Microbiological and chemical profile of Lebanese *qishta* (heat-coagulated milk)

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المرتسم المكروبيولوجي والكيميائي للقشدة اللبنانية (اللبن المتخثر بالحرارة)

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الخلاصة: إن القشدة هي أحد منتجات تخثر اللبن الرائجة في الشرق الأوسط، وهي تحضَّر بعملية التسخين والقشد المعتادة. وتسعى هذه الدراسة اللبنانية إلى تقييم المرتسم المكروبيولوجي والكيميائي لهذا المنتج. وقد اختيرت عينات من 31 موقعاً مختلفاً للتصنيع والبيع، وجرى تحليل العينات بالطرق المعيارية. وكان عدد المكروبات المختلفة في المزارع الجرثومية غير مقبول أو يكاد بالنسبة للإشريكية القولونية، والسالمونيلة، واللستيرية الـمُسْتَوْحِدة (إذ اكتشفت في 32٪، و7٪، و42٪ من العينات المفحوصة على التـرتيب). أما من الناحية الكيميائية، فقد كان متوسط محتوى الرطوبة [67.5 (بانحراف معياري قَدْرُه: 2.6) غرام/ 100 غرام] والباهاء (6.53) مرتفعَيْن نسبياً. إن العدد الكبير من المكروبات الممرضة والمحدثة للفساد، وطبيعة التـركيب الكيميائية للقشدة يجعلها سريعة العطب.

ABSTRACT *Qishta* is a popular Middle Eastern coagulated cream product, prepared using a traditional heating and skimming process. This study in Lebanon aimed to assess the microbiological and chemical profile of the product. Samples selected from 31 different manufacturers and outlets were analysed using standard methods. The plate counts for the various microorganisms were either of borderline acceptability or unacceptable for *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes* (detected in 32%, 7% and 42% of the analysed samples respectively). Chemically, the mean moisture content [67.5 (SD 2.6) g/100 g] and pH (6.53) were relatively high. High counts of spoilage and pathogenic microorganisms and the nature of its chemical composition make *qishta* highly perishable.

Profil microbiologique et chimique de la *qishta* libanaise (lait coagulé par traitement thermique)

RÉSUMÉ La *qishta* est un produit crémeux coagulé très populaire au Moyen-Orient, préparé selon un procédé traditionnel de chauffage et d'écrémage. Cette étude réalisée au Liban visait à évaluer le profil microbiologique et chimique de ce produit. Les échantillons choisis auprès de 31 fabricants et points de ventes ont été analysés à l'aide de méthodes standardisées. Le comptage sur plaque des divers micro-organismes a fourni des valeurs soit à la limite de l'acceptabilité, soit non acceptables pour *Escherichia coli*, *Salmonella* spp. et *Listeria monocytogenes* (détectées dans 32 %, 7 % et 42 % des échantillons analysés, respectivement). Sur le plan chimique, la teneur moyenne en eau (67,5 g/100g [ET 2,6]) et le pH (6,53) sont apparus relativement élevés. Compte tenu du nombre important de micro-organismes pathogènes et provoquant l'autolyse qu'elle contient et de la nature de sa composition chimique, la *qishta* est une denrée très périssable.

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Introduction

Outbreaks of disease in humans have been traced to the consumption of milk and milk products from both unpasteurized and pasteurized milk. Pathogens such as *Listeria monocytogenes* can survive and multiply after pasteurization, leading to recontamination of dairy products [1,2]. Although strict microbiological standards have been set for milk and dairy products, traditional dairy products in many countries are still produced under poor hygienic conditions with different manufacturing technologies that require extensive handling of perishable ingredients [3,4]. Identifying the reservoirs of a pathogen is vital for the control of isolated outbreaks and epidemics.

