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Colour and Fatty Acids Analysis of Fermented and Canned African Oil Bean (Pentaclethra macrophylla Benth) Seeds

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Abstract
The variations in the colour and fatty acid profiles as influenced by the processing steps and changes in canning media during thermal processing of fermented African oil bean (*Pentaclethra macrophylla* Benth) seeds were investigated. The seed cotyledons were prepared as slices and fermented at 30°C for 72 h. The fermented product was then filled into cans containing the selected media and processed using conventional canning procedures. Cooking and canning steps slightly darkened the light brown colour of the cotyledons. However, overall, thermal processing did not significantly ($p > 0.05$) affect the colour profile of the fermented seed product '*ugba'* as shown from a*/b* ratios, hue and chroma values. Although fermentation significantly ($p > 0.05$) affected the composition and concentrations of fatty acids in the seed oil, the effect of canning of the fermented seed product in three different media (tomato sauce, refined groundnut oil and brine solution) on the fatty acid profiles of the seed oil was not significant ($p > 0.05$). Linoleic acid, which was the major fatty acid in the raw seed oil (67.20 wt.-% of total fatty acids), increased significantly during fermentation, but further thermal processing in lacquered cans did not affect the fatty acids profile.
Keywords: Oil bean seed; fermented product; thermal processing; colour; fatty acids

INTRODUCTION

The African oil bean tree *Pentaclethra macrophylla* Benth (*Leguminosae,* sub-family *mimosoidae*) is a large leguminous, nodule-forming multipurpose tree species occurring naturally in the humid lowlands and some parts of the sub-humid zones of West and Central Africa (Enujiugha, 2008). The edible part of the plant is the seed which is commonly consumed as fermented condiment in soups and porridges (Enujiugha and Olagundoye, 2001; Enujiugha *et al.*, 2008). A previous study centered on shelf life elongation of the fermented product by optimizing the variables for thermal processing in lacquered cans (Enujiugha and Akanbi, 2010). However, one major parameter that is important in the consumer acceptance of the fermented product is colour and it is worthwhile to examine the effect of thermal processing on the product's colour and visual appearance. Vision and appeal are important in consumer acceptance of any processed food product (Makanjuola and Enujiugha, 2015).

An important component of visual appearance is colour, which is three-dimensional, based on responses of three different receptors (red, green, and blue) in the human eye. The inherent subjectivity and lack of reproducibility of visual colour specification led to development of instrumental colour measurement, based on the principle that a colour can be mathematically described as a combination of the three primary colour intensities. Specific colour measurement instruments, such as tristimulus colorimeters and spectrophotometers, are effectively used in determining the colour of food materials. The application of tristimulus colorimetry offers an objective form of colour assessment, because it is based on the consideration of the whole visible spectrum, and makes it possible to obtain the real chromatic profile of each compound or food product. The chromatic parameters usually considered are: lightness (L*), an attribute related to the transmission of light observed in the spectra; hue (h_{ab}), the qualitative expression of chromaticity; and chroma (C^*_{ab}), the quantitative component of chromaticity (Montes *et al.*, 2005). McDonagh *et al.* (2005) observed that the CIE L* and %R (% reflectance) measurements have the potential to replace or support subjective methods of assessing product quality.

Browning during storage and processing is a significant problem in the food industry and is believed to be one of the main causes of quality loss during post-harvest handling of legumes (Nagai and Suzuki, 2003). Polyphenol oxidase in the presence of oxygen catalyzes the oxidation of phenolic substances to quinones. These quinones spontaneously polymerize to form a brown pigment. The properties of polyphenol oxidase in African oil bean seeds has been studied by Chilaka *et al.* (1993). Enzymatic browning is a major problem in African oil bean seed processing. The desirable light-brown colour of the fermented product is most often replaced by a dark brown and sometimes blackish pigment. This undesirable browning is usually experienced during the natural inoculation stage. In the traditional fermentation, prepared seed slices (cooked and soaked) are spread on matted surfaces for upwards of 16 h to provide enough inoculum for the subsequent fermentation (Enujiugha and Olagundoye, 2001). By the time fermentation sets in, the colour would have changed. Apart from enzymatic browning, some undesirable colours are also imparted, if the product is fermented, by wild microbial contaminants. An example is the reported greenish colour in *ugba* imparted by the action of *Pseudomonas chlororaphis* isolated from the fermenting seed slices (Mbajunwa *et al.*, 1998). Moreover, Maillard-type

reactions may occur during the thermal processing of legumes, and these contribute to colour change.

