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Chemical and Biochemical Changes in African Locust Bean (*Parkia biglobosa*) and Melon (*Citrullus vulgaris*) Seeds During Fermentation to Condiments

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Abstract: The chemical (proximate composition, pH, total tocopherol, acid and iodine values, total and reducing sugars) and biochemical (α-amylase, sucrase and protease) changes occurring during the fermentation of locust bean and melon to the condiments - *iru* and *ogiri* respectively were monitored. Processing locust bean to the condiment involved boiling for 12 hours, soaking the seeds in water, dehulling, boiling for another 6 hours and fermentation for 3-4 days while melon is boiled for 6 hours, cooled and fermented. Boiling, soaking (in water) and dehulling of locust bean resulted in reduction of ash, crude fibre (CF) and total tocopherol but increased ether extract. Fermentation increased the pH value, crude protein (CP), reducing sugar, α-amylase, sucrase, protease and free amino acid, decreased the total sugar in both samples and also increased the CF of locust bean sample but had no effect on the CF in melon. Fermentation had no effect on total tocopherol content in both samples. Heat treatment and fermentation employed in processing both samples increased the acid value with a corresponding decrease in iodine value. High negative correlation was observed between acid and iodine values while a high positive correlation was observed between tocopherol and iodine value.

Key words: Proximate composition, tocopherol, sugar, free amino acids, sucrase, amylase, protease

Introduction
Fermentation is one of the oldest methods of food preservation known to man. In Africa, the art of fermentation is widespread including the processing of fruits and other carbohydrate sources to yield alcoholic and non-alcoholic beverages, the production of sour-tasting *ogi* - the fermented cereal products, which provide instant energy in breakfast and convalescent diets (Adewusi *et al.*, 1991; 1992). Oil seeds such as African locust bean, melon seed, castor oil seed, mesquite bean and soybean are also fermented to give condiments.

The production of condiments is largely on a traditional small-scale, household basis under highly variable conditions (Odunfa, 1985). In addition, the fermentation is usually carried out in a moist solid state, involving contact with appropriate inocula of assorted microorganisms and is accomplished by the natural temperatures of the tropics. The desired state of fermentation of the condiments is indicated by the formation of mucilage and overtones of ammonia produced as a result of the breakdown of amino acids during the fermentation (Omafuvbe, 1998). The characteristic ammoniacal odour and flavour of condiments enhance the taste of traditional soups and sauces especially the various soups used as accompaniment to the starchy root and tuber diets. Condiments are also known to contribute to the calorie and protein intake (Simmons, 1976; Umoh and Oke, 1974) and are generously added to soups as low-cost meat substitute by low-income families in parts of Nigeria (Odunfa, 1985).

Some research has been carried out on the production of fermented condiments-*iru*-from African locust bean (Eka, 1980; Odunfa, 1986), melon seed fermented *ogiri* (Odunfa, 1981; Barber and Achinewhu, 1992) and soybean produced *daddawa* (Omafuvbe *et al.*, 2000; 2002). Oil seeds are generally processed to yield condiments but so far, no investigation has been carried out on the changes in the oil component of these seeds as a result of fermentation. Furthermore, comparative chemical and biochemical changes occurring during fermentation have been neglected especially in the fermentation of the two most popular condiments *iru* and *ogiri*. The main objective of the present study is to compare the proximate composition of the raw African locust bean and melon seeds fermented for the production of *iru* and *ogiri* and the chemical and biochemical changes occurring during the fermentation process. The nutritive value of *iru* and *ogiri* is also compared with that of monosodium glutamate based salts.

