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Effect of Ash, KOH and Millet on the Fermentation of *Parkia biglobosa* Seeds to Form a Condiment

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Abstract: *Parkia biglobosa* seeds were collected from the local market and processed into 'dawadawa', into a local condiment. The process was followed by adding millet, ash and ground potassium hydroxide to determine the effect of these on the fermentation for six days. The food types including, crude protein, fat, carbohydrate, crude fibre, ash and moisture content were determined. Only carbohydrate content was determined by difference. The results showed that pH, crude protein, fat and moisture increased during the period of fermentation, while carbohydrate, crude fibre and ash decreased in content. The mean content of protein was 32.43%, fat (32.43%) and pH (7.68) from the millet treated sample while the others were, carbohydrate (42.73), ash (1.86%) and moisture content (6.53%) from KOH-treated and CF (3.66%) from the raw sample,. The KOH-treated sample produced the least amount of protein and fat but the highest carbohydrate content. In terms of protein content, the rank was millet-treated> ash-treated>raw>KOH-treated. It was found that KOH had a negative effect on the conversion to protein even though it provided an alkaline medium for the process. The effect of millet and ash on fermentation provided the reason for the local people using either but due to relative cost, ash is usually preferred.

Key words: Ash, crude protein, fat, KOH

INTRODUCTION

A food condiment locally known as 'dawadawa' in Ghana, 'iru' in Nigeria and Benin and 'soumbala' in Burkina Faso is obtained from fermented dried seeds of the African locust bean *Parkia biglobosa*. This plant is a perennial leguminous tree which belongs to the sub-family *Mimosoideae* and family *Leguminosae*. It grows in the savannah region of West Africa up to the southern edge of the Sahel zone 13^{o}N (Campbell-Platt, 1980). The tree is not normally cultivated but can be seen in population of two or more in the savannah region of West Africa (Hopkins, 1983). The *Parkia* tree plays vital ecological role in cycling of nutrients from deep soils, by holding the soil particles to prevent soil erosion with the aid of its roots. It also provides shade where it is found (Campbell-Platt, 1980). It is used as timber for making pestles, mortars, bows, hoe handles and seats (Irvine, 1961; Hagos, 1962).

The *Parkia* species is usually preserved by the inhabitants where it grows because it provides valuable sources of food, especially the pulp of the seeds which serve as good nutrition for humans. The seed has also been found to be a useful ingredient for consumption. It has been reported that the husks and pods are good for livestock (Douglas *et al*., 1976; Obiozoba, 1998).

The high cost of animal protein has directed more interest towards that from plant origin since they are other sources of vegetable protein for human and livestock. This is because of the negative effects of cholesterol and other diseases associated with animal-derived foods. Vegetarian diet has become popular in recent years in an attempt to reduce high cholesterol and fat deposits in humans. Among the plant species, grains and legumes are considered as the major sources of dietary proteins. They are consumed world wide, especially in developing and under-developed countries where consumption of animal protein is limited as a result of economic, social, cultural or religious factors (Latunde-Dada, 1997). African locust bean seeds are rich in protein and usually fermented to a tasty food condiment called *dawadawa* which is used as a flavour intensifier for soups and stews and also adds protein to a protein-poor diet (Ikenebomeh *et al*., 1984; Odunfa, 1986; Dike and Odunfa, 2003). In Ghana, the dawadawa trees can be found in Volta, Brong Ahafo, Northern, Upper East and Upper West Regions. The condiment was initially consumed by only people of northern extraction in Ghana and some West African countries such as Burkina Faso, Benin and Nigeria but due to its high nutritious value and other medicinal values, such as lowering of high blood pressure, it has become more popular. It is evident that *dawadawa* has played a major role in the food habits of people in the country particularly among the five regions mentioned above. It serves as a nutritious non meat protein substitute, as a condiment and a flavouring agent in soups.

The objective of this work was to determine the effect of adding selected substances during the fermentation by considering the level of nutritional and anti-nutritional food factors. These selected substances are millet, ash and potassium hydroxide (KOH).

Literature review: Fermentation is one of the most important technological processes in the food industry. It converts the less to the more nutritious forms of the seed by the use of oxidizing agents such as bacteria. The process enhances the nutritional value of processed foods, adds a variety of flavours and extends the shelf life. In Ghana the traditional fermented foods are derived from roots, tubers, cereals, legumes and dairy products such as milk. Alcoholic beverages are also prepared from fermented cereal juice from fruits or liquids from trees (Achi, 2005).

