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Determination of Lipase Activities and Lipid Peroxidation Level of Fermented Oil Bean Seeds (Ugba, *Pentaclethra macrophylla*), Castor Oil Seeds (Ogiri, *Ricinus communis*) and Millet Seeds (Kunu, *Eleusine coracana*)

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Abstract Lipases are glycerol ester hydrolases which hydrolyze esther linkages of glycerides at water-oil interface. The activities of lipase in fermented oil bean seeds (ugba), fermented castor oil seeds (ogiri) and kunu were determined with respect to the levels of free fatty acids produced from each sample. The samples were prepared by local adoption of processing oil bean and castor seeds. The seeds were cooked (100°C) in cooking pot for 4hr, chopped into pieces, washed with clean tap water and covered in a stainless pot and allowed to ferment at room temperature. The lipid peroxidation and lipase activity were also determined on the fermented products using standard methods. The total free fatty acid determined were 349± 7, 1026± 5 and 94± 5mg/ml for ugba, ogiri and kunu respectively. The level of lipid hydroperoxide concentrations of fermented ugba, ogiri and kunu were $(2.5 \pm 0.3) \times 10^{-4}$, $(1.57 \pm 0.05) \times 10^{-4}$ and $(4.5 \pm 0.3) \times 10^{-4}$ mg/ml malondialdehyde respectively. It was observed that the higher the peroxidation or concentration of malondialdehyde in a given sample, the least the lipase activity as determined by the level of free fatty acids. The findings of this research indicate that fermented ugba, ogiri and kunu could be good sources of lipase for industrial application.

Keywords: *lipase activity, malondialdehyde, free fatty acid, fermentation, lipid peroxidation*

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1. Introduction

Food fermentation is important aspect of diet worldwide. Fermentation constituted a substantial percentage of African regimens for decades [1,2]. Some of the advantages of fermentation are the preservation of significant amount of food and biological fortification of food substrates with vital nutrients [2].

'Ugba' is a prevalent native fermented condiments made from legumes and oil seeds by fermenting melon seeds (*Citrullus vulgaris*), fluted pumpkin (*Telfairia occidentlis*) and castor oil seeds (*Ricinus communis*) [3]. Different varieties exist depending on the raw material used [3].

Kunu is a popular nonalcoholic drink made from grains and consumed mainly in the northern part of Nigeria [4]. The beverage drink is mostly produced from millet, sorghum or maize depending on the local availability [5]. Although a refreshing drink, the locally processed beverage drink has as a challenge of having short shelf life and thus needs appropriate preservatives to improve its shelf-stability and keeping value [4]. Spicy ingredients like ginger and saccharifying agents are added depending on the locality and taste [6,7]. To improve the shelf life of kunu and present it in a hygienic state different processing methods and studies have been adopted [8,9,10].

The term 'lipase' represents the enzyme system that is commonly called 'lipases' [11]. They are hydrolase enzymes that catalyze the hydrolytic cleavage of ester carboxyl bonds in acylglycerol into their constituent free fatty acids and glycerols; which may be employed as structural material and energy source by the organism [11]. Lipases are the most common biocatalysts that have

significant applications in stimulating several biochemical methods in the industry [12]. They are effective in catalyzing several processes related to the food, pharmaceutical, leather [13], cosmetic, detergent, [14], medical diagnostics, diary, beverage, fatty acid and paper industries [15].

Lipid hydroperoxides are non-radical intermediates derived from unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol itself. Their formation occurs in enzymatic or non-enzymatic reactions involving activated chemical species known as reactive oxygen species (ROS) which are responsible for toxic effect in the body via various tissue damages. These reactive oxygen species include among others hydroxyl radicals, lipid oxyl or peroxyl radicals, singlet oxygen and peroxynitrite formed from nitrogen oxide, all free radicals.

Significant contributions have been made in microbiology and biochemistry of fermentation of legumes and oil seeds leading to production of fermented condiments such as ugba from African bean seed, ogiri from castor seed or melon seed [16]. Because continuous intake and production of lipase in our daily life is paramount, this research work is therefore, aimed at examining food samples especially fermented food stuffs like ugba, ogiri and kunu to determine their malondialdehyde concentration of free fatty acid through lipase activities.

