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CAMEL MILK AND MILKING

Enhancement of β-galactosidase activity of lactic acid bacteria in fermented camel milk

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ABSTRACT

It is known that, the main problem associated with the production of fermented camel milk is the lower growth rate of lactic acid bacteria which caused many quality problems in the final product. The aim of this research was to enhance of β-galactosidase enzymatic activity of *Lactobacillus delbrueckii ssp. bulgaricus* DSMZ 20081, *Streptococcus thermophilus* ATCC 19258 and *Lactobacillus acidophilus* DSMZ 20079 by cell-rupture method to release their intracellular β-galactosidase and used the ruptured cell cultures as the source of the β-galactosidase enzyme. The results show that the bacterial growth rate in fermented camel milk made by using mixed cells of *L. delbrueckii ssp. bulgaricus*, *S. thermophilus* and *L. acidophilus* was 2 h faster than whole cells of the same strains. Moreover, the significant ($P<0.05$) faster dropping in pH-value to about 4.6 was observed in fermented camel milk made by using mixed cells of *S. thermophilus, L. acidophilus* and *L. delbrueckii ssp. bulgaricus* it was 4.65 ± 0.02, 4.64 ± 0.01and 4.63 ± 0.01 during 4 h of fermentation time, respectively. Furthermore, the highest significance ($P<0.05$) activity of β-galactosidase was obtained during 4 h of fermentation in all fermented camel milk made by using mixed cells of *L. delbrueckii ssp. bulgaricus, L. acidophilus* and *S. thermophilus* it was about 1.97 ± 0.12, 1.77 ± 0.06 and 1.70 ± 0.01 IU/ml, respectively. On the other hand, more rapid and efficient utilization of lactose was observed in fermented camel milk made by using mixed cells of *S. thermophilus, L. acidophilus* and *L. delbrueckii ssp. bulgaricus* during the first 2 h of incubation time and the higher degree of lactose hydrolysis reached the maximum approximately 37.69, 38.39 and 40.56 % at the end of 10h of incubation time, respectively. The present results revealed that enhance of microbial growth rate in fermented camel milk can be simultaneously achieved by a suitable rupture-cell method.

Keywords: β-galactosidase activity; Lactose hydrolysis; Fermented camel milk

INTRODUCTION

Camel milk is considered a high nutritive value food and plays an important role in ensuring food security for the consumer in the in arid rural communities around the world. Due to an increasing human population in the deserts and semi-deserts zones, it is a great exigency to develop and optimize dairy food supplies and it is evident that the camel is the best candidate for a domesticated animal in this region. During the last decade, there is an increasing trend all over the world in the consumption of camel milk and milk products as a result of its nutritional and therapeutic properties (Khalesi et al., 2017). Camel milk contains an excellent nutritional profile, along with high-quality protein and fat milk, contains many vitamins, especially Vit. (C, B1 and B2), also contains higher amounts of minerals such as sodium, potassium, manganese, calcium, phosphorus, zinc and about ten times higher in iron than in cow milk, which made it superior to cow milk in terms of nutrients (Ibrahim and Khalifa, 2015). Moreover, several researchers reported the role of camel milk in the promotion of health and disease particularly for tuberculosis and dropsy, jaundice, anemia and piles (Mal et al., 2001). Also, used to treat diabetes type-1 and reduced insulin dose (Agarwal et al., 2005).

Although there are several commercial farms in the world, producing camel milk and milk products such as UAE and Saudi Arabia etc., a limited amount of traditional fermented products is available in local markets except in Central Asia (Konuspayeva and Faye, 2011) or in the Horn of Africa (Ahmed et al., 2016). Yet, making fermented milk is relatively easy and the technology is well described (Algruin and Konuspayeva, 2015). However, Hashim et al. (2009) and Mohamed et al. (1990) indicated that fermented camel milk failed to reach a gel-like structure and the curd formation

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fragile after 18-h of fermentation. Also, (Attia et al., 2001) showed that the lactic acid starter cultures take longer time for lag phase and an earlier decline phase resulting in a fragile curd and heterogeneous structure. That was attributed to the presence a variety of protective properties factor against different microorganisms such as lysozyme, lactoferrin; lactoperoxidase and immunoglobulin prevent the growth of lactic acid bacteria. These antimicrobials factors were present in camel milk compared with those in other milk (El-Agamy, 2000; Barbour et al., 1984).

Camel milk can prevent the growth of lactic acid bacteria, and that caused quality problems. Nevertheless, there are several traditional fermented camel milk in several parts of the world. They differ according to the method of processing as well as on the ecological localities where they have been produced e.g. *gariss* is popular in Sudan (Abdelgadir et al., 1998). *Susa* is fermented camel milk produced in Kenya and Somalia (Lore, et al., 2005). Chal or Shubat beverage is camel's sour milk from Kazakhstan (Shori, 2012). Kefir is the Caucasian fermented camel's milk produced by the fermentation activity of kefir grains (Vedamuthu, 1982). *Oggt* is dried fermented camel milk produced in Syria and Jordan (Al-Ruqaie et al., 1987).

On the other hand, to optimize growth conditions required is the best strategy for camel milk processing (Hassaïne et al., 2007). The basic technological purpose of lactic acid bacteria in milk fermentation is acidification. However, the rates of lactic acid formation dependent on the strains viability and metabolic activity (Tamime and Robinson, 2007; Sobowale et al., 2011).

The hydrolysis degree is dependent on the source and activity of *β*-galactosidase. The cells of lactic acid bacteria do not metabolize lactose directly, but by using lactose permease, which is responsible for transporting lactose into the bacterial cell where it is hydrolyzed to glucose and galactose, resulting in the glucose metabolized into lactic acid (Neves et al., 2005).

β-galactosidase (*β*-D-galactosidase galactohydrolase, EC 3.2.1.23), which are also referred as lactases. Furthermore, *β*-galactosidase enzymes are present in numerous microorganisms, among *β*-galactosidase sources found in abundant in biological systems and microorganisms such as yeasts, mold, and extensively in bacteria, bacterial sources are preferable for dairy industrial application due to ease of fermentation, high enzyme activities, and good stability. Further, bacterial enzyme sources are a GRAS-organism (generally regarded as safe) widely used in the production of fermented dairy foods (Al-Jazairi, 2015; Matijević et al., 2011). Among lactic acid bacteria, yogurt bacteria (*S. thermophilus*, *L. delbrueckii ssp. bulgaricus* and *L. acidophilus*) are the highest *β*-galactosidase producers with high specific activity (Vasiljevic and Jelen, 2001; Akolkar et al., 2006).

During the last three decades, the application of *β*-galactosidase for hydrolysis of lactose in the dairy industries increased. The enzyme was used to improve the technological and sensorial characteristics of dairy products: produce lactose-free milk or fermented products, which is a suitable approach for treating individuals suffering from lactose intolerance affecting humans worldwide (Vasileva et al., 2016; Harju et al., 2012).

Recent studies have shown that the bacterial growth rate and lactic acid produced can be increased in the milk medium by addition *β*-galactosidase enzymatic hydrolysis. Several researchers have reported that stimulation of acid production by lactic acid bacteria in milk cultures after the addition of *β*-galactosidase to hydrolyze the milk lactose. Vénica et al., (2013); Nagaraj et al. (2009) and Shah and Jelen, (1991) noticed that addition of β-galactosidase to the milk had greatest positive effect on the starter cultures growth rate and showed decrease in coagulation time and more amount of lactic acid production in fermented milk treated with *β*-galactosidase due to a higher strains viability and their metabolic activity.

It is known that *β*-galactosidase enzyme is intracellular and whole microbial cells exhibit very little extracellular *β*-galactosidase activity. However, in order to achieve an acceptable of lactose hydrolysis rate and acid development, the bacterial cells must be disrupted in order to release and increase *β*-galactosidase enzyme (Bury et al., 2001; Shah and Jelen, 1991). However, the activity of *β*-galactosidase can be increased several times by isolation of cellular contents using cell disruption methods that included the mechanical such as bead mills, homogenizer and sonicator, or non-mechanical methods. In each case, the cell membrane may be totally disrupted (Geciova et al., 2002; Kula and Schütte, 1987).

Although the rupturing of lactic acid bacteria by bead milling reduce the viable count without killing all cells, but also increased the levels of *β*-galactosidase produced. Furthermore, Bury et al. (2001); Vasiljevic and Jelen (2002); Shah and Jelen (1991) suggested the possibility of using rupture yogurt bacteria in lactose-reduced fermented milk production and showed that the rate of lactose hydrolysis and acid development showed the greater increased than that of whole cells bacterial.

