HC1 was added. It was made up to mark with distill water to 100ml. It was filtered and taken for the AAS analysis. The optical system was set up with hollow cathode lamp for elements (analyte), with the appropriate slit and wavelengths selected for the elements. The prepared solution of known concentration of the analyte (standard) was aspirated and the absorbance reading was noted.

Determination of Tannic Acid

The tannic acid was determined according to the method of Maga[53] 2g of each sample were weighed into a beaker. Each was soaked with solvent mixture 80ml of acetone and 20ml of glacial acetic acid for 5hrs to extract tannin. The mixture was filtered and each filtrate was placed in water bath for 4hrs, after which the filtrate were removed and then filtered again with double layer filter paper to obtain the filtrate. A set of standard solution of tannic acid was prepared ranging from 10ppm to 50ppm. The absorbance of the standard solution as well as that of the filtrates was read at 700nm on a spectromic. Percentage of tannin was calculated using the formula:

$$\% \, Tannin = \frac{Absorbance \times Absorbance \times Average \ gradient \times Dilution \ \ factor}{100}$$

Where;

Dilution factor = Total amount of solvent (water) used Mass of tannic acid dissolved in 1000ml (1000 ppm).

Determination of Phytic Acid

The phytic acid was determined using the procedure described by Lukas and Markakas.[54] 2g of each was placed in a 250ml conical flask. 100mls of 20% concentrated hydrochloric acid was used to soak each sample in conical flask for 3hrs. This was filtered through a double layer of hardened filter papers. 50mls of each filtrate was placed in 20mks beaker and 107mls of distilled water was added in each to give proper acidity.

100mls of 0.3% ammonium thiocyanate solution was added into each solution as indicator. This was titrated with standard iron, chloride solution, which contains 0.00195g iron per ml. The end point was slightly brownish yellow persisted for 5 min. The percentage phytic acid was calculated using the formula.

% Phytic acid = V x 1.198 x 100

Where; x = Titre value x 0.00195 g.

Oxalate Estimation/Determination:

2g of sample was boiled in 40ml of water for 30mins in a reflux condenser; 10ml of 20% Na_2CO_3 was added and boiled for another 30mins. The liquid was extracted and the residue was washed with hot water until the wash water stopped showing any alkaline reaction. The combination of wash water and filtrate were concentrated to a small volume and cooled. With constant stirring, HC1 was added (1:1) drop wise until the acid concentration after neutralization was about 1% at which stage, a heavy precipitate appeared (which was allowed to flocculate). The extract was carefully filtered into a 250ml flask and made-up to mark. It was kept overnight, and then the supernatant liquid was filtered through a dry filter paper in a dry beaker.

An aliquot of this filtrate was taken into a 400ml beaker, diluted with water to 200ml and just ammonia cal and rectified with acetic acid. In the cold medium, 10ml of 10% calcium chloride

solution was added and stirred well to induce calcium oxalate precipitate to appear and it was allowed to settle overnight. The clear supernatant liquid was carefully decanted off through Watchman No 42 filter paper, without disturbing the precipitate. The precipitate was dissolved in HC1 (1:1). Oxalate acid was re-precipitated by adjusting the pH with ammonium hydroxide solution. Contents were boiled and allowed to settle overnight. Oxalate acid was determined by titrating against 0.05N $KMnO_4$ solution dissolved in HCl (1:1). Oxalate acid was re-precipitated by adjusting the pH with ammonium hydroxide solution. Contents were boiled and allowed to settle overnight. Oxalate acid was determined by titrating against 0.05N $KMnO_4$ solution.

Calculations

1ml of 0.05 N $KMn0_4$ = 0.00225 a hydrous oxalic acid.
% oxalic acid = Titre value x 0.00225/2
=T.Ux0.1125

DETERMINATION OF SAPONINS

The method used was that of Obadoni and Ochuko (2001). The samples were ground and 20g of each were placed in a conical flask and 20% aqueous ethanol was added. The samples were heated over a hot water bath with continuous storing at about 55°C The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^0C. The concentrate was transferred into 250ml separately funnel and 20ml of diethyl ether was shaken vigorously. The aqueous layer was discarded. The purification process was repeated.

