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Full Length Research Paper

Exploring the effects of *OWOH* consumption on rat enzymatic and haematological profiles: A Nigerian cotton seed-derived condiment

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The proximate, energy, microbial load of two different *owoh* samples were determined using standard methods. The enzymatic, haematological and nutritional changes in rats fed *owoh* samples were also determined. The bacterial loads in the two *owoh* samples ranged between 4.5×10^6 and 7.5×10^7 cfu/g in *Bacillus* fermented *owoh* (BFO) and, 5.7×10^6 and 6.6×10^7 cfu/g in naturally fermented *owoh* (NFO), respectively. The microbial load in the samples reached the peak value at 72 h of fermentation. Rats fed on diet supplemented with BFO and NFO had average consumption of 10.42 and 8.05 g/rat/day, respectively, while the control (soy meal supplemented) groups recorded 8.49 g/rat/day. At the end of the feeding experiment, the test diets had lower body weight gain compared to the control; though the difference was not significant ($p < 0.05$). Compared with the control, the rats in the dietary groups had lower organ weight. NFO group had the least amount of packed cell volume (PCV), haemoglobin concentration and red blood cell (RBC) count with the values of 34%, 9.78 g/dl and 6.92×10^6/mm^3, respectively. The alanine transaminase (ALT) and aspartate transaminase (AST) ratio were 0.05 and 0.91 for both NFO and BFO, respectively. Test groups had increased amount of AST compared with the control. The values recorded for BFO and NFO were not significantly different from the control.

Key words: *Owoh*, condiment, cotton seeds, transaminases, phosphatases, haematological parameters, proximate analyses.

INTRODUCTION

Fermented foods are food substrates that have undergone a desirable change due to the action of invading microorganisms. They form a major portion of the diets especially West Africa (Odunfa, 1987; Aderiye and Adebayo, 1999; Tatsadjiue et al., 2004). Fermented foods constitute vast quantities of nutritious foods for infants and adults (Steinkraus, 1997; Aderiye and Laleye, 2003). Their nutritional benefits include a reduction in food toxicity, easy digestibility and a prolonged shelf life (Aderiye and Adebayo, 1999). Fermented foods especially condiments are very rich in vitamins A and D (Oyenuga, 1968). Fermented soup condiments have been reported to have high protein (Edem et al., 1990; Balogun and Fetuga, 1986).

Cotton (*Gossypium* spp) is the world's most important non-food agricultural commodity and one of the vegetable fibres. Apart from its use for textile purposes (Kochlar, 1986). Its seeds are also fermented to produce a soup condiment popularly known as *owoh* in the Western part of southern Nigeria *Owoh* unlike *iru*, *tempeh* and *soy-iru* which could not be fried and consumed as snacks after fermentation (Sanni and Ogbona, 1991). High level of phytate in unfermented cotton decreases the bioavailability of minerals (Linera, 1973) and protein digestibility. Due to its pleasant aroma and taste, *owoh* enjoys a wide acceptance. Fermented cotton seed is highly cherished as a soup condiment in some part of South Western of Nigeria (Onazi, 1988).

To the best of our knowledge, there is no report in the

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literature of the safety level of *owoh* which enjoy a wide acceptance among the local people in south western Nigeria. Consumption of owoh could produce effects in experimental animals; hence, this work aims at determining the safety level and nutritional potential of fermented cotton seeds.

MATERIALS AND METHODS

Sample collection

Dehulled cotyledons of cotton seeds (*Gossypium hirsutum*) samples used for this research work were bought from Oja Oba (a main market) in Akure, Ondo State, Nigeria. The seeds were collected in a clean polythene bag and transported to the laboratory.

Preparation of *owoh*

The cottonseeds were sorted and washed in clean water three times to obtain clean seeds. They were put into a sieve to drain off the water. The seeds were carefully wrapped in clean aluminum foil and boiled for 3 h. The water was drained off and the seeds were allowed to cool. The sample was inoculated with starter (mixed) cultures of organisms from a commercial '*owoh*' sample. The inoculated sample was divided into seven portions and each portion was carefully wrapped in clean aluminum foil, placed in an airtight container and fermented at 37°C for a period of 120 h.

