



## Identification of lactic acid bacteria from fermented camel cheese produced in Saudi Arabia

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### Abstract

The current study aimed to identify probiotic strains of lactic acid bacteria isolated from fermented camel cheese produced in Saudi Arabia Region -Arar-by placing pieces of unpasteurized soft white camel cheese with pieces of green pepper in soured and salty camel milk in tightly closed glass jars and keeping at room temperature. The microbiological and biochemical characteristics of the isolates from fermented camel cheese were studied after 12 weeks, where the averages of  $\log_{10}$  (CFU/g) of the aerobic plate count (APC) and lactic acid bacteria count (LAB) for the five batches of cheese were 8.25, 6.88, 7.22, 6.49, and 6.94 and 6.94, 5.67, 5.90, 5.82 and 6.77 respectively. Thirty five isolates were tentatively characterized as LAB; these bacteria were gram positive rods or cocci, catalase and oxidase negative, non- motile and non spore-forming bacteria. The isolates were distributed into nine group according to the common characteristics they had and subjected to further biochemical tests using API50 CH system. The results were compared, and it was concluded that *Lactobacillus acidophilus*, *Lactobacillus delbrueckii sub sp. bulgaricus*, *Lactobacillus rhamnusus*, *Streptococcus thermophilus* and *Lactococcus lactis sub sp. cremoris*, species that were identified as probiotics were associated with this fermentation process. The results showed that panelists had preferred the sensory properties of fermented camel cheese. So it was concluded that this newly processed cheese was a rich source of LAB especially probiotic that may be involved in many food industries and may have positive effect on health.

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## Introduction

Lactic acid bacteria (LAB) are involved in both spontaneous and large-scale fermentation processes for the preservation and transformation of many raw food materials such as milk, meat, fish, cereals, tubers and vegetables. Miriam *et al.* (2001) also studied the commensal inhabitants of the gastrointestinal tract in humans and animals where they contribute to the complex interactions between the intestinal microbiota and the host (Kalliomäki *et al.*, 2001).

Today, LAB are a focus of intensive international research for their essential role in most fermented food (Bintsis, 2018), for their ability to produce various antimicrobial compounds promoting probiotic properties including antitumoral activity (Hilde *et al.*, 2003; Pilar *et al.*, 2008), reduction of serum cholesterol and they may present significant beneficial clinical effects in preventing and treating diarrhea (Axelsson *et al.*, 2004), and in improving the digestion of lactose by lactase-deficient individuals and stimulation of the immune system (Isolauri *et al.*, 2001; Nettles and Barefoot, 1993), stabilization of gut microflora. LAB strains that produce exopolysaccharide (ESP) are employed in the manufacture of fermented milk to improve its texture and viscosity. Some LAB strains are known to produce mannitol which is claimed to have several health promoting effects (Wisselink *et al.*, 2002; Azadnia *et al.*, 2011; Pilar, 2003).

New sources of nutrients should be more exploited for varying the human diet and also to benefit from new functional ingredients and natural food components. Arab countries, where the breeding conditions for camels are severe and fastidious, can get over this situation (Ohrisand Joshi, 1961; Hamed and Elattar, 2013; Nafiseh *et al.*, 2015). The beneficial microbiota of camel milk represented by LAB is a potential source of biological materials to be used in dietary clinical purposes and dairy technology (Ashmaig *et al.*, 2009; Beg *et al.*, 1986). It is commonly reported that processing camel milk into cheese is a difficult one. In this research, white camel cheese was processed then naturally fermented for

the first time in Saudi Arabia (Arar –Northern Border Region) using conventional cheese making method which is aimed to identify probiotic strains of lactic acid bacteria through studying their microbiological and biochemical characteristics.