The oil content of the African oil bean seed has been reported to be 52.28 % (Enujiugha and Ayodele-Oni, 2003) with the following percent by weight of the different components: triglyceride, 39.9 %; diglyceride, 2.1 %; monoglyceride, 1.6 %; free fatty acids (FFA), 32.9 %; and sterol, 23.5 %. In the fermented-product oil, the other components with exception of sterol are hydrolyzed to the free fatty acids (triglycerides become 4.89 % while free fatty acids become 73.85 %) (Enujiugha, 1990). The analysis of fatty acid composition of the seed oil of the African oil bean by gas-liquid chromatography and mass spectrometry (Jones *et al.*, 1987) showed that linoleic acid (56.6 %) was the major fatty acid, with oleic (16.1 %) and lignoceric (10.5 %) acids making significant contributions. The work of Ikediobi (1981) revealed a high level (73 %) of the principal fatty acid, linoleic acid, and a very low level (3.9 %) for the essential fatty acid, linolenic acid. Achinewhu (1982) reported the presence of behenic and lignoceric acids which must have contributed to formation of an insoluble soap by the seed oil. No work has hitherto been done on the effect of thermal processing of the fermented product on the fatty acids profile of the seed. The purpose of this study was to examine the effect of the steps involved in canning fermented oil bean slices on colour and fatty acids profiles.

MATERIALS AND METHODS

Preparation of samples

Mature African oil bean (*Pentaclethra macrophylla* Benth) seeds obtained from local farmers at Nri in eastern Nigeria were subjected to controlled laboratory fermentation using a mixed bacterial starter as described previously (Enujiugha *et al.*, 2008). Parboiling of whole seeds was done for 4 h using heating plate, and after dehulling, the cooking of sliced cotyledons was carried out for 6-8 h. Slicing of the cooked seed cotyledons was done manually with a kitchen knife. The cooked seed slices were soaked overnight, washed in five changes of water, and sterilized using laboratory autoclave. The prepared seed slices were then inoculated with 10^{-1} dilution of the starter culture (*Bacillus subtilis* and *Bacillus licheniformis* at ratio 1:1) using sterile syringe at 3 % v/w. Fermentation was carried out at 30 ^{o}C for 72 h. Samples were drawn from the raw seeds, cooked slices and fermented slices for analysis.

The fermented samples were canned in three different media (tomato sauce, brine, and refined groundnut oil) using medium size cans as described in an earlier work (Enujiugha and Akanbi, 2008). The fermented oil bean seed slices were canned using 300 x 208 kk lac/lac cans with 300 kk lined ends (Carnaud Metal Box Nig. Plc., Ikeja – Lagos, Nigeria) in three different media (reflecting the use to which the fermented product is put); namely, tomato sauce, refined groundnut oil and brine solution. The tomato sauce used in the present study was prepared from the following ingredients: tomato puree (30% solids), 75%; flour, 5%; sugar, 14.5%; salt, 3%; vinegar, 1%; and spices, 1%; to give a total solids content of 14.0 ± 0.5%. The brine solution used in the study contained 1.5% sodium chloride and 3% sucrose (w/v). The refined groundnut oil used was a commercial brand purchased from a local market at Ile-Ife, Nigeria. Portions of the fermented ugba slices (about 100 g each) were placed in the cans and the appropriate medium added, with a headspace of about 15 mm. The cans were covered with lids, exhausted in a steam chamber for 15 min, and sealed immediately by means of a semi-automatic sealer (Dixie Model 23-500, MD-75, USA). Thermal processing was carried out at 116 ^{o}C for 60 min in a still retort and the cans were cooled under running water at 38 ^{o}C and stored in a cool place (about 15 ^{o}C) prior to analysis.

Measurement of pH

A 10 g portion of the sample was homogenized with 40 ml of distilled water. The pH of the homogenate was measured using a digital membrane pH meter (Hanna Instruments Model HI 8314).

Colour determination

Colour of the raw, cooked, fermented and canned samples was determined using the methods of Skrede *et al.* (1992) and Yeh *et al*. (2002) with some modifications. 4 g of each sample was extracted for 12 hrs with 100 ml of 60% ethanol. The sample extracts were filtered using Whatman no.2 filter paper and subsequently used for the colorimetric measurements. Instrumental colour of the samples, described by Hunter L* (lightness/darkness), a* (red/green), b* (yellow/blue) and hue values, was obtained using a 1-cm cell in a Corning Colorimeter Model 253 (Corning Ltd, Halstead, Essex, UK). Hunter L*, a*, b* values were calculated from the transmission spectra between 400 and 700 nm (50 nm intervals) by means of the weighted-ordinate method (Hunter and Harold, 1987). All measurements were done in triplicates, and the mean values were reported in the data. The equations for calculation in this system are:

$L^* = 100Y^{1/2}$,

$a^* = 175(1.02X - Y)/Y^{1/2}$, and

$b^* = 70(Y - 0.847Z)/Y^{1/2}$

where X, Y and Z were the chromaticity coordinates

Light source A (incandescent light) was used for calculations. The psychometric colour terms, chroma and hue angle, were calculated from the a* and b* values using formula: Chroma = $[(a^*)^2 + (b^*)^2]^{1/2}$ and Hue angle = $\tan^{-1} b^*/a^*$. Also the total colour difference $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ and a*/b* ratio as a measurement of the yellow (<1) or red (>1) colour was calculated from the data.