Qishta is a Middle Eastern handmade heat-coagulated cream product that resembles the clotted or scalded cream that is made principally in the west of England. It is prepared from powdered or pasteurized liquid milk in small dairy plants or in large-scale bakeries to be consumed fresh as a dessert or used as a filling in a number of traditional sweets. Unlike many other coagulated dairy products, *qishta* is not fermented or coagulated by chemical or microbial methods and, despite recent increases in the volume of production, the process remains quite traditional. Milk is heated in a large tilted shallow pan until it boils. This allows the proteins to coagulate and entrap the fat particles while they float to the surface and move towards the colder part of the pan at temperatures around 60 °C. The process takes over 3 hours for the collection of the final product. *Qishta* is then cooled in bulk to 45 °C at room temperature for 2 hours before it is stored under refrigeration (process as described by Refaat Hallab, Tripoli, Lebanon). The shelf-life of *qishta* is only 24 hours at room temperature and 4 days at 2–5 °C.

Personal contacts and information from hospitals suggest a high incidence of foodborne diseases associated with *qishta* in various regions of Lebanon. Since to our knowledge there are no published studies on *qishta*, the present study aimed to assess the microbial quality and chemical constituents of the product. It was hoped that the data would be applicable to other coagulated milk products in the region, with a view to enhancing the process, extending the shelf-life of the product and improving public safety.

Methods

Samples

Triplicate samples of *qishta* (250 g each) were collected from 31 different manufacturers and outlets in the north and the south of Lebanon and the Beirut area between April and November 2007. The samples were transported in sterile plastic bags to the laboratory under aseptic and refrigerated conditions. Microbiological and chemical analyses were performed within 1–2 hours after purchasing.

Microbiological analysis

Product samples were analysed microbiologically and identified according to standard methods for total mesophilic bacteria, total coliforms and faecal coliforms, Enterobacteriaceae, *Salmonella* spp., *L. monocytogenes*, *Staphylococcus aureus* and yeasts and moulds [5].

In the procedure, 10 g of *qishta* were homogenized with 90 mL of sterile 0.1% peptone water (356-4684) in a stomacher (Seward 400, Seward, London) for at least 2 minutes. Serial dilutions were prepared in 0.1% peptone water (356-4684) and samples of 0.1 mL of each of the 10^{-2}, 10^{-4} and 10^{-6} dilutions were spread on appropriate media in duplicates and 1 mL was used for the pour-plate technique. Total mesophilic bacteria were enumerated on plate-count agar (356-4475) at 30 °C for 48 hours while total coliforms and *Escherichia coli* were differentiated and enumerated on RAPID'E.coli 2 agar (356-4024) by the pour-plate technique and plates were incubated at 37 °C for 24 h. Total Enterobacteriaceae and *Sta. aureus* were respectively detected on violet-red bile dextrose agar (256-4584) and Baird-Parker agar supplemented with egg-yolk–tellurite emulsion (356-4814) and incubated at 37 °C for 24 h. *Sta. aureus* were further confirmed biochemically using rabbit plasma (355-6352). Yeast and moulds were identified on yeast glucose chloramphenicol agar (256-4104) with plates incubated at 25 °C for 5 days.

For the isolation of *Salmonella* spp. and *L. monocytogenes*, the pre-enrichment/enrichment selective plating method was used [5]. For *Salmonella* spp., selective enrichment was performed in rappaport-vassiliadis-soya broth (256-4324) to be incubated at 41.5 °C. After 24 h of incubation, a 0.1 mL sample was plated on RAPID *Salmonella* agar (356-4705) and plates were incubated at 37 °C for 24 h (± 2 h) and another 0.1 mL was transferred onto xylose–lysine–desoxycholate agar plates (356-9124) that were incubated at 37 °C for 24 h.

Salmonella spp. colonies were identified biochemically by the lysine iron agar (B211363) and tryptic sugar iron agar (D4402) 2 slants biotyping technique. Additional confirmation for positive *Salmonella* spp. colonies was done by the API 20E bacterial identification test strip (Biomérieux, France). For *L. monocytogenes*, Fraser ½ broth (356-4616) was used in the selective enrichment and after incubation for 1 h at 20 °C, 0.1 mL of the homogenate was transferred onto RAPID'L. mono agar (356-3694) plates to be incubated at 37 °C for 24–48 h. Typical *L. monocytogenes* colonies were afterwards selectively identified and enumerated.