## Materials and methods

### *Camel Cheese Processing*

Five Cheese making trials from different camel milk sources in Saudi Arabia - Arar - were conducted at Applied Medical Science Laboratory of Northern Border University. The conventional cheese making process was followed as shown in

### *Production of Fermented Camel Cheese*

In this study, five different unpasteurized white camel cheese with salted aged camel milk was prepared from different places (different batches), the same treatment for all camel cheese batches was produced under hygienic conditions as shown in figure 2. Samples of the study was collected from the fermented camel cheese after three months (12 weeks).

### *Sampling*

Samples of fermented camel cheese was collected from each preserved glass jar after 3 months of production (12 weeks). Each twenty five grams of cheese was transferred separately under aseptic conditions into a sterile stomacher bags examined directly after collection.

### *Microbiological testing*

A pre-sterilized knife was used to prepare 25 g of equal amount sample taken from three different pieces of the five identified glass jars for each batch into presterilized bag, and 225 ml of buffer peptone water was added.

The bag was placed and locked into stomacher (Inter science bag Mixer, Germany) and mixed for two min. Appropriate serial dilutions was made from  $10^{-2}$  to  $10^{-7}$  by aseptically pipetting mixed sample into 0.1% peptone water (Oxoid, UK) sterilized at  $120 \pm 1^\circ\text{C}$  for 15 minutes as diluents for all microbiological tests.

#### *Aerobic plate count (APC)*

Aerobic plate count was executed according to Laird *et al.* (2004). Plates count agar was prepared and sterilized according to manufacturer's directions. One ml of each sample dilution, was inoculated in presterilized plastic petri dish. The medium was poured at 45 °C of about 12-15 ml for each plate above the sample dilution, and then mixed gently until dispersed. The dishes was incubated at 32 ±1°C for 48 ± 3 h.

#### *Lactic acid bacteria count*

Pour plating of sample dilutions was done by using preprepared sterilized MRS agar, after cooling to 45 ±1°C, plates was incubated at 37±1°C for 48 ± 3 h under reduced aerobic conditions (using anaerobic jars). Individual colonies was retested for catalase reaction, Gram reaction (gram stain kit from Delta lab, Spain), and cell morphology. The count was reported as CFU/g cheese (Corsetti *et al.*,2001; Frank and Yousef, 2004).

#### *Catalase test*

Catalase test was conducted where a small drop of normal saline was placed on a clean glass slide, a loop from MRS agar plate was scraped across the growth of several colonies with a sterilized and cooled inoculating loop. One or two colonies on the drop was emulsified to make a smooth suspension, where the test smear should be about the size of a pea seed. A Pasteur pipette was used to place one drop, amount of 0.5 ml of 3 % hydrogen peroxide (Sigma Aldrich, UK) over the test smear. Effervescence was an indication of a positive test by observing the fluid over the smears for the appearance of gas bubbles (Whittenbury, 1964).

#### *Biochemical characterization*

The ability of the new isolates to produce acids from carbohydrates fermentation was determined using API 50 CH kits and CHL media (Biomérieux, France). The API 50 test strips was prepared according to the manufacturer's instructions. Ten ml of pure water were dispensed into the incubation box where the identification strips was placed. Then bacterial

cultures was introduced into 5 ml API 50 CHL medium.

The wells of the API 50 strips was then inoculated by the test isolate and topped by sterile mineral oil. Results was scored after incubation for 24 and 48 h at 37°C.

The results of 49 carbohydrates fermentation test was joined to the API Web <sup>TM</sup> identification software, which uses the phenotypic data to predict species identity for each isolates (Pelienscu *et al.*, 2009).

#### *Sensory Analysis*

Samples of fermented camel cheese was cut into approximately 5x5 cm pieces and placed on white plates and presented at ambient temperature (20 ± 2° C) to panelists from both Academic teaching staff, technicians and students who are familiar with the cheeses and were asked to judge the quality of the cheese. Sensory evaluation was assayed on a Hedonic Scale of 1 to 9 points (1: low value; 9: high value), with five sensory attributes (appearance, flavor or smell, taste, texture and overall acceptability) was used for the evaluation, each panelist was provided with water for rinsing. The samples was given codes before being tested (Clark *et al.*, 2009).