2. Materials and Methods

2.1. Collection of Samples

African oil bean seeds (*Pentaclethra macrophylla*), castor seeds (*Ricinus communis*) and millet seeds (*Eleusine coracana*) were purchased from local market in Abia State, Nigeria.

2.2.1. Processing of Ugba

The African oil bean seeds (300g) were boiled in water (100°C) over an open fire for 5hours according to the method described by Odunfa and Oyeyiola [17]. The cotyledons were removed from the seed coats and washed after which it was boiled over a low flame for 12hours. It was allowed to cool, drained several times to remove bitter components in the cotyledons and soaked in water for a period of 6 hours. The cotyledons were then cut into long thin slices which were mixed with salt (4.0g), put in a clean pot, covered and fermented for 5 days at room temperature.

2.2.2. Processing of Ogiri.

Castor seeds (300g) were boiled for 12 hours and allowed to cool for 10 minutes. The seeds were de-hauled by pressing between palms and then the cotyledons were boiled in water for 1hour and allowed to cool after which they were sliced to desirable sizes. The sliced cotyledons were wrapped in jute bag and allowed to ferment for 48hours [3].

2.2.3. Processing of Kunu

This was done as described by Adeyemi and Umar [9]. Millet seeds (300g) were soaked in distilled water for three days at room temperature, spread in a flat surface and covered with jute bag for another 3 days to enable the seeds germinate. The germinated seeds were blended with sweet potatoes (*Ipomoea batatas*) (50g), ginger(*Zingiber officinale*) (5g) and pepper (*Capsicum annuum*) (1g) to form a sweet paste. The paste was divided into two, one part was placed in a vessel and had boiling water poured on it and stirred to give a mixture. The other part of the paste was then added to the mixture and stirred very well. The mixture was left for 2 days at room temperature for the grain husk to settle. The husk and other sediments were filtered out of the mixture using silk sieve and the filtered liquid is bottled for consumption as kunu.

2.3. Determination of Lipid Peroxidation of Fermented Ugba, Ogiri and Kunu

The lipid peroxidase activities of fermented ugba, ogiri and kunu were determined by the method of Maduka [18]. Aliquot (1ml) of dissolved samples (in distilled water) each was added to test tube and incubated with 3ml KCI buffer (0.02m) pH 7.4 for 30minutes. After the incubation, 0.12ml of 5NHCI was added and mixed thoroughly, followed by addition of 0.35ml of 2% Na-TBA solution (thiobarbituric acid) together with 0.35ml TCA. The tubes were covered with cotton wool and boiled for 10 minutes, allowed to cool and then the color absorbance read at 532nm using UV-visible spectrophotometer.

2.4. Determination of Lipase Activities of Fermented Ugba, Ogiri and Kunu

Each sample (10g) was dissolved in 50ml of distilled water in 250ml conical flask and 4 drops of phenolphthalein indicator was added as described by Maduka [18]. The mixture was titrated against 0.1M NaOH and was constantly shacked until a pink color which persisted for fifteen seconds was obtained. The absorbance was read at 715nm.

2.5. Statistics

Results obtained were subjected to analysis using SPSS version 20.0. The results were presented as mean ± SD and difference in mean were compared using ANOVA at a probability threshold P=0.05.

3. Results

3.1. Lipid Peroxidation of Fermented Ugba, Ogiri and Kunu

The results of this study are shown in Table 1. It was observed from the research that kunu has the highest peroxidase activity followed by ugba and finally ogiri ($P<0.05$). This suggests that fermentation causes some level of deterioration of local stored food preparations. The level of lipid peroxidation was statistically higher.

