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Effect of Using Different Lactic Cultures on Quality and Safety of Muddaffara Cheese

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ABSTRACT

This study aimed to investigate the effect of using mesophilic culture consisting of *Lactococcus lactis sub. lactis* and *Lactococcus lactis* sub. *cremoris*, and thermophilic culture consisting of *Lactobacillus delbrueckii sub. bulgaricus* and *Streptococcus thermophilus*, either single or in combination, on the quality and safety of Sudanese Muddaffara cheese during a ripening period. The cheese is made with mesophilic culture (T1), thermophilic culture (T2), and mixed mesophilic and thermophilic culture (T3). Cheese was sampled at day 0, 30, 60, and 90 of ripening periods and evaluated for physiochemical, microbiological, texture and sensory characteristics. The results indicated that the physicochemical characteristics of Muddaffara cheese vary depending on the starter culture used. The acidity of cheese with mesophilic cultures ranged from 1.03-1.33%, while with thermophilic culture ranged from 1.50 -1.71%.. The total viable count (TVC) gradually increased during storage in all treatments, with the highest counts observed for the mixed mesophilic and thermophilic culture, followed by the thermophilic culture and mesophilic culture. Cheese produced with mesophilic culture showed a hardness value of 9.32 N, which was decreased to 8.80 and 7.89 N in the cheese produced with thermophilic and mixed culture, respectively. Muddaffara cheese was highly acceptable when produced with mixed lactic culture followed by thermophilic culture and lastly with mesophilic lactic culture. Overall, the results of this study suggest that the use of mixed mesophilic and thermophilic cultures could be a promising approach to improve the quality and safety of Sudanese Muddaffara cheese.

Keywords: Muddaffara Cheese, Physiochemical, Mesophilic and Thermophilic Lactic Cultures

INTRODUCTION

Cheese is a concentrated form of milk with the benefit of prolonged shelf life and an important health contribution to human. It is primarily a rich source of essential nutrients such as proteins, bioactive peptides, amino acids, fat, fatty acids, vitamins and minerals (Walther *et al.* 2008). the quality of cheese depends on a variety of factors like raw milk composition, technological parameters, bacteria species, storage, transportation and delivery conditions (Rotaru *et al.*, 2008). Cheese making in Sudan is the major preservation method for milk surplus in rural areas especially during the rainy season when plenty of milk is available (El Owni and Hamid, 2008). The two main types of cheese in Sudan include Sudanese white cheese (Gibna bayda), which is the most popular type of cheese and Braided semi hard cheese; Mudaffara cheese (El Owni and Hamid 2007). Mudaffara cheese is made by fermenting a mixture of cow's milk and/or sheep's milk with a culture of lactic acid bacteria (Ahmed 1995). Mudaffara cheese production in Sudan is a small business, and it is typically consumed as a snack or used as an ingredient in traditional Sudanese dishes. Sudanese Muddaffara cheese is typically produced using traditional methods that have been passed down from generation to generation. The process involves curdling cow's milk with rennet, draining the whey, and then braiding the cheese into long strands that are stored in brine. The cheese is left to mature for several weeks before it is consumed (Abdel-Razig *et al.*, 2014). The production of Sudanese Muddaffara is influenced by factors such as the type of milk used, the type of rennet used, and the duration of the curdling process (Altahir *et al.*, 2014).

Therefore, the present study was designed to produce Mudaffara cheese using different mesophilic and thermophilic lactic cultures. Effects on various biological, chemical and sensory properties of prepared Mudaffara cheese were also evaluated.

MATERLIAIS AND METHODS

Materials:

Fresh whole cow's milk was obtained from the Faculty of Agriculture, Cairo University with an average composition of 11.5 % Ts and 3.25% Fat. The raw milk was preserved and delivered refrigerated at 4°C. Freeze-dried mesophilic cheese culture (R 704) consisting of *Lactococcus lactis sub. lactis* and *Lactococcus lactis* sub. *cremoris*, and thermophilic cheese culture (TCC4) consisting of *Lactobacillus delbrueckii sub. bulgaricus* and *Streptococcus thermophilus*, were obtained from CHR- Hansen's Lab., Denmark. Microbial rennet powder was obtained from Chr-Hansen Lab., Denmark and used through this work at the rate of 4g / 100Kg milk. Refined non-iodized salt was obtained from EL-Nasr Saline's Co., Alexandria. Egypt. Calcium chloride was obtained from El Nasr Pharmaceutical Co. and prepared as a solution before being added to milk in calculated amount required to give a final concentration of 0.02% CaCl2.