RESULTS AND DISCUSSION

Both the raw and fermented seeds were investigated for moisture, ash, crude fibre, crude protein, crude fats, carbohydrates, minerals, sodium, potassium, calcium, magnesium, copper and zinc, phytic acid, oxalates, tannins and saponins content, were analyzed and the results were recorded and discussed as follows:

Table 1: Proximate Composition (g/100g dry weight) of *Cassia tora*

Constituents	Raw	Fermented
Moisture content	11.50%	27,00%
Ash	4.69%	7.99%
Protein	13.79%	17.21%
Lipids	16.01%	18.36%
Crude fibre	7.78%	11.80
Carbohydrates	58.33%	35.25%

The proximate compositions of raw and fermented seeds of *cassia tora* are shown in Table 1. The seed is a good source of protein, lipids, crude fibre, and carbohydrates. The crude protein content was found to be (13.79 % and 17.21 %) for the raw and fermented seeds respectively. The seed is an excellent source of crude fibre (7.78 % and 11.80 %) for the raw and fermented seeds respectively and Ash (4.69 % and 7.99 %), for the raw and fermented seeds respectively while it contain Low carbohydrates of 33.47% and 21.80%, for both the raw and fermented seeds respectively. The seeds also contain a low level of crude fats (16.08% and 18.36%) for the raw

and fermented seeds respectively. The moisture content was found to be (11.50% and 27%) for both the raw and the fermented respectively. There is a significant increase of about 24.8% of the protein observed in the fermented seeds. Moisture content increased in the fermented seeds of with about 13.4%, while an increase of about 14.68% of crude lipid content was observed. A very significant decreased (about 39.57%) for total carbohydrates were observed with the fermentation. On the other hand, *ash* content showed a substantial increase in fermented seeds of about 59.58 % and the crude fibre increased by 51.37% with fermentation.

Cooking and fermentation fielded a significant increase in protein. That could be explained by bacteria metabolism involving in prateogenesis during fermentation ().[41] Similar proteins increment was reported locust bean *(Parkia bighbosa)* fermentation (Ibrahim and Antai 1986). Bengaly, 2007 reported an increase of 5% of crude protein during fermentation of H *sabdarrifa* to produce Bikalga. In addition to having high protein content, various antioxidants, anti mutagenic and antibacterial properties have been reported in *C. tora* seeds (Choi 1997). Ibrahim and Antai (1986) reported an increase of lipid content during the fermentation of African of locust beans for soumbala production; this is due to a selective utilization of carbohydrate by the microflora during the fermentation.

In fact, a very significant decreased (about 50%) for total carbohydrates were observed after fermentation which collaborated with the observation of Yagoub *et al.,* 2004 and Ibrahim and Antai (1986). This decreased might by mainly linked to two factors: the long cooking where a certain amount of soluble carbohydrates was lost in the cooking water and the fermentation where carbohydrates hydrolyzed into reducing sugars easily utilizable by the microorganisms as source of energy (Odunfa 1985, Yagoub *et al*., 2004). Ash content showed a substantial increase in fermented seeds which reflects mainly the mineral contribution made by liberal add of ash-leachate. The moisture content increased in the fermented seeds probably related to the long cooking period.

Table 2: Mineral Composition of Raw and Fermented Seeds *of Cassia tora*

Element	Raw Sample (mg)	Fermented Sample (mg)
Potassium	0.00085+/_0.01	0.0016+/-0.01
Sodium	0.005+/- 0.2	0.0033+/- 0.0l
Calcium	0.0022+/- 0.01	0.0024 +/- 0.01
Magnesium	0.0028+/-0.02	0.0024+/-0.02
Iron	0.0045+/- 0.01	0.0019+/-0.01
Copper	0.255 +/- 0.01	0.17+/-0.01
Zinc	0.003 +/-0.001	0.0045+/- 0.001

Table 2, shows the results of some of the mineral composition of *Cassia tora* seeds. The seed is a good source of copper, (0.255 +/- 0.01 and 0.17 +/- 0.01) for both raw and fermented seeds. The results indicated a low level of potassium, calcium and magnesium, which is different from what (Siriguri) found who recorded a high level of the metals, The differences could be attributed to the differences in the soil where the weeds grow.[45] There are variations between the values of the raw and the fermented seeds, although the variation is not highly significant. Except for calcium, potassium and zinc which showed a significant increase with fermentation. Some literature

recorded that fermentation resulted in 37% increase in calcium levels in mouldy sorghum with addition of *C. tora* seeds. This may be attributed to degradation of phytates, tannins, trypsin inhibitors and oxalates that are known to bind minerals and decrease their bioavailability. Earlier studies on lactic fermentation of whole wheat flour showed degradation of phytic acid and greater solubility of calcium and magnesium, thus improving their availability.

