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ORIGINAL PAPER

Biochemical changes during the fermentation of *Prosopis africana* seeds for *ogiri-okpei* production

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Abstract Biochemical changes during fermentation of seeds of *Prosopis africana* for production of *ogiri-okpei*, a food condiment popular among people of West Africa were studied. Fermentation resulted in a net increase in concentrations of total soluble sugars and free amino acids, both reaching a peak after 72 h of fermentation but declining thereafter. Corresponding increases were observed in amylase and protease activities, respectively. Lipase activity was observed to be very strong, increasing throughout the duration of fermentation. Analyses of amino and fatty acid composition using an amino acid analyzer and gas liquid chromatography, respectively, revealed a wide variety of amino acids including glutamine > cystine > arginine and the fatty acids stearic > Arachidic > linolenic > linoleic in the unfermented seed in the highest concentrations. Fluctuations in the concentrations of these compounds were observed during the fermentation. At the end of 96 h fermentation, glutamine > cystine > lysine and an unidentified fatty acid > arachidic > linolenic acids were found in the highest concentrations. Marked increases in composition with increasing period of fermentation were observed for Ca, P, K, Mn, and Z. Transformations of amino acids, fatty acids, and minerals during the fermentation of this seed revealed during this study will contribute towards the development of an industrial process for *ogiri-okpei* as well as an understanding of its contribution to the nutrition of its consumers.

Keywords *Prosopis africana* · Fermentation · Biochemical changes

Introduction

Ogiri-okpei is a dark brown food condiment popular among peoples of West Africa. It is prepared by fermentation of boiled leguminous oil seeds of *Prosopis africana* (mimosaceae) (Guill and Perr Syn. P. Oblonga Benth), a savannah tree that grows to about 40–60 feet high and 7 feet in girth [9]. In brief, preparation of *ogiri-okpei* involves the following process. Cleaned seeds are boiled to soften the hard testa, which is then manually removed. The cotyledons are washed and rid of excess water by heating for a few minutes. The seeds are then packed into shallow containers, covered with leaves, and allowed to undergo fermentation for about 3–4 days. The fermenting seeds may be occasionally exposed to the sun to accelerate this process. The fermentation has been severally described. It is mixed, spontaneous, and alkaline and is reported to involve most especially, various species of *Bacillus*, *Staphylococcus*, and *Micrococcus* species [1, 2, 14]. The fermented seeds are ground into a paste, molded into balls, and sun-dried before it is consumed or sold.

Ogiri-okpei is mainly used as a seasoning agent and meat substitute in soups and sundry dishes. In this respect, this condiment is in many ways comparable to *dawadawa*, another seasoning produced by fermentation of African locust bean (*Parkia* sp.) or soybean seed by a similar microflora [7, 18]. *Dawadawa* is now industrially produced by Nestle and sold throughout Africa [27]. The realization

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of this potential for *ogiri-okpei* requires an understanding of the biochemical changes that take place during its fermentation. Furthermore, legumes contribute significantly to protein intake in the developing countries of the world [19]. Thus, the influence of biochemical transformations during fermentation on the concentrations of proteins, essential amino acids, essential fatty acids, and minerals among others in this food will also have implications for nutrition.

There are some reports on the biochemical changes that occur during the fermentation of this condiment [2, 26]. However, these reports do not contain details of transformation of specific types of compounds. In this study, we report details of transformations of amino acids, fatty acids, and minerals. Knowledge of this will contribute towards the development of an industrial process for *ogiri-okpei* as well as an understanding of its contribution to nutrition.

Materials and methods

Prosopis africana seeds and processing into *ogiri-okpei*

Prosopis africana seeds were obtained from markets in Awka, Anambra State of Nigeria. The traditional method of *ogiri-okpei* production reported by Odibo et al. [14] was adopted. About 800 g of seeds were cleaned, washed, and then boiled for 8 h to soften the hard testa, which were then removed by mashing between the palms. About 350 g of cotyledons were recovered, washed, and subsequently heated over fire for 15 min to evaporate excess water. The seeds were packed into a plastic basket, lined with washed plantain leaves, previously wilted over fire. The basket was covered with the same leaves and the seeds were fermented for 96 h at room temperature in our laboratory, with exposure to the sun for about 1 h daily. The fermented seeds were ground in a mortar into a smooth paste and then sun-dried.

