

Effect of period of fermentation on nutrients of Castor oil seed (*Ricinus communis*)

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Research Paper

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Castor oil seed (*Ricinus communis*) was dehulled, boiled for 2 h and wrapped in a banana leave. The wrapped cotyledon was placed in a basket and allowed to ferment over a four day period. On daily basis, the package was unwrapped to remove 250 g of the fermenting cotyledon which was directly exposed to steam for 1 h and dried at 60°C for 24 h in order to terminate the fermentation process. The fermented samples were labeled BF, BF₂, BF₃ and BF₄ respectively. Raw seed (R) served as control sample. The samples were analysed for proximate composition, minerals, available lysine and glutamic

acid. Samples exhibited increase in moisture, crude protein, crude fibre, carbohydrate, K and Zn with increase in period of fermentation. The available Lysine content significantly increased (P<0.05) only on the fourth day of fermentation. The fermented product (Ogiri) possibly owes its culinary role as a traditional flavouring condiment in Eastern Nigeria to the presence of sodium and glutamic acid.

Key words: Castor oil seed, period of fermentation, proximate composition, lysine, glutamic acid.

INTRODUCTION

Fermented foods, whether from plant or animal origin, are an intricate part of the diet of people in all parts of the world. Fermented food plays a very important role in the socio-economics of developing countries. Each nation has its own types of fermented food, representing the staple diet and the raw ingredients available in that particular place. Fermented foods contribution to the protein requirements of the rural population. According to Steinkraus (1995), the traditional fermentation of foods serves several functions, which includes: enhancement of diet through development of flavour, aroma, and texture in food substrates, preservation and shelf-life extension through lactic acid, alcohol, acetic acid and alkaline fermentation, enhancement of food quality with protein, essential amino acids, essential fatty acids and vitamins, improving digestibility and nutrient availability,

detoxification of anti-nutrient through food fermentation processes, and a decrease in cooking time and fuel requirement. This is necessary therefore to improve the prevailing cases of malnutrition in Nigeria (Steinkraus, 1995).

Reduction in anti-nutritional and toxic components in plant foods by fermentation was observed in a research (Achinewhu, 1985) which showed " Cereals, legumes, and tubers that are used for the production of fermented foods may contain significant amounts of anti-nutritional or toxic components such as phytates, tannins, cyanogenic glycosides, oxalates, saponins, lectins, and inhibitors of enzymes such as alpha-amylase, trypsin, and chymotrypsin. These substances reduce the nutritional value of foods by interfering with the mineral bioavailability and digestibility of proteins and

carbohydrates. In natural or pure mixed-culture fermentations of plant foods by yeasts, molds, and bacteria, anti-nutritional components (e.g. phytate in whole wheat breads) can be reduced by up to 50%; toxic components, such as lectins in tempe and other fermented foods made from beans, can be reduced up to 95%.

Plant protein and oil seeds used in livestock feed formulation over the years have suffered serious competition between man, livestock and agro-industries. Hence, there is a need to explore alternative protein sources to reduce the competition. Castor oil bean (*Ricinus communis*) is grown in tropical and temperate regions, where the dehulled seeds are processed for use as flavour-enhancer in sauce and soup.

Fermented foods are essential components of the diet in many countries and are consumed either as main dishes or as condiments (Steinkraus, 1996). Indigenous fermented foods were developed through traditional technologies which were preserved over the years in order to maintain their uniqueness and identity (Valyasevi and Rolle, 2002). They are prepared from both plant and animal materials, using processes in which microorganisms play active roles in the physical, nutritional and organoleptic modification of the starting materials (Aidoo, 1994).

Among traditional condiments used in Eastern part of Nigeria include: fermented products of *Ricinus communis* (Ogiri-Igbo), *Pentaclethra macrophylla*, (ugba/ukpaka), and *Parkia biglobosa* (ogiri-okpi). Also spices used traditionally include: *xylopia aethiopica* (Uda), *Piper guineense* (Uziza) and *Monodora myrsitica* (ehuru). The bulk of the indigenous fermented condiments of Nigeria are found in the southern states of Nigeria. Interstate trade and relocation has however, widened the scope of the spread throughout the country and beyond (Iwuoha and Eke, 1996). „Iru“ and „Ogiri“ have played major roles in the food habits of communities in the rural regions serving not only as a nutritious non-meat protein substitute but also as condiments and flavouring agents in soups and sauces (Achi, 2005). They have potential good uses as protein supplement and as a functional ingredient. Soups are the main sources of protein and minerals and one of the ways to improve the diet is to improve the nutrient content of soups, According to (Steinkraus, 1985), the traditional fermented foods contain high nutritive value, better digestibility and developed a diversity of flavours, aroma and texture in food substrates.