In this paper, we describe a new simple and potentially economically approach method, involving growth and harvest some lactic acid bacterial cultures, rupturing the cells to release the intracellular *β*-galactosidase enzyme,

and using the ruptured cell cultures as the source of the enzyme to improve fermented camel milk.

However, there is essentially no information regarding about the effects of the addition ruptured cells of *S. thermophilus*, *L. delbrueckii ssp. bulgaricus* and *L. acidophilus* on *β*-galactosidase activity, survival and microbial growth, the degree of lactose hydrolysis and acid development of camel milk during the fermentation period.

Thus, the research work in this paper has been carried out with an objective to evaluate the influence of added the ruptured and mixed (whole and ruptured 1:1% v/v) cells of a single strain of *S. thermophilus* ATCC 19258, *L. delbrueckii ssp. bulgaricus* DSMZ 20081 and *L. acidophilus* DSMZ 20079 cultures to camel milk during fermentation on *β*-galactosidase activity, survival, and microbial growth, the degree of lactose hydrolysis and acid development compared to traditional fermented camel milk.

MATERIALS AND METHODS

Camel's milk source

Fresh camel milk of healthy Maghrebi camel's (*Camelus dromedarius*) was purchased from a local farm in Al-Nigella areas, Matrouh Governorate, North West Coast, Egypt, immediately cooled and stored at 4±1°C during transportation to the laboratory until further use. The composition of the camel milk was (fat 3.9, protein 3.75, lactose 4.6, total solids 12. 25%, titratable acidity 0.18% and pH 6.8) as determined by using a Lactoscan milk analyzer, (Model Lactoscan SL, Milkotronic Ltd, Bulgaria) calibrated for camel milk.

Bacterial strains source

Three bacterial strains of *Streptococcus thermophilus* ATCC® 19258™ and *Lactobacillus delbrueckii ssp. bulgaricus* DSMZ® 20081™ and *Lactobacillus acidophilus* DSMZ® 20079™ were obtained from the Egyptian microbial culture collection of Cairo MIRCEN, Faculty of Agriculture, Ain-Shams University, Egypt.

Rupturing of bacterial strains

According to the method of Shah and Lankaputhra (1997) the bacterial strains were grown separately in the 2 L working volume of MRS broth (De-Man, Rogosa, and Sharpe) for *L. acidophilus* and *L. delbrueckii ssp. bulgaricus*, or M17 broth for *S. thermophilus* at 43°C for 16 hours (overnight incubation) and the cells were harvested by centrifuging at 6000 rpm at 4±1°C for 30 min using a refrigerated ultracentrifuge (Beckman Avanti J-25i Floor-model, JA-14 rotor Beckman Instruments, Palo Alto, CA). The cell pellet was washed by suspending in 100 ml sterile phosphate-buffered saline and re-centrifuged. The cell pellet was suspended in 50 ml of sterile saline solution and 10 ml of glass beads (0.1 mm in diameter) were added. The cell suspension cooled to 4±1°C and glass beads was placed in a 60 ml sterile glass and mechanical vibration was applied using a Braun homogenizer (Biotech- Potter- S -homogenizer, Sartorius, Germany) at 3000 rpm for 30, 60, 90 or120 second in order to rupture the cells to release the intracellular enzyme. The glass beads were removed by centrifuging at 6000 rpm for 1 min to remove the glass beads and then, the treated ruptured cells were placed in an ice bath with occasional shaking for 10 min and filtered with sterile cellulose membrane filters. The cell suspension solution samples were used before and after cell ruptured to enumerate viable counts and measure the *β*-galactosidase attributed to a gram of dried cell suspension. Before the experiment, each of all strain cells suspension solution was mixed with (120 g/l) (w/v) reconstituted non-fat dry milk at a 1:1 ratio prior to inoculation of camel milk fermentation as an experimental medium.

Experimental design

The bulk of camel milk was heat-treated at 95°C for 5 min in a water bath and cooled immediately to 4±1°C in an ice bath. The experiments fermentation of each type of fermented camel milk was carried out in duplicate sterile flasks (150 ml). Camel milk was equilibrated for one hour at the fermentation temperature (42ºC) in a water bath before inoculation with the single strain starter cultures. Camel milk was divided into three lots for each treatment where, the first portions, camel milk was inoculated with 3 % of whole cell cultures of *L. delbrueckii ssp. bulgaricus*, *S. thermophilus* and *L. acidophilus*, activated and propagated in sterilized reconstituted skim milk (120g/L) as a control. The second portions of camel milk were inoculated with 3% of ruptured cell cultures of *L. delbrueckii ssp. bulgaricus* ruptured for (60 s), *S. thermophilus* ruptured for (60 s) and *L. acidophilus* ruptured for (90 s). The third portions of camel milk were inoculated with 3% of mixed cells contain (mixed of each whole and ruptured cells of the same strain at a ratio of 1:1) and the contents were thoroughly mixed and incubated. Each treatment of the whole, ruptured and mixed cells of all strains were incubated at 42°C for 10 h. The fermentation samples were taken during fermentation for microbiological and biochemical tests at two-hour intervals for measurement of lactose concentrations (%), pH values, viable cell counts of lactic acid bacteria and for determination of *β*- galactosidase activity.

Measuring *β*-galactosidase activity

Preparation of fermented camel milk samples

The bacterial strain cells in fermented camel milk samples were disrupted, according to the method of Ustok et al.

(2010) by adding one milliliter of a 1:10 dilution (w/v) of fermented camel milk in 0.05 M (pH 7.0) sodium phosphate buffer to a tube containing 0.2 ml of egg white lysozyme (50 mg/ml buffer) (EC 3.2.1.17 ≥ 40000 U/mg protein, Sigma); mixed and held in an ice-water for 30 min to lysing bacterial cells by hydrolyzing the peptidoglycan present in the bacterial cell walls. Cell lysis suspensions were centrifuged at 6000 rpm for 10 min at 4±1°C to remove debris and whole cells. The bacterial cell disruption solution was immediately cleared according to the method of Chowdhury et al. (2008) one milliliter of bacterial cell disruption solution of each strain was added to 0.5 ml of a mixture of organic solvents and detergents Clarifying Reagent® (sigma-Aldrich-UK) solution and shaken vigorously for 20 min to solubilize camel milk protein and fat to access to a clear solution for measured *β*-galactosidase activity absorbance.

Assay of β-galactosidase activity

The activity of the *β*-galactosidase enzyme was determined as described by Mahoney et al. (1975). The reaction mixture was composed of 0.1 ml of each cleared solution was added to 4 ml of the chromogenic substrate (prepared by solving 300 mg ortho nitrophenol-*β*-D-galactopyranoside (o-NPGal) in 100 ml of 100 mM phosphate buffer solution pH 7.0), the mixture was incubated at 37°C for 10 m in a shaker water bath. After the desired incubation time, 2 ml of 0.625 M sodium carbonate Na_2CO_3 was added to stop the reaction. Then, by considering the yellow color which is a result of o-NPGal hydrolysis, the absorbance was measured at A_{420} nm with a spectrophotometer using a scanning double-beam spectrophotometer Jenway 6850 spectrophotometer (Jenway Instruments, Beacon Road, Stone, Staffordshire, ST15 OSA, UK) against a reagent blank. The number of moles of o-nitrophenol released was based on the relationship of the A_{420} to a standard curve using o-NPGal as the standard. One unit (IU) of enzyme activity was defined as the amount of the enzyme that needed to hydrolyze μmol of o-nitrophenol per minute (1U =1 μmol of o-NPGal/min/ml or g) under the assay measurement conditions (37°C, pH 7, for 10 min). Assays were carried out at least in duplicate and the data were given are an average of these results.

Lactose determination

Lactose concentration of fermented camel milk samples was determined according to the method of (Martins et al., 2014). The fermented samples were taken at time intervals and cleared by mixing 1ml of fermented milk with 0.1 ml 6 N HCl and centrifuge at 10000 rpm for 5 min then filtered using a 12.5 cm Whatman® filter paper. Approximately 0.3 ml of the supernatant was transferred into a clean tube and neutralizes with 50 μL 6 N Na OH. The neutralized supernatant was diluted with distilled water (dilution factor 1.36). The lactose concentration was estimated enzymatically by using Lactose/D-Galactose determination kit UV method (Boehringer Mannheim Co., Germany) with absorbance reading at A_{340} nm by using a scanning double-beam spectrophotometer (Jenway 6850 spectrophotometer-UK), according to the instructions of the manufacturer. For each fermented camel milk sample, the assay was carried out in duplicate and their averages were taken. The degree of lactose hydrolysis was calculated by using the hydrolyzed lactose concentration to total lactose concentration.