Determination of microbial load in *owoh* samples

One gram of the fermented cotton seeds was aseptically weighed and homogenized in 9 ml of 0.1% sterile peptone water for 5 min. Thereafter, 10 fold serial dilutions were carried out. One milliliter of appropriate dilution was aseptically plated, using pour plate technique, on Plate Count Agar (Oxoid). The plates were incubated at 40°C for 24 h.

Composition of diets

The composition of test diets differed only in the protein supplement but each diet contained the same amount of appropriate protein sources. Vitamin and minerals mixture in the diet was done in ratio 1 to 4. The mixture contained (g/kg diets): thiamine (0.02), riboflavin (0.03), pyridoxine (0.10), vitamin B12 (0.00003), niacin (0.001), calcium pantothenate (0.10), p-aminobenzoic acid (0.01) Vitamin A acetate (0.04), ergocalciferol (0.4) and choline-HCl (2.0). Minerals mixture contained (g/kg diet) $CaCO_3$ (15.56) $CaCl_2.6H_2O$ (0.001), $CuSO_4.5H_2O$ (0.019), $FeSO_4.7H_2O$ (1.078), $MgSO_4$ (2.292), and $MnSO_4.2H_2O$ (0.025). The diets were composed as shown in Table 1. The feeding experiment lasted for six weeks.

Experimental animals

Thirty male albino Wistar male rats were obtained from the Pre-clinical Animal House, University of Ibadan, Nigeria. The animals were acclimatized for five days and fed with grower's mash (TopFeed®) and adequately supplied with distilled water *ad libitum*. The rats were housed in stainless steel cages in a well-ventilated room at about 27 ± 2°C. Lighting regimen was about 13:11 h of light and dark, respectively. The animals were randomly assigned (in cages) into three groups: naturally fermented owoh, (NFO), bacillus fermented owoh (BFO) and a control. All animal management and experimental procedures were performed in strict accordance with the requirements of the National Research Council's Guide for the Care and Use of Laboratory Animals (NRC, 1985).

Table 1. Percentage composition of experimental diets (g/100 g).

Component	Diets		
	BFO	NFO	Control
Maize	28.5	28.5	28.5
Wheat offal	30.0	30.0	30.0
Fish meal	12.5	12.5	12.5
Bone meal	12.5	12.5	12.5
Oyster shell	1	1	1
Mineral (salt)	0.25	0.25	0.25
Vitamin (premix)	15	15	15
BFOw	15	--	--
NFOw	--	15	--
Soya meal	--	--	15
Total	100	100	100

Collection of blood from the experimental animals

Blood was collected from the rat by cardiac puncture after a deep anesthesia. During the terminal blood collection the rats were held by the scruff of skin above the shoulders so that its head is up and its rear legs were down. A 1 ml syringe and a 22 gauge needle was inserted 5 mm from the center of the thorax towards the animal's chin, 5 to 10 mm deep, the syringe was held at 25 to 30° away from the chest. Blood drawn from the animals were used for the haematological and enzymatic examinations.

Determination of absolute weight and relative organ weight

The weight of each rat in the diet groups was taken, with sensitive balance, and recorded twice a week. The difference between the initial and the final weight was taken to be the weight gained by the rat. The average weight gained by the rats in each diet group was determined. At the end of the experiment (21 days), the rats were sacrificed and blood samples were collected for analysis. Different organs: heart, kidneys and liver were carefully removed, rinsed in 0.25 M sucrose solution, and weighed.

Determination of haematological parameters

Haematological parameter determined include the packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb), mean cell haemoglobin concentration (MCHC), mean cell volume (MCV) and mean cell haemoglobin (MCH) as described by Cheesbrough (2003).