Enzyme assays

Samples of *ogiri-okpei* were taken 0, 24, 48, 72 and 96 h after commencement of fermentation, ground into paste in a mortar, and extracted in 0.2 M phospate buffer, pH 7.0, as described by Odunfa [15]. Extracts were stored in a deep freezer throughout the duration of the assays. Amylase activity was measured using the method of Bernfeld [6]. Absorbance was measured at 540 nm and one unit of amylase activity was defined as amount of enzyme, which liberated 1 mg of maltose per minute under the assay conditions. The technique of Upton and Fogarty [28] was adopted to determine proteinase. The reaction mixture consisted of 1 ml of 1% (w/v) casein in 0.2 M phosphate buffer, pH 7.0, and 1 ml of enzyme solution. Absorbance was read at 620 nm and one unit of protease activity was defined as amount of enzyme, which released 1 mg of tyrosine from casein per minute under the experimental conditions. Lipase was assayed using the method of Ota et al. [21], in which the reaction mixture consisted of 2 ml of 0.2 M phosphate buffer, pH 7.0, 1 ml of 0.1 M $CaCl_2$, 0.5 ml of olive oil, 2 ml distilled water, and 1 ml of extract. One unit of lipase activity was defined as the amount of enzyme, which liberated 1 µg of oleic acid per minute under the prevailing experimental conditions.

All reagents used during enzyme assays and chemical analysis were of analytical grade. Spectrophotometric readings were performed in a Spectronic 21UVD spectrophotometer**.** Values reported for all assays were mean of three determinations.

Chemical analyses

Samples for chemical analyses were taken simultaneously with samples for enzyme assays and prepared as already described. For the determination of total sugars and free amino acids, 5 g samples were then extracted in 20 ml of 80% ethanol using methods described by Odunfa [16]. Samples for fatty acid analysis were first dried at 100°C for 5 min, then 10 g extracted in petroleum ether [4]. Total sugars and free amino acids were determined by the Anthrone method [23] and the Ninhydrin method of Rosen [25], respectively.

The residue from fatty acid extraction was used for determination of amino acid composition. For this analysis, 20 mg samples were further defatted by extracting with chloroform-methanol mixture in a soxhlet and after sealing in glass vials containing 6 M HCl were digested at 105 ± 5°C for 22 h. The amino acid composition of the hydrolysates was analyzed in a Technicon Sequential Multisample (TSM) amino acid analyzer. A standard amino acid mixture (Serva, Feinbiochemica Heidelberg, Germany) was prepared as above and used as a quantitative standard.

The method of Kyriakidis and Dionysopoulos [10] was employed for gas liquid chromatography (GLC) of fatty acids of extracted oil using the Carlo Erba Fractovap 2350 Gas liquid chromatograph (Carlo Erba Strumentazione, Milan). The column was 2 × 7.5 cm, 5% DIDP and 3% PEG, 400 on Diatomite C80-100 mesh at a temperature of 190°C. The methyl esters of the fatty acids were identified by comparing their retention times with those of authentic standards (capric, lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, arachidic, and linolenic acids), all from Serva, Feinbiochemica Heidelberg, Germany.

Mineral compositions of samples were determined using the Pye Unicam SP9 atomic absorption spectrophotometer and methods described by the AOAC [4].

Results and discussion

Amylase activity and changes in composition of sugars

Results of analyses of total soluble sugars show a net increase in the concentration of these compounds, reaching a peak after 72 h of fermentation but declining thereafter (Fig. 1). The increase observed in the concentration of these compounds correlated positively with increases observed for amylase activity (Fig. 2). Similar increases in concentrations of sugar/amylase activity have been reported for fermented seeds of *egusi* [16], locust bean [17], and *Prosopis africana* [2, 13, 14]. Odunfa [16] has explained that increase in total sugars result from hydrolysis of oligosaccharides by the action of amylases.

Generally, the increases in activities of all enzymes assayed during this fermentation are a consequence of increase in total microbial populations as has been severally reported [1, 2, 12, 14]. The solubilization of carbohydrates during *ogiri-okpei* fermentation is significant in terms of availability of nutrients for consumers of this food.