Measurement of pH

The pH of the fermented camel milk samples was measured directly into a homogenized sample with a digital pH meter equipped with a glass electrode (model pH 211; Hanna Instruments).

Enumeration of microorganisms

Serial dilutions of fermented camel milk or bacterial suspension cell before and after cell-rupture sample solution (1 ml) were homogenized for one minute in 9 ml of 0.1% (w/v) a Ringer's solution (Oxoid, Unipath, Basingstoke, Hampshire, UK). From these samples, serial decimal dilutions were prepared in sterile 0.1% Ringer's solution. *S. thermophilus* was counted in M17 agar (Difco Laboratories) and aerobically incubation at 37°C for 72 h according to Torriani et al. (1996), whereas acidified MRS (pH 5.2) agar (Oxoid Ltd., Cambridge, England) was used for enumeration *L. delbrueckii ssp. bulgaricus* and anaerobically incubation using AnaeroGen in plastic anaerobic jars (Oxoid, England) at 43°C for 72 h according to Dave and Shah (1996). MRS agar (Oxoid Ltd., Cambridge, England) with 0.20% oxgall (Difco Laboratories) was used a selective medium for *L. acidophilus* and anaerobically incubation using AnaeroGen in plastic anaerobic jars (Oxoid,England) at 37° C for 72 h according to Marshall, (1992). Colonies in plates with 25 to 250 colonies were counted and viable counts in (log^{10} CFU/ml) according to Torriani et al. (1996).

Statistical analysis

Experimental data were statistically analyzed as one-way ANOVA with a 95% confidence level according to SPSS Statistics package (SPSS V.18, 2012) for Windows. Differences were considered significant at (P<0.05). Results are expressed as average ±SD of all available data.

RESULTS AND DISCUSSION

Results regarding change in viable cell counts and *β*-galactosidase activity of *S. thermophilus* ATCC 19258, *L. delbrueckii ssp. bulgaricus* DSMZ 20081 and *L. acidophilus* DSMZ 20079 starter cultures before and after cell-rupture

(Table 1) indicated that the viable cell counts of all strain cultures tested were decreased gradually with increasing ruptured time and showed the same tendencies. However, the minimum viable cell that can be used throughout this study was identified after the 60s of cell rupture treatment for *S. thermophilus* and *L. delbrueckii ssp. bulgaricus*; it was 6.66±0.57 and 6.75±0.12, respectively, while for *L. acidophilus* it was 6.25±0.61 Log CFU/ml after the 90s of cell rupture. The higher survival cells of *L. acidophilus* at 90 s of cell rupture processing may indicate a more resistant cell wall material compared to the other organisms tested.

The amounts of *β*-galactosidase released out the cells by the rupture processing of all strain cultures was increased significantly ($P<0.05$) with increasing the cell- rupture time. Furthermore, it can be seen that the most suitable *β*-galactosidase activity which marked by higher *β*-galactosidase activity and can be used throughout this study, was 366 ±5.7 for *S. thermophilus* at the 60 s, 479±5.7 for *L. delbrueckii ssp. bulgaricus* at 60 s and 418±19.8 IU/O-NPGal/min per g at 90 s of cell rupture for *L. acidophilus*. Shah and Lankaputhrab (1997); Bury et al. (2001) reported that the *β*-galactosidase activity in the medium can be greatly increased by rupturing cells.

However, before cell-rupture, the amount of *β*-galactosidase was minimal quantity, but after ruptured cells, the *β*-galactosidase activity was increased rapidly. Furthermore, this difference in the amount of *β*-galactosidase enzymes released from bacterial strains could be attributed to the difference in cell wall structures (Carević et al., 2015). *L. acidophilus* after the 60s of ruptured cells due to the fact that *L. acidophilus* has a high portion of peptidoglycan layer, which gives strength to the cell wall and prevents the release of intracellular *β*-galactosidase (Jafarei and Ebrahimi, 2011).

On the other hand, the results showed that *β*-galactosidase activity of *L. delbrueckii ssp. Bulgaricus* was at relatively high levels in comparison to *S. thermophilus* and *Lactobacillus acidophilus*. Bury and Jelen (2000) and Gaudreau et al. (2005) indicated that the L. delbrueckii ssp. bulgaricus had the highest levels of β-galactosidase enzyme activity in comparison to other dairy cultures especially using skim milk.

Regarding the changes in viable cell counts of the whole, ruptured and mixed cells of *S. thermophilus*, *L. delbrueckii ssp. bulgaricus* and *L. acidophilus* during camel milk fermentation for 10 hours (Table 2) throughout fermentation, the mixed cells had significantly ($P<0.05$) fastest growth rate after 2 h of incubation while all whole and ruptured cells of all strain cultures showed slow growth rate and did not initiate rapid growth until 4-6 h of incubation and reached a maximum after 10 h of the incubation period.

However, although rupture processing decreased the viable cell counts, it did not destroy the propagation ability of the surviving bacterial cells. The viable cell counts in fermented camel milk made by using ruptured cells of all strain cultures increased again after 4 h of incubation and reached stationary phase after approximately 8 h of growth. These results are in agreement with those obtained by Shah and Lankaputhrab (1997) who noticed that the viable counts of ruptured cells of yogurt bacteria was increased again after 4 h of the fermentation period. The phenomena could be explained by a portion of lactose in the camel milk medium used by the free *β*-galactosidase enzyme than by the intracellular *β*-galactosidase due to the lack of mass transfer resistance from the cellular membrane and helped stimulate the bacterial culture growth. Therefore, enhancement of this mechanism would improve metabolic activities and the growth rate of these strains significantly in camel milk. This finding is in agreement with many researchers which confirm our findings (Shah and Lankaputhrab, 1997; Vénica et al., 2013 and Hsu, et al., 2005).

In all experiments, the bacterial growth rate in fermented camel milk made by using mixed cells of all strain cultures was 2 h faster and the final viable cell counts were approximately one log cycles higher when compared with that made with whole cells of the same strain cultures. This means that ruptured cells of all strains are able to propagate

Table 1: Change in viable cell counts and *β*-galactosidase activity of *streptococcus thermophilus* ATCC 19258, *lactobacillus delbrueckii ssp. bulgaricus* DSMZ 20081 and *lactobacillus acidophilus* DSMZ 20079 starter cultures before and after cell-rupture

Bacterial strains	Before cell-rupture	After cell-rupture (second)			
Viable counts (Log^{10} CFU/ml)	Zero time	30 s	60 s	90 s	120 s
S. Thermophilus	9.24^{a}±0.53	7.05^{b}±0.11	6.66^{b}±0.57	4.67^{c}±0.11	2.73^{d}±0.23
L. Delbrueckii ssp. bulgaricus	9.31^{a}±0.51	7.34^{b}±0.26	6.75^{b}±0.12	4.40^{c}±0.61	2.28^{d}±0.04
L. Acidophilus	9.62^{a}±0.54	7.79^{b}±0.04	7.01^{c}±0.03	6.25^{d}±0.61	3.89^{e}±0.03
β-galactosidase activity * (1U/g)					
S. Thermophilus	25^{e}±2.8	227^{d}±4.2	366^{c}±5.7	388^{b}±8.5	429^{a}±12.7
L. Delbrueckii ssp. bulgaricus	43^{d}±2.8	277^{c}±9.9	479^{b}±5.7	501^{b}±12.7	585^{a}±9.9
L. Acidophilus	36^{e}±1.4	253^{d}±8.5	360^{c}±2.8	418^{b}±19.8	551^{a}±14.1

*1U=1 μmole of o-NPGal/min per g of cell suspension. [a, b,c]. Means of each strain in the same column with different letters are significantly different at ($P<0.05$) by DMRT

Table 2: Change in viable cell counts (log^{10} CFU/ml) of *streptococcus thermophilus* ATCC 19258, *lactobacillus delbrueckii ssp. bulgaricus* DSMZ 20081 and *lactobacillus acidophilus* DSMZ 20079 during camel milk fermentation with whole, ruptured and mixed cells