#### *Statistical Analysis*

The statistical analysis was performed using the Statistical Analysis System (SAS, 2008) version 9. Analysis of Variance (ANOVA) with t-test was used to determine significant differences between the means at P< 0.05 (Steel and Torrie, 1980). Values in the tables are expressed as mean ± standard error of the mean, where all experiments were duplicated.

## **Results**

#### *Aerobic plate count*

Aerobic plate count ranged between 6.49 and 8.25 - at the end of week 12 – for five different fermented camel cheese batches. There were significant differences (p < 0.05) in APC among different batches. APC ranged between 8.25, 6.88, 7.22, 6.49 and 6.94 in samples respectively as shown in table 1.

**Table 1.** Comparison of microbial cheese count (log CFU/g).

Sample	Log <sub>10</sub> (CFU/g)		Temperature (°C)
	APC	LAB	
1	8.25 <sup>a</sup> ± 0.33	6.94 <sup>a</sup> ± 0.04	20.53 <sup>d</sup> ± 0.30
2	6.88 <sup>cd</sup> ± 0.02	5.67 <sup>b</sup> ± 0.16	22.63 <sup>b</sup> ± 0.03
3	7.22 <sup>bc</sup> ± 0.10	5.90 <sup>b</sup> ± 0.38	20.07 <sup>a</sup> ± 0.26
4	6.49 <sup>d</sup> ± 0.05	5.82 <sup>b</sup> ± 0.07	20.22 <sup>d</sup> ± 0.15
5	6.94 <sup>cd</sup> ± 0.11	6.77 <sup>a</sup> ± 0.35	21.37 <sup>c</sup> ± 0.03

Means in the same column have the same letters are not significantly different using Least Significant Difference (LSD) at 0.05.

#### LAB count

LAB count was ranged between 5.67 and 6.94 - at the end of week 12 - for fermented camel cheese samples. It was found that there is significant differences ( $p < 0.05$ ) in LAB count among different batches of fermented camel cheese. LAB ranged between 5.67, 5.82, 5.90, 6.77 and 6.94 in samples respectively as shown in table 1.

#### Isolation and identification of LAB

##### Isolation and purification of LAB

Fermented camel cheese was used as an isolation source. Thirty five strains were isolated checked for

the purity by streaking on MRS agar and then kept in MRS broth plus 20% glycerol at -20°C, where the working cultures on MRS agar plates were used for identification tests.

#### Morphological identification

Nine isolates were tentatively characterized as LAB; these bacteria were gram positive rods or cocci, catalase and oxidase negative, non- motile and non spore-forming bacteria. The isolates were distributed into groups according the common characteristics they had and subjected to further biochemical identification tests (Table 2).

**Table 2.** Lactic acid bacteria strains isolated from fermented camel cheese.

Sample no.	Weeks
	12
1	2RLAB 1SLAB
2	2 RLAB
3	1 SLAB
4	1RLAB
5	2 RLAB
Total	9

SLAB: Spherical Lactic Acid Bacteria

RLAB: Rods Lactic Acid Bacteria

#### Biochemical identification of LAB isolates

Based on the results of API 50 CH system and testing for gas production from glucose, five LAB isolates

were found to belong to defined species as summarized in table 3.

**Table 3.** LAB species isolated from fermented camel cheese.

Identification by API kit	Sample no.
<i>Lactobacillus acidophilus</i>	1 and 5
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	2
<i>Streptococcus thermophilus</i>	1
<i>Lactobacillus rhamnusus</i>	2, 4 and 5
<i>Lactococcus lactis</i> subsp. <i>cremoris</i>	3