In addition 'iru' and 'ogiri' contribute protein, minerals and calories in the diets (Fetuga et al., 1973). Legumes and oil seeds are fermented by allowing the microorganisms to act on them through enzymatic activity to yield condiments by the extensive hydrolysis of carbohydrate and protein components (Fetuga et al., 1973) and (Eka, 1985). Apart from reduction in the anti-nutritional factors, fermentation markedly improved the digestibility, nutritive value and flavours of the raw

seeds (Odunfa, 1995) and (Reddy et al., 1986). Although „iru“ and „ogiri“ condiments constituted significant proportion of the diet of many people, they are associated with some problems such as having a short shelf life, objectionable packaging material, the characteristic putrid odour.

These spices and condiments apart from their culinary role as flavouring agents in foods have other functional uses as: raw materials for industries and pharmaceutical uses. The fermented product of castor oil seed (ogiri-Igbo) is used as a condiment in bitter leave soup, oha soup and in source used for preparing African salad (abacha). Castor oil bean is a poisonous seed of the castor bean plant, *Ricinus communis* and belongs to the class of a plant called *Euphorbiaceae* and family *Angiosperm*. The castor oil plant probably indigenous to the south-eastern Mediterranean region and parts of East Africa is today widespread throughout the tropical regions of the world (Ibe and Orabuiké, 2009). The castor oil bean is inedible because the seed contains a toxic protein, ricin and other toxic constituents, ricinine and ricinoleic acid but these can be removed by fermentation (Odunfa and Oyeyiola, 1985). The fat content of the seeds of *R. communis* is about 15 to 25% and consists of about 40-53% of fixed oil comprising glycosides of ricinoleic, isoricinoleic, stearic and dihydroxy stearic acids (Lin and Areinas, 2007). Also the seeds contain about 25% protein with 10-20% carbohydrates, 2.2% ash and 5.1-6.5% moisture (Verscht et al., 2006). Castor oil seed, *Ricinus communis*, is one of the well-known oil seeds in Africa which forms important part of the diet.

It was also reported that the toxic ricin in the castor oil meal is eliminated by fermentation (Lugmer, 1952; Odunfa, 1985). Other advantages of fermentation includes: increase in bioavailability of the nutrients evident in the fermented African Locust bean seed (Eka, 1980). Moreover, the digestibility, the protein efficiency ratio (PER), net protein utilization (NPU) and biological value (BV) of fermented locust bean were higher than that of the raw locust bean (Umoh and Oke, 1974).

It was observed that the entire microorganism isolated in the fermented castor oil seed were proteolytic in their activities with *Bacillus subtilis* as the most predominant (Odunfa, 1985). Also *Bacillus subtilis* group has frequently been found to be involved in the fermentation of similar vegetable proteins notably African locust bean and African oil bean (Odunfa, 1986).

The aim of this present study is to monitor the changes in the proximate composition, minerals, available lysine and glutamic acid contents during the fermentation of castor oil seed into ogiri.

MATERIALS AND METHODS

Source of raw material

Castor oil seed (*Ricinus communis*) used for this study

was bought and certified at the Crop Science Department of the University of Nigeria, Nsukka.

Sample preparation

The samples were prepared by the modification of the traditional method of fermenting vegetable proteins described by Odunfa (1985). The castor oil seeds were dehulled and then sorted and then sorted to remove bad and unwanted materials. The cotyledons were separated from the shells by handpicking, and boiled for 2 h. After boiling, the cotyledons were wrapped in banana leaves, kept under tropical room temperature (27-28°C) and fermented. On daily basis, 250 g was removed from the wrap and labeled BF₁, BF₂, BF₃, and BF₄ have been fermented for 1, 2, 3 and 4 days respectively. Fermentation was terminated in each sample by steaming the fermented seeds for 1 min. The fermented seeds were then dried in an air oven at a temperature of 60°C for 24 h. The fermented-dried seeds were then put into different sample bottles and stored in a refrigerator at 4°C until required for analysis.