Fatty Acids Analysis

Fatty acids were determined using gas chromatography, as previously described (Enujiugha, 1990). The fatty acid methyl esters were obtained quantitatively from the oil by direct transesterification with methanolic sodium hydroxide at room temperature, followed by subsequent methylation with 14% boron trifluoride (BF_3) – methanol (Enujiugha, 2003). The component fatty acids were determined with a Johnson Q94 gas chromatograph with flame ionization detector.

Esterification with boron trifluoride:

The oil extracted from each sample was homogenized by heating gently in a water bath until a clear sediment-free oil was obtained. About 2-3 drops (ca. 250 µg) of the oil was put in a screw-capped test tube and 1.2 ml of 0.4N sodium hydroxide in methanol was added to saponify the oil. The test tube with content was heated on water bath at 60°C for 10 minutes to dissolve all the fat globules. The mixture was then neutralized with 1.2 ml 0.7N HCl to release the fatty acids. About 2-3 ml of 14% boron trifluoride in methanol was added to methylate the fatty acids. Test tube with content was again heated for another 10 minutes at 60°C.

Approximately 3 ml of petroleum ether (b.p. 40-60°C) was added and the test tube was shaken vigorously for about 5 minutes to effect distinct phase separation. The upper petroleum ether layer was decanted, dried over anhydrous sodium sulphate, and concentrated.

As a control, approximately 0.2 g of fatty acid standards was methylated instead of the oil sample.

Chromatographic analysis:

Exactly 1µl of methylated sample was injected into gas liquid chromatograph with a micro syringe. The fatty acid methyl esters were analyzed by GLC using Q94 gas chromatograph with JCL 6000 For Windows 2.0 Chromatography Data System (Johnson Chromatography Ltd) under the following conditions: Column, glass; stationary phase, 10% bisethyleneglycol succinate polyester (DEGS); support, 60-80 mesh chromosorb W; carrier gas, nitrogen; inlet pressure, 20 psig; injection temperature, 200°C; detector, hydrogen flame ionization (FID); sensitivity, $1x10^{-9}$A; chart speed, 5 mm/min,; hydrogen pressure, 15 psig; oxygen pressure, 7 psig.

The separated fatty acid methyl esters were identified by comparing their relative retention times with those of known standards and using the usual semi log plot of relative retention value versus equivalent chain length. The identified fatty acids were quantitated by multiplying peak areas by appropriate response factors.

Statistical Analysis

Data collected from the study were subjected to analysis of variance (ANOVA) as described by Snedecor and Cochram (1976). Differences among means were separated using Duncan's multiple range test; significances were accepted at 5% level ($p < 0.05$). The statistical software used was SPSS 10.0 for windows.

RESULTS AND DISCUSSION

The changes in pH and colour during processing

The changes in pH and colour during the canning process are presented in Table 1. The results of pH measurements indicate that *ugba* production is classified under alkaline fermentation, and agree with the report of Mbajunwa *et al.* (1998). However, the higher acidity of the canned sample compared with the alkaline fermented *ugba* could possibly be a result of leaching of basic elements into processing medium.

The colour intensity index (L*) shows that cooking slightly darkened the brown colour of the seed cotyledons. The a*/b* ratio reveals that, overall, the colour was not affected significantly ($p>0.05$) by the processing conditions. The higher hue angle of fermented *ugba* is reflective of the much lower a*, which shows that canning slightly darkened the *ugba* colour. Hue angle is the characteristic associated with the conventional perceived colour name. An angle of 90° represents a yellow hue. Objects with higher hue angles are greener while lower angles are more orange-red. Loss of brightness (or chroma) in the processed samples was primarily as a result of decrease of b*, which implies that processing darkened the colour of the seed cotyledons. Enzymatic browning, especially due to polyphenol oxidase, may not be entirely ruled out. It is also likely that changes in acidity contributed to the observed slight colour changes.

Profile of Fatty Acids

The results of the present study (Table 2) show that the African oil bean seed oil is of the drying type. Drying oils are composed of triglycerides whose fatty acid components contain mainly unsaturated bonds. Such oils are found in olive oil, linseed oil, poppy seed oil, tung oil, etc. and they are important because they help in lowering cholesterol and thus contribute to reducing the risks of heart attack (Heaton, 1984). In the present study, the raw

seed oil contained 91.45% unsaturated fatty acids expressed as FAME (fatty acids methyl esters), while the fermented product oil contained 94.40% unsaturated fatty acids, which represents an insignificant increase of only 1 percent. Onwuka *et al.* (1984) reported unsaturated fatty acid content of about 85% for the unprocessed African oil bean seed oil. In the present study, further thermal processing in three different media tomato sauce, refined groundnut oil and brine solution) did not significantly ($p>0.05$) affect the fatty acids profile of the seed oil.

