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Effect of Fermentation on Physicochemical and Antibacterial activity of Cottonseed Oil

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Oil from cottonseeds (*Gossypium hirsutum* L) and its fermented product ('owoh') were investigated for their physicochemical and antibacterial properties using standard methods. Antibacterial property of the oils was carried out against five clinically important pathogens: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella* sp, and *Salmonella* sp. The iodine value (81.49) and specific gravity (0.8276) of oil from 'owoh' was lower than the substrate (109.11) and (0.8621) in that order. Saponification value increases after fermentation (from 209.00 to 196.00 mgKOH/g). This indicates that the molecular weight decreases after fermentation. Fermentation affects the keeping quality of the samples. Refractive index of oil from 'owoh' (0.8276) was slightly lower than the unfermented sample (0.8621). The antibacterial property of the oil was not significantly affected by fermentation. *Pseudomanas aeruginosa* was resistant to the oils even at higher concentration. The two investigated samples were effective against both Gram-positive and Gram-negative pathogenic bacteria. The results show that fermentation does not affect the edibility of cottonseed oil and the oils can be used against enteric pathogens especially *Salmonella typhii* and *E. coli*.

Keywords: Cottonseed oil, antibacterial, fermentation, pathogens, physicochemical properties.

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 bioavalability of minerals such as Ca^{2+} and Fe^{2+} (Linera, 1973) and protein digestibility by forming complexes with trypsin and pepsin, hence cottonseeds are not consumed raw (Adams, 1990). Cottonseed could be fermented to produce highly cherished soup condiment in some part of South Western zone of Nigeria (Onazi, 1988).

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Oils of plant origin have been reported to possess both antibacterial (Reineccius, 1994; Circella *et al.,* 1995) and antifungal properties (Bauer *et al.*, 1996). Fermentation leads to significant increase in essential fatty acids due to the breakdown of protein (Omaefuvbe, 2004). This might translate to significant alteration in the properties of the oil.

The objective of this work is to determine the effect of fermentation on the physicochemical and antibacterial properties of the cottonseed oil.

MATERIAL AND METHODS

Collection of Samples

Cottonseed samples were purchased from the local market in Akure, Ondo State. It was stored in a polythene bag at room temperature.

Methods of 'Owoh' Preparation

The cottonseeds 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 carefully wrapped in clean aluminum foil, placed in an airtight container and incubated at 37°C for a period of 120 hours.

Extraction of Oils

Oils from the samples were extracted from it by steam distillation of the samples as described by Dusk and Gokel (1987).

Determination of Physicochemical Properties of Oil samples

Saponification value, Wijs iodine value peroxide value, free fatty acid were determined as described by AOAC (1990). The specific gravity and refractive index were determined using a universal hydrometer and refractometer respectively.

Source of Test Organisms and Inocula Standardization

The test organisms were obtained from the Department of Microbiology, University of Ado-Ekiti, Nigeria. The organisms used were *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Klebsiella* sp. They were grown (in separate tubes) at 37°C in Mueller-Hilton (Oxoid) broth for 16-18h with shaking and diluted to an optical density of 0.1 (0.5 McFarland Standard) at optical activity of 625nm with Mueller-Hilton (Oxoid) broth and stored at 4°C to arrest further bacterial multiplication.

Determination of Antibacterial activity

From each of the samples, 0.2ml was transferred into the sterile test tube containing 9.8ml of 10% aqueous Tween 80 (diluents). Serial dilutions of the samples were made until a concentration of 0.0625% was achieved. Dilution tubes were stored at 4°C until use. Different concentrations (2.00, 1.00, 0.5, 0.25 and 0.125%) were then filled into separate holes, of 6.0mm diameter each, cut with a sterile cork borer into the Hilton-Hilton (Oxoid) agar seeded with test organism. The plate was refrigerated at 4°C for 30min before being incubated at 37°C. Zones of inhibition were measured after an incubation period of 24h. The oil was found to produce zones of inhibition against all the test organisms.

RESULTS AND DISCUSSION

Table 1 shows varying degrees in the physicochemical properties of the samples. The refractive indexes (at 29°C) of both fermented and non-fermented sample are very close. The specific gravities (at 20°C) of both oils were lower than palm oil (0.891) (Cocks and Van-Rede, 1966). The acid values of both samples are lower than that of African almond oil with acid value (Agatemor, 2006). Free fatty acid (FFA) is an index of keeping quality of an oil sample. It is also represents the extent to which the glycerides in the oil have been

Table 1. Effect of fermentation on the physicochemical properties of cottonseed oil.

Physicochemical Properties of oils from cotton seeds	Samples	
	Fermented	Non fermented
Refractive index (at 29°C)	1.462	1.460
Specific gravity (at 20°C)	0.828	0.862
Acid value(Mg KOH/	6.86	2.10
Free fatty acid (as oleic acid)	3.91	2.12
Saponification value (Mg KOH/g)	184.69	175.41
Iodine value (Wijs)	81.49	109.11
Peroxide value (Mg KOH/g)	12.95	5.18

Data are the mean values of three determinations

Table 2. Antibacterial activity of cottonseed oils on medically important pathogens after 48 h of incubation. (zone of inhibition in mm)

Pathogens	Concentrations									
	2.00%		1.00%		0.50%		0.25%		0.125%	
	FC	NFC	FC	NFC	FC	NFC	FC	NFC	FC	NFC
Escherichia coli	12	15	12	11	11	10	10	10	9	10
Klebsiella sp	12	16	10	15	9	9	9	8	9	6
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-
Salmonella typhi	12	12	11	11	11	11	11	11	10	11
Staphylococcus aureus	10	11	10	10	9	10	9	9	9	8

Data shown are the modal values of three determinations.
FC = fermented cotton seeds oil; NFC = non fermented cotton seeds oil; - = No growth.

decomposed (Pearson, 1975). The FFA increases with fermentation. This may have been as a result of the lipase elaborated by the fermenting organisms. Saponification values of fermented cottonseed oil was higher than cottonseed oil. This revealed that the molecular weight of the oil from fermented sample is lower than the one from unfermented samples (Cook and Van-Rede, 1966; Ong *et al.,*1992).

The iodine value decreased after fermentation of cottonseed. This showed that the degree of saturation in oil of fermented cottonseed was higher than the oil from cottonseed (Williams, 1966). Peroxide value of non-fermented sample (12.95 mg KOH/g) was higher as compared to the fermented sample (5.18 mg KOH/g). This gives an indication that the fermentation has greatly enhanced rancidity (Pearson, 1976).

Oil from both samples showed no varying degrees of antibacterial properties. Oils from cottonseed (fermented and unfermented) have no effect on *Pseudomonas aeruginosa* as against the oil from pepper fruit (Ejechi and Akpomeda, 2006). The zone of inhibition was most pronounced on *Salmonella typhi* even at a lower concentration. Fermentation has not contributed to the antibacterial properties of cottonseed oils (general overview). The antimicrobial effect of these oils may be due to the disruption the membrane structure of bacteria (Koyama *et al*., 1997). There was no difference in the activity of the oils on both Gram-positive and Gram-negative bacteria as noted for the essential oil obtained from the sweet basil (*Ocimum basilicum* L) and other *Ocimum* species (Lachowick *et al.,*1989).

Fermentation has little or no effect on the antimicrobial activity of the oil. Both samples possess antibacterial activity against gram-positive and gram-negative bacteria. Though fermentation has a pronounced effect on the physicochemical properties of cottonseed oil, oil from fermented cotton is still good for consumption.

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