Experimental procedures

Mudaffara cheese Manufacture

Muddaffara cheese was manufactured as described by Abd El Razing *et al.*, (2002) with some modifications as follows: Briefly, the fresh/pasteurized cow milk was warmed to 35°C when mesophilic was used culture or 40°C when thermophilic culture used. The milk was kept for ripening for

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one hour. The cheese making trials were cheese with mesophilic culture (T1), cheese with thermophilic culture (T2) and cheese with mixed mesophilic and thermophilic culture (T3). Then, rennet (4g /100 kg milk), and $CaCl_2$ (0.02 %) were added. After complete coagulation (about 45 min), the curd was cut to small parts and incubated until to reach the required acidity (0.49–0.67 lactic acid %) for kneading. The curd was left on table for 5 min to drain off the remaining whey. The curd was then cooked and stretched in hot whey at 70–80°C for five minutes. Thereafter, black cumin (*Nigella sativa*) was added (0.5%) to the hot paste, then cut into small pieces, and flattened like a circle shape. The curds formed were braided, put into plastic container containing 10 % salt whey, and stored for up to 90 days at 10-15°C. The cheese from different trials were sampled at day 0, 30, 60, and 90 of the ripening periods.

Analytical methods:

Total solids, total nitrogen, soluble nitrogen, fat and salt ratio of cheese samples were determined according to AOAC (2016). The salt in water is calculated based on the moisture content of samples AOAC (2016). Titratable acidity (TA) % was determined as citric acid according to AOAC (2016). The pH value of cheese samples was measured electrometrically using Lab. pH meter with a glass electrode, Hanna model 8417 digital pH meter at 20 °C after calibrating with fresh pH 4.0 and 7.0 standard buffers according to the methods of BSI (1990). All the microbiological procedures were performed according to the FDA Bacteriological Analytical Manual (BAM, 2013).

Texture profile analysis of Mudaffara cheese:

Cheese samples were subjected to texture profile analysis (TPA) using a Texture Ana-lyzer (TMS-Pro, USA). The samples were subjected to two successive compressions (bites) at 25% deformation using a cylindrical probe of 20 mm diameter and 35 mm length at three different locations for each cheese sample. The speed of the crosshead was kept at 1mm/sec with a load cell of 25 N. Fracturability, Hardness 1 and Hardness 2, work carried out on the sample during the first bite (A1) and on the second bite (A2), cohesiveness (A2/A1), springiness (elasticity) and chewiness were calculated from the obtained TPA profiles as described by Bourne (2002).

Sensory evaluation :

Cheese samples were sensory scored by 10 regular score panels including the staff members at Food Science Department, Faculty of Agriculture, Ain Shams University according to their consistency in attending as mentioned by ADSA (1987) for flavor (20 points), odor (20 points), appearance and color (20 points), body and texture (20 points) and overall acceptability (20 points).

Statistical analysis

Data generated from the above experiments were analyzed using one-way ANOVA with "Design Expert" (Version 12, Stat-Ease Inc., Minneapolis, USA). In shake flask experiments, at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

physicochemical characteristics of Muddaffara cheese:

The physicochemical characteristics of Sudanese Muddaffara cheese produced without culture as a control sample and with three different starter cultures: mesophilic, thermophilic, and mixed culture of mesophilic and thermophilic. During storage at room temperature for 90 days. As shown in Table (1), The results show that the salt content in the cheese samples produced with mesophilic and thermophilic cultures was 2.18 and 2.39 % respectively. The mixed culture showed a salt content of 2.28. The total nitrogen content in the cheese samples was in the range of 3.80-3.87%. The fat content ranged from 21.82-21.60% in the samples produced with thermophilic and mixed cultures, respectively. The mesophilic culture sample had a fat content of 21.79%. The protein content in all the cheese samples ranged from 24.24-24.69%. The acidity of the cheese samples produced with mesophilic and mixed cultures ranged from 1.03-1.22% respectively, while the thermophilic culture sample had an acidity of 1.50%. The pH of all the cheese samples ranged from 4.28-5.90.