Determination of enzyme activity in the serum of experimental animals

Alkaline phosphatase (ALP) and acid phosphatase (ACP) were determined according to the method of Bergemeyer and Brent

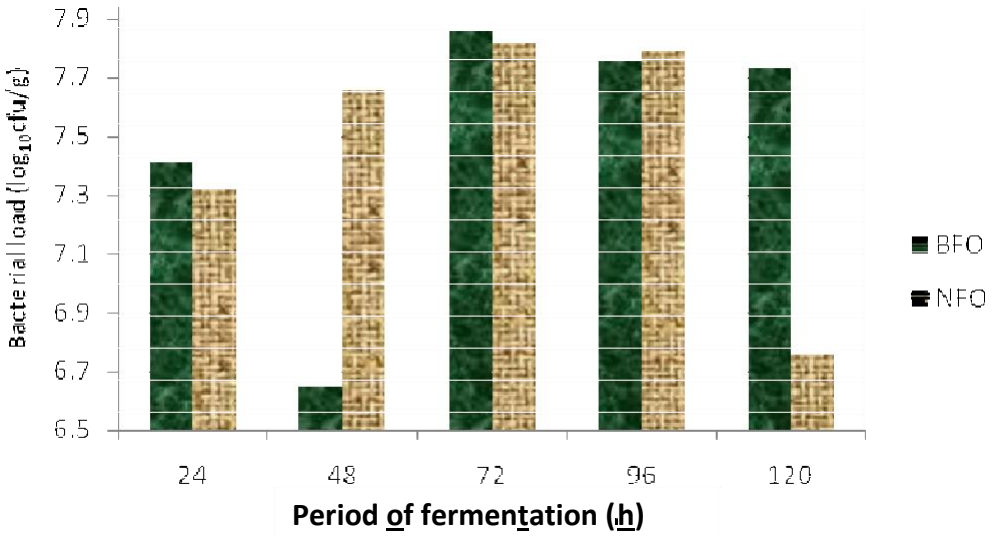

Figure 1. Bacterial load of bacillus- and naturally fermented *owoh.* BFO, *Bacillus* fermented *owoh*; NFO, Naturally fermented *owoh*.

Table 2. Average feed intake and average energy consumed by the rat fed *owoh* diets (g).

Week	Diets					
	BFO		NFO		Control	
	AFI	AEC	AFI	AEC	AFI	AEC
1	8.52 ± 1.02	106.00 ± 2.33	6.76 ± 32	89.07± 2.43	6.58 ± 1.90	80.28 ± 2.49
2	9.96 ± 0.32	124.98 ± 12.47	7.73 ± 3.99	101.86 ± 23.02	7.57 ± 3.12	92.13 ± 8.41
3	10.19 ± 2.90	127.86 ± 2.41	7.69 ± 4.10	101.33 ± 23.82	6.78 ± 3.11	82.51 ± 9.47
4	11.03 ± 0.21	138.40 ± 4.1	7.99 ± 3.01	105.28 ± 4.21	8.46 ± 0.23	102.92 ± 3.99
5	11.37 ± 3.01	142.67 ± 3.11	8.42 ± 1.34	110.95 ± 3.24	10.73 ± 3.11	130.59 ± 3.45
6	11.46 ± 2.11	143.80 ± 9.36	9.72 ± 4.1	128.08 ± 9.34	10.79 ± 2.14	131.32 ± 9.43
Mean	10.42 ± 2.01	130.62 ± 5.10	8.05 ± 2.13	106.10 ± 41.34	8.49 ± 2.83	103.29 ± 3.56

Vales are mean ± SD; AFI, average feed intake; AEC, average energy consumed.

(1974), while aspartate transaminase (AST) and alanine transaminase (ALT) were determined using Randox® enzyme kits.

Statistical analyses

Data were analyzed with statistical package for the social science (SPSS) version 11 software. The level of significance was determined at $p > 0.05$.

RESULTS AND DISCUSSION

The load of the fermenting bacteria with the period of fermentation is shown in Figure 1. After 48 h of fermentation, the total bacterial load in BFO was drastically reduced from 2.6×10^7 cfu/g and 4.5×10^6 cfu/g. The microbial load in the samples peaked at 72 h of fermentation. The total bacterial count recorded the least (5.7×10^6 cfu/g) in NFO at the 120 h of fermentation. The reduction may be as a result of depletion in the matabolizable nutrients in the substrate as noted by Yong and Wood (1976) and Falegan and David (2007).