Materials and Methods
Materials: African locust bean and melon seeds were bought at the local market in Ile-Ife. Seasoning salts were bought off the shelf in supermarkets or from the local market also in Ile-Ife, Nigeria. The processing of both African locust bean and melon seeds to condiments were carried out in the local factories of the

two major producers of these condiments in Ile-Ife, Nigeria as outlined below:

Production of *iru*: Raw African locust bean was boiled for 12 hours and further soaked in the boiling water for another 12 hours (preferably overnight). Excess water was drained off and the seeds were dehulled by marching the seeds by foot in a large wooden mortar and further removal of the seed coat was achieved by rubbing the cotyledons between the palms of the hand and washing with water. The cotyledons were again cooked for another 6 hours, the hot boil water was drained off and the cotyledons were then spread into calabash trays, covered with wooden trays, wrapped with jute sacks and fermented for 3-4 days to produce *iru*.

Production of ogiri: Shelled melon seeds were sorted out to remove grit, dirt and decomposing seeds; rinsed with warm water twice and boiled for one hour in 10 times its volume of water. The boil water was then drained and replaced by cold water and boiled again for 6 hours to softness. The melon seeds were thereafter transferred into a clay pot and covered with *Thaumaloccus demoelli* leaves and wrapped in sack clothe for 3 days. The fermented product is then ground with stone mortar and pestle to almost a smooth paste sold as *ogiri*.

Sampling: The raw seeds, samples collected at the different processing stages and the products thus produced were kept in a freezer at -17°C until required for analysis.

Analytical technique: Proximate composition was determined by the Association of Official Analytical Chemists (AOAC, 1990) method.

Tocopherol content was estimated by the method of Contreras-Guzman and Strong (1982a,b) using cuproine (2, 2-biquinoline) as the complexing agent for the colour formation. All rac-α-tocopherol (Merck) was used as a standard. Solvents used for the extraction and subsequent assay were purified and redistilled as per Contreras-Guzman *et al.* (1982).

Iodine and Acid values were determined by the AOAC (1990) method.

pH of the fermented products was determined using a PYE Unicam pH meter (Model 290 MK2).

Determination of sugars and free amino acids: The raw seeds and samples collected at the different processing stages were dried in hot air oven at 70°C, ground and defatted. The soluble sugars and free amino acids in the defatted samples were extracted with 80% ethanol (v/v) following the method of Odibo *et al.* (1990). The free amino acid in the ethanolic extract was estimated by the ninhydrin calorimetric method (Rosen, 1957) using

glycine as standard. The total soluble sugar was determined by the anthrone reagent method of Morris (1948) while reducing sugar was estimated by the calorimetric method (Somogyi, 1945) using glucose as a standard.

Extraction of extracellular enzymes: Extracellular enzymes (α-amylase, sucrase and protease) in the fermenting material (5.0 g) were extracted with appropriate buffer (50 ml) as previously described (Omafuvbe *et al.*, 2000).

α-Amylase activity was determined by the blue value method of Fuwa (1954) on 0.1 M potassium phosphate buffer (pH 6.0) extract of the samples. One unit of α-amylase activity was defined as the amount that produced a 10% reduction in the intensity of blue colour of starch - iodine complex under the experimental conditions.

Protease activity was measured on 0.1 M sodium phosphate buffer (pH 6.5) extract of the samples. One unit of proteolytic activity was defined as the amount that produced 100 μg of tyrosine in 1.0 ml of trichloroacetic acid soluble peptides under the assay conditions (Omafuvbe *et al.*, 2000).

Sucrase activity was measured on 0.05M sodium citrate (pH 6.5) extract of the samples. One unit of enzyme activity was defined as the amount of enzyme that produced 100 μg of glucose under the assay conditions (Omafuvbe *et al.*, 2000).

Results and Discussion

Proximate composition and pH value of raw and fermented African locust bean (*Parkia biglobosa)* and melon seeds (*Citrullus vulgaris*) are presented in Table 1. The moisture content of raw African locust bean and melon was 8.6 and 5.3% respectively. There was an increase in the moisture content of processed African locust bean and processed melon, which ranged between 51.9 and 56.7% and 43.0 and 44.1% for both samples respectively as a result of boiling in water followed by further soaking in water in the case of African locust beans.