Treatments	Incubation time (hours)					
	Streptococcus thermophilus					
	Zero time	2 h	4 h	6 h	8 h	10 h
Whole cells	$6.54^{bA}\pm0.42$	$6.70^{bB}\pm0.36$	$7.60^{aB}\pm0.48$	$7.87^{aB}\pm0.07$	$8.12^{aB}\pm0.14$	$8.18^{aB}\pm0.02$
Ruptured cells	$6.66^{cA}\pm0.57$	$6.70^{cB}\pm0.26$	$7.64^{bB}\pm0.11$	$7.94^{abB}\pm0.10$	$8.26^{aB}\pm0.03$	$8.30^{aB}\pm0.15$
Mixed cells*	$6.98^{cA}\pm0.02$	$8.37^{bA}\pm0.38$	8.55^{ab}A±0.23	$8.83^{abA}\pm0.73$	$9.19^{aA}\pm0.07$	$9.21^{aA}\pm0.14$
Lactobacillus delbrueckii ssp. bulgaricus						
Whole cells	$6.93^{cA}\pm0.09$	$7.04^{cC}\pm0.04$	$7.87^{bB}\pm0.36$	$7.94^{bB}\pm0.07$	$8.31^{aB}\pm0.02$	$8.47^{aC}\pm0.10$
Ruptured cells	$6.75^{bB}\pm0.12$	$7.89^{aB}\pm0.05$	$8.07^{aAB}\pm0.18$	$8.12^{aB}\pm0.49$	$8.45^{aB}\pm0.46$	$8.57^{aB}\pm0.04$
Mixed cells*	$7.01^{dA}\pm0.02$	8.46cA±0.39	$8.60^{bcA}\pm0.25$	8.95bA±0.06	$9.55^{aA}\pm0.11$	$9.62^{aA}\pm0.04$
Lactobacillus acidophilus						
Whole cells	$6.79^{cAB}\pm0.34$	$7.00^{bcB}\pm0.33$	$7.57^{bB}\pm0.47$	$8.19^{aB}\pm0.21$	$8.32^{aB}\pm0.37$	$8.36^{aB}\pm0.03$
Ruptured cells	$6.25^{bB}\pm0.61$	$7.63^{aAB}\pm0.59$	$7.70^{aB}\pm0.18$	$8.24^{aB}\pm0.08$	$8.36^{aB}\pm0.31$	$8.39^{aB}\pm0.03$
Mixed cells*	$7.39^{cA}\pm0.36$	$8.46^{bA}\pm0.41$	$8.54^{bA}\pm0.43$	$9.03^{abA}\pm0.40$	$9.29^{aA}\pm0.22$	$9.32^{aA}\pm0.02$

$^{A, B,C.}$ Means of each strain in the same column with different letters are significantly different at (P<0.05) by DMRT., $^{a, b.c.}$ Means of each strain in the same column with different letters are significantly different at (P<0.05) by DMRT. * mixed cells=mixed of each whole and ruptured cells of the same strain 1:1% v/v.

after incubation, even when some microorganisms are damaged. Moreover, with increases in cell viability, the fermentation time of the fermented camel milk decreased. This is an important aspect from the industrial viewpoint since it would not increase the usual production time of fermented camel milk.

On the other hand, the longer fermentation time and the slow growth rate of fermented camel milk made by using whole or ruptured cells of all strain cultures, it could be due to an imbalance between the number of killed bacteria by natural antibacterial activity in camel milk towards bacterial starter cultures and the cells destroyed by the rupture process and between attempted to growth and surviving again. El-Hatmi et al. (2006); Benkerroum et al. (2004); Levieux et al. (2005) reported that camel milk lower activity and viability of the lactic acid starter cultures. Thus, in fermented camel milk made by using whole cells of all strain cultures, the acidification rate was lower and takes a longer time to develop. It could only be explained by the presence of higher concentrations of natural protective proteins when compared with cow's milk.

Moreover, at any given time of fermentation, the viable cell counts of mixed cells of *L. delbrueckii ssp. bulgaricus* have been always more numerous than the other strain cultures tested, while the viable counts of the whole cell cultures of *S. thermophilus* were the least numerous. These could be due to that *Lactobacilli* alter metabolic pathways based on the carbohydrates (Vinderola et al., 2000; Gueimonde et al., 2002). Rahman et al. (2009) found that the Lactobacilli have been always more numerous than the streptococci during camel milk fermentation at 42°C for 6 h. While (Abdel Moneim et al., 2006; Lore et al., 2005) indicated that the main genus of lactic acid bacteria in Garris and Suusac product was Lactobacilli.

The change in β-galactosidase amounts (or activities) of *S. thermophilus*, *L. delbrueckii ssp. bulgaricus and L. acidophilus* during camel milk fermentation by using whole, ruptured and mixed cell cultures durig10 h of incubation (Table 3) cannot be easily compared with results of the literature as not much data is available on the β-galactosidase activity of pure starter cultures during camel milk fermentation. Thus, comparisons will be made with results of researchers who studied fermented milk from other animal's milk. β-galactosidase from lactic acid bacteria is a type of endo-enzyme, primarily bound to cell walls. As expected from the above experiment, the initial β-galactosidase activity in fermented camel milk made by using ruptured or mixed cells of all strain cultures was higher than that made by using the whole cells of all strain cultures, there was a 7-10-fold increase in the initial β-galactosidase activity after the addition as a result of rupture processing or mixed cells of strain cultures. These are in agreements with previous reports by Shah and Lankaputhrab (1997).

On the other hand, the highest significant (P<0.05) β-galactosidase activity was obtained after 4 h of fermentation in fermented camel milk made by using all ruptured or mixed cells of *L. delbrueckii ssp. bulgaricus, L. acidophilus* and *S. thermophilus*. It was 1.87±0.06, 1.63±0.06 and 1.70±0.01 for the ruptured cell cultures and 1.97±0.12, 1.77±0.06 and 1.70±0.01 IU/ml for the mixed cell cultures, respectively. This activity declined as the pH decreased through fermentation period. These results are comparable with those found by Shah and Lankaputhrab (1997) who reported that the highest β-galactosidase activity reached about 1.31-2.0 units per gram in fermented milk made with whole and ruptured cells during 5 h of fermentation, respectively. The decreased in β-galactosidase activity after 4 h of incubation probably due to the acidification

Table 3: Change in the β-galactosidase activity of *streptococcus thermophilus* ATCC 19258, *lactobacillus delbrueckii ssp. bulgaricus* DSMZ 20081 and *lactobacillus acidophilus* DSMZ 20079 20079 during camel milk fermentation with whole, ruptured and mixed cells

Treatments	Incubation time (hours)					
	Streptococcus thermophilus					
	Zero time	2 h	4 h	6 h	8 h	10 h
Whole cells	0.10^{eC}±0.01	0.41^{dC}±0.01	0.58^{cC}±0.06	0.73^{bC}±0.06	0.87^{aC}±0.06	0.90^{aC}±0.06
Ruptured cells	1.07^{cA}±0.06	1.33^{bB}±0.12	1.50^{aB}±0.10	1.47^{abB}±0.06	1.43^{abB}±0.06	1.40^{abB}±0.01
Mixed cells*	0.70^{bB}±0.10	1.57^{aA}±0.12	1.70^{aA}±0.01	1.68^{aA}±0.08	1.67^{aA}±0.06	1.63^{aA}±0.06
Lactobacillus delbrueckii ssp. bulgaricus						
Whole cells	0.17^{eC}±0.01	0.60^{dC}±0.06	0.77^{cB}±0.01	0.83^{bB}±0.01	0.93^{abC}±0.04	0.97^{aC}±0.02
Ruptured cells	1.53^{cA}±0.06	1.70^{bB}±0.10	1.87^{aA}±0.06	1.77^{abA}±0.06	1.73^{abB}±0.06	1.73^{abB}±0.11
Mixed cells*	1.02bB±0.08	1.90^{aA}±0.10	1.97^{aA}±0.12	1.93^{aA}±0.15	1.91^{aA}±0.09	1.90^{aA}±0.08
Lactobacillus acidophilus						
Whole cells	0.11^{dC}±0.01	0.48^{cC}±0.08	0.60^{bC}±0.05	0.70^{aB}±0.06	0.85^{aC}±0.01	0.93^{aC}±0.10
Ruptured cells	1.27^{cA}±0.06	1.43^{bB}±0.06	1.63^{aB}±0.06	1.62^{aA}±0.08	1.60^{aB}±0.01	1.60^{aB}±0.01
Mixed cells*	0.82^{cB}±0.02	1.63^{bA}±0.06	1.77^{aA}±0.06	1.74^{abA}±0.05	1.73^{abA}±0.06	1.73^{abA}±0.12

