Enhancement of $\beta$-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 $\beta$-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 $\beta$-galactosidase and used the ruptured cell cultures as the source of the $\beta$-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 ± 0.01 and 4.63 ± 0.01 during 4 h of fermentation time, respectively. Furthermore, the highest significance ($P<0.05$) activity of $\beta$-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: $\beta$-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 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...
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). *Guta* 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). Ogutt is dried fermented camel milk produced in Syria and Jordan (Al-Ruqai et al., 1987).

On the other hand, to optimize growth conditions required is the best strategy for camel milk processing (Hassain 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 \textit{S. thermophilus}, \textit{L. delbrueckii ssp. bulgaricus} and \textit{L. acidophilus} on $\beta$-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 \textit{S. thermophilus} ATCC 19258, \textit{L. delbrueckii ssp. bulgaricus} DSMZ 20081 and \textit{L. acidophilus} DSMZ 20079 cultures to camel milk during fermentation on $\beta$-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 (\textit{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 \textit{Streptococcus thermophilus} ATCC® 19258™ and \textit{Lactobacillus delbrueckii ssp. bulgaricus} DSMZ® 20081™ and \textit{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 \textit{L. acidophilus} and \textit{L. delbrueckii ssp. bulgaricus}, or M17 broth for \textit{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 $\beta$-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 (42°C) 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 \textit{L. delbrueckii ssp. bulgaricus}, \textit{S. thermophilus} and \textit{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 \textit{L. delbrueckii ssp. bulgaricus} ruptured for (60 s), \textit{S. thermophilus} ruptured for (60 s) and \textit{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 $\beta$ galactosidase activity.

**Measuring $\beta$-galactosidase activity**

\textit{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±1ºC 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 Na₂CO₃ 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 A₄₂₀ 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 A₄₂₀ 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 centrifuged 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 A₃40 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 Oxoid (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 solubilize camel milk protein and fat to access to a clear solution for measured β-galactosidase activity absorbance.

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
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 (Jafari and Ebrahim, 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 yoghurt 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 \textit{Streptococcus thermophilus} ATCC 19258, \textit{Lactobacillus delbrueckii} ssp. \textit{bulgaricus} DSMZ 20081 and \textit{Lactobacillus acidophilus} DSMZ 20079 starter cultures before and after cell-rupture

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Viable counts (Log^{10} CFU/ml)</th>
<th>Before cell-rupture</th>
<th>After cell-rupture (second)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Zero time</td>
<td>30 s</td>
</tr>
<tr>
<td>(S.\ Thermophilus)</td>
<td>9.24±0.53</td>
<td>7.05±0.11</td>
<td>6.66±0.57</td>
</tr>
<tr>
<td>(L.\ Delbrueckii ssp. bulgaricus)</td>
<td>9.31±0.51</td>
<td>7.34±0.26</td>
<td>6.75±0.12</td>
</tr>
<tr>
<td>(L.\ Acidophilus)</td>
<td>9.62±0.54</td>
<td>7.79±0.04</td>
<td>7.01±0.03</td>
</tr>
<tr>
<td>β-galactosidase activity * (1U/g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(S.\ Thermophilus)</td>
<td>25±2.8</td>
<td>227±4.2</td>
<td>366±5.7</td>
</tr>
<tr>
<td>(L.\ Delbrueckii ssp. bulgaricus)</td>
<td>43±2.8</td>
<td>277±9.9</td>
<td>479±5.7</td>
</tr>
<tr>
<td>(L.\ Acidophilus)</td>
<td>36±1.4</td>
<td>253±8.5</td>
<td>360±2.8</td>
</tr>
</tbody>
</table>