The ash content of raw African locust bean and melon seeds was 5.4 and 3.3% respectively. The value reported for African locust bean agreed favourably with 5.1% reported earlier by Eka (1980). Boiling, soaking in water and dehulling of African locust beans led to a loss of 41% ash. This means that about 41% of the total mineral content of African locust bean may reside in the hull of the seed and or leached during processing. When the African locust bean seed was boiled for additional 6 h during processing, 13% of the mineral was further leached into the broth. Fermentation seemed to have increased the ash content of African locust bean seed by 29% in direct agreement with the observation of Eka (1980) of about 30% increase in ash

Table 1: Proximate Composition and pH Value of Raw and Fermented African locust bean (*Parkia biglobosa*) and Melon Seeds (*Citrullus vulgaris*). % dry sample

	Moisture* (%)	Ash (%)	Crude fiber (%)	Ether Extract (%)	Crude Protein (%)	CHO (%)	pH
African locust bean							
Raw	8.6 ± 0.6	5.4 ± 0.3	11.7 ± 0.2	17.7 ± 0.2	31.0 ± 0.7	35.0 ± 0.5	6.2
Boiled 12 h and Dehulled	51.9 ± 0.7	3.2 ± 0.3	4.6 ± 0.1	20.9± 0.3	31.4 ± 0.2	42.0 ± 0.4	6.3
Boiled 6 h	56.7 ± 0.7	2.8 ± 0.0	4.4 ± 0.9	28.4 ± 0.1	31.1 ± 0.9	33.3 ± 0.5	6.4
Fermentation Period (h)							
24	55.7 ± 0.8	3.6 ± 0.1	5.7 ± 0.1	32.6 ± 0.7	31.7 ± 0.4	25.1 ± 1.1	8.3
48	55.5 ± 0.4	3.5 ± 0.1	5.4 ± 0.1	35.2 ± 0.1	31.3 ± 0.2	21.0 ± 0.4	8.4
72	52.0 ± 5.0	3.6 ± 0.1	4.0 ± 0.1	37.2 ± 0.2	32.9 ± 0.1	16.3 ± 0.8	8.4
Melon seed							
Raw	5.3 ± 0.2	3.3 ± 0.2	15.8 ± 0.6	37.5 ± 0.6	19.3 ± 0.4	24.1 ± 0.2	7.0
Fermentation** period (h)							
24	43.1 ± 0.8	3.2 ± 0.2	20.7 ± 0.5	34.1 ± 2.7	20.6 ± 0.4	21.4 ± 0.7	7.2
48	43.0 ± 0.7	3.2 ± 0.3	20.0 ± 0.7	35.3 ± 0.4	19.5 ± 0.7	22.0 ± 0.9	7.5
72	44.1 ± 0.8	3.0 ± 0.0	15.6 ± 0.4	36.3 ± 0.5	19.9 ± 0.8	25.2 ± 1.2	7.9

*Moisture content was determined on the fresh materials.
**Fermentation of melon seed was carried out after boiling for 6 h (See Experimental for details).

Table 2: The Tocopherol, Acid and Iodine Values of African locust bean and Melon Seeds during Different Stages of Processing to Condiments in Comparison to the Seasoning Salts

African locust bean	Total tocopherol (mg / 100 g)	Acid Value	Iodine Value
Raw	21.6 ± 0.0	1.06	148.4
Boiled (12 h) & Dehulled	18.3 ± 0.1	1.23	142.8
Scaled & Boiled (6 h)	18.2 ± 0.1	1.40	137.3
Fermentation period (h)			
24	17.2 ± 0.0	1.46	134.5
48	17.1 ± 0.0	1.57	131.7
72	17.2 ± 0.1	1.63	125.2
Melon seed			
Raw	25.0 ± 0.1	1.68	127.8
Fermentation period (h)			
24	20.7 ± 0.1	1.85	124.3
48	20.8 ± 0.1	1.96	114.1
72	20.9 ± 0.1	2.19	111.3
Seasoning salts			
Knoor cube	1.7 ± 0.0	ND	ND
Royco cube	1.3 ± 0.0	ND	ND
Doyin cube	0.6 ± 0.0	ND	ND

after fermentation. In contrast, boiling and fermentation of melon did not seem to have any significant effect on the ash content.