### Sensory evaluation of fermented camel cheese

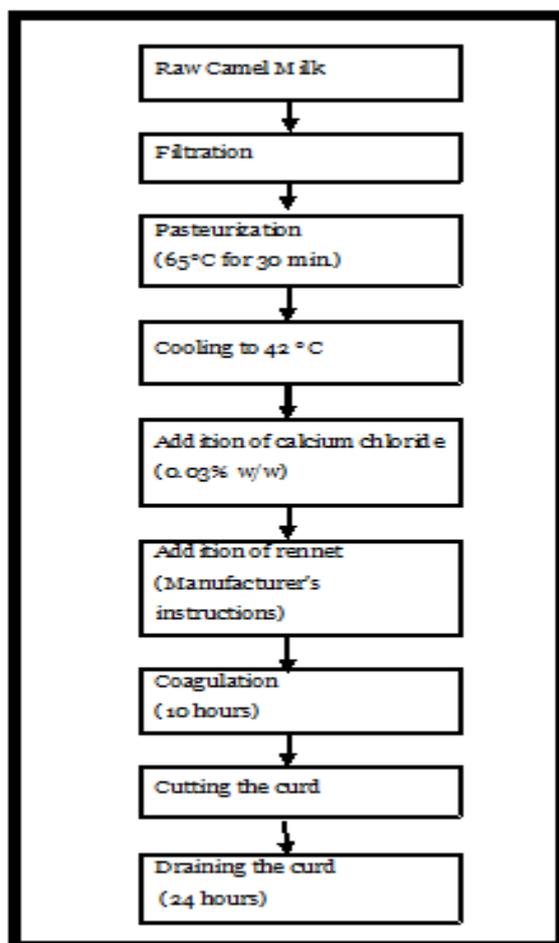
Results of sensory testing of the five different fermented camel cheese batches were shown in table 4. The panelists were requested to consider the appearance, flavour (smell), texture (consistency),

taste and overall acceptability according to hedonic scale, also they were asked to list any defects. Significant differences were observed between all sensory parameters among all samples.

**Table 4.** Comparison of sensory parameters between the five batches.

Sample	Appearance	Flavor (Smell)	Texture (Consistency)	Taste	Overall acceptability
1	7.80 <sup>a</sup> ± 0.26	8.12 <sup>a</sup> ± 0.27	7.92 <sup>a</sup> ± 0.32	7.80 <sup>a</sup> ± 0.32	8.04 <sup>a</sup> ± 0.27
2	6.72 <sup>c</sup> ± 0.31	7.08 <sup>b</sup> ± 0.44	6.56 <sup>b</sup> ± 0.39	6.72 <sup>ab</sup> ± 0.44	6.96 <sup>bc</sup> ± 0.36
3	7.48 <sup>ab</sup> ± 0.29	7.00 <sup>b</sup> ± 0.32	7.56 <sup>a</sup> ± 0.26	6.80 <sup>ab</sup> ± 0.42	7.56 <sup>ab</sup> ± 0.31
4	4.52 <sup>c</sup> ± 0.39	5.88 <sup>c</sup> ± 0.43	6.16 <sup>b</sup> ± 0.39	5.92 <sup>b</sup> ± 0.44	6.12 <sup>c</sup> ± 0.43
5	6.48 <sup>c</sup> ± 0.35	6.60 <sup>bc</sup> ± 0.35	6.20 <sup>b</sup> ± 0.33	6.00 <sup>b</sup> ± 0.40	6.32 <sup>c</sup> ± 0.41

Means in the same column have the same letters are not significantly different using Least Significant Difference (LSD) at 0.05, N= 30.