\*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\textsuperscript{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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Incubation time (hours)</th>
<th>Streptococcus thermophilus</th>
<th><em>Lactobacillus delbrueckii ssp. bulgaricus</em></th>
<th><em>Lactobacillus acidophilus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero time</td>
<td>2 h</td>
<td>4 h</td>
<td>6 h</td>
</tr>
<tr>
<td>Whole cells</td>
<td>6.54±0.42</td>
<td>6.70±0.36</td>
<td>7.60±0.48</td>
<td>7.87±0.07</td>
</tr>
<tr>
<td>Ruptured cells</td>
<td>6.66±0.57</td>
<td>6.70±0.26</td>
<td>7.64±0.11</td>
<td>7.94±0.10</td>
</tr>
<tr>
<td>Mixed cells(^*)</td>
<td>6.98±0.02</td>
<td>8.37±0.38</td>
<td>8.55±0.23</td>
<td>8.83±0.73</td>
</tr>
<tr>
<td><strong>Streptococcus thermophilus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole cells</td>
<td>6.93±0.09</td>
<td>7.04±0.04</td>
<td>7.87±0.36</td>
<td>7.94±0.07</td>
</tr>
<tr>
<td>Ruptured cells</td>
<td>6.79±0.12</td>
<td>7.89±0.05</td>
<td>8.07±0.18</td>
<td>8.12±0.49</td>
</tr>
<tr>
<td>Mixed cells(^*)</td>
<td>7.01±0.12</td>
<td>8.46±0.39</td>
<td>8.60±0.25</td>
<td>8.95±0.06</td>
</tr>
<tr>
<td><strong>Lactobacillus delbrueckii ssp. bulgaricus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole cells</td>
<td>6.79±0.34</td>
<td>7.00±0.33</td>
<td>7.57±0.47</td>
<td>8.19±0.21</td>
</tr>
<tr>
<td>Ruptured cells</td>
<td>6.25±0.61</td>
<td>7.63±0.59</td>
<td>7.70±0.18</td>
<td>8.24±0.08</td>
</tr>
<tr>
<td>Mixed cells(^*)</td>
<td>7.39±0.36</td>
<td>8.46±0.41</td>
<td>8.54±0.43</td>
<td>9.03±0.40</td>
</tr>
<tr>
<td><strong>Lactobacillus acidophilus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole cells</td>
<td>8.10±0.02</td>
<td>8.46±0.39</td>
<td>8.54±0.43</td>
<td>9.03±0.40</td>
</tr>
<tr>
<td>Ruptured cells</td>
<td>8.25±0.59</td>
<td>8.37±0.60</td>
<td>8.45±0.58</td>
<td>8.59±0.60</td>
</tr>
<tr>
<td>Mixed cells(^*)</td>
<td>8.39±0.36</td>
<td>8.46±0.41</td>
<td>8.54±0.43</td>
<td>9.03±0.40</td>
</tr>
</tbody>
</table>

\(^{a, b, c}\) Means of each strain in the same column with different letters are significantly different at (\(P<0.05\)) by DMRT. \(^{ab, ac}\) 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.

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 during 10 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Incubation time (hours)</th>
<th>Streptococcus thermophilus</th>
<th>Lactobacillus delbrueckii ssp. bulgaricus</th>
<th>Lactobacillus acidophilus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero time</td>
<td>2 h</td>
<td>4 h</td>
<td>6 h</td>
</tr>
<tr>
<td>Whole cells</td>
<td>0.10±0.01</td>
<td>0.41±0.01</td>
<td>0.58±0.06</td>
<td>0.73±0.06</td>
</tr>
<tr>
<td>Ruptured cells</td>
<td>1.07±0.06</td>
<td>1.33±0.12</td>
<td>1.50±0.10</td>
<td>1.47±0.06</td>
</tr>
<tr>
<td>Mixed cells*</td>
<td>0.70±0.10</td>
<td>1.57±0.12</td>
<td>1.70±0.01</td>
<td>1.68±0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole cells</td>
<td>0.17±0.01</td>
<td>0.60±0.06</td>
<td>0.77±0.01</td>
<td>0.83±0.01</td>
</tr>
<tr>
<td>Ruptured cells</td>
<td>1.53±0.06</td>
<td>1.70±0.10</td>
<td>1.87±0.06</td>
<td>1.77±0.06</td>
</tr>
<tr>
<td>Mixed cells*</td>
<td>1.02bB±0.08</td>
<td>1.90±0.10</td>
<td>1.97±0.12</td>
<td>1.93±0.15</td>
</tr>
</tbody>
</table>

*1U=1 μmole of o-NPGal/min per ml released culture.*  
* 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, Guéimonde 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.

