JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2007. Vol. **1**(1), p. 39-44

The Effect of Fermentation on Nutritional Composition of Cottonseeds (*Gossypium hirsutum* Linn) for Production of 'Owoh'

C.R. Falegan and O.M. David*

Department of Microbiology, University of Ado-Ekiti PMB 5363, Ado-Ekiti, Nigeria.

(Received: 03 February 2007; accepted: 29 March 2007)

Effect of fermentation on the nutritional status of cotton seeds (*Gossypium hirsutum* Linn) was assessed by determining the Wende's composition, mineral contents, anti-nutritional factors and amino acid profile. There was reduction in fat content from 19.63 to 12.49g/100g, fibre content from 6.45 to 2.59g/100g, ash content from 2.40 to 0.25g/100g and crude protein from 18.91 to 16.97/100g at zero hour and 120hour respectively. Increases were observed in moisture content from 47.88g/100g to 60.07g/100g and carbohydrate content from 4.75g/100g to 7.63g/100g. pH decreased from 5.57 to 4.51 while titratable acidity increased from 1.75% to 2.23%. Increases were observed in minerals such as sodium, potassium, zinc and phosphorus. There were decrease in calcium, magnesium and iron as fermentation continued to 120 hours. Phytin, tannin and oxalate reduced from 28.84 to 13.59mg/g, 14.2 to 12.1 mg/g and 0.86 to 0.50mg/g respectively after fermentation. Increases were observed in some amino acid such as lysine, histidine, threonine, glycine, cystine, leucine and tyrosine.

Keywords: Cottonseeds, Fermentation, Amino acids, Owoh.

Apart from the pleasant aroma, condiments serve as good sources of protein and calorie intake in many countries such as Africa and India where protein calorie malnutrition is a major problem (Odunfa, 1985a; Sarkar *et al.*, 1993). In addition, the presence of vitamins A and D in most fermented seeds for condiments is an added advantage over seasoning salts (Oyenuga, 1968).

Fermentation leads to significant increase in essential fatty acids (Keshiro, 1983) due to the breakdown of protein (Omaefuvbe, 1998). Odunfa (1986) reported that 'iru' contains higher amount of riboflavin than the raw locust beans.

Cotton (*Gossypium* spp) is the world's most important non-food agricultural commodity and one of the vegetable fibres mainly used for textile purposes (Kochlar, 1986). It is also fermented to produce 'owoh' which unlike 'iru', tempeh and 'soy-iru' could not be fried and consumed as snacks after fermentation (Sanni and Ogbona, 1991). High level of phytate in cotton decreases the bioavailability of minerals such as Ca^{2+} and Fe^{2+} (Linera, 1973) and protein digestibility by forming complexes with trypsin and pepsin (Reddy and Pearson, 1994). Cottonseed is highly cherished as a soup condiment in some part of South Western zone of Nigeria (Onazi, 1988). The objective of this work is to determine the effect of fermentation on the proximate composition and biochemical properties of 'owoh'

MATERIALS AND METHODS

Sample Collection

Dehulled cotyledons of cottonseed (*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.

* To whom all correspondence should be addressed.
E-mail: davidgenerationng@yahoo.com

Preparation of 'Owoh'

The cottonseed were 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 hours. 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 incubated at 37°C for a period of 120 hours.

Determination of Proximate Composition of 'Owoh'

The standard method of AOAC (1990) was used to determine titratable acidity, moisture content, crude protein, crude fibre, ether extract (crude lipid). The method of Pearson (1976) was used to determine the protein content of the samples. Carbohydrate was determined by difference. The pH of the samples was determined by digital pH meter (ELE 256 model). Minerals were analyzed using the solutions obtained by dry ashing the samples at 550°C and dissolving it in 25ml of 10% HCI and 2ml of 5% lanthanum chloride. Deionized water was added to make the final concentration after boiling and filtration. Mn, Cu, Co, Zn, Fe, Mg and Ca were determined with a Buck atomic absorption spectrophotometer (Buck Scientific, Model 200A/200, Inc. East Norwalk, Connecticut, U.S.A.). Na and K were measured with a Corning 405 flame photometer (Corning, Halstead, Essex, UK, Model 405). The detection limits had previously been determined using the methods of Varian-Techtron (1975) as Mn 0.01, Cu 0.005, Co 0.05, Zn 0.005, Fe 0.02, Mg 0.002, Ca 0.04, Na 0.001 and K 0.005 ppm (all for aqueous solutions). The optimum analytical range was 0.5-10 absorbance units with coefficient of variation of 0.05-0.40%. Phosphorus was determined by the phosphovanado-molybdate method using a Spectronic 20 colorimeter (Galenkamp, London, UK) (AOAC, 1990).

Determination of Energy Value and Anti-Nutritional Content

The energy values were calculated by adding up the values obtained for carbohydrates (x 17 kJ), crude protein (x17kJ) and crude fat (x37kJ) for each of the samples (Kilgour 1987). Calcium/phosphorus (Ca/P) and sodium/potassium (Na/K) ratios were calculated for all the samples as described by Nieman *et al*. (1992). The Phy:Zn, Ca:Phy and Ca x Phy:Zn values were calculated according to the method of Wyatt and Triana-Tejas (1990). Tannin and oxalate were determine by the methods of Markkan and Goodchild, (1996) and Day and Underwood, 1986) respectively.