Table 1. Lipid peroxidation and lipase activity (as free fatty acid) of fermented ugba, ogiri and kunu

Sample	Malondialdehyde concentration ($\times 10^{-4}$mg/ml)	Free fatty acid value (mg/ml)
Ugba	2.5 ± 0.3^{b}	349 ± 32.59^{b}
Ogiri	1.57 ± 0.05^{c}	1026 ± 2^{a}
Kunu	4.5 ± 0.3^{a}	94 ± 2^{c}

Values are mean ± SD of triplicate readings (P<0.05). Values with different superscript in a column are significant to each other.

3.2. Lipase Activity of Fermented Ugba, Ogiri and Kunu

Following the result of this research, ogiri had the highest lipase activities followed by ugba and finally kunu from the free fatty acid determined (P<0.05) (Table 1). The sample with the highest malondialdehyde concentrations exhibited the least lipase activity suggesting a relationship between malondialdehyde and concentration of fatty acids.

4. Discussion

The fermented foods namely African oil bean seed commonly called ugba, fermented melon commonly called ogiri and fermented local sorghum beverage (kunu) were subjected to biochemical investigation in nutrition. Lipid peroxidation as determined by malondialdehyde was evaluated to access their extent of deterioration and the effect of fermentation as local food preparatory method. The samples contain varied concentration of malondialdehyde, a degradation product of lipid peroxidation pathway. As reviewed by the results kunu contain the highest values. This is fallout of past nutritional evaluation of most of the fermented local samples earlier reported [19,20,21]. It is also necessary to acknowledge the fact that since malondialdehyde appears to be endogenous DNA adduct in human beings which contribute significantly to cancer linked to lifestyle and dietary factors, it should be at the barest minimum concentration to ensure good health in our society.

The lipase activities as determined by free fatty acid were also investigated in the samples. All the samples contained copious amounts of free fatty acids showing reasonable lipase activities. Lipases catalyze hydrolysis of free fatty acids from triacylglycerides [12] and can be used in industrial purpose as the source of flavor in production of foods. The catalytic activities are used in the industrial preparation of detergent, pharmaceutical products, agrochemical, cosmetics, bioremediation and biosensoring [13]. In the research being reported, reasonable lipase activities have been implicated and interplaying in the fermentation processes of the local ugba, ogiri and kunu which are all consumed locally as nutritional supplements and locally nutritional supplements and novel sources of proteins, lipids and vitamin [22].

The samples with the highest MDA concentrations exhibited the least lipase activities suggesting a relationship between MDA and concentrations of fatty acids.

5. Conclusion

The study revealed the fact that fermented food samples can be exploited as source of lipase which can be used for industrial purposes.

Conflict of Interest

The authors have no conflict of interest with regards to this publication.

References

[1] Achi OK. (2005). Traditional fermented protein condiment in Nigeria. *African .Journal of Biotechnology*. 4(12):1612-1621.

[2] Falegan A. (2014). Enzymatic and haematological changes in rats (*Rattus norvegecus*) fed with defated ogiri (fermented *Citrullus vulgaris*) and melon seeds. *Journal of Natural Sciences Research.* 4 (17): 133-140.

[3] Ibeabuchi JC, Olawuni IA, Iheagwara MC, Ojukwu M and Ofoedu CE. (2014). Microbiological evaluation of 'iru' and 'ogiri-isi' used as food condiments. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT).* 8(8): 45-50.

[4] Ogungbemi K, Alejo AO, Ilesanmil FF, Ishola DT, Afolabi AA, Oyelakin MO, Zaka KO, Ajanwachukwu CN. (2017). Sensory, shelf-life and nutritional evaluation of kunu (Nigeria non-alcoholic beverage) produced from different grains. *International Journal of Research Studies in Agricultural Sciences (IJRSAS).*3(9): 20-25.

[5] Abulude FO, Ogunkoya MO, Oni VA. (2006). Mineral composition, shelf-life and sensory attributes of fortified "kunuzaki" beverage. *Acta Sci. Pol. Technol Aliment*. 5(1): 155-162.

[6] Onuorah SI, Adesiyun AA, Adekeye JO. (2012). Survival and multiplication of Staphylococcus aureus and Echerichia coli in Nigerian ceral drink(Kunu zaki). Effect of spices, pH and temperature. *Journal of Food Agriculture*. 1: 169-173.