Methods of analysis

Mineral determination

The method of AOAC (2005) was used. Two grammes of sample was weighed into a crucible and ashed in a muffle furnace at 550°C for 6 h. The ash was cooled and 6N HCl added and boiled for 10 min, cooled and filtered into 100 ml volumetric flask. The crucible was washed with distilled water and the washings added to the ash filtrate. The ash filtrate was then made up to 100 ml with distilled water. An aliquot of the filtrate was aspirated into the atomic absorption spectrophotometer (Pye Unicam) and the absorbance values corresponding to the different minerals recorded against standard solutions of Na, K, Fe, Cu, Zn and P. The percentage of the elements in the samples was calculated from the absorbance values of the samples and standard solutions.

Available lysine content determination

The effect of boiling at different time and the effect of boiling at various temperatures on the available lysine content of the 2 day fermented sample were investigated. 500 mg of milled sample was weighted into different test tubes and 10 ml of distilled water added into each tube. The samples were boiled at different time that varied between 20 to 60 min in a water bath. Also 500 mg of milled sample was weighted into different test tubes and 10ml of distilled water added into each tube, and heated for 30 min at temperature ranging from 40 to 100°C in a water bath. After heating the tubes were cooled and shaken at a medium speed (280-300 cycles per min)

using Eberbach 2- speed automatic shaker for 40 min. The tubes were centrifuged at 2000 rpm for 10 min. The supernatant obtained were pipetted into different clean dry test tubes and the available lysine determined (Obi, 1982).

Glutamic acid content determination

A modification of the method described by Bailey and Swift, (1970) was used. Two grammes of sample flour was extracted with 40 ml of distilled water in a 500 ml volumetric flask by shaking the mixture continuously for 40 min at a medium speed (280-300 cycles per min) in an Eberbach 2 – speed automatic shaker. The mixture was filtered and the filtrate centrifuged at 2000 rpm for 20 min using SME high speed centrifuge to obtain the supernatant. Standard solution of glutamic acid was prepared by dissolving 100mg of glutamic acid powder with a little quantity of distilled water in a 100 ml volumetric flask, and the volume made up to 100ml mark with distilled water. This prepared solution contains 1mg/ml of glutamic acid. An aliquot was taken from the stock solution above and diluted 0-10 times using distilled water.

About 0.01 ml aliquot of the sample water extract and standard solution of glutamic acid were spotted at different points (3 cm apart) at the origin of a prepared chromatographic paper and air-dried. The spotted paper was then developed for 2 h in a mixture of n-propanol water (7:10) solvent. After development the chromatogram was air-dried at room temperature (25°C) for 1 h, sprayed with Ninhydrin stain and allowed to dry. The chromatogram was further dried in an air-oven at 75°C for 15 min. The R_f values of the developed spots were calculated. Two different methods were used to quantify the glutamic acid content of the sample. The method described by Shellard, (1968), and the absorbance technique. The area of the spot was calculated, the major and the minor axes of the spot were measured and their product taken as an estimate of the area of the spot which is directly proportional to the concentration of glutamic acid in the spot of the sample. The concentration of glutamic acid was extrapolated from the standard curve of glutamic acid.

Statistical analysis

Data obtained from the study were subjected to analysis of variance (ANOVA) using a statistical software SPSS version 17.0 and the means were separated using Fisher LSD and judged significantly different at 95% confidence level (i.e. P<0.05).

RESULTS AND DISCUSSION

The proximate composition of the sample is shown in

Table 1. Proximate composition (%) of the raw and boiled – fermented castor oil seed.