Fermented vegetable proteins have potential uses as protein supplements and functional ingredients in fabricated foods. The local food flavouring condiments are normally prepared by traditional methods which are uncontrolled resulting in extensive hydrolysis of the protein and carbohydrate components (Fetuga *et al.*, 1974; Eka, 1979). Apart from increasing the shelf -life and reduction in the anti-nutritional factors fermentation improves the digestibility, nutritive value and flavours of the raw seeds (Odunfa 1985; Reddy and Pierson, 1999; Barimala and Antai, 1989).

In the household or cottage industry preparation of *dawadawa*, the techniques employed are simple and non-sterile materials are used. This fermentation relies on natural inocula under uncontrolled fermentation conditions. This uncontrolled fermentation can lead to inconsistent products and shorter shelf life (Latunde-Dada, 1997). However, in modern industrial processing, processes are more closely monitored and controlled with direct inoculation with isolated and purified microorganisms. In this case longer shelf life is obtained for the commercial product. The studies of Antai and Ibrahim (1986) and Odunfa (1985) found several microorganisms associated with *dawadawa* but the most abundant and the major agent of fermentation after 72 h was *Bacillus subtilis*. These bacteria have also been identified as the agent for the fermentation of soybean into Japanese *natto*. Other microorganisms that were present after fermentation included *Leuconostoc mesenteroides and Staphylococcus spp*. (Antai and Ibrahim, 1986). Fermenting to produce the condiment was found to be exothermic and the pH also increased during the period (Konlani *et al.*, 1999). During fermentation soluble amino acids, namely glutamic acid is liberated. The salt of this amino acid, monosodium glutamate is used widely as an additive to enhance flavour (Odunfa, 1985). *Dawadawa* is also an important source of Vitamin B in the form of riboflavin which is generally deficient in most African diet. D*awadawa* contains the highest riboflavin content when compared to 33 common plant foods, about 0.80 mg per 100 g, (Campbell-Platt, 1980).

MATERIALS AND METHODS

Analysis of physicochemical parameters: Very healthy African locust bean seeds were obtained from the market since the season for harvesting had run out. Damaged seeds were hand picked and the good and healthy ones were then washed, cleaned and soaked in distilled water for 24 h to remove any inhibitory materials. They were then boiled for another 24 h with constant replacement of water. After cooling, the seeds were gently pounded in a porcelain mortar and washed with water to remove the testa from the cotyledons. The cotyledons were then parboiled for another 4 h and then allowed to cool to a temperature of 30°C. The pounded material was divided and put into four different sacks labeled Ash, Mil, KOH and R with the following treatment. A quantity of wood ash was sprinkled on the pounded seeds in sack labeled Ash, millet flour in another labeled Mil and KOH powder in the last labeled KOH. These were added as inoculants for the fermentation process. Sack R was to serve as a control where nothing was added. The sacks were then kept in a warm dark place for fermentation at room temperature. Small quantities were fetched from each sack everyday for 6 days and ground into paste for the analysis. The parameters analyzed included pH moisture content, Crude Protein (CP), Crude Fibre (CF), ash, carbohydrate and fat content.

The pH was determined by grinding 10 g and dissolving in 10 ml of distilled water and left for about 30 min before reading.

For the moisture content, about 2.0 g of each of the samples obtained from the sacks were accurately weighed into dried and pre-weighed glass crucibles. They were then dried in an oven at 105°C overnight until constant weights were obtained. Difference in weight between dried and original samples was taken and converted into percentage values.

An automated solvent extraction machine using diethyl ether as solvent for recovering the fat and oil was employed for the extraction of the fat and oil. The equipment used was the solvent recovery extractor for fats and oils.

