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Biochemical Effects of Dietary Consumption of Raw and Fermented Seeds of African oil Bean (*Pentaclethra macrophylla Benth*) in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author ODA designed the study and did laboratory analysis. Authors ACF and CMO carried out the statistical analysis. Authors ACF, CMO and UIU managed the literature search and interpretation of the data, while author ACF wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Studies have reported the microbial role, biochemical changes and nutritional quality of fermented African oil bean seed, known as Ugba in the eastern Nigeria; however, assessment of possible health impact of this food is lacking in scientific literature. The study investigated the biochemical effects of raw and fermented seed of African oil bean incorporated in diet of albino rats on plasma lipids and liver function indices.
Methods: Rats were divided into control and experimental groups and fed with diet containing casein, raw seeds or fermented seeds of African oil beans for 21 consecutive days. Plasma obtained after the experimental period were used to analyze for total cholesterol, triglyceride, HDL-C, LDL-C, total protein, albumin, conjugated and total bilirubin and liver enzymes.

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Results: Fermented seed diet significantly reduced lipoprotein lipids, liver function proteins, aminotransferases, ALP, and bilirubin (p<0.05), while raw seed-incorporated diet significantly increased the parameters but not significantly for liver enzymes and conjugated bilirubin (p>0.05), as compared to control.

Conclusion: Our data suggest beneficial biochemical effects of fermented seeds of African oil beans, as demonstrated by reduced lipoprotein cholesterol, triglyceride and improved hepatic status.

Keywords: Africa oil bean; Pentaclethra macrophylla; fermentation; fermented foods; ugba; aminotransferases.

1. INTRODUCTION

Fermented foods are prepared from plant and animal materials by microbial processes which play an important role in modifying the substrate's physical, natural and sensory properties [1]. Fermented foods are consumed by a large number of people in different climes of the world. Over the years, these foods have become a part of the cultural and traditional norm among the indigenous communities in Africa. Different parts of West Africa are renowned with their own favorite food that has evolved over centuries, depending on the customs, tradition and religion [2]. Some of these are seeds and nut foods which are cooked, fermented or roasted to be consumed as snacks, soup or sauce ingredients, including African oil bean seeds [1].

African oil bean (*Pentaclethra macrophylla Benth*) is a member of the family *Leguminosae*. It is popular in Nigeria where it is known as Apara in Yoruba, Ukana in Efik, and, the most prominent, Ugba in Igbo in local parlance [3]. Ugba, an indigenous Nigerian fermented food, is the Igbo name for the fermented seed of African oil bean, estimated to be consumed by about 15 million people in the south-eastern Nigeria [4]. It is a popular Igbo condiment and delicacy made from traditional household solid state fermentation of African oil bean seed. The fermentation is a mixed culture alkaline process involving a variety of microorganisms among which *Bacillus spp.* has been found to be necessary for the development of the aesthetic and organoleptic appeal in the daily diet [3,5]. Prominently among the Igbo ethnic group in Nigeria, it is served as snacks, side dish or as a food condiment. It is an essential food item for various traditional ceremonies where it is mixed with slices of boiled stock fish garnished with boiled vegetables and consumed by all socio-economic classes [6]. The fermentation methods are not standardized and that may be responsible for different nutritional qualities and flavours in the food. However, in the basic method, seeds are boiled (up to 12 hours), dehulled, and the cotyledon sliced. The slices are washed in water, then wrapped in plantain leaves and allowed to ferment for 2-4 days depending on the environmental condition, the needed use of the fermented product (soup or salad making), the thickness of the slices and the extent of cooking [7]. The oil bean seed is mainly composed of proteins (42%), lipids (43%) and carbohydrates (15%) [1,8].

Previous studies on Ugba have reported on microorganisms involved, the biochemical changes that occur during fermentation and the nutritional quality [1]. However, the possible effects of changes in nutritional quality during fermentation on health indices are sparsely reported. A study [9] reported that Ugba decreased the level of plasma cholesterol in rats in a duration-dependent manner, with highest reduction by Ugba fermented for 4 days. Chidozie et al. has shown that consumption of fermented seed as a food supplement is associated with reduced risk of cancer and tobacco-related diseases [10]. The seed is rich in oil containing 25% saturated and 75% unsaturated long chain fatty acids [4] with minimal activity of lipase on them during fermentation [5,11]. This is important for the role of dietary fats in cholesterol perturbation well reported to be associated with cardiovascular diseases [12]. Furthermore, oxidative stress and nephrotoxic potentials of raw seeds of African oil bean have been suggested in experimental studies due to its adverse effect on catalase, malondialdehyde, urea and creatinine levels [13,14]. Although fermented seeds of African oil bean have reduced levels of toxic anti-nutritional factors [15], the effects of the processing method on lipids and liver function are sparsely reported. Thus, the study aimed to investigate the effects of raw and fermented seeds of African oil bean on plasma lipids and liver functions in rats.