Sample	Moisture	Fat	Protein	Ash	Crude fibre	Carbohydrate
R	17.00 ^a	22.67 ^a	23.00 ^a	2.98 ^a	6.85 ^a	27.50 ^a
BF ₁	27.00 ^b	20.76 ^b	21.40 ^b	3.07 ^b	6.42 ^b	21.35 ^b
BF ₂	29.00 ^b	21.25 ^b	22.00 ^b	3.26 ^c	6.10 ^b	18.39 ^c
BF ₃	30.00 ^b	21.70 ^a	22.50 ^b	3.37 ^b	5.25 ^c	17.18 ^d
BF ₄	31.00 ^b	22.30 ^a	22.90 ^a	3.47 ^e	5.15 ^c	15.18 ^e

The values are means of triplicate determinations. Means on the same column bearing different superscripts are significantly different ($p < 0.05$).

(Table 1). The moisture content of the product increased as the period of fermentation was increased from day one to day four as evident from the (Table 1). The increase in the moisture content after boiling the seeds and fermenting for one day could be attributed to the moisture absorbed by the seeds during the boiling process. Moreover, the subsequent increase in moisture content as fermentation progressed could be attributed to hydrolytic decomposition of the product during fermentation. From the statistical analysis the unboiled - unfermented sample (R) exhibited a significant difference in moisture value compared with other samples ($p < 0.05$). However, the difference in the moisture content of the fermented samples were not significant ($p > 0.05$). The result of moisture content agrees closely with the values reported by (Odunfa, 1985).

The crude fat content of the product decreased by about 1.81% after boiling and fermenting the product for one day. However, the decrease in the fat content after boiling and fermenting for one day may be attributed to loss due to leaching during boiling.

However, the increase in the fat content as the length of fermentation was increased may be associated with the increased activity of lipolytic microorganism which occurred at the later stage of fermentation of castor oil seed (Odunfa, 1986). This observed increase in fat content of the product is similar to the observation made by Okeoma and Oyeleke, (1988) when African locust bean seeds were fermented for the production of 'iru'. The high lipid content of this product is of some nutritional significance considering the high calorific value of lipids. Moreover, castor oil bean product is known to be rich in Linoleic and oleic acids (Adefarati, 1985). The product could possibly serve as a source of these essential fatty acids in addition to enhancing the flavour of the soups and sauces prepared with it.

Castor oil seed showed similar value in protein content with most dry legume seeds. Its protein content that decreased from 23.00% in the raw seeds to 22.90% in the boiled-fermented product compares with the protein content of Bambara groundnut and soybean (Oke, 1965; FAO, 1969). The protein content of castor oil seed decreased from 23.00% in the raw seed to 21.40% in the boiled-one day fermented seed but subsequently a gradual increase in the protein content was observed with increase in periods of fermentation. However, statistical

analysis indicated non significant ($p > 0.05$) change in protein between samples R, BF₃, and BF₄. The initial low protein content of the product is in agreement with the result reported on castor oil seed that was cooked and fermented for 3 days (Anosike and Egwuatu, 1981). The initial decrease in the protein content may be associated with nutrient loss due to leaching. According to Anosike and Egwuatu, (1981) the decrease in the protein was only determined at the end of the 3 day fermentation period. However, they did not monitor what happened to the protein content at different days of fermentation, and thus suggested that the microorganisms responsible for fermenting the seed must have used part of the protein of the seed for their metabolic activities. It was observed that the crude protein content started increasing gradually as the length of fermentation increased from day two to day four. However, after 4 days fermentation the crude protein content of the fermented seed was still lower than that of the unfermented seed but the difference was non significant ($p > 0.05$). The observed gradual increase in the protein content with increase in length of fermentation may be due to the activities of predominant proteolytic microorganisms that hydrolysed the product to different free amino acids (Odunfa, 1985). Also the microorganism that brought about the fermentation being protein in nature may have contributed to the observed increase in the protein content of the product. The high protein content of the product is noteworthy since it could contribute to dietary protein in addition to its flavouring role.

Total ash was also observed to increase with fermentation period.

Table 2 represents the mineral analysis of the sample. The mineral analysis indicated that some major trace elements occur in different concentrations. Some elements like sodium, iron, copper and phosphorus increased as the period of fermentation increased, while potassium and zinc decreased.

Fermentation markedly improves digestibility, nutritive value and flavour of raw seeds or the seeds cannot be consumed in their raw state. In Africa, fermented foods play a major role in the diet, whereby many staple foods undergo fermentation before they are consumed (Kpikpi et al., 2009). Fermented food condiments enhance flavour and improve the protein content, essential amino acids and fatty acids of foods.