For the analysis of crude protein about 2.0 g of each sample was digested using 0.9 g of Na_2SO_4, 0.8 g of $CuSO_4$ and concentrated H_2SO_4 on a hot plate in a fume chamber for 2 h. After complete digestion, the sample was transferred into a 100 ml volumetric flask and made up to the mark. About 20 ml were pipetted and distilled in Kyedahl apparatus and repeated three times. The %N for each sample was obtained and multiplied by 6.25 to obtain the percentage protein. Each of the defatted samples was transferred into different 750 ml Erlenmeyer flasks and 0.5 g of asbestos was added to each of them. About 200 ml of boiling 1.25% H_2SO_4 was added to the content in each of the flask and immediately placed onto a hot plate. After 30 min of boiling, the flasks were removed from the hot plate and the content in each of the flask was filtered using linen cloth in a funnel and washed back into the flask with 200 ml boiling 1.25% NaOH. The boiling was repeated one more time. The residue was transferred into a dried and weighed porcelain crucible and dried in an oven at 100°C for 1 h and later cooled in a desiccator and weighed again. The crucible and its content were ignited in an electric furnace at 600°C for 30 min cooled and reweighed. The loss in weight was reported as percentage crude fibre.

Two grams (2.0 g) of each sample was accurately weighed into a pre ignited and weighed porcelain crucible placed in a muffle furnace and ignited for 2 h at 600°C. After ashing, the crucibles were cooled to about 105°C in a forced convection oven before cooling them further to room temperature in a desiccator. The crucibles and their contents were weighed. The carbohydrate content of the product was obtained by subtracting percentage ash, fibre, protein, fat and moisture from 100.

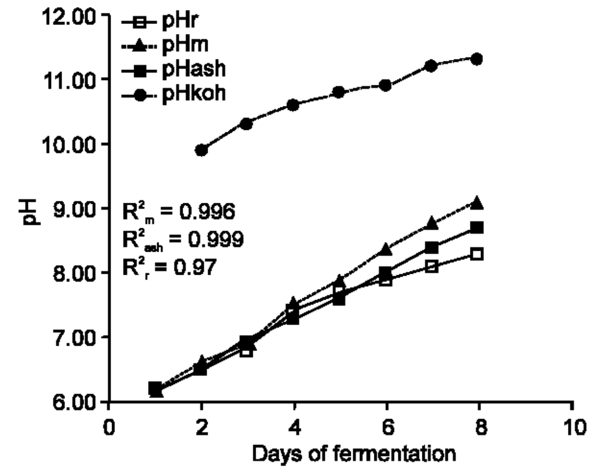

Fig. 1: Variation of pH with fermentation period

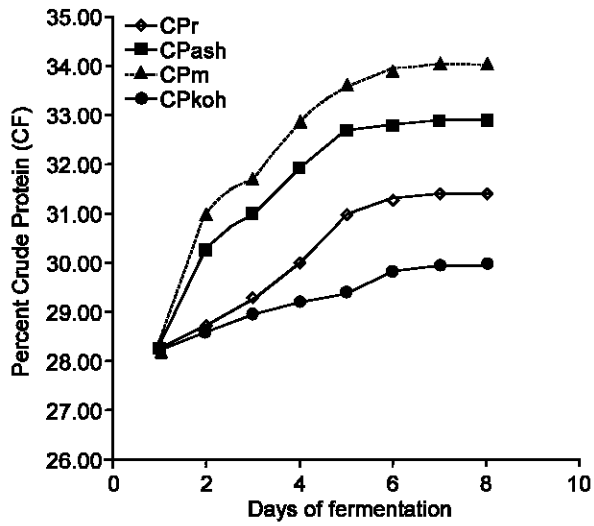

Fig. 2: Variation of Crude Protein with fermentation period

RESULTS

There was a general increase in pH, (6.2-9.1) protein and fat content in all the samples during the fermentation period with all the three treated and the non treated samples. However carbohydrate, fibre, ash and moisture contents of the seeds decreased during the fermentation period. The increase in pH is more pronounced in the last days of fermentation than the beginning. Figure 1 shows the variation of pH with fermentation period..

The effect of KOH on pH is observed by its movement of the lines to a definite distance and parallel to them. There is an increase of pH with period of fermentation even with the addition of KOH. Even though KOH is highly alkaline and could have some suppression effect, the continuous increase in pH suggests that a reaction is occurring resulting into the release of ammonia which reacts with water to increase the pH. This could be termed an alkaline reaction. The ash-treated sample was next to the millet while the raw sample followed in that order. There was a general increase in crude protein from the first to the last day. The seeds treated with KOH produced the least amount of protein per day while the millet-treated sample produced the highest.

This suggested that the KOH sample had a negative effect on the conversion of the seed to protein. The highest percentage values for protein from the various samples were; 29.98% (KOH), 31.40% (raw), 32.90% (ash) and 34.07% (millet). Comparable fat contents were observed in the samples with ash, millet and the raw sample.