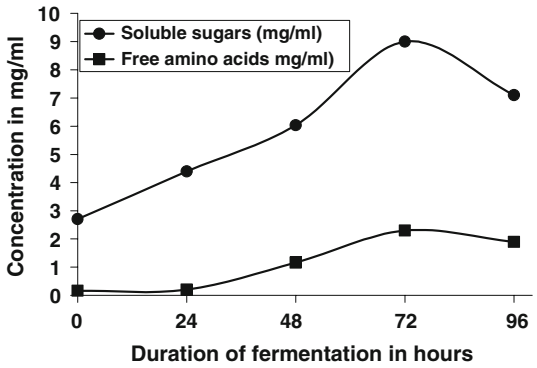

Fig. 1 Changes in the chemical composition of fermenting seeds

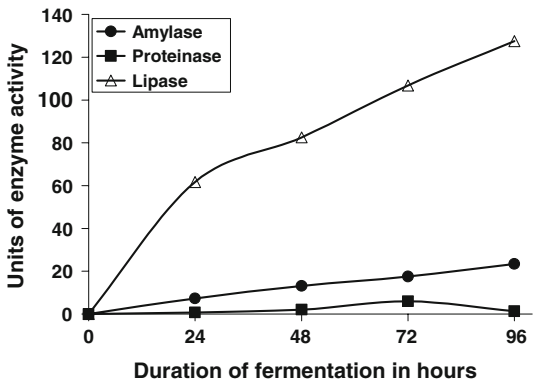

Fig. 2 Changes in the activities of enzymes during fermentation of *ogiri-okpei*

Protease activity and changes in composition of amino acids

Results of analyses of free amino acids show a net increase in their concentration. As with soluble sugars, peak concentration of free amino acids was observed at 72 h of fermentation, after which it also declined (Fig. 1). The increase observed in concentration of amino acids also correlated positively with increases observed for protease activity (Fig. 2). Similar increases in concentrations of free amino acids and protease activity has been reported for fermented seeds of *egusi* [16], locust bean [17], and *Prosopis africana* [2, 13, 14]. Proteolysis has been reported to be the main metabolic activity during the fermentation of African locust bean [3, 17], *Prosopis africana* [13]. During these fermentations, degradation of proteins contributes to the development of the texture and flavor of the fermented products.

Amino acid analyses of fermenting *Prosopis africana* seeds reveal the occurrence of a wide range of amino acids before and during the process (Table 1). The most abundant amino acids in the unfermented seeds were glutamine, cystine, and arginine, while lysine, asparagine, and leucine occurred in medium quantities. The concentration of individual amino acids fluctuated during fermentation. However, most of the amino acids reached their highest levels of concentration after 72 h of fermentation. Continued fermentation up to 96 h appeared to lead to a general reduction in the composition of amino acids. The amino acids found in the highest concentrations at the end of 96 h fermentation were glutamine, cystine, lysine, and arginine. It is significant that glutamine is one of the amino acids present in appreciable quantities at the end of fermentation. The sodium salt of this compound is the principal ingredient in seasonings such as Nestle Maggi cubes [27]. The increase in concentration of lysine resulting from fermentation is also important from the nutrition point of view. Lysine is an essential amino acid, which many plant proteins are deficient in.

The general reduction in the concentration of most amino acids at the end of fermentation suggests active metabolism of these compounds by bacteria responsible for this fermentation. This process is important for the development of aroma and character of this food. According to Allagheny et al. [3], amino acids are consumed as an energy source by these organisms releasing ammonia. This reaction is responsible for the increase in pH and alkaline nature of these fermentations. Ammonia is an important component of the aroma of *ogiri-okpei.*

Table 1 Changes in amino acid composition during the fermentation of *ogiri-okpei*

Amino acid g/16 g N_2	Duration of fermentation in hours				
	0	24	48	72	96
Lysine	6.60	6.94	9.89	13.43	9.60
Histidine	2.30	3.11	1.87	2.55	2.50
Arginine	12.15	12.45	11.50	11.33	7.64
Asparagine	6.53	11.22	9.88	10.04	6.69
Threonine	2.57	3.49	2.57	3.13	1.47
Serine	3.82	3.28	2.81	3.22	2.54
Glutamine	19.25	23.78	14.68	19.62	13.18
Proline	5.20	4.65	3.50	5.04	3.52
Glycine	3.13	3.01	2.31	3.44	1.57
Alanine	2.53	3.30	1.99	3.68	1.90
Cystine	13.60	15.29	11.13	20.23	10.29
Valine	5.45	5.56	3.05	4.15	1.85
Methionine	3.93	3.44	3.62	5.98	1.45
Isoleucine	3.01	2.15	1.77	4.04	1.41
Leucine	6.89	7.55	4.11	7.15	2.14
Tyrosine	1.46	1.12	1.31	2.01	1.15
Phenylalanine	2.07	1.58	1.09	1.45	1.53

Lipase activity and changes in composition of fatty acids

Lipase activity during this study was observed to be very strong, increasing throughout the duration of fermentation (Fig. 2). This observation is expected, since microorganisms would only resort to the utilization of fats following the decline of preferred sources of carbon and energy such as carbohydrates and proteins. However, our observation is in disagreement with earlier reports of low activities of this enzyme during the fermentation of seeds of *Prosopis africana* [13, 16]. Ouoba et al. [22] have noted that similar contradictions have also been reported for esterase and lipase activity against African locust bean oil. According to these authors, these contradictions arise because there is a pronounced variability between species and within species of *B. subtilis* in lipase and esterase activity. Thus, observations during a particular study depend on the strains of *Bacillus* sp. dominant. Low lipase activity has been considered advantageous [13, 16], because it was expected to minimize the development of rancidity and off-flavors. However, other workers including Odunfa and Adesomoju [20] and Beaumont [5] during studies on African locust bean consider that an adequate lipolytic activity could probably be a good characteristic, because liberation of free fatty acids was required for the development of desired aroma characteristics.