Table 2. Mineral content (%) of raw and boiled-fermented samples.

Sample	Moisture	Fat	Protein	Ash	Crude fibre	Carbohydrate
R	0.0123	0.6139	0.0025	0.0197	0.5190	0.1107
BF ₁	0.0133	0.5244	0.0030	0.0193	0.5190	0.1464
BF ₂	0.0153	0.5003	0.0037	0.0173	0.5236	0.1524
BF ₃	0.0162	0.4980	0.0038	0.0172	0.5318	0.1821
BF ₄	0.0171	0.4625	0.0040	0.0168	0.5319	0.2286

Table 3. Lysine extractable by ethanol – sodium hydroxide mixture and lysine extractable by water.

Sample	Ethanol-sodium hydroxide extract		Distilled water extract	
	Lysine (%)	L/P ratio	Lysine (%)	L/P ratio
R	1.27 ^a	5.52	1.22 ^a	5.20
BF ₁	1.06 ^b	4.90	0.82 ^b	3.84
BF ₂	1.08 ^b	4.91	0.87 ^b	3.96
BF ₃	1.11 ^b	4.93	0.99 ^b	4.41
BF ₄	1.26 ^a	5.50	1.09 ^c	4.76

Date represents means of triplicate determinations. Means on the same column bearing different superscripts are significantly different ($p < 0.05$).

Table 4. Concentration of glutamic acid in the raw-unfermented and boiled-fermented samples.

Sample	Concentration by areas of the spot (%)	Concentration by absorbance of the spot (%)
R	0.92 ^a	1.032 ^a
BF ₁	0.68 ^b	0.865 ^b
BF ₂	1.36 ^c	1.460 ^c
BF ₃	1.92 ^d	1.950 ^d
BF ₄	2.28 ^e	2.340 ^e

Date represents means of triplicate determinations. Means on the same column bearing different superscripts are significantly different ($p < 0.05$).

Proteolysis is the most principal and complex biochemical event occurring during the preparation of some legume based fermented condiments. The degradation products, amino acids, not only have a considerable influence on the nutritional values, but also contribute directly to the taste characteristics, in some cases serving indirectly as precursors of aromatic products (Han et al., 2004). Fermented foods also contribute to food security in developing countries like Nigeria.

The result of the available lysine and lysine protein ratio (L/P) is presented in (Table 3). The lysine content of the sample increased significantly ($p < 0.05$) as the period of fermentation increased. The sample exhibited a decrease in the content of available lysine after boiling and fermenting for one day. This decrease may be attributed to loss due to leaching. However, the lysine content increased gradually with increased length of fermentation but the increase was not significant ($p > 0.05$) until after 4 day fermentation period. The gradual increase in the content of available lysine with fermentation may be due to the presence of the microorganism that brought about fermentation in the product which is protein in nature. Also the proteolytic microorganisms must have

hydrolysed the protein to different amino acids including lysine. The lysine observed in the product is noteworthy considering that lysine is one of the limiting amino acids in cereals and root/tuber crops (Ihekoronye and Ngoddy, 1985). Therefore since the product is used in preparing soups and sauces eaten with roots/tubers and cereal based diets, the lysine in the product would supplement the limiting lysine in such diets. The lysine protein ratio (L/P) of the sample increased with increased length of fermentation. This suggests that the protein content of the sample showed a similar trend with the available lysine content. Sodium hydroxide – Ethanol mixture extracted more lysine from the product than distilled water.

The glutamic acid content of the samples significantly increased as the period of fermentation increased ($p < 0.05$). Monosodium glutamate (MSG) is used to flavour foods (Onajomo, 1983). Since the level of sodium increased with increased period of fermentation as evident from (Table 2) and concentration of glutamic acid also increased with increased length of fermentation, there is the possibility of the sodium salt of glutamic acid increasing in the product as the period of fermentation increased (Table 4).

Conclusion

Fermentation increased the nutrient composition of castor oil seed, which increased as the period of fermentation increased. Also, the glutamic acid content had a similar increase, which possibly imparted the characteristic flavour to the fermented product.

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