*1U=1 μmole of o-NPGal/min per ml released culture., $^{A, B,C.}$ Means of each strain in the same column with different letters are significantly different at (P<0.05) by DMRT., $^{a, b,c.}$ Means of each strain in the same column with different letters are significantly different at (P<0.05) by DMRT., * mixed cells=mixed of each whole and ruptured cells of the same strain 1:1% v/v.

produced by the starter cultures. This is due to the fact that the ruptured or mixed cell strain cultures reduced the pH of the fermented camel milk from 6.83 to less than 5.0 after 4 h of incubation accompanied by a reduction in the enzyme activity. These observations agree with Wang et al. (1996) who reported that the activity of β-galactosidase depends on pH; it decreases rapidly at lower pH values. Also, Gueimonde et al. (2002); Carević et al. (2015); Ustok et al. (2010) demonstrated that Lactobacillus acidophilus, L. delbrueckii ssp. bulgaricus, and *S. thermophilus* displayed significantly higher levels (P< 0.05) of β-galactosidase activity at pH 6.3 (activity over 90% of maximum) with an optimum at pH 6.0 -7.0 and then lose in activity as the pH decreased.

In contrast, the β-galactosidase activity was increased slowly in fermented camel milk made by using whole cells of *L. delbrueckii* ssp. bulgaricus, *L. acidophilus* and *S. thermophilus* and reached about 0.97±0.02, 0.93±0.10 and 0.90±0.06 IU/ml during 10 h of incubation, respectively. Although the β-galactosidase activity of whole cells of all strain cultures seem to be low, this range was nearly to that obtained by Cesca et al. (1984); Lin et al. (1989); Greenberg et al. (1982) reported that β-galactosidase activity of the pure strains of *S. thermophilus* vary from 0.1 to 1.1 IU/ml, while β-galactosidase activity from L. delbrueckii ssp. bulgaricus strains have a substantially higher 0.8 to 4.0 IU/ml. On the other hand, Carević et al. (2015); Murad et al. (2011) proved that the highest β-galactosidase activity obtained from *L. acidophilus* varies from 0.671 to 2.54 IU/ml.

In our study, the lowest activity of β-galactosidase in fermented milk made by using the whole cells of all strain cultures, obviously due to lower levels of live bacterial cultures this may be attributed to reducing of final β-galactosidase production. Several investigators have described that the activity of β-galactosidase was independent of the of strain growth rate (Lin et al., 1989; Greenberg et al., 1982). Barbour et al. (1984); Gassem and Abu-Tarboush (2000) all showed that dromedary milk failed to reach a gel-like structure (typically of cow milk) after 18 h of incubation due to the presence of growth-inhibiting factors.

Moreover, the present results revealed that the strain of *L. delbrueckii* ssp. bulgaricus showed the highest β-galactosidase activity in all fermented camel milk samples in comparison to *S. thermophilus* and *L. acidophilus* cultures which might be due to higher viable counts. This result is in agreement with that observed by Shah and Jelen (1991) and Bury and Jelen (2000) who reported that L. delbrueckii ssp. bulgaricus 11842, capable to produce relatively high levels of β -galactosidase to hydrolyze twice as much lactose to produce the same amount of energy as those capable of utilizing galactose in comparison to other dairy cultures.

The changes in pH values in fermented camel milk made by using whole, ruptured and mixed cells of *S. thermophilus*, *L. delbrueckii ssp. bulgaricus* and *L. acidophilus* during 10 hours incubation are shown in Table 4. For commercial utility, the pH values of most fermented milk are expected near to pH 4.6. As can be seen, the pH values of the camel milk medium were dropped gradually because of the accumulation of lactic acid. The significant (P<0.05) maximum drop in pH values to about 4.6 was found in fermented camel milk made by using mixed or ruptured cells of all strain cultures at the first of 4-6 h of incubation time, respectively with minor decreased later. Furthermore, the maximum decrease in pH values in all fermented camel

Table 4: Change in pH values of fermented camel milk made by using whole, ruptured and mixed cells of *streptococcus thermophilus* ATCC 19258, *lactobacillus delbrueckii ssp. bulgaricus* DSMZ 20081 and *lactobacillus acidophilus* DSMZ 20079 during 10 hours of incubation

Treatments	Incubation time (hours)					
	Streptococcus thermophilus					
	Zero time	2 h	4 h	6 h	8 h	10 h
Whole cells	6.73^{aA}±0.06	6.43^{bA}±0.06	5.43^{cA}±0.06	5.23^{dA}±0.06	5.13^{dA}±0.06	4.97^{eA}±0.06
Ruptured cells	6.77^{aA}±0.01	6.20^{bB}±0.01	5.27^{cB}±0.06	4.65^{dB}±0.01	4.62^{deB}±0.02	4.58^{eB}±0.03
Mixed cells*	6.79^{aA}±0.01	5.27^{bC}±0.06	4.65^{cC}±0.02	4.65^{cB}±0.03	4.60^{cdB}±0.01	4.58^{dB}±0.03
Lactobacillus delbrueckii ssp. bulgaricus						
Whole cells	6.74^{aA}±0.05	6.33^{bA}±0.06	5.27^{cA}±0.06	5.13^{dA}±0.06	4.97^{eA}±0.06	4.83^{fA}±0.06
Ruptured cells	6.79^{aA}±0.01	6.13^{bB}±0.06	5.10^{cB}±0.01	4.64^{dB}±0.02	4.60^{deB}±0.01	4.56^{eB}±0.05
Mixed cells*	6.76^{aA}±0.05	5.10^{bC}±0.01	4.63^{cC}±0.01	4.60^{cB}±0.01	4.57^{cdB}±0.06	4.53^{dB}±0.03
Lactobacillus acidophilus						
Whole cells	6.77^{aA}±0.05	6.23^{bA}±0.06	5.23^{cA}±0.12	5.17^{cA}±0.06	4.97^{dA}±0.09	4.90^{dA}±0.10
Ruptured cells	6.76^{aA}±0.01	6.17^{bA}±0.06	5.17^{cA}±0.06	4.62^{dB}±0.07	4.63^{dB}±0.01	4.55^{dB}±0.04
Mixed cells*	6.75^{aA}±0.05	5.13^{bB}±0.12	4.64^{cB}±0.01	4.62^{cB}±0.02	4.63^{cB}±0.06	4.52^{dB}±0.02

$^{A, B.C.}$ Means of each strain in the same column with different letters are significantly different at (P<0.05) by DMRT., $^{a, b.c.}$ Means of each strain in the same column with different letters are significantly different at (P<0.05) by DMRT., * mixed cells=mixed of each whole and ruptured cells of the same strain 1:1% v/v.

milk experimental was found in fermented camel milk made by using mixed cells of *S. thermophilus, L. acidophilus* and *L. delbrueckii ssp. bulgaricus* samples. It was 4.65±0.02, 4.64±0.01 and 4.63±0.01 during 4 h of fermentation, respectively.

According to our results, the higher *β*-galactosidase activity released to camel milk medium by rupture processed can induce more rapid and efficient utilization of lactose hydrolysis and that was appeared to be the strongest factor responsible for the more rapid pH decrease. This assumption was in agreement with data reported by Wang, et al. (1996) who noticed that β-galactosidase released to the medium by rupture processed caused a significant increased in lactose hydrolysis activity compared to that in the cells, and hence more lactic acid is produced. Also, this finding agreed with Gilliland et al. (1972) who observed that the increased acid production by lactic bacteria in milk cultures in the presence of β-galactosidase might be due to the conversion of a greater percentage of lactose to acid in end products.

Moreover, the results indicated that the fermented camel milk made by using mixed cells of L. delbrueckii ssp. bulgaricus showed a maximum significantly (P<0.05) decreased in pH values during all incubation time in comparison to fermented camel milk made by using of *S. thermophilus*, *L. acidophilus*. For each strain, the lower pH values corresponded to the cases with high cell viability and the availability of more quantity of easily fermentable sugar (Baeve, 1981; Nagaraj et al., 2009).