The sample with KOH had the least fat content and the least protein after the seven days fermentation period but with the highest carbohydrate content of 42.44%. The millet-treated sample had the least amount of carbohydrate (39.58%) but the highest protein (34.07%) among all the four samples were; 32.90% (ash) and 34.07% (millet). From the means, the samples can be ranked as KOH-treated< Raw<Ash-treated <Millet-treated samples. This supports the reason for traditional methods employing millet as an inoculant. Ash-treated sample ranked next to millet and because it is cheaper, it is usually preferred as an inoculant.

High correlation coefficients were observed for all the different treatments.

A correlation coefficient of over 0.95 was observed between the protein and carbohydrate contents in all the samples.

The sample with the millet recorded the highest correlation coefficient of 0.985 followed by the sample with ash (0.982) and the raw sample (0.968). The sample with KOH had the least correlation coefficient of 0.962.

These high correlation coefficients indicate that the increase in the protein content during the fermentation

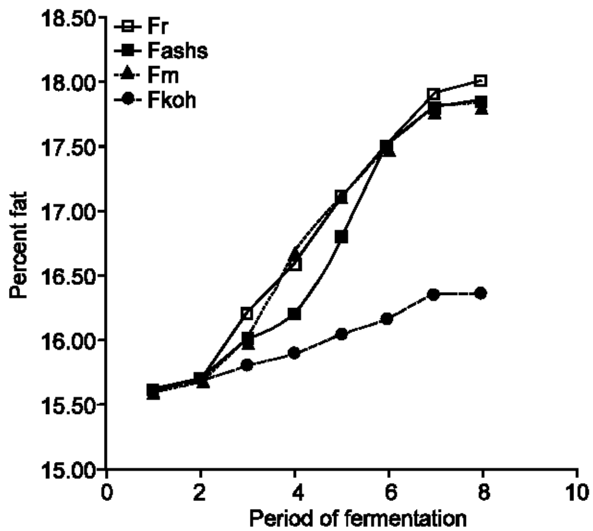

Fig. 3: Variation of fat content with fermentation period

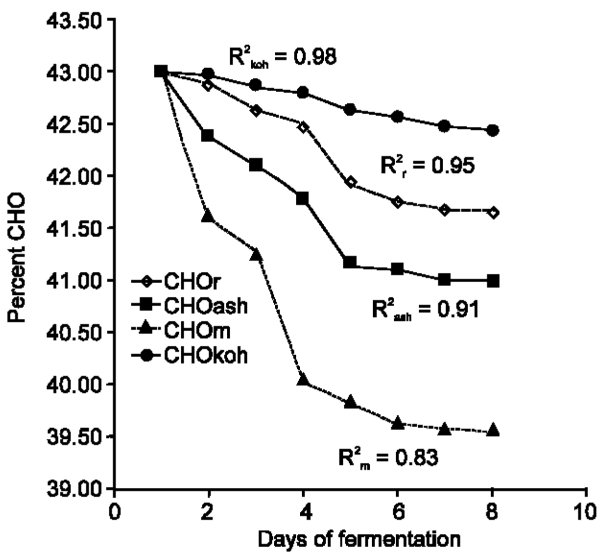

Fig. 4: Variation of Carbohydrate with fermentation period

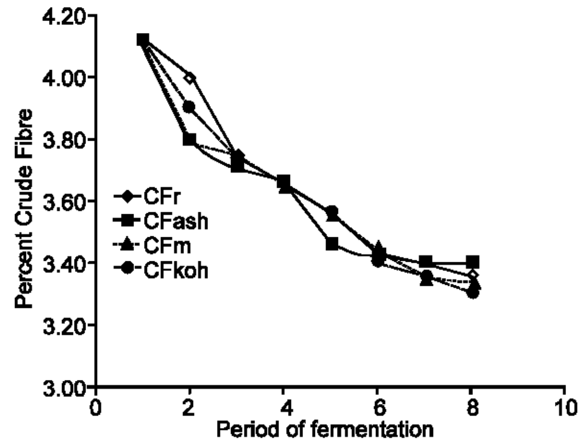

Fig. 5: Variation of Crude fibre with fermentation period

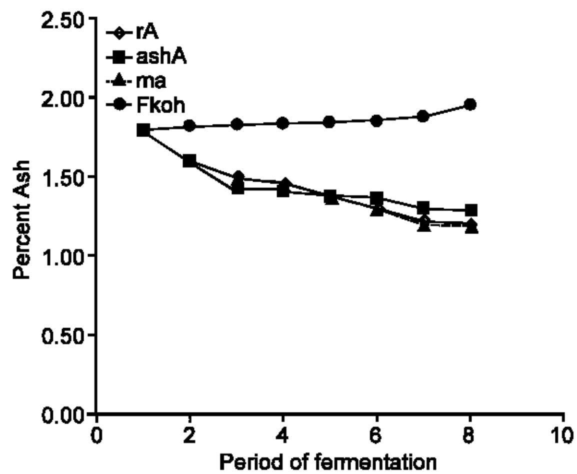

Fig. 6: Variation of Crude fibre with fermentation period

period was related to the amount of carbohydrate in the seed.

Possibly the carbohydrate could have been used as a binding agent so that as the intensity of fermentation increased, more of the bound food nutrients were released. Similarly high correlation coefficients were observed between pH and protein content in all the samples. The raw sample had the highest coefficient of 0.974 followed by the sample with the millet (0.821) and the sample with the ash (0.816). The ash-treated sample produced similar levels of protein and other food nutrients. Ash provides a weaker alkaline medium which is more preferred for fermentation than the KOH.

The KOH-treated sample had the least coefficient of 0.678 which re-enforces the suggestion that a very high alkaline medium provided by KOH may not be conducive to the fermentation process.

However, ash is weakly alkaline but produced similar levels of protein and other food nutrients and therefore supports the fact that the process may require an alkaline medium to some extent.

DISCUSSION

The increase in pH during the six days period was in agreement with earlier findings by others (Konlani *et al.*, 1999). Samples treated with KOH had the highest increase in pH than the others. The KOH treated sample increased in pH was due to the fact that it is a highly basic compound and the plots show a direct shift of its addition. However, the increase in pH of the other samples is attributed to the conversion of the amino acid in the fermented material where ammonium ion is released and causes an increase in the pH of the medium as indicated below:

$$RCH(NH_3)CO_2^- \rightarrow {}^-O_2CCH_2CH_2CH(NH_3^+)CO_2^- \rightarrow NH_4^+$$