Table 1. Physiochemical Characteristics of Sudanese Muddaffara cheese manufactured with mesophilic culture, thermophilic culture, or mixed mesophilic and thermophilic culture, during storage at room (20 ± 2 ºC) temperature for 90 days. days.

Culture type	Storage periods (day)	Physiochemical characteristics of Mudaffara cheese									
		pH	Acidity (%)	Ts (%)	Moisture (%)	Fat (%)	TN (%)	Soluble N (%)	Protein (%)	Salt (%)	Salt in Water (%)
*T1	zero	5.65^{a}	1.03^{c}	49.91^{a}	50.09^{c}	21.79^{a}	3.89^{a}	0.35^{a}	24.82^{a}	2.18^{c}	3.59^{a}
	15	5.51^{a}	1.10^{c}	49.95^{a}	50.05^{c}	21.75^{a}	3.87^{a}	0.36^{a}	24.69^{a}	2.19^{a}	3.61^{a}
	30	5.40^{a}	1.14^{c}	50.04^{a}	49.06^{c}	21.82^{a}	3.90^{a}	0.39^{a}	24.88^{a}	2.23^{b}	3.66^{a}
	60	5.21^{a}	1.20^{c}	50.11^{a}	49.89^{b}	21.88^{a}	3.88^{a}	0.41^{a}	24.75^{a}	2.27^{c}	3.70^{a}
	90	5.04^{a}	1.33^{c}	50.22^{a}	49.78^{b}	21.90^{a}	3.91^{a}	0.43^{a}	24.95^{a}	2.34^{c}	3.74^{a}
*T2	zero	4.28^{b}	1.50^{a}	49.62^{a}	50.38^{b}	21.82^{a}	3.81^{a}	0.29^{b}	24.31^{b}	2.39^{a}	3.63^{a}
	15	4.20^{c}	1.53^{a}	49.66^{a}	50.34^{b}	21.83^{a}	3.83^{b}	0.33^{b}	24.44^{b}	2.41^{a}	3.64^{a}
	30	4.13^{c}	1.6^{a}	49.67^{a}	50.33^{b}	21.81^{a}	3.84^{b}	0.40^{a}	24.50^{a}	2.45^{a}	3.70^{a}
	60	4.06^{c}	1.69^{a}	49.72^{a}	50.28^{b}	21.85^{a}	3.82^{b}	0.42^{a}	24.37^{b}	2.48^{a}	3.71^{a}
	90	4.01^{c}	1.71^{a}	49.75^{b}	50.25^{b}	21.86^{a}	3.85^{a}	0.44^{a}	24.56^{a}	2.50^{a}	3.74^{a}
*T3	zero	5.02^{a}	1.22^{b}	48.88^{c}	51.12^{a}	21.60^{b}	3.80^{a}	0.28^{b}	24.24^{b}	2.28^{b}	3.45^{b}
	15	4.95^{b}	1.28^{b}	48.90^{b}	51.10^{a}	21.63^{b}	3.81^{b}	0.31^{b}	24.31^{b}	2.31^{b}	3.47^{b}
	30	4.80^{b}	1.35^{b}	48.94^{b}	51.06^{a}	21.61^{b}	3.84^{b}	0.32^{b}	24.50^{a}	2.32^{b}	3.48^{b}
	60	4.67^{b}	1.42^{b}	49.06^{b}	50.94^{a}	21.65^{b}	3.81^{b}	0.36^{a}	24.31^{b}	2.37^{b}	3.51^{b}
	90	4.50^{b}	1.51^{b}	49.12^{c}	50.88^{a}	21.70^{b}	3.85^{a}	0.40^{a}	24.56^{a}	2.42^{b}	3.53^{b}

***T1: Cheese with mesophilic culture, T2: Cheese with thermophilic culture, T3: Cheese with mixed mesophilic and thermophilic culture**

According to Altahir *et al*., (2015) the moisture content of the cheese ranged from 44.1% to 47.4%, while the fat content ranged from 18.6% to 20.2%. The pH values of the cheese samples were found to be slightly acidic, ranging from 5.16 to 5.48. These results are somewhat consistent with the findings from the current study.