All media were supplied by Bio-Rad Laboratorios, California, USA. Microbial counts were reported as geometric means of colony-forming units (CFU) per g of *qishta*, except for *Salmonella*

spp. and *L. monocytogenes* which were reported as present or absent.

Chemical analysis

The pH of *qishta* was measured using a pH meter with a glass electrode (Orion, USA). Moisture content, salt, proteins, carbohydrates and fat contents were determined according to the Association of Official Agricultural Chemists (AOAC) standard methods for proximate analysis of dairy products [6]. Samples were analysed in duplicate and each proximate analysis was repeated twice.

Statistical analysis

Data were analysed by analysis of variance using *SPSS*, version 8.0 for Windows and means were separated by the Duncan multiple range test. Significance was defined at $P < 0.05$ and numerical results are given as means and standard deviations (SD).

Results

The mean plate counts of the various microorganisms in the analysed *qishta* samples are shown in Table 1. The mean total aerobic count was 4.42 (SD 0.10) $\log_{10}$ CFU/g and total coliforms were 3.23 (SD 0.09) $\log_{10}$ CFU/g. The mean plate counts of *E. coli*, Enterobacteriaceae and *Sta. aureus* also ranged from 3–4 $\log_{10}$ CFU/g (Table 1). Of the tested *qishta* samples 9/31 (29%) had mesophilic bacteria counts in the range of 3–4 $\log_{10}$ CFU/g while 15/31 (48%) had yeast and mould levels within that range. *Salmonella* spp. and *L. monocytogenes* were present in 2/31 (7%) and 13/31 (42%) of the samples respectively (detailed identification of *Salmonella* spp. will be reported in a future study).

The results of chemical analyses (moisture, acidity and pH and fat, proteins and ash content) are shown in Table 2. There was a high mean moisture content at 67.5 (SD 2.6) g/100 g. The mean fat content was 13.0 (SD 2.4) g/100 g (Table 2). The mean pH of the samples was 6.53.

Table 3 compares the mean microbial counts of *qishta* samples from large-scale manufacturers and small-scale manufacturers/retailers. There were no significant differences between the 2 categories of suppliers in any of the parameters studied ($P > 0.05$) (Table 3).

Discussion

The chemical composition of *qishta* as determined in this study, along with other procedural aspects in the manufacturing process, may have contributed to the high microbial incidence encountered in the analysed samples and the likelihood of rapid multiplication if contamination did occur. Most of the microbial counts in our samples of *qishta* were relatively high when compared with the international microbiological criteria [7], on the upper margin for rejecting products. The high incidence of pathogenic and spoilage microorganisms in *qishta* may be accounted for by contamination during manufacturing or post-processing and cross-contamination in plants and in refrigerators or retail stores.

Because the total mesophilic bacteria and yeasts and moulds are indicators of spoilage, their numbers are essential in deciding the shelf-life of the product. This may explain the observed short shelf-life of *qishta* in the Lebanese market. In addition, the bitter taste in *qishta* which develops during prolonged refrigerated storage may be attributed to the high mould counts. Hence, the results of this study agree with similar work performed on dairy products in Turkey [8]. Milk-coagulated products such as *qishta* are rich in nutrients and have high moisture content (in this study almost 70%), which is conducive to the growth of spoilage and pathogenic microorganisms [9].

The presence of coliforms in the various samples is indicative of insufficient sanitary conditions during manufacturing and storage. Coliforms such as *E. coli*, Enterobacteriaceae and *Sta. aureus* are markers for unsafe foods, due to their pathogenic nature. Hence the high mean microbial counts obtained

Table 1 Microbial plate counts of 31 *qishta* samples from various manufacturers in Lebanon

Microorganism	No. of positive samples/total no. of samples analysed	Mean (SD) plate counts ($\log_{10}$ CFU/g)	Minimum	Maximum
Total aerobic count	31/31	4.42 (0.10)	2.41	7.01
Total coliforms	31/31	3.23 (0.09)	1.39	5.43
Escherichia coli	10/31	3.01 (0.60)	2.30	4.03
Enterobacteriaceae	31/31	3.67 (0.06)	1.74	5.66
Yeasts & moulds	31/31	4.54 (0.08)	2.19	7.27
Staphylococcus aureus	30/31	3.03 (0.10)	0.05	4.63
Salmonella spp.	2/31	–[a]	–	–
Listeria monocytogenes	13/31	–[a]	–	–

[a] Salmonella *spp and* L. monocytogenes *are reported as present or absent.*
SD = standard deviation; CFU = colony-forming units.