In contrast, using the whole cells of *S. thermophilus*, L. delbrueckii ssp. bulgaricus and *L. acidophilus* was showed slowly decreased in pH values and had the highest pH values at the end of 10 h of incubation time. It was 4.97±0.06, 4.90±0.10 and 4.83±0.06, respectively. According to Damin et al. (2009); Toba et a1. (1990), a rapid decrease in pH values is expected as a result of the higher metabolic activity of starter cultures during fermentation. When using the whole cells of *S. thermophilus*, L. delbrueckii ssp. bulgaricus and *L. acidophilus*, only a small decrease rate in pH values was obtained during camel milk fermentation because of the low viability of the whole cells (Benkerroum, 2008).

On the other hand, the pH values of the all fermented camel milk made by using of *L. delbrueckii ssp. bulgaricus* had the highest decrease in pH values until the end of incubation time. As it was expected, *L. delbrueckii ssp. bulgaricus* has a higher *β*-galactosidase activity and total viable cells which is probably the cause of faster dropping in pH-value of camel milk medium. Previous studies support these findings (Abu-Tarboush, 1996; Dirar, 1993; Rahman et al. 2009).

Less time was required for decreased pH value to 4.6 in fermented camel milk made by using mixed cells of all strain cultures than in the whole cells of the same strain cultures. The rapid drop in the pH values in fermented camel milk made by using mixed cells of all strain cultures would allow a shorter fermentation time to be used. So, 4 h of incubation periods was optimum for incubation and can be employed in the manufacture of fermented camel milk by using the mixed cells of strain cultures.

To the best of our knowledge, there are no works that summarized the results about the degree of lactose hydrolysis by pure strains of starter cultures in fermented camel milk during the fermentation period. The stimulating effects of lactose consumption occurred immediately after

the fermentation started by all strain cultures (Table 5). The rapid degree of lactose hydrolysis was observed approximately during 2 h of incubation time in fermented camel milk made by using a mixed or ruptured cell of all strain cultures, which were maintained with a little significant change (P>0.05) until the end of the 10h of incubation time. On the other hand, the rapid decrease in lactose during the first 2 h of fermentation that may be due to lower pH values attained during fermentation, that probably caused inactivation of the β-galactosidase enzyme at that fermented camel milk pH value, where the higher activity of intracellular and cell-free β-galactosidase enzyme was independent in pH range. This finding was in agreement with those reported by Toba et al. (1990) who noticed that the maximum lactose hydrolysis was obtained between 3 and 4 h of incubation for L. delbrueckii ssp. bulgaricus B-6 and the β-galactosidase production reached a maximum after 3-4 h of bacterial growth. Also, Wang et al. (1996); Lin et al. (1989) reported that the relative efficiency and activity of a β-galactosidase enzyme for hydrolysis of lactose in milk depends on pH.

In comparison to that observed in the case of whole cells of strain cultures, the lactose hydrolysis level was increased slowly with increasing incubation time and reached the maximum at the end of 10 h of the incubation period. As well known, the decrease in the lactose content in fermented camel milk by using whole cells of strain cultures tested was produced only by the activity of starter cultures and the slower decreased in lactose hydrolysis in this case can be explained by the lower of both bacterial growth rate and the lower in amount and activity of β-galactosidase enzyme achieved during conventional camel milk fermentation.

As expected, the significant (P<0.05) degree of lactose hydrolysis in fermented camel milk made by using mixed or ruptured cells of *S. thermophilus, L. acidophilus* and *L. delbrueckii ssp. bulgaricus* was observed during 2 h of the incubation period and reached the maximum approximately 37.69 - 38.39 and 40.56 per cent and about 33.31 - 35.47 and 38.40 per cent after 10 h of incubation time, respectively. These percentages of lactose hydrolysis were approximately the double that those obtained in fermented camel milk made by using the whole cells of all strain cultures tested. This is probably because a portion of milk lactose was consumed more easily by the free β-galactosidase enzyme in camel milk medium rather than in the bacterial cells during fermentation.

In contrast, the degree of lactose hydrolysis in conventional fermentation by using the whole cells of *S. thermophilus, L. acidophilus* and *L. delbrueckii ssp. bulgaricus* was increased slowly with increasing in incubation time and reached the maximum approximately 17.37, 20.27 and 22.44 per cent during 10 h of incubation time, respectively. Therefore, the rate of lactose hydrolysis in conventional fermentation is dependent only on the viable cell count and the activity of β-galactosidase. So, we will be needed to enhance the lactose hydrolysis for those strain cultures during manufacture of fermented camel milk.

However, the information available on lactose hydrolysis in fermented milk made by using the whole cells of pure strain cultures is scarce and very variable. However, our study was lower than the values reported by Fuquay et al. (2011); Batista et al. (2008); Tamime and Robinson (2007) who noticed that a greater consumption of lactose was metabolized up to ~20–30% by bacterial of yogurt culture through 2 h of incubation in fermented milk. In contrast, Ustok et al. (2010); Kreft et al. (2001) using L. delbrueckii ssp. bulgaricus ATCC 11842 and *S. thermophilus*95/2 pure strains, indicated up to 60% of lactose hydrolyzed at the

Table 5: Change in lactose concentrations (%) of fermented camel milk made by using whole, ruptured and mixed cells of *streptococcus thermophilus* ATCC 19258, *lactobacillus delbrueckii ssp. bulgaricus* DSMZ 20081 and *lactobacillus acidophilus* DSMZ 20079 during 10 h of incubation

Treatments	Incubation time (hours)					
	Streptococcus thermophilus					
	Zero time	2 h	4 h	6 h	8 h	10 h
Whole cells	4.60^{aA}±0.10	4.23^{bA}±0.06	3.97^{cA}±0.06	3.93^{cdA}±0.06	3.83^{deA}±0.06	3.80^{eA}±0.01
Ruptured cells	4.60^{aA}±0.10	3.37^{bB}±0.06	3.20^{cB}±0.01	3.10^{cdB}±0.01	3.07^{dB}±0.06	3.07^{dB}±0.06
Mixed cells*	4.60^{aA}±0.10	3.13^{bC}±0.06	3.03^{bcC}±0.06	2.97^{bcB}±0.12	2.90^{cC}±0.10	2.87^{cC}±0.12
Lactobacillus delbrueckii ssp. bulgaricus						
Whole cells	4.60^{aA}±0.10	4.03^{bA}±0.06	3.83^{cA}±0.06	3.77^{cA}±0.15	3.70^{cd}A±0.10	3.57^{dA}±0.06
Ruptured cells	4.60^{aA}±0.10	3.13^{bB}±0.06	3.03^{bB}±0.06	2.90^{cB}±0.10	2.87^{cB}±0.06	2.83^{cB}±0.06
Mixed cells*	4.60^{aA}±0.10	2.97^{bC}±0.06	2.87^{bcC}±0.06	2.83^{cdB}±0.06	2.77^{cdB}±0.06	2.73^{dB}±0.06
Lactobacillus acidophilus						
Whole cells	4.60^{aA}±0.10	4.13^{bA}±0.06	3.87^{cA}±0.06	3.80^{cdA}±0.10	3.73cdA±0.06	3.67^{dA}±0.12
Ruptured cells	4.60^{aA}±0.10	3.23^{bB}±0.06	3.13^{bcB}±0.06	3.10^{bcB}±0.10	3.03cdB±0.06	2.97^{dB}±0.06
Mixed cells*	4.60^{aA}±0.10	3.07^{bC}±0.06	2.97^{bcC}±0.06	2.93^{bcB}±0.06	2.90cB±0.10	2.83^{cB}±0.06

$^{A, B.C.}$ Means of each strain in the same column with different letters are significantly different at (P<0.05) by DMRT., $^{a, b.c.}$ Means of each strain in the same column with different letters are significantly different at (P<0.05) by DMRT., * mixed cells=mixed of each whole and ruptured cells of the same strain 1:1% v/v.

end of 4 h of incubation. Likewise, Linko et al. (1998) reported that only about 45–68% of the lactose in the medium was utilized by *S. thermophilus*11F under the experimental conditions used.

Generally, the present results revealed that the strain of L. delbrueckii ssp. bulgaricus showed the highest degree of lactose hydrolysis during all incubation time in comparison to *S. thermophilus* or *L. acidophilus* which might be due to higher viable cell counts and higher β-galactosidase activity. Shah and Jelen (1991) showed that the addition of L. delbrueckii ssp. bulgaricus 11842 to milk was effective in hydrolyzing the lactose than *S. thermophilus* ST20 and the rate of lactose hydrolysis is dependent on the activity of β-galactosidase, and the starter cell viability.