This process is called deamination and it is catalyzed by enzymes called aminotransferases where there is transfer of α-amino groups from α-amino acids to form α-ketoacid (Berg *et al.*, 2006). During the deamination processes various volatile compounds are released together with ammonia which results in producing odour characteristic of the fermented product. Increase in the fermentation of the seed, to an increase in the amount of NH_3 released and a resultant increase in pH. The release of ammonia and other gases may be responsible for the characteristic odour of the fermented product.

The percentage Crude Protein (CP) of the seed increased through out the fermentation period. The KOH treated sample produced the least crude protein while the millet treated sample was maximum followed by the ash-treated and the raw samples. This suggests that millet had a more enhanced effect on the fermentation of the seed followed by ash. The mean percentage crude protein of 3.43% was observed in the millet-treated sample at mean pH of 7.68 at a corresponding value of 29.98% at pH of 9.9 for KOH-treated sample. The millet treated sample was 13.64% higher than the KOH-treated . The ash-treated sample was 32.9% at pH value of 8.7 and was about 9.70% higher while the raw was over 4.74% higher than the KOH-treated sample. This suggested that the effect of adding KOH rather reduced the amount of CP. From these results the added millet flour provided a better working environment needed for the fermentation process than the others. This was followed by the ash.

The results of this work have shown that *dawadawa* is a rich vegetable food material. The mean range values of the food nutrients are contained in Table 1.

The CP values compare favourably with results by Alabi *et al.* (2005) who obtained mean value of 34.02%. Other workers had similar values earlier, Omafuvbe *et al.* (2004) had 30.2%, Okpala (1990) had 31.60%, Alabi (1993) had 35.00% and Obiozoba (1998) also had 34.3%. The protein and fat contents of *dawadawa* is comparable to that of soybeans. *Dawadawa* is made up 34.07% protein and 18% fat whilst soybeans are made up of 36.5% protein and 19.9% fat. The carbohydrate content of all the samples decreased during the period of fermentation. The KOH-treated sample contained the highest amount of carbohydrate while the millet- treated sample contained the least. The highest carbohydrate obtained in this work was 42.73% but ranged from 40.58% from millet-treated to 42.73% (KOH-treated) samples. Alabi *et al.* (2005) also obtained the carbohydrate content as 20.7% and 25.66% soluble sugar making a total of 46.36%. Millet by its chemical composition has carbohydrate which could be an additional source to enhance its quantity in the seed when added but this seemed not to be the case. On the other hand, ash is mildly alkaline but its effect on the fermentation was more pronounced than that of the KOH. This suggests that the fermentation process does not require highly alkaline conditions such as the KOH medium. This provides further evidence that KOH negatively affects the fermentation of the *P. biglobosa* seed. This probably explains the reasons why traditional people use these as their fermenting agents to enhance the process.