2. MATERIALS AND METHODS

2.1 Animals

Six weeks old albino rats obtained from the animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria were used for the experiment. The animals were kept in well-ventilated cages at room temperature of about 28ºC with a 12 hour light/dark cycle at the Department of Biochemistry, Ambrose Alli University. Normal feed and clean drinking water was provided to the animals until the time of experiment. The animals were allowed 1 week acclimatization before the experiment and ethical rules guiding the use of laboratory animals according to Zimmerman [16] was strictly followed.

2.2 Preparation of Raw and Fermented Seed (Ugba)

Seeds of African oil bean were purchased from a public local market of Owerri, Imo State and stored in a cool dry place. The oil bean seeds were dehulled and the outer coat removed to obtain the raw seeds, milled and used to compound the raw African oil bean diet. The fermented diet was prepared according to the traditional method described by Odunfa and Oyeyiola [17] with a slight modification. Seeds of African oil bean were boiled in water for 5 hours. The seeds were then de-hulled and the cotyledons removed and washed in sterile water to be boiled again for 2 hours. It was allowed to cool, drained and washed several times to remove the bitter components in the cotyledons. The cotyledons were sliced longitudinally using sharp table knife and wrapped in banana leaves to ferment for 2 days at room temperature (28±2ºC). The fermented seeds were open-air dried and milled to make fermented African oil bean seed diet.

2.3 Experimental Design

After acclimatization, rats were randomly assigned into three groups of three rats each. Group 1 served as control and rats were fed with casein-incorporated diet. Group 2 was given a diet prepared from raw oil bean seeds. Group 3 was fed with a diet prepared from fermented oil bean seeds. Each diet preparation contains 75 g of casein, raw or fermented seed of African oil bean mixed with other nutritional compositions (Table 1). The feeding lasted for 21 consecutive days. On the 22nd day, after an overnight fasting period, the rats were sacrificed and blood samples collected by cardiac puncture into heparinized bottles. The blood was centrifuged at 3000 rpm for 10 mins at the Department of Biochemistry, Ambrose Alli University. The plasma obtained was stored at -4ºC for analyses of lipid profile and liver function indices.

2.4 Biochemical Analyses

2.4.1 Determination of plasma lipids

Total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric methods as described previously [18,19], and HDL-cholesterol was determined enzymatically after precipitation of other lipoprotein as described by Warnic et al. [20], while LDL cholesterol was determined using Friedewald equation [21].

2.4.2 Determination of liver function indices

Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), conjugated and total bilirubin were determined using test kits (Randox Laboratories, UK) in accordance with manufacturer's instructions. Albumin was determined by colorimetric bromocresol green

Table 1. Formulation of synthetic diet

Diet g/100 g weight	Group 1	Group 2	Group 3
Corn starch	5.1	5.1	5.1
Groundnut oil	10.20	10.20	10.20
Casein	75.0	_	_
Raw milled seed	_	75.0	_
Fermented milled seed	_	_	75.0
Vitamin mixture	2.0	2.0	2.0
Mineral mix	3.0	3.0	3.0
Carboxymethylcellulose	4.7	4.7	4.7

method as described previously [22], and total protein concentration using test kits (Randox Laboratories, UK). Gamma-glutamyl transferase (GGT) was determined by the method of Persijn and Van der Slik [23].

2.5 Statistical Analysis

Differences between obtained values (mean±SD) were carried out by ANOVA followed by Student's t test using SPSS version 16.0. A *p*-value less than 0.05 was taken as a criterion for statistical significant difference.

3. RESULTS

3.1 Plasma Lipid Profile

Table 2 presents the effects of two diets incorporated with raw and fermented seeds of African oil bean on lipid profile measured in control and experimental groups in the study. Consumption of raw seeds in the diet significantly increased ($p<0.05$) levels of lipid parameters of the animals in comparison to control. Conversely, diet that contained fermented seeds decreased lipid parameters significantly, compared to control ($p<0.05$). Furthermore, the levels of plasma lipids were found to be significantly lower in the fermented seed diet when compared with the raw seed diet (Table 2).

3.2 Liver Function Parameters

The effects of the diets on proteins and bilirubin are presented in Table 3. While consumption of the raw and fermented seed diets had no significant effect on conjugated bilirubin ($p>0.05$), significant differences were observed for the effect of the diets as compared to control. Fermented seed in the diet significantly decreased total protein, albumin, and total bilirubin when compared to control and raw seed group animals.

From Table 4, diet that contained raw seed of African oil bean increased liver enzymes, although non-significant effects were statistically observed ($p>0.05$), when compared to control. The fermented seed diet significantly lowered ($p<0.05$) ALT and ALP when compared with the levels obtained for the enzymes in control animals. GGT was not affected significantly by the diets.