CONCLUSION

Although rupture processing decreased the viable cell count of all strain cultures examined, it did not completely destroy the propagation ability of the surviving cells, where the viable cell count increases again after the incubation. Fermented camel milk made by using mixed cells all strain cultures had more and rapid decrease in pH values to 4.6 during 4h of incubation compared to the whole cells of all strain cultures more than 10 h. The rapid drop in the pH values would allow a shorter fermentation time to be used in the manufacture of fermented camel milk. On the other hand, the information obtained from these experiments may provide a basis for selection of strains of lactic acid starter cultures that would produce fermented camel milk with highest *β*-galactosidase enzyme activities. This technique may have application in a variety of fermented camel milk and a potential alternative to the conventional processing. Thus, with the approach outlined in this study, the use of a mixed cell strain of starter cultures could improve the manufacture of fermented camel milk.

REFERENCES

Abdel Moneim, E. S., A. I. Abdalla and E. E. Ahmed. 2006. Chemical and microbiological quality of Garris, Sudanese fermented camel's milk product. Int. J. Food Sci. Technol. 41: 321-328.

Abdelgadir, W. S., T. K. Ahmed and H. A. Dirar. 1998. The traditional fermented milk products of the Sudan. Int. J. Food Microbiol. 44: 1-13.

Abu-Tarboush, H. M. 1996. Comparison of associative growth and proteolytic activity of yogurt starters in whole milk from camels and cows. J. Dairy Sci. 79: 366-371.

Agrawal, R. P., R. Beniwal, D. K. Kochar, F. C. Tuteja, S. K. Ghorui, M. S. Sahani and S. Sharma. 2005. Letter to the editor: Camel milk as an adjunct to insulin therapy improves long-term glycemic control and reduction in doses of insulin in patients with type-1 diabetes: A 1-year randomized controlled trial. Diabetes Res. Clin. Pract. 68: 176-177.

Ahmed, A. I., B. E. Mohamed, N. M. Eyousif, B. Faye. and G. Loiseau. 2016. Effect of starter cultures on various classes of fatty acids in Sudanese fermented camel milk (*Camelus dromedarius*) *gariss*. Emir. J. Food Agric. 28: 353-358.

Akolkar, S. K., A. D. Sajgure and S. S. Lele. 2006. *β*-Galactosidase from *Lactobacillus acidophilus* isolated from fermented ragi (*Eleusine coracana*). Ind. J. Biotechnol. 5: 184-188.

Algruin, K. H. and G. Konuspayeva. 2015. Making laben with camel milk. In: G. Proc. of 4th Conference of ISOCARD, "Silk Road Camel: The Camelids, Main Stakes for Sustainable Development", June 8-12, 2015. Vol. 02. Almaty, Kazakhstan: Special Issue of Scientific and Practical Journal Veterinariya, Poster presentation. p. 393

Al-Jazairi, M., S. Abou-Ghorra, Y. Bakri. and M. Mustafa. 2015. Optimization of *β*-galactosidase production by response surface methodology using locally isolated *Kluyveromyces marxianus*. Int. Food Res. J. 22: 1361-1367.

Al-Ruqaie, I. M., H. M. El-Nakhal. and A. N. Wahdan. 1987. Improvement in the quality of the dried fermented milk product. Oggtt. J. Dairy Res. 54: 429-435.

Attia, H., N. Kherouatou. and A. Dhouib. 2001. Dromedary milk lactic acid fermentation: Microbiological and rheological characteristics. J. Ind. Microbiol. Biotechnol. 26: 263-270.

Baeve, V.S. 1981. Possibility of producing low lactose milk products for infant feeding using *β* -gal. Vopeory Itaniya. 2: 63-65.

Barbour, E. K., N. H. Nabbut, W. M. Frerichs and H. M. Al-Nakhla. 1984. Inhibition of pathogenic bacteria by camel's milk: Relation to whey lysozyme and stage of lactation. J. Food Prot. 47: 838-840.

Batista, A. L., R. Silva, A. G. Da Cruz, J. A. F. Faria, M. R. L. Moura. and L. M. J. Carvalho. 2008. Lactose intolerance: Possibility of ingesting fermented dairy products. Milchwissenschaft. 63: 364-366.

Benkerroum, N. 2008. Antimicrobial activity of lysozyme with special relevance to milk. Afr. J. Biotechnol. 7: 4867-4856.

Bury, D. and P. Jelen. 2000. Lactose hydrolysis using a disrupted dairy culture: Evaluation of technical and economic feasibility. Canad. Agric. Eng. 42: 75-80.

Bury, D., P. Jelen. and M. Kalab. 2001. Disruption of *Lactobacillus delbrueckii* ssp. *Bulgaricus* 11842 cells for lactose hydrolysis in dairy products: A comparison of sonication, high-pressure homogenization and bead milling. Innov. Food Sci. Emerg. Tech. 2: 23-29.

Carević, M., M. Vukašinović-Sekulić, S. Grbavčić, M. Stojanović, M. Mihailović, A. Dimitrijević. and D. Bezbradica. 2015. Optimization of *β*-galactosidase production from lactic acid bacteria. Hemijska Ind. 69: 305-312.

Cesca, B., de M. M. Nadra, A. S. De Saad, A. P. De Ruiz Holgado and G. Oliver. 1984. *β*-D-galactosidase of *Lactobacillus* species. Folia Microbiol. 29: 288-294.

Chowdhury, B. R., R. Chakraborty. and U. Raychaudhuri. 2008. Study on *β*-galactosidase enzymatic activity of herbal yogurt. Int. J. Food Sci. Nutri. 59: 116-122.

Damin, M. R., M. R. Alcântara, A. P. Nunes. and M. N. Oliveira. 2009. Effects of milk supplementation with skim milk powder, whey protein concentrate and sodium caseinate on acidification kinetics, rheological properties and structure of nonfat stirred yogurt. LWT Food Sci. Technol. 42: 1744-1750.

Dave, R. I. and N. P. Shah. 1996. Evaluation of media for selective enumeration of *Streptococcus thermophillus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus acidophilus* and *bifidobacteria*. J. Dairy Sci. 79: 1529-1536.

Dirar, H. A. 1993. Gariss. Dairy Products. The indigenous fermented foods of the Sudan and Nutrition. A Study in African Food and Nutrition. 1st ed. University Press, UNIDO, Cambridge.

El-Agamy, E. I. 2000. Effect of heat treatment on camel milk proteins with respect to antimicrobal factors: A comparison with cows' and buffalo milk proteins. Food Chem. 68: 227-232.

El-Hatmi, H., A. Levieux. and D. Levieux. 2006. Camel (*Camelus dromedarius*) immunoglobulin G, α-lactalbumin, serum albumin and lactoferrin in colostrum and milk during the early post-partum period. J. Dairy Res. 73: 288-293.

Fuquay, J. W., P. F. Fox, P. L. H. McSweeney. 2011. Enzymes Exogenous to Milk in Dairy Technology, *β* D-Galactosidase: Encyclopedia of Dairy Science. 2nd ed. Academic Press, London.

Gassem, M. A. and H. M. Abu-Tarboush. 2000. Lactic acid production by *Lactobacillus delbrueckii* ssp. *bulgaricus* in camel's and cow's wheys. Milchwissenschaft. 55: 374-378.

Gaudreau, H., C. P. Champagne, P. Jelen. 2005. The use of crude cellular extracts of *Lactobacillus delbrueckii* ssp. *bulgaricus* 11842 to stimulate growth of a probiotic *Lactobacillus rhamnosus* culture in milk. Enzyme Microb. Technol. 36: 83-90.

Geciova, J., M. Giesova. and P. Jelen. 2002. Disruption of *Streptococcus thermophilus* 143 culture by three mechanical methods for increased *β* -galactosidase activity. Milchwissenschaft. 57: 509-511.

Gilliland, S. E., M. L. Speck and J. R. Woodard. 1972. Stimulation of lactic streptococci in milk by *β*-galactosidase. Appl. Microbiol. 23: 21-25.

Greenberg, N. A. and R. R. Mahoney. 1982. Production and characterization of *β*-galactosidase from *Streptococcus thermophilus*. J. Food Sci. 47: 1824.

Gueimonde, M., N. Corzo, G. Vinderola, J. Reinheimer. and C. G. De los Reyes-Gavilán. 2002. Evolution of carbohydrate fraction in carbonated fermented milks as affected by *β*-galactosidase activity of starter strains. J. Dairy Res. 69: 125-137.

