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A note on Microbiological and Biochemical Evaluation of 'Omtigania', a Sudanese Fermented Food Based on Peanut Seed Cake

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Abstract: The objectives of this study aimed to evaluate the microbiological and biochemical changes during the fermentation of peanut (*Arachis hypogaea*) seed cake. The microflora during fermentation was dominated by a homo fermentative lactic acid bacterium (*Streptococcus sp* and *lactobacillus sp*) initially, the yeasts and mould and acetic acid bacteria were also present. The accumulation of acids led to the decline of the pH of the mixture from 5.7 to 3.6 on the seventh day of fermentation. Dried 'Omtigania' possessed a crude protein content of 67 %. The microbiological analysis showed that the microbial population is mainly bacteria.

Key words: seed cake**,** *arachis hypogaea***,** 'Omtigania', Biochemical analysis.

Fermented foods are defined as "foods or beverages produced through controlled microbial growth, and the conversion of food components through enzymatic action". Many foods have historically undergone fermentation, including meat and fish, dairy, vegetables, soybeans, other legumes, cereals and fruits (Marco *et al.* 2017). Fermentation is also used to enhance the organoleptic properties (e.g., taste and texture), with some foods, such as olives, being inedible without fermentation that removes bitter phenolic compounds (Dimidi *et al.* 2019). In the Darfur region of western Sudan, the seed cake remaining after extraction of oil from peanut seed (*Arachis hypogaea*) is fermented by the people into a food product known as

'Omtigania' (Elfaki *et al*. 1991). The usage of the term 'Omtigania' in this study will be confined to the fermented product derived from peanut seed cake. Therefore, this study aimed at detection of microorganisms that led to 'Omtigania' fermentation, detection of pathogenic microorganisms, and the knowledge of the nutritional value.

The samples of peanut seed cake and plant ash remains 'kambo' were collected from various locations of Khartoum State. The study was conducted at the Microbiology Laboratory in Faculty of Agriculture, University of Khartoum, Shambat, Khartoum, Sudan. The protocol followed in the laboratory fermentation was as closely as possible to that used by the indigenous people of western Sudan. Seedcake (1 kg) was pounded and made to a paste with tap water (1000 ml) and packed into an aluminum container. In the experiment plant ash 'kambo' was mixed into the paste prior to fermentation at a rate of 10 g kg^{-1} peanut seed cake. A piece of polyethylene was tied tightly over the top of the container and the vessel was incubated at 30°C for 7 days. At two days intervals during the incubation period, the fermenting material was remixed and samples were taken for analyses. After 7 days, the fermented material was molded by hand into small balls (15 to 25 g) and dried in sunlight for 2 weeks. Microbiological analyzes were performed on the peanut seed cake before, after and during fermentation as well as the plant ash "kambo" samples. Total viable count and microbes, microbes that contributed to the fermentation (yeast and mould, lactic acid bacteria and acetic acid bacteria), serial dilution was performed and microorganisms were detected; the pathogenic microbes (total Coliform, *E.coli*, *Staphylococcus* sp, fecal *Streptococcus* and *Salmonella* sp) were detected using Harrigan method (1998). *Salmonella* was detected using the method described by ISO (2002). Proximate analysis was performed according to the AOAC (2016); to determine the moisture content, ash content, protein, fiber, fat and carbohydrate. The energy was calculated, pH and *in vitro* protein digestibility (IVPD) was measured according to Baur and Ensminger (2000). ANOVA was carried out to test for significant differences among means of the treatments. Duncan's Multiple Range Test (DMRT) was

used for further multiple comparisons among the means according to the method described by Montgomery and Douglas (2001).

The microflora of peanut seed cake prior to fermentation was consistently dominated by *Streptococcus* sp and yeast species (Table 1). Other species such as *Bacillus sp* appeared in sufficient numbers to be detected by the sub sampling procedure. The two bacterial genera *Streptococcus* and *Lactobacillus* remained to dominate the microflora throughout the rest of the fermentation. The TVC increased with time of fermentation; this could be attributed to the group of microorganisms playing an important role in the fermentation process. Yeasts and mould of the raw material peanut seed cake was 4.0×10^3 cfu/g, while on seventh day of fermentation was 6.2×10^4 cfu/g until they disappeared in the final fermented product after drying. The increment of yeasts and moulds count could be attributed to the role they played in the fermentation process. Acetic acid bacteria were absent prior to fermentation but it started to appear during fermentation and then disappeared again in the final fermented product, maybe due to sun light drying. Pathogenic bacteria were also detected in peanut seedcake *ie*; *Staphylococcus* sp. appeared prior to and during fermentation, but its numbers began to decrease from the fifth day of fermentation, which may be due to a decrease in the pH. Such type of *Staphylococcus* sp. often is *Staphylococcus epidermidis* because it does not ferment the mannitol salt agar, according to detection and enumeration of pathogenic and toxigenic organisms' methods by Harrigan (1998) which considered not pathogenic; and its presence may be attributed to the fact that the sample was contaminated with *Staphylococcus*. Coliform bacteria were not detected before and during fermentation; the high amount of acids produced during fermentation may retard the growth of coliform bacteria to the minimum (Yagoub and Ahmed 2012). *Escherichia coli* were not detected before and during fermentation.