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.

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 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.

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 \textit{streptococcus thermophilus} ATCC 19258, \textit{lactobacillus delbrueckii ssp. bulgaricus} DSMZ 20081 and \textit{lactobacillus acidophilus} DSMZ 20079 during 10 hours of incubation

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Incubation time (hours)</th>
<th>Streptococcus thermophilus</th>
<th>\textit{lactobacillus delbrueckii ssp. bulgaricus}</th>
<th>\textit{lactobacillus acidophilus}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h</td>
<td>4 h</td>
<td>6 h</td>
<td>8 h</td>
</tr>
<tr>
<td>Whole cells</td>
<td>6.73±0.06</td>
<td>6.43±0.06</td>
<td>5.43±0.06</td>
<td>5.23±0.06</td>
</tr>
<tr>
<td>Ruptured cells</td>
<td>6.77±0.01</td>
<td>6.20±0.01</td>
<td>5.27±0.06</td>
<td>4.65±0.01</td>
</tr>
<tr>
<td>Mixed cells*</td>
<td>6.79±0.01</td>
<td>5.27±0.06</td>
<td>4.65±0.02</td>
<td>4.63±0.03</td>
</tr>
<tr>
<td>Whole cells</td>
<td>6.74±0.05</td>
<td>6.33±0.06</td>
<td>5.27±0.06</td>
<td>5.13±0.06</td>
</tr>
<tr>
<td>Ruptured cells</td>
<td>6.79±0.01</td>
<td>6.13±0.06</td>
<td>5.10±0.01</td>
<td>4.64±0.02</td>
</tr>
<tr>
<td>Mixed cells*</td>
<td>6.76±0.05</td>
<td>5.10±0.01</td>
<td>4.63±0.01</td>
<td>4.60±0.01</td>
</tr>
</tbody>
</table>

|        | Zero time              | 2 h                      | 4 h                        | 6 h                       | 8 h                             | 10 h                           |
|Whole cells                  | 6.77±0.05               | 6.23±0.06                 | 5.23±0.12                   | 5.17±0.06                 | 4.97±0.09                       | 4.90±0.10                       |
| Ruptured cells              | 6.76±0.01               | 6.17±0.06                 | 5.17±0.06                   | 4.62±0.07                 | 4.63±0.01                       | 4.55±0.04                       |
| Mixed cells*                | 6.75±0.05               | 5.13±0.12                 | 4.64±0.01                   | 4.62±0.02                 | 4.63±0.06                       | 4.52±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. * mixed cells=mixed of each whole and ruptured cells of the same strain 1:1% v/v.

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 \textit{S. thermophilus}, \textit{L. acidophilus} and \textit{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 Damin et al. (2009); Toba et al. (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 \textit{S. thermophilus}, \textit{L. delbrueckii ssp. bulgaricus} and \textit{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 \textit{L. delbrueckii ssp. bulgaricus} had the highest decrease in pH values until the end of incubation time. As it was expected, \textit{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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Incubation time (hours)</th>
<th>Streptococcus thermophilus</th>
<th>Lactobacillus delbrueckii ssp. bulgaricus</th>
<th>Lactobacillus acidophilus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero time</td>
<td>2 h</td>
<td>4 h</td>
<td>6 h</td>
</tr>
<tr>
<td>Whole cells</td>
<td>4.60±0.10</td>
<td>4.23±0.06</td>
<td>3.97±0.06</td>
<td>3.93±0.06</td>
</tr>
<tr>
<td>Ruptured cells</td>
<td>4.60±0.10</td>
<td>3.77±0.06</td>
<td>3.20±0.01</td>
<td>3.10±0.01</td>
</tr>
<tr>
<td>Mixed cells*</td>
<td>4.60±0.10</td>
<td>3.13±0.06</td>
<td>3.03±0.06</td>
<td>2.97±0.12</td>
</tr>
</tbody>
</table>

* 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 \textit{S. thermophilus}\textsubscript{11F} under the experimental conditions used.

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


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Ibrahim


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