Table 1 shows the composition of experimental diets. All the diets are the same except for the fermented *owoh* samples used as supplement. Soy meal was used to supplement the control diet. The diets were fed to the experimental animals for the period of six weeks and the animals were observed for changes. Table 2 shows average feed intake and average energy consumed by the rat fed *owoh* diets. The groups on BFO and NFO supplemented diets had average consumption of 10.42 and 8.05 g/rat/day respectively, while the control groups recorded 8.49 g/rat/day. The average energy consumed (AEC) followed the same pattern with the average feed intake (AFI). Based on the AFI, the quality of the BFO

Table 3. Performance of rats fed with BFO and NFO supplemented diets (g).

Parameter	Diets		
	BFO	NFO	Control
Initial weight	40.60 ± 3.45	39.68 ± 7.01	38.12 ± 4.46
Final weight	88.86 ± 3.12	84.18 ± 2.91	86.76 ± 3.45
Weight gained	50.28 ± 3.42	44.50 ± 3.00	53.64 ± 1.93
Food intake	62.52 ± 2.45	48.06 ± 3.80	50.94 ± 4.85
% weight gained	56.58 ± 5.91	52.86 ± 6.01	61.83 ± 6.92
Protein consumed	83.46 ± 12.10	61.03 ± 3.01	63.82 ± 3.90
Protein efficiency ratio	0.60 ± 0.01	0.72 ± 0.01	0.84 ± 0.02

Values are mean ± SD.

Table 4. Relative weight of the organ of experimental rats fed different *owoh* diets (mean).

Organs	Diets					
	BFO		NFO		Control	
	Weight	% body	Weight	% body	Weight	% body
Liver	3.74 ± 0.12	4.21 ± 0.13	3.64 ± 1.05	4.32 ± 0.91	4.0 ± 0.51	4.61 ± 1.98
Heart	0.34 ± 0.00	0.38 ± 0.06	0.33 ± 0.01	0.39 ± 0.19	0.37 ± 0.11	0.43 ± 0.2
Spleen	0.44 ± 0.01	0.50 ± 0.12	0.36 ± 0.01	0.42 ± 0.08	0.68 ± 0.01	0.78 ± 0.23
Lungs	0.78 ± 0.00	0.89 ± 0.31	0.81 ± 0.38	0.96 ± 0.21	0.84 ± 0.11	0.97 ± 0.01
Kidney	0.65 ± 0.16	0.73 ± 0.46	0.64 ± 0.10	0.76 ± 0.01	0.80 ± 0.09	0.92 ± 0.05

Values are mean ± SD.

supplemented diet was better compared to both NFO supplemented and the control. It had been reported that poor quality of protein impedes the nutritional benefits (weight gain and stability of the appetite) anticipated of animals that are continuously supplied with food and water (Aniagu et al., 2005).

The food intake, body weight and body weight gains (BWG) of the rats on the various experimental diets was shown in Table 3. The final weights of the rats ranged between 84.18 and 88.86 g. At the end of the feeding experiment, the test diets had lower body weight gain compared to the control; though the difference was not significant ($p < 0.05$). The diet containing BFO had higher percentage weight gain of 56.58%, while soy meal supplemented diet had the highest value of 61.83%. BFO supplemented diet supported the growth of the rats than NFO supplemented diet. The daily protein consumption was highest in BFO (83.46 g) while protein consumption of rats fed NFO containing diets (61.03 g) was comparable to those of the control (63.82 g). Falegan and David (2008) reported that *Bacillus* species produced a better *owoh*. However, the protein efficiency ratio was highest in the control group (0.84) followed by NFO (0.72).

The relative weight of the organ of experimental rats fed different *owoh* diets varied among the experimental diets and the control as shown in Table 4. Compared with the control, the rats in the dietary groups (BFO and NFO supplemented diets) had lower organ weight.

The significant decrease in the relative organs weight of the test groups reveals the non-toxic effect of the diets to the organs (Aniagu et al., 2005). The WBC which was drastically lower in *owoh* fed rats may be due to cytotoxic, tissue-destructive potential or bone marrow depression (Leendertse et al., 2009).

Table 5 showed the haematological changes in rats fed *owoh* diets. NFO group recorded the least amount of packed cell volume (PCV), haemoglobin concentration and red blood cell (RBC) count with the values of 34%, 9.78 g/dl and $6.92 \times 10^6/mm^3$, respectively. However, the values of PCV, haemoglobin concentration and RBC count were highest in BFO group with 40%, 12.12 g/dl and $8.02 \times 10^6/mm^3$, respectively. A low PCV value has been ascribed low or poor formation of RBC which eventually resulted in anaemia (Cheesbrough, 2003).