**Fig. 1.** Camel cheese processing method.

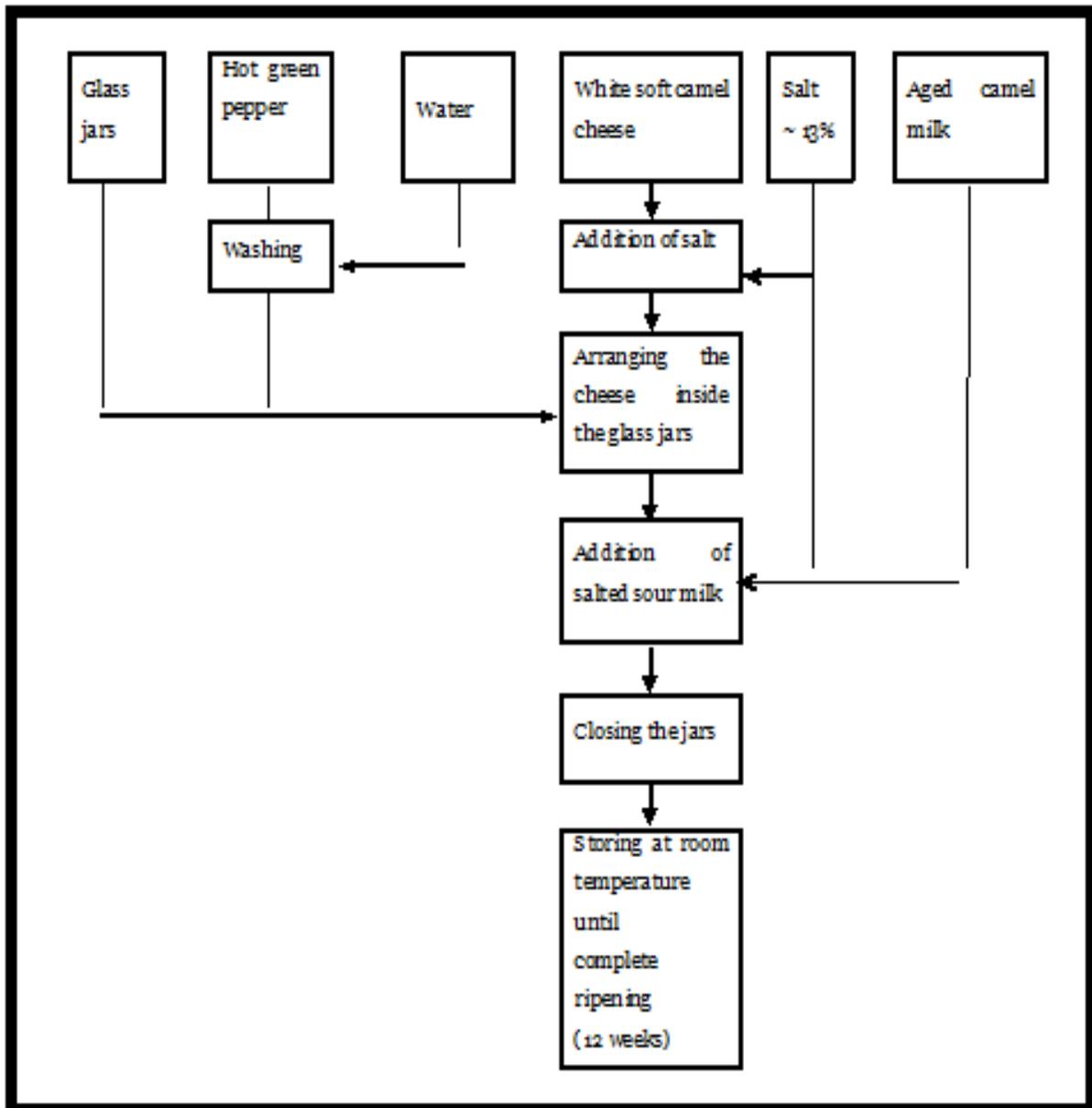
According to the sensory evaluation results, Sample no. 1 was found to be the most preferred cheese type

in terms of appearance, flavour (smell), texture (consistency), taste and overall acceptability, compared with the overall cheese samples that processed in same conditions. Also significant differences were observed between all sensory parameters among all batches, where (sample no. 1) got the highest value for all sensory parameters. These results can be justified by controlled conditions of the main source of the raw camel milk were offered.