Determination of Amino Acid Profile

The amino acid profile in the known sample was determined using methods described by Spackman *et al*., (1958). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-sample Amino Acid Analyzer (TSM).

RESULTS

The pattern of changes in the proximate chemical composition of the cottonseed during fermentation into 'owoh' is shown in Table 1. The moisture content increased significantly from 47.88% in the unfermented seeds to 60.07% in the fermented 'owoh' at 120 hours of fermentation. There was also a decrease in the fat content of the substrate from 19.63% at 0 hour to 12.49% at 120 hour. The same trend was observed with the ash and crude fibre content. There was only a slight variation in the crude protein.

The carbohydrate content also show similar trend it decreased from 4.75% at 0 to 7.63% at 120 hour.

The pattern of changes in the mineral composition of cottonseed during fermentation is presented in Table 2. There was a significant change in the sodium ion that increased from 78.79mg/100g to 85.07mg/100g. The same trend was observed in the potassium ion that increased from 123.33mg/100g at 0 hour to 141. 56mg/100g at 120 hour. The calcium ion deceased from 0.51/100g in the unfermented cottonseed to 0.31mg/100g in the fermented sample. The pH decreased from slightly acidic to more acidic that is 5.57 to 4.51. The percentage titratable acidity steadily increased from 1.75 at zero hour to 4.04 at 72 hour and a decrease was observed later to 2.23 at 120 hour.

Table 1. The pattern of changes in proximate composition during fermentation.

ParametersPeriod of fermentation (Hr)	0	24	48	72	96	120
Moisture (g/100g)	47.88±0.04	51.08±0.02	54.51±0.31	58.26±0.10	60.13±0.15	60.07±0.01
Fatty acid (g/100g)	15.70±0.15	13.67±0.04	13.06±0.02	12.96±0.02	12.50±0.01	9.99±0.01
Fibre (g/100g)	15.70±0.15	6.40±0.01	5.75±0.01	3.82±0.01	3.13±0.03	2.59±0.02
Ash (g/100g)	2.40±0.01	1.93±0.01	1.69±0.02	1.49±0.01	0.31±0.01	0.25±0.01
Crude protein(g/100g)	18.91±0.82	19.39±0.00	21.36±0.42	19.71±0.42	16.48±0.00	16.97±1.92
Carbohydrate (g/100g)	4.75±0.72	4.13±0.04	0.36±0.07	0.50±0.39	4.33±0.04	7.63±1.91
Energy (kJ/100g)	1128.531	1032.17	973.45	943.71	932.08	880.33
pH	5.57	5.45	4.86	4.47	4.67	5.51
Acidity (Lactic acid)	12.90	14.40	24.00	24.00	26.30	16.40
Titratable acidity	1.75	1.96	3.26	4.04	3.58	2.23

Values are the means of triplicate determinations

Table 2. Changes in mineral composition during fermentation (mg/100g)

Fermentation period (Hr)	Na	K	Ca	Mg	Zn	Fe	P	Na/K	Ca/P
0	78.79	123.33	0.51	0.78	0.82	5.31	0.12	0.6389	4.25
24	78.82	126.41	0.32	0.30	0.52	4.33	0.11	0.6235	2.91
48	81.04	132.30	0.39	0.54	0.64	4.56	0.12	0.6125	3.25
72	83.36	140.25	0.55	0.47	0.89	4.58	0.15	0.5944	3.67
96	75.42	138.33	0.24	0.53	0.40	5.02	0.15	0.5452	1.67
120	85.07	141.56	0.31	0.51	0.93	5.06	0.14	0.6009	2.21

Values are the means of triplicate determinations

Table 3. Effect of fermentation on anti-nutritional factors in cottonseeds (mg/g).

Parameter	Unfermented cottonseed (0h)	Fermented cotton seed (120h)	Percentage increase (%)
Phyt ate	28.84	13.59	45.86
Tannin	14.20	12.10	14.79
Oxalate	0.86	0.50	41.86
Phy:Zn[a]	0.29	0.38	-31.03
Ca:Phy[b]	3.48	1.45	58.33
[Ca][Phy][c][Zn]	0.04	0.01	75.00

[a]mg of Phytate/MW (molecular weight) of Phytate: mg of Zn/MW of Zn; [b]mg of Ca/MW of Ca:mg of Phytate/MW of Phytate; [c][mol/kg Ca] x [mol/kg Phytate]/[mol/kg Zn]

There was a slight decrease in the level at magnesium ion from 0.78mg/100g and Iron from 5.31mg/100g at zero hour to 0.51mg/100g and 5.06mg/100g at 120hour respectively.

The Antinutritional Factors

In Table 3 the phytic acid content decreased from 28.84mg/g of the unfermented cottonseed to 13.59mg/g in 'owoh'. The tannin content reduced

to 12.1 mg/g from 14.2 mg/g in unfermented cottonseed while oxalate content reduced from 0.86mg/g in unfermented cottonseed to 0.50mg/g of the 'owoh' produced.