[7] Fapohunda SO and Adeware A. (2012). Microbial load and keeping quality of kunu under various preservative regimes. *Journal of Nutrition and Food Sciences*. 2: 4.

[8] Tahir F, Oyewole O. (1993). Bacteriological studies of kunuzaki. *Nigeria Journal of Microbiology.* 9: 47-53.

[9] Adeyemi IA, Umar S. 1994. Effect of methods of manufacture on quality characterization of "kunuzaki", a millet based beverages. *Nigeria Food Journal.* 12: 33-40.

[10] Bankole MO, Akpan I, Atanda OO. (1999). The anti-microbial effect of spices in kunutsamiya. *Nigeria Journal of Microbiol*. 13: 95-97.

[11] Gureeva NV. (2009). Pancreatic lipase activation, inhibition, and relationship to peroxidation of lipids. *Pharmaceutical Chemistry Journal*. 43(11): 619-624.

[12] Gitlesen T, Bauer M and Adlercreutz P. (1997). Adsorption of lipase on polypropylene powder. *Biochimica et Biophysical Acta*. 1345: 188-196.

[13] Gulati R, ISAR Jasmine, Kumar V, Parmar VS, Sexana R.K. (2005). Production of a novel alkaline lipase by *Fusarium globulosum* using Neem oil and its applications. *Pure and Applied Chemistry*. 77(1): 251-262.

[14] Gombert AK, Pinto AL, Castilho LR, Freira DMG. (1999). Lipase by *Penicillium restrictum* in solid state fermentation using Babassu oil cake as substrate. *Process Biochemistry*. 35(1):85-90.

[15] Nipon S, Revathi D, Sheelu G, YamunaRani K, Sridhar S, Mehtab V, Suman C. (2018). Recent advances on sources and industrial applications of lipases. *Biotechnology Progress*. 34(1): 5-28.

[16] Odunfa SA. (1985). Biochemical changes in fermenting African locust bean (*Parkia bigiobosa*) during iru fermentation. *Journal of Food Technology*. 20: 295-303.

[17] Odunfa SA and Oyeyiola GE. (1985). Microbiological study of the fermentation of 'ugba'- a Nigerian indigenous fermented food flavor. *Journal of Plant Foods*. 6: 155-163.

[18] Maduka HCC. (2008). Effect of time course administration of bergenin an isolate of *Sacogloths gabonensis*, Nigerian alcoholic

beverage additive on lipid peroxidation and natural antioxidant defenses during normal biological oxidation in weaning rats *in vivo*. *Nigerian Journal of Botany*. 21(1): 109-121.

[19] Maduka, H.C.C., Chukwu, N.C., Ugwu, C.E., Dike, C.C., Okpogba, A.N., Ogueche, P,N and Maduka, A.A. (2014). Assessment of commercial bottled table and sachet water commonly consumed in Federal University of Technology, Owerri (FUTO), Imo State, Nigeria using microbiological indices. *IOSR Journal of Dental and Medical Sciences*. 13,(1):86-89.

[20] Maduka HCC, Mohammed IH, Okpogba AN, Ugwu CE, Dike CC, Maduka AA, Ogueche PN, Gadaka MA. (2014). Biochemical assessment of some common commercial fruit juices consumed in Maiduguri Metropolis, Borno State Nigeria. *Journal of Natural Sciences Research*. 4(10): 94-98.

[21] Maduka HCC, Ugwu CE, Dike CC, Aguoru CU, Okpogba AN, Ogueche PN, Maduka AA, Gadaka MA, Alike SO, Okonkwo CO. (2014).Assessment of some commercial fruit juices commonly consumed in Federal University of Agriculture Makurdi, Benue State Nigeria, for lipid peroxidation intermediates and antioxidant vitamin. *Advances in Life Science and Technology*. 24: 49-52.

[22] Steinkraus KH. (2002). Fermentations in world food processing. *Comprehensive Reviews in Food Science and Food Safety*. 1: 23-32.