### Discussion

Camel milk is gaining more rapidly now a days, because of its high nutritional value (Yagil, 1987; Mehaia, 1996; Imenet *et al.*, 2017). In Arabian Gulf countries, camel milk; is mostly consumed fresh or when got aged and soured, fresh camel milk has a low acid content of 0.03% and a pH of 6.5-6.7. Although no study suggest that fermented camel cheese could be used as probiotic, some resercherers believe that synergistic effect exist between components in dairy foods and probiotic culture and that there are components in milk that turn on the beneficial genes in probiotic bacteria (El-Amin and Welcox, 1992;Kasimogluet *al.*, 2004; Rodgers, 2008; Carol and Leon, 2010;Alegríaet *al.*, 2016).

There are different varieties of white brined cheeses varying from their original milk sources or the degree of softness (Milica *et al.*, 2008).



**Fig. 2.** Process flow chart of fermented camel cheese production.

The cheeses are preserved or stored in brine of high salt concentration (Tamimeet *al.*, 1991). Since the Middle East countries are characterized by warm climate, the shelf life of milk is short and cheese deteriorates before its ripening. Therefore, the fermentation of cheeses is a great importance since it elongates the shelf life of the cheese (Abd El-Salam and Alichanidis, 2004; Portilla-Vázquez *al.*, 2016).

Cultured dairy products are an important part of the diet of many societies. These dairy products were initially develop Fed as means to preserve milk, and they have desirable sensory characteristics

(Małgorzata *et al.*, 2010). Fermentation by the microbial starter cultures preserves the product through the production of lactic acid and contributes to the development of characteristic flavour compounds (Urbach, 1993; Molimard and Spinneler, 1996). These products are now recognized for their nutritional benefits (Lim and Dong-Soon, 2009).

Haddad and Yamani (2017) examined 30 soft white cheese samples from Amman, Al-Balqa, Jerash and Ma`daba Governorates and found that the average  $\log_{10}$  of LAB was 7.9, also it was noticed that a significant positive correlation (0.90) between SPC

and LAB count, which indicated that most of SPC are LAB, where these results are harmonized with our study which shows the average  $\log_{10}$  of APC and LAB of fermented camel cheese samples among all sources were around 7.16 and 6.22, respectively (Tzanetaki, 1990; Johnson *et al.*, 1990; Guessaset *al.*, 2004).

Probiotics are obtained by the action of microorganisms, usually LAB which are already isolated from fermented camel cheese. Those microorganisms are useful in assisting the gastrointestinal tract by breaking down sugars and carbohydrates to promote good digestion, boost the immune system and maintain proper intestinal pH. Symbiotic forms when probiotics and prebiotics are combined (Giraffa, 2012; Domingos-Lopes *et al.*, 2017).

This study suggests that camel milk is a potential source for the isolation of probiotic LAB strains and can be considered good for health with antibacterial properties against pathogenic bacteria because of the presence of bacteriocin-producing strains such as *Enterococcus spp.*, *Weissella spp.* and *Pediococcus spp.* (Leisner *et al.*, 1999; Merteau *et al.*, 2001, Zübeyde, *et al.*, 2006; Mutlag, 2013; Marco *et al.*, 2017).

The strains which were isolated from fermented camel cheese after twelve weeks of fermentation are of industrial uses in dairy plants: *Lactobacillus acidophilus*, *Lactococcus lactis subsp. cremoris*, *Lactobacillus rhamnosus*, *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* are used as probiotic bacteria in production and preparation several types of fermented dairy products (De Rodas *et al.*, 1996; Shah, 2002; Granato *et al.*, 2010; Akhmetsadykova *et al.*, 2015).

Sameen *et al.* (2010) studied that the homemade yoghurt (dahi) was used as the source of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp bulgaricus* culture (Samelis *et al.*,

1994; Soukoulis *et al.*, 2007).

## Conclusions

In conclusion, this study emphasized that fermented camel cheese is a good source of probiotics. Moreover, studies could be done to link between microflora population and variation factors as species or regions to know its effects on organoleptic properties.

In addition it's recommended that these species be further investigated according to selection criteria like stimulation of immunological system adhesion to epithelial tissue and additional efforts should enhance consumption of camel cheese as a fermented product.

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