Harju, M., H. Kallioinen, O. Tossavainen. 2012. Lactose hydrolysis and other conversions in dairy products: Technological aspects. Int. Dairy J. 22: 104-109.

Hashim, I. B., A. H. Khalil. and H. Habib. 2009. Quality and acceptability of a set-type yogurt made from camel milk. J. Dairy Sci. 92: 857-862.

Hassaïne, O., H. Zadi-Karam. and N. E. Karam. 2007. Technologically important properties of lactic acid bacteria isolated from raw milk of three breeds of Algerian dromedary (*Camelus dromedarius*). Afr. J. Biotechnol. 6: 1727-1720.

Hsu, C. A., R. C. Yu. and C. C. Chou. 2005. Production of *β*-galactosidase by bifidobacteria as influenced by various culture conditions. Int. J. Food Microbiol. 104: 197-206.

Ibrahim, A. H. and S. A. Khalifa. 2015. Effect of freeze-drying on camel's milk nutritional properties. Int. Food Res. J. 22: 1438-1445.

Jafarei, P. and M. T. Ebrahimi. 2011. *Lactobacillus acidophilus* cell structure and application. Afr. J. Microbiol. Res. 5: 4033-4042.

Khalesi, M., M. Salami, M. Moslehishad, J. Winterburn. and A. A. Moosavi-Movahedi. 2017. Biomolecular content of camel milk: A traditional superfood towards future healthcare industry. Trends Food Sci. Technol. 62: 49-58.

Konuspayeva, G. and B. Faye. 2011. Nutritional value of camel milk and shubat. IDF Int. Symp. On sheep, goat and other non-cow milk, Athens, Greece, Poster No. S4-7.

Kreft, M. E., L. Roth. and P. Jelen. 2001. Lactose hydrolysing ability of sonicated cultures of *Lactobacillus delbrueckii* ssp. *bulgaricus* 11842. Le Lait. 81: 355-364.

Kula, M.R. and H. Schütte. 1987. Purification of proteins and the disruption of microbial cells. Biotechnol. Progress. 3: 31-42.

Levieux, D., A. Levieux, H. El-Hatmi. and J. P. Rigaudiere. 2005. Immunochemical quantification of heat denaturation of camel (*Camelus dromedarius*) whey proteins. J. Dairy Res. 72: 1-9.

Lin, W. J., D. A. Savaiano. and S. K. Harlander. 1989. A method for determining *β*-galactosidase activity of yogurt cultures in skim milk1, 2. J. Dairy Sci. 72: 351-359.

Linko, S., S. Enwald, Y. H. Zhu. and A. Mäyrä-Mäkinen. 1998. Production of *β*-galactosidase by *Streptococcus salivarius subsp thermophilus* 11F. J. Ind. Microbiol Biotechnol. 20: 215-219.

Lore, T. A., S. K. Mbugua. and J. Wangoh. 2005. Enumeration and identification of microflora in Suusac, a Kenyan traditional fermented camel milk product. Lebensmittel Wisseschaft Technol. 38: 125-130.

Mahoney, R. R., T. A. Nickerson. and J. R. Whitaker. 1975. Selection of strain, growth conditions, and extraction procedures for optimum production of lactase from *Kluyveromyces fragilis*. J. Dairy Sci. 58: 1620-1629.

Martins, A. R., A. P. Manera, J. Burkert. and C. Burkert. 2014. Improving galacto-oligosaccharide content in the production of lactose-reduced yogurt. Int. J. Eng. Res. Appl. 4: 84-89.

Matijević, B., K. Lisak, R. Božanić. and L. Tratnik. 2011. Impact of enzymatic hydrolyzed lactose on fermentation and growth of probiotic bacteria in whey. Mljekarstvo. 61: 154.

Mohamed, M. A., M. L. Raznikiewicz. and M. U. Mohamud. 1990. Hard cheese making from camel milk. Milchwissenschaft. 45: 716-718.

Murad, H. A., R. I. Refaea. and E. M. Aly. 2011. Utilization of UF-permeate for production of beta-galactosidase by lactic acid bacteria. Pol. J. Microbiol. 60: 139-144.

Nagaraj, M., B. Sharanagouda, H. Manjunath. and M. Manafi. 2009. Standardization of different levels of lactose hydrolysis in the preparation of lactose hydrolyzed yoghurt. Iran. J. Vet. Res. 10: 132-136.

Neves, A. R., W. A. Pool, J. Kok, O. P. Kuipers. and H. Santos. 2005. Overview on sugar metabolism and its control in *Lactococcus lactis*-The input from *in vivo* NMR. FEMS. Microbiol. Rev. 29: 531-554.

Rahman, I. E. A., H. A. Dirar. and M. A. Osman. 2009. Microbiological and biochemical changes and sensory evaluation of camel milk fermented by selected bacterial starter cultures. Afr. J. Food Sci. 3: 398-405.

Shah, N. P. and P. J. Jelen. 1991. Lactase activity and properties of sonicated dairy cultures. Milcliwissenschaf. 46: 570-573.

Shah, N. P. and W. E. Lankaputhra. 1997. Improving viability of *Lactobacillus acidophilus* and *Bifidobacterium* spp. in yogurt. Int. Dairy J. 7: 349-356.

Shori, A. B. 2012. Comparative study of chemical composition, isolation and identification of micro-flora in traditional fermented camel milk products: Gariss, Suusac, and Shubat. J. Saudi Soci. Agric. Sci. 11: 79-88.

Sobowale, A., M. Efuntoye. and O. Adesetan. 2011. Energy sources of yoghurt bacteria and enhancement of their galactose uptake. Afr. J. Biotechnol. 10: 4457-4463.

SPSS, Version 18. 2012. Software Package for Social Science for Windows SPSS Package (SPSS v.18), Chicago, USA.

Tamime, A. Y. and R. K. Robinson. 2007. Tamime and Robinson's Yoghurt: Science and Technology. CRC Press, Boca Raton, USA, pp. 535-607.

Toba, T., I. Hayasaka, S. Taguchi. and S. Adachi. 1990. A new method for manufacture of lactose- hydrolysed fermented milk. J. Sci.

Food Agric. 52: 403-407.

Torriani, S., F. Gardini, M. E. Guerzoni. and F. Dellaglio. 1996. Use of response surface methodology to evaluate some variable affecting the growth and acidification characteristics of yoghurt cultures. Int. Dairy J. 6: 625-636.

Ustok, F. I., C. Tari. and S. Harsa. 2010. Biochemical and thermal properties of *β*-galactosidase enzymes produced by artisanal yoghurt cultures. Food Chem. 119: 1114-1120.

Vasileva, N., Y. Ivanov, S. Damyanova, I. Kostova. and T. Godjevargova. 2016. Hydrolysis of whey lactose by immobilized *β*-galactosidase in a bioreactor with a spirally wound membrane. Int. J. Biol. Macromol. 82: 339-346.

Vasiljevic, T. and P. Jelen. 2001. Production of *β*-galactosidase for lactose hydrolysis in milk and dairy products using thermophilic lactic acid bacteria. Innov. Food Sci. Emerg. Technol. 2: 75-85.

Vasiljevic, T. and P. Jelen. 2002. Lactose hydrolysis in milk as affected by neutralizers used for the preparation of crude *β*-galactosidase extracts from *Lactobacillus bulgaricus* 11842. Innov. Food Sci. Emerg. Technol. 3: 175-184.

Vedamuthu, E. R. 1982. Fermented milks. In: A. H. Rose, (Ed.), Fermented Foods. Economic Microbiology. Vol. 7. Academic Press, London. 199-255.

Venica, C., C. Bergamini, C. Zalazar. and C. Perotti. 2013. Effect of lactose hydrolysis during manufacture and storage of drinkable yogurt. J. Food Nutr. Disord. 2: 1-7.

Vinderola, C. G., M. Gueimonde, T. Delgado, J. A. Reinheimer. and G. De los Reyes-Gavilan. 2000. Characteristics of carbonated fermented milks and survival of probiotic bacteria. Int. Dairy J. 10: 213-220.

Wang, D., M. Sakakibara, N. Kondoh. and K. Suzuki. 1996. Ultrasound-enhanced lactose hydrolysis in milk fermentation with *Lactobacillus bulgaricus*. J. Chem. Technol. Biotechnol. 65: 86-92.