Crude fibre (CF) content of 11.7 and 15.8% are now reported for raw African locust bean and melon seeds respectively (Table 1); these values were higher than the 3.6% value reported for cowpea (Ojimelukwe *et al.,* 1999); 0.2% crude fibre (but 10.9% total dietary fibre) for soybean (Suarez *et al.,* 1999) but close to the 8.8% value reported for African locust bean seed by Oyenuga (1968). Boiling and dehulling the African locust bean seed reduced its CF by 61% but fermentation increased the CF by 30% during the first 48 hours and then reduced the amount by about the same level (30%) during the last 24 hours. This pattern agrees with the observations of Gle (1992). At the end of the boiling process, the boil water of the African locust bean was more viscous than it was at the beginning of the process. This is an indication of the presence of mucilaginous materials in the boil water, which would explain in part the reduction of the crude fibre content of the African locust bean seed on boiling. On the other hand, the crude fibre content of melon seed increased by 31% after the boiling process probably due to soluble compounds in the seed being leached out. The crude fibre content of melon seed like that of the African locust bean seed decreased during the last 24 hours of fermentation (by 22%) probably due to the production of extracellular enzymes.

The ether extract of raw African locust bean seed

Table 3: Some Biochemical Properties of Fermenting African locust bean and Melon Seeds for *Iru* and *Ogiri* Production respectively

Sample	Total soluble sugar		Reducing sugar		Free amino acids	
	mg glucose g^{-1} dry wt				mg glycine g^{-1} dry wt	
	African locust bean	Melon***	African locust bean	Melon***	African locust bean	Melon***
Raw undehulled seeds	57.0±0.6	33.4±0.5	11.8±0.2	4.9±0.1	47.8±0.4	15.8 ± 0.2
Cooked dehulled seeds*	10.9 ± 0.3	NA	6.0 ± 0.3	NA	22.9 ± 0.3	NA
Cooked dehulled seeds**	9.3 ± 0.2	16.2 ± 0.3	3.3 ± 0.2	2.1 ± 0.1	12.9 ± 0.2	9.0 ± 0.224
h fermentation	7.5 ± 0.3	11.4 ± 0.2	4.5 ± 0.2	2.0 ± 0.3	23.5 ± 0.2	10.4 ± 0.248
h fermentation	8.1 ± 0.2	8.5 ± 0.2	7.6 ± 0.3	3.0 ± 0.1	49.2 ± 0.4	12.3 ± 0.172
h fermentation	9.0 ± 0.4	7.5 ± 0.1	8.2 ± 0.5	1.5 ± 0.1	55.6 ± 4.2	30.0 ± 0.396
h fermentation	9.9 ± 0.3	NA	8.8 ± 0.4	NA	61.6 ± 0.3	NA

NA - Samples not available. *Raw African locust bean was cooked for 12 h and left in the boil water for another 12 h. **African locust bean seed was dehulled and cooked again for 6 h before fermentation. ***Melon was shelled to give the raw sample, cooked for 6 h to give the second set of samples and then fermented for 72 h.