Table 2 Chemical composition, pH and acidity of the 31 samples of *qishta* analysed

Parameter	Mean (SD)	Minimum	Maximum
Moisture (g/100 g)	67.5 (2.6)	62.4	72.0
Fat (g/100 g)	13.0 (2.4)	9.2	18.3
Protein (g/100 g)	12.9 (1.9)	9.8	16.2
Ash (g/100 g)	1.8 (0.14)	1.6	2.2
Acidity[a] (g/100 g)	0.3 (0.001)	0.11	0.5
pH	6.53 (0.14)	6.28	6.82

[a]As lactic acid.
SD = standard deviation.

in this study suggest that the product may be hazardous for human consumption. This result agrees with work in other countries on different products that have similar characteristics to *qishta* [10–13]. Mandokhot et al. [12] and Gill et al. [13] in studies from different parts of India indicated that *khoa* (partially desiccated milk) is often contaminated with pathogens such as *Sta. aureus* and *Bacillus cereus*. Furthermore, Rajorhia et al. [10] and Kumar et al. [11] also reported that *paneer* (a coagulated milk product) is often contaminated with *Sta. aureus* and coliforms. The high counts of *S. aureus* in particular may be due to extensive handling by personnel during the elaborate multi-stage processing. Dairy products are a rich medium and can enable the growth of this and many other pathogens. Hence, Halpin-Dohnalek et al. reported that both sweet and neutralized sour cream products support the growth of all strains of *Sta. aureus* [14].

The *qishta* samples also tested positive for *Salmonella* spp., while *L. monocytogenes* was detected in 45% of samples. According to the microbiological criteria for milk-based products in the European Union those 2 high-risk pathogens should be absent for a product to be considered safe for human consumption [15]. There are several explanations for the results confirming the presence of those 2 pathogens in the final product. The heating step during processing or the contact time at that boiling temperature may be insufficient to eliminate the pathogens. Another possibility is the existence of heat-resistant *Salmonella* and *Listeria* strains and this hypothesis will be investigated in another study. On the other hand, the high microbial and pathogen counts observed in *qishta* are indicative of possible contamination before, during and after processing. Several researchers have noted the prevalence of these pathogens in milk-based products. For instance, Szwarcbort de Tamsut et al. reported the presence of *Sal. typhimurium* and other pathogens such as *Sta. aureus* and *Shigella* spp. in pasteurized milk creams in Venezuela [16].

As for *L. monocytogenes*, several researchers in the Middle Eastern region reported the presence of this pathogen in raw milks and pasteurized milk products [17–20]. *L. monocytogenes* was isolated from cheese products in Turkey, some of which were either ripened or brine-salted [17,21]. Rudolf et al. also found *L. monocytogenes* in European red smear cheese [22], while Cordano et al. found *Listeria* spp. in soft cheeses samples [23]. Contamination might also occur post-processing from environmental sources and cross-contamination in the dairy plant and/or retail stores or inadequate processing [22,24,25]. It has been noted by Sergelidis et al. that contamination can also occur because of colonization of *L. monocytogenes* in refrigerators in retail stores [26]. *L. monocytogenes* can survive a number of processes and can remain viable in the final product for a considerable length of time [19,27]. Therefore, in most cases the contamination sources are likely to be insufficient hygiene during the milking and manufacturing process.

The microbial counts were mean values of the total samples collected and analysed from the various manufacturers and retailers. Theoretically, the range of microbial counts should be the result of differences between major manufacturers who apply hazard analysis critical control points (HACCP) procedures or have a controlled sanitation programme and the small retail stores and/or manufacturers who do not abide by any strict sanitation procedures. However, there were no significant differences in the microbial counts between large and small suppliers. This could be attributed to the fact that the larger manufacturers supply the small retailers with the product and the shelf-life of the product is short.