The fat content of the raw sample and on the first day was 15.6% and it increased with fermentation period for all the different treatments. The raw sample registered the highest fat content during the period of seven days. The fat content of the raw sample of this study was 28.25% which is higher than that found by Omafuvbe *et al.* (2004) who had a value of 20.9% on the day before fermentation started but the other values (29.17-32.43%) were comparatively similar to their study (20.9-37.2%). The fat content of the millet-treated and that of the raw samples were similar from the beginning until towards the end suggesting that their effect was similar unlike the KOH which might have had a negative effect. The highest mean fat value obtained was 16.83% in the raw sample followed by that treated with millet. These values were similar to those found by Alabi *et al.* (2005) who obtained about 16.86% while Omafuvbe *et al.* (2004) obtained 17.7% from the raw but a higher value (37.2%) after three days for the fermented seed. However, the KOH-treated sample produced the least amount of fat. The increase in fat content could have been enhanced by the heating and soaking since this caused the weakening of the bonds in the seeds thereby making the extraction by solvent easier.

Crude fibre generally decreased from a maximum of 4.12-3.30%. However, the crude fibre of the raw seeds was 7.0%. Soaking, boiling and removal of the testa would have reduced it to 4.12% and fermentation would have further reduced it to a lower value of 3.30%. Crude fibre found by Omafuvbe *et al.* (2004) also was in the same range as contained in this paper. These values are generally low and suggest that P. *biglobosa* seed may have low anti nutrient content since crude fibre is also one measure of the amount of anti-food nutrient content. The raw sample had the highest crude fibre and this could be attributed to the fact that some of the fibre might have been converted to other food substances by the action of some enzymes.

It was observed that the moisture content also varied with the period of fermentation. The KOH-treated sample had the highest moisture content while the other samples treated with ash and millet were the lowest. The untreated sample was slightly above the ash and millet. Potassium hydroxide absorbs moisture from the atmosphere and this could have accounted for the high moisture content observed. The moisture contents of the

Table 1: Range of means of nutrients of the various treated samples

Item	% Range	Sample with minimum	Sample with maximum
Crude Protein	29.27-32.43	KOH-treated	Millet-treated
Carbohydrate	40.58-42.73	Millet-treated	KOH-treated
Fat	15.99-16.83	KOH -treated	Raw
Crude Fibre	3.62-3.66	Ash-treated	Raw
Ash	1.43-1.86	Raw and Millet-treated	KOH-treated

sample treated with millet and the untreated sample were similar from the beginning to the end of the experiment suggesting that the moisture content was not affected by the addition of the ash and millet during the fermentation. However, moisture content of all the samples decreased with fermentation period.

The ash content was generally low, less than 2% and it decreased but to a lesser extent than that of the moisture content. However, the sample treated with KOH remained constant throughout the period suggesting that the KOH had no effect on the ash content. The others were relatively lower but there was a slight decrease with the period of fermentation.

The seed contains all the food nutrients but these are not available until it has undergone some process of conversion into the various nutrient types through the action of enzymes or catalysts and heat.

Conclusion: The results of this work have shown the effect of adding external agents as inoculants. The pH increased throughout the fermentation period irrespective of the type of agent used as inoculant. The *Parkia biglobosa* had mean values of 34% protein, 18% fat and 39.58% carbohydrate. The protein and fat contents of *dawadawa* are comparable to that of soybeans. It was found that millet and ash-treated samples had a greater effect on fermentation than KOH which had a negative effect. However, millet was found to be better than ash in producing protein by fermentation. But ash is usually preferred by most people because of its low cost, while one has to choose between using millet for food or for fermentation of *Parkia biglobosa* to make dawadawa.

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