Table 3: Anti- Nutritional Factors

	Raw	Fermented Sample
Tannin phytic acid	1.08%	0.698 %
	2.11%	1.11%
Oxalate	0.00 %	0.00 %
Saponins	7.20 %	6.71 %

Anti- nutritional factors present in both raw and fermented seeds of *Cassia tora* is represented in Table 3. The results revealed that saponins have the highest percentage content among the anti-nutritionals; it has (7.20% and 6.71%) for the unfermented and fermented seeds respectively. The oxalic acid has 0 % for both the unfermented and the fermented samples. The Sinning contain (1.08% and 0.1%), and the phytic acid contain (2.11% and 1.11%).There was a general reduction in the content of anti- nutritional factors as a results of processing the seeds, via cooking and fermentation. Tannins showed the least decrease (7.41%) with fermentation, phytic acid showed the highest reduction (47%) while the reduction in saponins was about (7.5%) This reduction is in agreement with the results obtained by Ijarotimi.[49] who reported a reduction phytic acid, tannin, oxalate and trypsin after the fermentation of African yam bean**.** Various studies have reported considerable reduction in anti-nutritional factors in traditional legumes by fermentation involving lactic acid bacteria.[4]

No literature was found to have recorded the effect of fermentation on anti nutritional factors in *C. tora* seeds, however, the values obtained in this study are comparably low in the fermented seeds when compared with the values obtained from the raw seeds and this could be attributed to the processing methods (boiling and fermentation) that were employed.

Table 4: Absorbance Working Standard in (ppm) of Serial Dilution of Tannins from Tannic Acid Salt Solution in 1000ppm at 720nm

S/No.	Serial Dilution (ppm)	Absorbance
1	10	0.003
2	20	0.006
3	30	0.053
4	40	0.020
5	50	0.009

From the results obtained the absorbance were indicated as follows 0.003ppm, 0.006ppm, 0.053ppm, 0.020ppm and 0.009ppm for the various concentrations; 10ppm, 20ppm, 30ppm, 40pprn, and 50ppm respectively. There is an increase in the absorbance as the concentration increase, except at 40 ppm where it decrease and rose up at 50ppm.

Fig. 6, is the Graph of absorbance against serial dilution of tannic acid, from 10ppm to 50ppm. The graph is a curve which indicates that the absorbance increased as the concentration increased but decrease around 40ppm concentrations.

Table 5: Absorbance (ppm) of Both Raw and Fermented Seeds of *Cassia tora*

Seeds Sample	Absorbance of Sample (ppm)	Average Gradient (ppm)
Raw Seeds	0.036	28.00
Fermented Seeds	0.025	24.00

From Table 5 above the raw sample indicated an absorbance of 0.036 ppm with an average gradient of 28.00 ppm while the fermented sample indicated an absorbance of 0.025 ppm with an average gradient of 24.00 ppm. These results revealed that there is a little decrease in tannin content in the fermented sample compared with the raw sample.

CONCLUSION

In conclusion, the aim of this research Chemical and Nutrient Analysis of raw and fermented seeds *of Cassia tora* seeds was achieved. The results of the studies have shown that the appreciable value of protein, carbohydrates, fats and minerals contents *of Cassia tora* seeds give it potential usefulness as a good protein source. Better values for protein, crude fats, crude fibre, and mineral and high percentage reduction in the anti nutritional factors for the fermented seeds have made the fermented seeds *of Cassia tora* re commendable as a better source of nutrients in feeds formulation. This also suggests that processing of the seed by boiling and fermentation could improve some nutritional values and enhance the reduction of some anti-nutritional factors.

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