Approximate analysis of peanut seed cake; (moisture, ash, protein, fiber, fat and carbohydrate contents), energy, IVPD and pH were determined. The results showed a significant ($p \leq 0.05$) difference between and within the samples (Table 2). The result of moisture content prior fermentation was

8.3 % and it increased during fermentation to 38.2 % -41.0 % due to processing and it decreased in the final product to 7.5 % due to the drying of final fermented product. The protein result prior fermentation showed the lowest percentage (55.9 %), and the percentage increased continuously during fermentation (60.9 %-75.0 %) which could be due to the action of extracellular enzymes produced by the fermentation microorganisms (Enujiugha 2003), protein content decreased slightly in the final fermented product to 67.1 % due to exposure of the product to sun light during drying that might denatured protein due to high temperature. The results of fiber, ash and carbohydrates prior fermentation were 9.3 %, 6.5 %, 11.7 %, respectively. These results are in accordance with those found by Bhatt *et al.* (2005) and Babiker *et al.* (2009) in some groundnut seed cakes.

It could be concluded from this study that the fermentation of 'Omtigania' occurs mainly by lactic acid bacteria and the predominant bacteria were identified as *Lactobacillus* and *Streptococcus*. Fermentation increases the nutritional value and indeed the high chemical score of 'Omtigania' protein implies a nutritionally well balanced protein which can largely substitute for meat in most circumstances.

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Table1. Numbers of microorganisms isolated at stages prior and during fermentation of 'Omtigania'

Sample	Total viable count of bacteria cfu/g	Yeast and mould cfu/g	Lactic acid bacteria		*Staphylococcus aureus* cfu/g	*Coliforms* MPN/g		Acetic acid bacteria cfu/g	Fecal *Streptococcus* MPN/g	Detection of *Salmonella*
			Lacto Bacillus cfu/g	*Streptococcus* cfu/g		Total coliform	*E.coli*			
Prior fermentation	4.0×10^3	4.0×10^2	0	8.0×10^5	2.0×10^3	0	0	0	23	-
Day 1	8.0×10^2	4.0×10^3	1.4×10^3	9.0×10^3	2.7×10^3	0	0	2.1×10^3	23	+
Day 3	6.8×10^4	1.5×10^4	9.0×10^4	5.5×10^5	4.5×10^5	0	0	3.0×10^3	< 3	+
Day 5	9.0×10^4	1.3×10^6	3.7×10^4	1.5×10^4	2.2×10^5	0	0	1.3×10^5	< 3	+
Day 7	9.9×10^4	6.2×10^4	3.2×10^6	3.6×10^6	1.3×10^4	0	0	5.0×10^5	< 3	+
Final fermented Product	1.5×10^5	0	1.5×10^3	1.2×10^3	1.5×10^3	0	0	0	< 3	-
Kambo	5.0×10^2	5.0×10^2	1.5×10^3	0	0	0	0	1.0×10^4	0	-

MPN: Most probable number

Table .2. Chemical composition, Energy, *in vitro* protein digestibility (IVPD) and pH at stages prior, during fermentation and in final product of 'Omtigania'

Sample	Ash %	Moisture %	Protein %	Fiber %	Fat %	Carbohydrate %	Energy kcal/g	IVPD %	pH
Prior fermentation	6.5^{ab}	8.3^{a}	55.9^{a}	9.3^{b}	7.9^{c}	11.7^{bc}	342.7^{a}	58.4^{a}	5.7^{cd}
Day 1	5.4^{a}	38.2^{b}	60.6^{b}	9.5^{b}	7.7^{bc}	16.5^{d}	378.5^{c}	60.3^{a}	7.1^{e}
Day 3	5.8^{a}	40.0^{cd}	63.9^{be}	9.0^{b}	7.5^{bc}	13.6^{cd}	378.5^{c}	63.9^{b}	5.9^{d}
Day 5	7.7^{b}	41.0^{d}	69.0^{d}	9.2^{b}	6.5^{ab}	5.4^{ab}	364.9^{b}	70.4^{c}	4.4^{b}
Day 7	6.8^{ab}	38.9^{b}	75.0^{e}	8.8^{b}	6.0^{ab}	3.0^{a}	367.2^{b}	76.7^{d}	3.6^{a}
Final product	6.1^{a}	7.5^{a}	67.1^{cd}	5.7^{a}	6.4^{ab}	7.0^{a}	383.3^{c}	88.2^{e}	5.5^{c}

Values with different letters in the same column are significantly different at level $p<0.05$ according to DMRT .

التقييم الميكروبيولوجي و الكيمواحيائي لأم تيجانية كغذاء مخمر من كعكة بذور الفول السوداني

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المستخلص: إستهدفت هذه الدراسة تقييم التغيرات الكيمواحيائية و الميكروبيولوجية أثناء تخمير إمباز الفول السوداني لإنتاج ام تيجانية، الغذاء التقليدي في دارفور. كانت الميكروفلورا السائدة أثناء التخميرهي بكتيريا حمض اللاكيتك متجانسة التخمر(الإستريبتوكوسس واللاكتو باسلس) في البدء أيضاً تواجدت الخمائر و الأعفان و بكتريا حمض الخليك. أدي تجمع الاحماض في اليوم السابع من التخمير لانخفاض الاس الهيدروجيني من 5.7 الي 3.6. تحتوي أم تيجانية الجافة على نسبة بروتين 67%. أظهرت التحاليل الميكروبية أن المحتوي الميكروبي السائد هي البكتريا.