Table 2. Effect of raw and fermented seeds of African oil bean on plasma lipid profile of albino rats expressed in mg/dl

Treatment	TC	LDL-C	HDL-C	TG
Control	183.33±2.08	114.33±4.04	53.00±2.65	143.00±2 .65
Raw seed	201.67±10.41*	135.67±2.08*	65.00±5.00*	160.00±5.00*
Fermented seed	164.67±5.03**#	96.67±2.89**#	41.00±3.61**#	129.33±1.16**#

*Values are mean±SD and significant level at p<0.05. *: significantly different from control; **: significantly different from control; #: significantly different from group 2 (raw seed)*

Table 3. Effect of raw and fermented seeds of African oil bean on plasma protein and bilirubin of albino rats

Treatment	Total protein (g/dl)	Albumin (g/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Control	7.50±0.50	4.17±0.29	1.53±0.06	0.20±0.10
Raw seed	8.73±0.25*	5.17±0.29*	2.27±0.32*	0.33±0.06
Fermented seed	6.10±0.36**#	4.07±0.12**#	0.90±0.10**#	0.13±0.06#

*Values are mean±SD and significant level at p<0.05. *: significantly different from control; **: significantly different from control; #: significantly different from group 2 (raw seed)*

Table 4. Effect of raw and fermented of African oil bean on plasma liver enzymes of albino rats expressed in IU/L

Treatment	ALT	AST	ALP	GGT
Control	17.67±0.58	18.00±1.00	16.33±0.58	14.33±0.58
Raw seed	19.67±1.53	19.00±1.00	17.00±1.00	15.47±1.06
Fermented seed	15.67±0.58**#	16.00±1.00#	14.33±0.58**#	13.33±0.57

*Values are mean±SD and significant level at p<0.05. *: significantly different from control; **: significantly different from control; #: significantly different from group 2 (raw seed)*

4. DISCUSSION

Proximate composition, biochemical and microbiological changes associated with fermenting African oil bean (*Pentaclethra macrophylla Benth*) seeds have been well reported. Here, we have reported *in vivo* biochemical effects of consumption of raw and fermented diets of African oil bean seeds in rats.

The present study has shown that fermentation of African oil bean seed influences the *in vivo* biochemical effects of this food. Fermented seed in the diet demonstrated beneficial role in lipid, bilirubin and liver enzyme status. Our findings do not suggest that it may improve the protein level, although the fermented seed of African oil bean, known as Ugba in the south-east, is traditionally claimed to be rich in protein [4]. Raw seeds in the diet increased cholesterol lipids, including triglycerides above the control. Raw seed are not edible and bitter until local fermentation is achieved [24]. Studies on chemical composition of raw seeds found higher concentration of anti-nutritional factors, in comparison to fermented sample, including saponins, alkaloids, tanins, and phytate [4,24], which may be involved in the hyperlipidemic effect of the raw diet, although saponins have been reported to have bitter taste and lowers plasma cholesterol [25,26]. On the other hand, fermented seed diet yielded significantly reduced levels of plasma lipids. Fermentation process of African oil bean seed has been reported to reduce anti-nutritional factors, improve taste, digestibility, and produce flavor, the quality index that play an important role in consumer acceptability [3,24]. Fatty acid analysis found that seeds contain 44-47% oil which has been found to contain mainly unsaturated oleic acid and linoleic acid [4] a polyunsaturated fatty acid associated with lower total and low density lipoprotein cholesterol [27] and reduce risk for coronary heart disease [28]. Conceivably, lipoprotein cholesterol reduction by fermented seed in the current study may be associated with the unsaturated fatty acids and reduced level of anti-nutritional factor. Although limited studies are available in this line, our observation here is in consonance with a previous report that associated consumption of fermented seeds (Ugba) with lower level of plasma cholesterol [9].

However, the observed opposite effects of the diets on liver proteins and bilirubin follows the reported effect of fermentation on protein content of cooked African oil bean seeds. In our study, raw seed increased total protein and albumin while fermented seed decreased the proteins, significantly. Tannin in food depresses growth by depressing protein quality, digestibility and utilization [4,24]. Although seeds of African oil bean losses about 73.49% of tannin content on fermentation [24], the residual amount may have important role in protein digestion and absorption. And currently, investigations on the presence or absence of these toxic substances in the fermented beans are lacking. Poor performance and low digestibility of protein found in a previous experimental rat study were attributed to toxic components in the seeds which impair protein utilization [29,30]. The effect of fermented seed on liver enzymes enhanced hepatic status as evidenced by their significantly reduced activities in plasma. Moreover, in this study it appears that fermented seed of African oil bean (Ugba) may mildly adversely affect liver synthetic function in relation to lower total protein and albumin. However, the enzymes levels suggest that the fermented seed may not be involved in liver injury. To our knowledge, there are no studies in this direction to compare the obtained data with. This lends more credence to the need for further studies in this area.

5. CONCLUSION

The present study demonstrated that consumption of fermented oil bean seeds, prominently known as Ugba in the south-eastern Nigeria, in diet has beneficial biochemical effects in albino rats, although the diet concomitantly reduced HDL-C. Our findings do not support that Ugba may nourish the body with protein, although anecdotal evidence suggest it. However, there is need for further studies to assess the effect of Ugba on biochemical indices, especially on liver functions and potential for oxidative stress.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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