The amino acid profile was determined in both the unfermented cottonseed and the fermented sample. As shown in Table 4 only slight increase was observed in lysine, Histidine, threonine, Glycine, cystine, leucine and tyrosine. While slight reductions were observed in all other, 10 amino acids determined.

Table 4. Amino acid composition of unfermented cottonseed and 'owoh' in g/100g protein

Amino acid	Unfermented cottonseed	Owoh
Lysine	5.66	6.17
Histidine	2.26	2.51
Arginine	7.09	6.58
Aspartic acid	10.10	9.95
Threonine	2.66	3.19
Serine	3.26	2.80
Glutamic acid	13.25	12.6
Proline	2.60	2.27
Glycine	3.96	4.17
Alanine	4.02	3.06
Cystine	0.66	0.77
Valine	4.45	4.06
Methionie	1.21	1.04
Isoleucine	3.46	3.26
Leucine	7.02	7.65
Tyrosine	3.28	3.65
Phenylalanine	4.92	4.44
Total Basic Amino Acid	15.67	16.77
Total Acidic Amino Acid	23.35	5.99
Total Neutral Amino Acid	40.92	39.92
Total Essential Amino Acid	34.33	34.84
Protein quality	2.375	2.622

Values are the means of triplicate determinations

DISCUSSION

There was an increase in the moisture content observed from 47.88% to 60.07% this is probably due to the hydrolytic decomposition by fermenting organisms (Ogueke and Aririatu, 2004; Ajuebor *et al.,*2004; Sanni and Ogbonna, 1991). However, a direct drying method under sun or salting may help to prolong its shelf life (Aderiye and Laleye, 2003). There was a considerable decrease in the fat content of the 'owoh' produced from 19.63% to 12.49% which must have been due to production of lipase enzyme during fermentation (Sanni and Ogbonna, 1991; Enujuigha, 2003).

The reduction in the ash content observed in this study from 2.40% in unfermented cotyledons to 0.25% in fermented 'owoh' may be as a result utilization by the fermenting organisms. This report contradicts some other findings on fermented soup condiments. Gernah *et al*. (2005) and Enujuigha (2003) reported increase ash content during fermentation of locust beans for 'dawadawa' (fermented locust beans) and 'ugba', a fermented product of Africa oil beans respectively.

The crude protein values in the fermented 'owoh' were lower compared to the unfermented cotyledons (18.91%) this study contradicts the findings of Enujuigba (2003) who reported an increase during production of ugba'.

The reduction could be due to nativity of proteolytic enzymes produced fermenting organisms as reported by Odunfa (1983).

The decreased carbohydrate content of the fermenting substrates observed agrees with the work of Gernah *et al.,* (2005) during the fermentation of locust beans. This was reported could be due to carbohydrate utilization by the organisms during fermentation (Enujuigha, 2003).

The decrease in the hydrogen ion concentration (pH) of cotton seed during fermentation (from 5.57 to 4.51) could as a result of organic acids (Enujuigha, 2003; Moat, 1979). Increased pH was observed during fermentation of 'ugba' (Ogueke and Aririatu, 2004; Odunfa and Oyeyiola, 1985), 'iru' (Gernah *et al*., 2005) and 'ogiri' (Odunfa, 1981).

Though not many momentous changes were observed in the mineral content that were determined slight increase were observed in Na, K, Fe and P. This supports the earlier work of Eka (1980a) that there were slight increases in elements such as K, Na, Zn, Mg, Ca, Cu, Fe and phosphorus.

Some added advantages in the nutritional quality of the 'owoh' produced are the Ca/P ratio that is greater than 1.0 and Na/K ratio that is also equal to 0.6. These values indicate that 'owoh' will aid calcium absorption and will not promote high blood pressure (Niema *et al.,* 1992).

Apena (2004) has earlier reported the presence of nutritional factors in the unfermented cottonseeds at higher amounts. Fermentation reduces the amount of tannin, oxalate and phytate in 'owoh' by 45.86, 14.74 and 41.86% respectively. This result supports the work of Eka (1980). Foods with a molar ratio of Phy:Zn less than 10 showed adequate bioavailability of Zn and when the value is greater than 15.00 the food is considered poor (Wise, 1983). The value increased in fermented sample but it was mush lower than 15.00 cut-off point.

Fermentation affects the amino acid composition of cottonseed just like other substrates (Young and Wood, 1977; Oyeyiola, 1988). There were slight reductions in 10 amino acids after fermentation. The most abundant amino acid in the unfermented cottonseed and 'owoh' is glutamic acid; this may contribute more to the flavour of 'owoh' as it does in 'iru' (Odunfa, 1985). In general the level of amino acid present in the 'owoh produced is more than that observed in 'Kirbwang' a fermented yellow pulp of *Parkia biglobosa* (Musa *et al*., 2005).

The 'owoh' produced is therefore safe for consumption as soup condiment and also preferred to other chemical soup additives and flavoring agents because of its nutritional values.

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