Table 4: The Activity of Extracellular Enzymes in Fermenting African locust bean and Melon Seeds for *Iru* and *Ogiri* Production Respectively

Processing Steps	α-Amylase		Sucrase		Protease	
	Enzyme activities expressed as unit ml^{-1}					
	African locust bean	Melon seed	African locust bean	Melon seed	African locust	Melon seed
Raw undehulled seeds	ND***	ND	ND	ND	ND	ND
Cooked dehulled seeds*	ND	NA	ND	NA	ND	NA
Cooked dehulled seeds**	0.28± 0.03	0.34 ± 0.04	0.00	0.00	0.00	0.0024
h fermentation	0.30± 0.02	0.54±0.03	0.47±0.03	0.25±0.02	0.23±0.01	0.14±0.02
48 h fermentation	0.66±0.05	0.72±0.06	0.81±0.09	0.31±0.03	0.31±0.02	0.19 ±0.03
72 h fermentation	ND	3.4 ± 0.1	ND	0.16 ± 0.02	ND	0.28±0.10
96 h fermentation	3.0±0.1	NA ****	0.45±0.04	NA	0.51±0.04	NA

*Raw African locust bean was cooked for 12 h and left in the boil water for another 12 h. **African locust bean seed was dehulled and cooked again for 6 h before fermentation. ***Melon was shelled to give the raw sample, cooked for 6 h to give the second set of samples and then fermented for 72 h. ***ND - Not determined. ****NA - Samples not available.

increased by boiling and fermentation in agreement with earlier findings (Eka, 1980) while that of melon seed decreased after boiling and thereafter increased by 3-5% every 24 hours during fermentation.

CP content was 30.2 and 19.3% for raw African locust bean and melon seed respectively. Fermentation also led to increase in CP for the African locust bean but not for melon (Table 1). The CP reported for African locust bean agreed favourably with 30.6% reported earlier by Eka (1979) while that of melon was close to the 18.4% value reported for another sample of melon by Adewusi *et al.* (1992). The increase in ether extract and CP with increase in fermentation period of locust bean seed was probably due to the reduction in the content of ash, CF and carbohydrate.

Fermentation of both African locust bean and melon seed samples led to increase in pH (Table 1) as earlier observed by Odunfa (1981).

Tocopherol is a fat soluble vitamin with antioxidant property which makes it indispensable for the maintenance of the cell integrity and prevent oxidation of lipid *in vivo* (Stephen *et al.*, 1996; Losonczy *et al.*, 1996). The total tocopherol content of the raw and processed products presented in Table 2 indicated that melon seed had the highest value of 25 mg/100 g tocopherol closely followed by African locust bean seed with 21.6 mg/100 g. These values are higher than 2 and 8 mg/100 g reported for lima bean and banana respectively (Cataldo *et al.*, 1999). Boiling and dehulling reduced total tocopherol of African locust bean by 15%. Fermentation for 24 hours reduced tocopherol by 5% while subsequent fermentation period had no effect on this vitamin. Boiling and fermentation of melon seed for the first 24 hours reduced tocopherol by 17% after which there was no effect with fermentation. The total tocopherol of seasoning salts used in recent times as a replacement for these local condiments ranged between 0.6 and 1.7 mg / 100 g (Table 2). Rural communities and

low-income families use *iru* and *ogiri* liberally often as a substitute for meat and thereby consume a fair amount of these condiments on a daily basis. 50 g of both condiments level will provide more than the Recommended Dietary Allowance (RDA) of 10 and 8 mg α-tocopherol for adult men and women respectively (FNB, 1979) while the seasoning salts cannot be regarded as a source of this essential vitamin.

Acid and iodine values are presented in Table 2. Heat treatment during processing of African locust bean increased acid value. Acid value in both African locust bean and melon seeds also increased with prolongation of the fermentation period. Heat treatment employed in the manufacture of the condiments reduced the iodine value in both African locust bean and melon seed. There was a high negative correlation between acid and iodine values (r = - 0.98 and - 0.94; p < 0.05 for African locust bean and melon seed respectively). A high positive correlation was also observed between the total tocopherol level and iodine value (r = 0.85 and 0.68; p < 0.05 for African locust bean and melon seed respectively).