The fatty acids present in the samples included capric C_{10}, lauric C_{12}, myristic C_{14}, palmitic C_{16}, stearic C_{18}, oleic $C_{18:1}$, linoleic $C_{18:2}$ and linolenic $C_{18:3}$. Arachidonic acid was not found in the oil, but in man, arachidonic acid is synthesized from linoleic acid, so that the absence of arachidonic acid could not constitute a nutritional disadvantage. With the exception of myristic acid, all the saturated fatty acids identified in the seed oil decreased as a result of fermentation. Further thermal processing brought about slight increases in lauric and myristic acids.

Linoleic acid was found to be the major essential fatty acid in the oil bean seed oil (constituting more than 66% by weight of the total fatty acids), followed by oleic acid and linolenic acid (two other unsaturated fatty acids). It is well known that dietary fat rich in linoleic acid, apart from preventing cardiovascular disorders such as coronary heart diseases and atherosclerosis, also prevents high blood pressure (Vles and Gottenbos, 1989). All studies on the raw African oil bean seed oil have shown that linoleic acid is the major fatty acid, followed by oleic acid (Ikediobi, 1981; Achinewhu, 1982; Enujiugha, 2003). Linoleic acid, alongside α-linolenic acid and docosahexaenoic acid (DHA), is essential to the well being, growth and development of children, especially breast-fed infants during the first six months (Innis, 1991). The African oil bean seed which is high in linoleic acid could therefore be usefully incorporated into infant formulae and weaning foods in developing countries where infant mortality rates are currently high due to protein-energy malnutrition (PEM). In a recent study (Enujiugha, 2006), African oil bean seed flour was incorporated into a maize-based infant weaning food, *ogi*, at ≤20% optimum substitution levels with remarkable results.

CONCLUSION

The present study has shown that the steps involved in canning of fermented African oil bean slices did not significantly ($p > 0.05$) affect the colour profile. Also, the African oil bean seed oil is of the drying type (with a high content of unsaturated fatty acids), and such oils are important because they help in lowering cholesterol and thus contribute to reducing the risks of heart attack. Thermal processing did not significantly ($p > 0.05$) affect the fatty acids profile of the fermented seed oil.

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Table 1: pH and colour changes during the production of canned *ugba* slices

Raw	Cooked	Fermented	Brine-canned	Sauce-canned	G/oil-canned	
pH	5.39±0.04	6.37±0.01	7.03±0.07	6.18±0.02	6.94±0.01	6.73±0.05
L*	64.58±0.10	64.03±2.05	64.42±1.22	64.42±3.01	64.38±2.13	64.41±0.19
a*	17.74±1.04	16.46±0.21	15.83±1.06	16.39±0.17	16.05±1.02	16.11±1.02
b*	35.10±0.07	32.88±0.04	33.96±0.15	34.14±0.09	34.07±0.10	34.20±0.12
a*/b*	0.51±0.02	0.50±0.01	0.47±0.01	0.48±0.03	0.47±0.07	0.48±0.01
Hue	63.19±0.17	63.41±0.13	65.01±0.08	64.36±0.10	64.91±0.03	64.67±0.15
Chroma	39.33±0.12	36.77±0.20	37.47±0.05	37.87±0.13	37.63±0.08	37.79±0.12

Values represent means ± s.d. of triplicate determinations

Table 2: Changes in fatty acids profile during fermentation and subsequent thermal processing of oil bean seed slices

	Fatty acids (percent by weight)						
Samples	Linoleic	Oleic	Palmitic	Stearic	Lauric	Linolenic	Myristic
Raw seed	67.20^{b}	21.05^{b}	3.81^{a}	2.21^{a}	1.50^{a}	3.20^{ab}	1.03^{b}
Cooked	66.18^{c}	22.16^{ab}	3.20^{a}	2.09^{a}	1.72^{a}	3.08^{b}	1.17^{ab}
Fermented	68.13^{a}	22.25^{a}	2.01^{ab}	1.29^{b}	1.14^{b}	4.02^{a}	1.16^{ab}
Brine-canned	68.09^{a}	22.28^{a}	2.00^{ab}	1.09^{c}	1.25^{b}	4.09^{a}	1.20^{a}
Oil-canned	68.01^{a}	22.36^{a}	1.97^{b}	1.14^{c}	1.24^{b}	4.12^{a}	1.06^{b}
Sauce-canned	68.14^{a}	22.29^{a}	1.90^{b}	1.12^{c}	1.30^{ab}	4.06^{ab}	1.19^{a}

Values in the same column with same following letters in superscript are not significantly different ($p>0.05$)