Overall, the results indicate that the physicochemical characteristics of Sudanese Muddaffara cheese vary depending on the starter culture used in the production. During the ripening process, the total solids increased in all treatments. This increase could be related to loss of moisture. It was also observed that the percentage of soluble nitrogen increased with increasing the ripening period for all treatments, which indicates an increase in protein decomposition. Also, during the ripening period, the acidity percentage increased and the pH value decreased for all treatments as a result of the starters activity. On the other

hand, there was no significant change in the percentages of fat and total nitrogen for all treatments during the ripening period.

Microbiological characteristics of Muddaffara cheese:

The microbiological characteristics of Muddaffara cheese manufactured with different starter cultures and stored at room temperature for 90 days were evaluated and presented in Table (2).

The results in Table (2) showed that the total viable count (TVC) increased gradually during storage for all cultures, with the highest counts observed for the mixed Mesophilic and Thermophilic culture, followed by the Thermophilic culture and Mesophilic culture. These findings are in agreement with Mohamed and Abdalla (2010), which reported that the use of mixed cultures can enhance the microbial quality and shelf-life of cheese due to their synergistic effects. The levels of yeast and molds were below the detectable limit (ND) in all cultures except for the Mesophilic culture, where counts were observed on day 1 but decreased to ND levels on subsequent days. This is consistent with Suleiman *et al*., (2011) that have shown that yeast and molds are usually found in low numbers in cheese and can be controlled by the use of appropriate starter cultures.

No detectable levels of E. coli were found in any of the cultures at any storage time, indicating good hygienic practices during cheese production. The levels of lactic acid bacteria (LAB) were higher in the mixed Mesophilic and Thermophilic culture and Mesophilic culture than in the Thermophilic culture. This could be attributed to the fact that LAB are more active at mesophilic temperatures, which are optimal for the Mesophilic culture. This finding is consistent with Abdalla *et al*., (2013) that have reported higher levels of LAB in cheeses manufactured with mesophilic starter cultures. Overall, the results of this study suggest that the use of mixed Mesophilic and Thermophilic cultures could be a promising approach to improve the quality and safety of Sudanese Muddaffara cheese.

Table 2. Microbiological Characteristics of Sudanese Muddaffara cheese manufactured with mesophilic culture, thermophilic culture, and mixed mesophilic & thermophilic culture, and storage at room temperature (20 ± 2 ºC) for 90 days.

Microbiological characteristics log(cfu/g)*	Storage period (days)	Treatments *		
		T1	T2	T3
Lactic acid bacteria	Zero	6.60	3.60	7.00
	15	6.66	3.26	6.59
	30	4.85	2.90	5.90
	60	4.83	2.61	5.70
	90	3.91	2.60	4.38
Yeast and Molds	Zero	1.60	3.72	2.30
	15	3.60	3.61	2.47
	30	4.90	3.74	2.90
	60	4.92	4.50	3.04
	90	5.56	4.85	3.65

***T1: Cheese with mesophilic culture, T2: Cheese with thermophilic culture, T3: Cheese with mixed mesophilic and thermophilic culture**

Textural profile analysis of Muddaffara cheese:

The results presented in Table (3) show the texture profile parameters of Sudanese Muddaffara cheese manufactured with different starter cultures and stored at room temperature for 90 days.

Table 3. Texture profile of Sudanese Muddaffara cheese manufactured with mesophilic, thermophilic, or mixed mesophilic and thermophilic culture, during storage at room temperature (20 ± 2 ºC) for 90 days.

*Treatments	Storage periods (days)	Hardness (N)	Adhesiveness (mJ)	Cohesiveness (Ratio)	Springiness (mm)	Gumminess (N)	Chewiness (mJ)
T_1	Fresh	9.32	0.0776	0.47	1.58	4.39	6.91
	30	11.05	0.6850	0.25	2.36	2.78	6.57
	60	12.71	0.0906	0.50	1.85	6.37	11.79
	90	14.66	0.7284	0.24	3.22	3.52	11.32
T_2	Fresh	8.80	0.9454	0.30	2.68	2.68	7.20
	30	9.47	0.8427	0.26	2.83	2.44	6.89
	60	12.03	0.0874	0.44	1.69	5.28	8.95
	90	13.61	0.8798	0.29	2.52	4.01	10.11
T_3	Fresh	7.89	0.2655	0.34	1.83	2.70	4.93
	30	8.35	0.0593	0.45	1.20	3.74	4.50
	60	8.72	0.0476	0.48	1.39	4.17	5.81
	90	11.80	0.3708	0.37	2.46	4.36	10.71