The pattern of change in the levels of soluble sugars and free amino acid content of African locust bean and melon seeds were quite different with processing and fermentation into *iru* and *ogiri* respectively (Table 3). Fermentation of both seeds resulted in substantial decrease in the total sugar and free amino acid levels. This was probably due to the removal of the hulls that contain higher level of sugar and amino acids. The cooking stage of the seeds that have been reported as the most important step in the preparation of raw materials for fermentation (Wang *et al.*, 1979) may have also resulted in partial loss of soluble solids in the cooking water. For example, Kanno *et al.* (1982) reported a partial loss of oligosaccharides in cooking and soak water during soaking and steaming of soybean for *natto* production. Further decrease in total soluble sugar level was observed with fermentation in melon seeds while the level decreased in the first 24 h of fermentation and increased thereafter in African locust bean. These patterns of change in soluble sugar level have been reported in similar fermented condiments (Omafuvbe and Oyedapo, 2000; Omafuvbe *et al.*, 2000).

The reducing sugar level decreased during the processing stages of both seeds but increased with fermentation in African locust bean and fluctuated in melon seeds (Table 3). The increased level of reducing sugar is a reflection of the activities of α-amylase and sucrase in the fermenting seeds (Table 4). Alpha amylase increased with fermentation in both seeds while sucrase increased, reached its peak at the 48th hour of fermentation and then dropped. This is however not unusual since similar pattern have been reported (Odunfa, 1983; Omafuvbe *et al.*, 2002). It is worthy of note that sucrase activity was higher in African locust bean than melon seeds. This may have been responsible for the high reducing sugar level in the fermented African locust bean seeds. The fluctuation in

the level of sugar with fermentation may be related to its utilization by the fermenting microorganisms for their metabolic activities.

The level of total free amino acids increased in both seeds with fermentation (Table 3). Similar increases in the level of free amino acids with fermentation have been reported in other seeds (Omafuvbe *et al.*, 1999, 2000). This rapid increase in the total free amino acids is a reflection of the increased protease activity observed in the fermenting seeds (Table 4). Protease activity has been reported to be abundant in the fermentation of similar protein rich foods (Sarkar *et al.*, 1993; Omafuvbe *et al.*, 2002). Of significant note is the high level of amino acids and protease activity in African locust bean though the protein content of both seeds are the same.

Conclusion: Fermentation of African locust bean and melon seeds, to their respective condiments *iru* and *ogiri*, is desirable nutritionally since the process increases the crude protein and ether extract content of the products. The liberal use of these condiments is expected to increase the intake of these essential dietary components appreciably. In addition, the presence of a high level of vitamin E in addition to vitamins A and D (Oyenuga, 1968) in these condiments is an added advantage over seasoning salts, which now tend to replace the local condiments in our kitchens.

References
Adewusi, S.R.A., B.O. Orisadare and O.L. Oke, 1991. Studies on Weaning Diets in Nigeria. 1. Carbohydrate Sources. Cereal Chem., 68: 165-169.

Adewusi, S.R.A., B.O. Orisadare and O.L. Oke, 1992. Studies on weaning diets in Nigeria: 2 protein sources. Plant Foods for Human Nutr., 42: 183-192.

Association of Official Analytical Chemists (AOAC), 1990. Official Methods of Analysis 15th edition, AOAC, Arlington, USA.

Barber, L.I. and S.C. Achinewhu, 1992. Microbiology of ogiri production from melon seeds (*Citrullus vulgaris*). Nig. Food J., 10: 129-135.

Cataldo, C.B., L.K. DeBruyne and E.N. Whitney, 1999. Nutrition and Diet Therapy Principles and Practice 5th edition. Wadsworth Publishing Company and International Thomason Publishing Company U S A. pp: 35-204.

Contreras-Guzman, E.S. and F.C. Strong III, 1982a. Determination of Tocopherols (Vitamin E) by reduction of Cupric ion. J. Assoc. off. Anal. Chem., 65: 1215-1221.

Contreras-Guzman, E.S. and F.C. Strong III, 1982b. Determination of Total Tocopherols in grains, grain products, and commercial oils, with only slight saponification, and by a new reaction with Cupric ion. J. Agri. Food Chem., 30: 1109-1112.