Table 3 Comparison of microbial plate counts between large-scale manufacturers and small-scale manufacturers/retailers in Lebanon

Microorganism	Mean (SD) plate counts (log_{10} CFU/g)	
	Large-scale producers (*n* = 13 samples)	Small-scale producers/retailers (*n* = 18 samples)
Total aerobic count	4.36 (1.34)	4.48 (1.02)
Total coliforms	3.14 (1.19)	3.29 (0.85)
Enterobacteriaceae	3.41 (1.16)	3.85 (0.99)
Yeasts & moulds	4.21 (1.70)	4.78 (1.14)
Staphylococcus aureus	2.89 (1.24)	3.13 (0.66)

No significant difference between large-scale and small-scale producers, P > 0.05.
SD = standard deviation; CFU = colony-forming units.

Such chemical characteristics of *qishta* are essential in determining the hazards associated with the product. The mean values show that *qishta* has a high moisture content which is comparable to thickened yoghurts and other types of clotted creams. Furthermore, the pH of *qishta* is higher than many other dairy products such as whipped creams, clotted creams and yoghurts [28]. Such findings explain the short shelf-life of the product. Furthermore, the combination of high moisture content and pH is a major factor in rendering the product susceptible to high microbial contamination and growth.

The proximate analysis results indicate that the product is nutritious and not excessively high in its fat composition, with a fat content (13.0 g/100 g) close to but less than sour cream (18%) or light creams (20%) and much less than whipping cream (30%) and other types of clotted cream (55%).

The labour-intensive and lengthy preparation, storage and cooling procedures and the poor hygienic environment observed in many of the facilities visited are concerning. An additional safety concern is that in some manufacturing locations, in an attempt to cool the product faster before refrigeration, fans without air filters are used. Furthermore *qishta* in many retail outlets, whether sold bulk or in desserts, is unpackaged and unprocessed. This amplifies the problem of contamination and cross-contamination. Furthermore, the problem of microbial safety is of course exacerbated during spring and summer months, when the ambient temperatures in Lebanon rise above 25 °C and most of the facilities have no or insufficient cooling systems.

Concerning the contamination with moulds, an entry airlock to avoid direct contact with the outside air or an area with positive air pressure where major parts of the process are carried out could alleviate this problem [29].

Conclusions

The current study shows that the perishable coagulated dairy product *qishta* is a high-risk product since its chemical composition makes it susceptible to growth of microorganisms and because most of the tested samples showed a high frequency of pathogenic and spoilage microbial contamination. Because *qishta* is consumed with no further processing to reduce any microbial load, the results are concerning. This study is important because *qishta* is consumed widely not only in Lebanon and the region but is becoming increasingly popular elsewhere.

In order to improve the safety and quality of the product, processing and storage needs to be carried out under good hygienic conditions and HACCP systems should be implemented. It is also imperative that the product should be stored continuously under refrigerated conditions to avoid post-heating contamination during cooling and packing procedures.

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FAO/WHO Expert Meeting on the Application of Nanotechnologies in the Food and Agriculture Sectors: Potential Food Safety Implications: meeting report

The advent of nanotechnology has unleashed enormous prospects for the development of new products and applications for a wide range of industrial and consumer sectors. Many countries have identified its potential in the food and agriculture sectors and are investing significantly in its applications to food production. However, owing to our limited knowledge of the human health effects of these applications, many countries recognize the need for early consideration of the food safety implications of the technology. In response to this request, FAO and WHO convened an Expert Meeting on the topic in order to identify further work that may be required to address the issue at a global level. Seventeen experts from relevant disciplines, such as food technology, toxicology and communication, met to discuss three main areas: the use of nanotechnology in food production and processing; the potential human health risks associated with this use; the elements of transparent and constructive dialogues on nanotechnology among stakeholders.

The above-mentioned publication reports the outcome of the meeting and is available online at: http://whqlibdoc.who.int/publications/2010/9789241563932_eng.pdf