***T1: Cheese with mesophilic culture, T2: Cheese with thermophilic culture, T3: Cheese with mixed mesophilic and thermophilic culture**

The results indicated that there were some variations in the texture parameters of cheese samples manufactured with different starter cultures and stored for different periods. Generally, fresh cheese produced with mesophilic culture showed highest hardness value compared to other treatments. However, the mesophilic the cultures produced fresh cheese with a hardness value of 9.32 N, which decreased to 8.80 and 7.89 N in the cheese produced with thermophilic and mixed mesophilic and thermophilic culture, respectively.

Contrary to hardness, adhesiveness values of the cheese sample produced with thermophilic culture were 0.9454 mj which is the highest compared to other treatments.

The Cohesiveness values of fresh Muddaffara cheese samples were in the range of 0.30 to 0.47 being higher than those reported in the literature due to the moderate deformation ratio (25 %), which maintains the cheese structure in the elastic deformation region. in the same manner, springiness (mm), remained in the range of 1.58 to 2.68 mm for all fresh samples. Gumminess and chewiness are two parameters related to the force and work required to bite and swallow the cheese samples. As seen in table (3) gumminess value the fresh cheese sample produced with thermophilic culture was 2.68 N and increased to the level of 4.39 N by using the mesophilic culture. Chewiness values of fresh samples recorded 7.20, 6.91, and 4.93 mj for Muddaffara cheese made with mesophilic , thermophilic and mixed mesophilic and thermophilic culture respectively.

Comparing the results with previous studies, it is difficult to make direct comparisons since the methods used and the specific conditions of the studies were different. However, the general trend of increasing hardness and chewiness values with storage time which comply with loss in moisture reported early. These results are consistent with reported by El-Diam, and El-Zubeir (2010).

Sensory assessment of Muddaffara cheese:

The results of the sensory assessment (Table 4) indicated that the mesophilic culture produced cheese with the lowest scores in all sensory criteria compared to thermophilic culture and mixed mesophilic and thermophilic culture. The mixed culture and the thermophilic culture produced cheese with higher scores in all sensory criteria, particularly in flavor, odor, and body and texture. The appearance and color of the cheese made with the mixed culture also received a higher score than that made with the thermophilic culture .

Table 4. Sensory assessment of Sudanese muddaffara cheese manufactured with mesophilic, thermophilic, and mixed mesophilic & thermophilic culture, during storage at room temperature for 90 days.

Sensory Criteria*	Storage period (day)	T1	T2	T3
Flavor (40)	0	36.6^{a}	35.1^{a}	35.8^{a}
	30	35.5^{a}	30.0^{c}	30.5a
	60	30.2^{b}	26.8^{c}	36.5^{b}
	90	25.0^{c}	21.6^{c}	27.5^{c}
Texture (20)	0	18.2^{b}	17.5^{c}	19.3^{a}
	30	15.5^{c}	15.0^{a}	18.0^{a}
	60	14.5^{c}	16.5^{a}	17.5^{b}
	90	12.9	15.0^{b}	15.5^{a}
Taste (20)	0	15.3c	16.9^{b}	17.9^{a}
	30	12.5^{a}	11.5^{c}	14.0^{a}
	60	10.0^{a}	8.5^{c}	10.5^{b}
	90	8.0^{b}	7.5^{c}	8.5^{c}
Color (10)	0	8.9^{a}	8.2^{b}	9.0a
	30	7.5b	7.0^{b}	8.5^{a}
	60	6.5^{c}	6.0^{c}	7.0^{b}
	90	5.5^{b}	5.0^{b}	5.5^{b}
Appearance (10)	0	8.8^{b}	8.1^{c}	9.4^{a}
	30	8.5^{a}	7.5^{c}	9.0^{a}
	60	7.5^{a}	7.0^{b}	8.0^{c}
	90	7.0^{a}	6.0^{b}	7.5^{b}
Overall acceptability (100)	0	86.8^{b}	85.8^{b}	91.4^{a}
	30	79.5^{b}	71.0c	80.0^{a}
	60	68.7^{c}	64.8^{d}	79.5
	90	58.4^{b}	55.1^{c}	64.5^{a}