Table 2 shows changes in fatty acid composition of fermenting *Prosopis africana* seeds. The fatty acids high in concentration in the raw seed included the saturated fatty acids, stearic and palmitic, and the essential fatty acids, oleic, linoleic, and linolenic acids. At the end of fermentation, however, fatty acids present in the highest concentrations included linoleic, arachidic, oleic, linolenic, and two unidentified fatty acids. It is nutritionally significant that fermentation with the exception of arachidic acid generally reduced the concentration of saturated fatty acids in seeds of *Prosopis africana*. Saturated fatty acids are implicated in incidence of atherosclerosis and coronary heart disease [24]. On the other hand, the high occurrence of linolenic, linoleic, and oleic acids in *ogiri-okpei* is desirable, because omega-3 and omega-6 fatty acids are derived from them. These essential fatty acids support the cardiovascular, reproductive, immune, and nervous systems of the body [24].

However, a disadvantage of high concentrations of unsaturated fatty acids in the product would be susceptibility to rancidity, since these fats are less stable than satu-

Table 2 Changes in fatty acid composition during the fermentation of *ogiri-okpei*

Fatty acid (%)	Duration of fermentation in hours					
	Notation	0	24	48	72	96
Capric acid	C10;0	1.707	1.902	1.687	0	0
Lauric acid	C12;0	0.732	0.411	0	0	0
Myristic acid	C14;0	2.927	6.477	6.365	2.781	3.478
Palmitic acid	C16;0	11.708	8.636	4.455	3.862	3.478
Palmitrelic acid	C16;1	6.635	5.963	3.564	4.635	1.304
Stearic acid	C18;0	17.953	14.60	11.965	13.596	4.638
Oleic acid	C18;1	12.938	12.337	10.852	12.051	6.089
Linoleic acid	C18;2	17.075	15.165	14.320	17.420	8.696
Arachidic acid	C20;0	7.903	8.328	11.456	10.429	10.870
Linolenic acid	C18;3	17.416	16.913	18.266	22.557	26.087
Unidentified fatty acid I		2.273	2.673	7.587	4.171	9.275
Unidentified fatty acid II		0.732	6.595	9.483	8.497	26.087

Table 3 Changes in mineral composition during the fermentation of *ogiri-okpei*

Minerals (mg/100 g dry weight)	Duration of fermentation in hours				
	0	24	48	72	96
Calcium	24.1	46.1	47.8	58.0	68.3
Phosphorus	131.3	234.8	159.4	159.4	540.0
Magnesium	60.2	63.0	64.4	62.4	63.0
Potassium	144.0	240.0	236.8	225.8	303.8
Sodium	36.0	37.5	45.0	42.0	40.5
Manganese	14.4	22.9	26.3	25.5	25.9
Zinc	4.6	5.0	8.2	29.3	10.1
Copper	1.5	2.1	2.3	2.1	1.8
Iron	16.8	21.0	16.8	58.8	16.8

rated fats. This fact should be taken into consideration in designing procedures for the preservation of this food. The presence of some unidentified fatty acids, one of which occurred at a very high percentage of the fats in *ogiri-okpei*, will require further research to elucidate their significance.

Changes in composition of minerals during the fermentation of *ogiri-okpei*

Legumes are important sources of minerals in the diet. Mineral elements are important in the human body because of their role as components of structures such as bones and as cofactors for some enzymes. The analysis of mineral composition of fermenting seeds of *Prosopis africana* reveals the occurrence of some important minerals in the raw seed (Table 3). Generally, fermentation resulted in an increase in the concentration of minerals present. Marked increases in composition with increasing period of fermentation were observed for Ca, P, K, Mn, and Z. Enijiugha [8] has reported similar increases in concentration of minerals measured as ash, of up to 35% for fermenting seeds of the African oil bean. According to Moat [11], increases in ash content of fermenting seeds may be due to the ability of *Bacillus* species to synthesize divalent metals.

Conclusion

It has been shown that during the fermentation of seeds of *Prosopis africana*, for the production of *ogiri-okpei*, remarkable changes occur in both the varieties and quantities of sugars, amino acids, fatty acids, and minerals present in the seed. These changes are aided by enzymes, which included amylases, proteinases, and lipases. Fermentation in addition to developing flavor of *ogiri-okpei* also improved the nutritional quality of this seed when used as food.

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