The MCHC which provides an index of the average haemoglobin was 0.30 in BFO and 0.8 in both NFO and control group. Low MCHC value indicates deficient haemoglobin synthesis (EMDEX, 2007). MCH value in BFO and NFO groups were 1.51 and 1.41. MCH gives an estimate of the average hemoglobin content of each red cell. The MCV value reflects the average volume of each red cell (Cheesbrough, 2003). NFO recorded the highest value of MCV.

Table 5. Haematological changes in rats fed *owoh* diets.

Parameter	Diets		
	BFO	NFO	Control
PCV (%)	40.00 ± 4.98	34.50 ± 2.46	38.56 ± 2.43
RBC (10^6)	8.02 ± 2.91	6.92 ± 2.01	7.80 ± 0.10
WBC (mm^3)	6500 ± 56	6580 ± 329	9760 ± 94
Haemoglobin (g/dl)	12.12 ± 1.89	9.78 ± 1.23	10.87 ± 1.83
MCH (10^{12}l)	1.51	1.41	1.39
MCHC (g/l)	0.30	0.28	0.28
MCV (10^{12}l)	5.00	5.18	5.02

Values are mean ± SD. Key: PCV, Packed cell volume; RBC, red blood cells; WBC, white blood cell; MCHC, mean cell hemoglobin concentration; MCH, mean cell hemoglobin; MCV, mean cell volume.

Table 6. Level of marker enzymes in the serum of the experimental animals during feeding experiment.

Parameter	Diets		
	BFO	NFO	Control
AST	163.20 ± 37.07	146.60 ± 31.33	160.4 ± 32.60
ALT	171.34 ± 42.07	293.9 ± 181.01	177.00 ± 17.49
AST/ALT	0.95	0.50	0.91
ALP	37.60 ± 8.07	41.74 ± 24.73	40.80 ± 10.00
ACP	45.75 ± 12.09	50.02 ± 28.31	39.39 ± 12.56

Values are mean ± SD. Key: AST, Aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; ACP, acid phosphatase.

There is no significant different in the amount of acid phosphatase and ALP in the test groups compared with the control (Table 6). This implies that the quality of the BFO and NFO did not affect the structural integrity of the membrane of the vital organs. Serum ALP is sensitive to both intra and extrahepatic bile obstruction in the liver and some bone diseases (Owu et al., 1998). However, the ALP levels observed in these studies were within the normal physiological range of 20 to 90 IU/L for rats (Harper, 1975; Kaneko et al., 1997). Thus it shows that the fermented samples used in the study did not adversely interfere with the calcification and other metabolic activities mediated by ALP this is in consonant with the report of David et al. (2007) and Singh et al. (2009).

The transamination reaction catalyzed by AST and ALT is essential for the protein synthesis in the liver (Murray et al., 2000). The levels of the enzymes in blood in the test groups were not significantly different from the control group. This indicates that there is no membrane damage in the cells due to degenerative changes (Shrivastava and Jha, 2010).

In the three samples, the amount of AST was lower than ALT which disagrees with the finding of Mayne (1996) who reported that the body cells contain more AST than ALT. The values were not significantly low to have adverse effect on protein synthesis and hence affect the cellular metabolism (Al-Attar, 2004).

Stroev (1989) reported that a high ALT/AST ratio indicates pathology involving the liver. The ALT/AST values recorded in the experiment ranged between 0.05 and 0.91 (for NFO and Control group, respectively). The least value was recorded for NFO diet group while BFO recorded 0.95. ALT/AST values greater than 1.00 indicate alterations involving the liver cells (Tietz, 1982).

The knowledge of biochemical, biological and nutritional effects of some of some Africa fermented condiments is necessary. Higher desirable qualities noted in the BFO called for controlled fermentation and development of starter cultures for traditional fermentations. Our study indicates that both BFO and NFO have different effects on the rats; however, the BFO appear to be nutritionally better than the NFO in term of body weight gain, haematological and enzymes indices.

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