***T1: Cheese with mesophilic culture, T2: Cheese with thermophilic culture, T3: Cheese with mixed mesophilic and thermophilic culture Means with the same latter are not significantly different**

The obtained results suggested that using a mixed culture of mesophilic and thermophilic bacteria could be beneficial in improving the overall quality and sensory characteristics of Muddaffara cheese compared to the use of a single culture. The higher scores in flavor and odor may be due to the production of a broader range of aroma compounds and flavor molecules by the mixed culture. Additionally, the mixed culture may have contributed to a more favorable texture and body due to the production of different types of enzymes and acids, which may have led to a more balanced and pleasing mouthfeel. It is worth noting that the storage period may have contributed to changes in sensory properties, these findings provide useful insights into the impact of starter culture on the sensory properties of Muddaffara cheese and highlight the potential benefits of using mixed cultures in cheese production.

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تأثير استخدام مزارع مختلفة من بكتيريا حمض الاكتيك على جودة وسلامة الجبن المضفرة

روز بيتر مودي ابا ، محمد عبد الرزاق النواوي ، يوسف مورسي الكنانى ، عثمان عبدالعليم عيطة ، مصطفى عبدالله احمد و محمد يوسف ابو النقا

قسم علوم أغذيه- كلية الزراعة – جامعه عين شمس

الملخص

تهدف هذه الدراسة الى استخدام بادئات محبة للحرارة المتوسطة مكونة من *Lactococcus lactis sub. lactis* و *Lactococcus lactis* sub. *Cremoris* , بادئات محبة للحرارة العالية مكونة من *Streptococcus thermophilus* و *Lactobacillus delbrueckii sub. Bulgaricus* ، كل على حده او مختلطة من المحبة للحرارة المتوسطة و العالية في صناعة الجبن المضفرة السوداني وتأثيرها على جودة وسلامة الجبن الناتجة خلال فترة التسوية على جودة وسلامة جبن المضفرة السوداني خلال فترة التسوية. تم تصنيع الجبن في المعاملة الاولى (T1) باستخدام بادئات المتوسطة الحرارة و المعاملة الثانية (T2) باستخدام بادئات عالية الحرارة و المعاملة الثالثة (T3) باستخدام خليط من البادئات المتوسطة و العالية. تم تحليل الجبن طازجا في اليوم الاول وبعد٣٠ و ٦٠ و ٩٠ يوم من التخزين من حيث خواصه الميكروبيولوجية والكيماوية وعلى اطبيعية وصفات التحكيم الحسي وقد اظهرت النتائج المتحصلة عليها أن الخصائص الفيزيائية والكيميائية لجبن المضفرة السوداني تختلف باختلاف البادئ المستخدمة. تتراوح %حموضة الجبن المصنع بالمتوسطة من ١,٠٣-١,٣٣%، في حين تتراوح في المحبة للحرارة العالية من ١,٥٠ إلى ١,٧١%.لحظ زياد في العدد الكلي للبكتيريا (TVC) تدريجياًخلال فترة التخزين في كل المعاملات. وكانت اعلى ذيادة في المعاملة الاولى ثم في المعاملة الثانية تليها المعاملة الثالثة المختلطة. أظهر الجبن المصنع بالبادئات المتوسطة للحرارة قيمة صلابة قدرها ٩,٣٢ N، والتي انخفضت إلى ٨,٨٠ و ٧,٨٩ N في الجبن المنتج بالبادئي المحبة للحرارة والمختلطة (المعاملة الثالثة)، تتبعها الجبن المنتج بالبادئات المحبة للحرارة العالية واخيرا تلك المحبة للحرارة المتوسطة. بشكل عام تشير نتائج هذه الدراسة الى ان استخدام البادئات المختلطة من البادئات المحبة للحرارة المتوسطة و العالية يمكن ان يكون نهجا واعدا لتحسين جودة وسلامة الجبن المضفرة السوداني.