Effect of camel's milk processing on Orotic acid stability

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**ABSTRACT**

The objective of this study was to evaluate orotic acid stability throughout the manufacture and storage of camel milk products. The results observed that, freezing camel milk for 3 months at -18°C led to stability of orotic acid, pH and acidity did not change during storage. Thermal treatment carried out on camel milk at three treatments (63°C/30 min, 72°C/15 s and 80°C/20 s). The results showed that, the treatment at 80°C caused less change in microbial load, pH, acidity and a 16.74% decrease in orotic acid during storage than that of other treatments. Then we were advised to make fermented camel milk (heated at 80°C/20 s) with a traditional starter and another commercial probiotic starter. The results showed that, probiotic fermented camel milk significantly affect the stability of orotic acid compared to the traditional starter during fermentation and storage at 5°C.

**Keywords:** freezing, heat treatment, fermentation

**INTRODUCTION**

Camel milk's nutritional and therapeutic properties have been employed to promote human health (Gader & Alhaider, 2016), because it has higher concentrations of essential fatty acids, immunoglobulins, lactoferrin, lactoperoxidase and other antimicrobial components as well as insulin-like protein than milk from other animals (Yadav et al., 2015). Although the exact mechanism underlying camel milk's hypocholesterolaemic effects is still unknown, several suggestions have been put out, such as the reduction of cholesterol caused by interactions between bioactive peptides produced from camel milk proteins and cholesterol (Li and Warthesen, 1989; Navder et al., 1990). Additionally, as per (Larson and Hegarty 1979 and Suzuki et al. 1986), the amount of soluble whey solids and the degree of fermentation influence the amount of orotic acid in cultured dairy products. It is crucial to measure this amount as well as, it can serve as a reliable gauge of a cow's quality (Ferreira, 2003; Kavaz and Bakirci, 2014). This study investigated the effect of freezing, different heat treatments and storage, fermentation time and storage of fermented camel milk on orotic acid content and stability.

**MATERIALS AND METHODS**

**Materials**

Camel milk (total solids 11.0%, protein 3.2%, fat 3.4%, acidity 0.15% and pH 6.71) were obtained from Matrouh station, Desert research center Egypt., was used for preparing fermented camel milk.

Direct vat set (DVS) classic (national) yogurt culture (L. delbrueckii subsp. bulgaricus and Streptococcus thermophilus YoFlex® Premium 4.0, 1:1). The bacterial strains contained 7.7 and 7.4 Log CFU/g of freeze-dried culture.

ABT-5 culture (L. acidophilus La-5, Bifidobacterium spp. and S. thermophilus TH-4, 1:1:1). The bacterial strains contained 8.2, 9.5 and 7.3 Log CFU/g of freeze-dried culture; respectively, was purchased from Chr. Hansen, Hoersholm, Denmark, by Misr Food Additives (MIFAD), Egypt.

The study was performed in three stages:

**First study:** comparable estimate orotic acid in local animals' milk (goat, sheep, cow and camel milk).

**Stage 1:** The samples of raw camel milk were freezing at -18°C during 3-month of storage and analysis stability of orotic acid during 1, 2 and 3 months.

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Stage 2: The samples were heated in glass vials in a water bath. The heat treatment consists of three sets of treatments: 63°C for 30 min, 72°C for 15 s and 80°C for 20 s. It was immediately cooled to 5°C and stored for 15 days for analysis, and then determined number of bacteria, pH, acidity and orotic acid after 1, 5, 10 and 15 days.

Stage 3: Fermented milk was prepared by the best heat treatment results. Camel milk was divided to two portions, the first portion, with traditional yogurt culture and second portion was ABT-5 culture. Raw camel milk was heated to 80°C for 20 s, cooled to 37°C, and then inoculated with a fermented milk culture and 0.02% (w/v) probiotic bacteria (ABT-5) and set in a plastic container until pH 4.7±0.05. Fermented milk samples were stored at 5±2°C and measured during fermentation (2, 4, 6, 8 and 10 h) and after cooling for total bacterial count, stability of orotic acid, acidity and pH during storage period (1,7,15 and21 days).

Methods of analyses
Camel milk samples were chemically analyzed according to (AOAC, 2019) for protein, fat, total solid contents, acidity (as lactic acid %) and pH value.

Orotic acid by HPLC methods:

Extraction of Orotic acids:
Orotic acid in camel milk and fermented camel milk was extracted using 0.13 N H₂SO₄ and the milk samples were centrifuged at 7000 xg for 7 min at 5°C. The top layer is Whatman No. 1 after analysis using filter paper; the filtrate was passed through a 0.45 μm Millipore PVDF Millipore (Billerica, MA, USA) syringe filter. High performance liquid chromatography equipment was used to perform the separation.

HPLC conditions:
An Agilent 1260 HPLC series was used for the study. The separation was done with Kromasil 60-5-Hilik-D (4.6 mm x 100 mm). 30% sodium phosphate (pH = 6.7 with phosphoric acid) + ACN (3:7) is the mobile part and the flow rate is 1 ml/min. An isovolumetric linear regression method was used to sequentially lyse the mobile phase. Monitoring at 214 nm is the DAD detector. An injection volume of 2 μl was used for all sample solutions. The column temperature was maintained at 35°C. Calibration curve data points were subjected to least squares regression analysis (Güler, 2014).

Microbiological analyses:
Viable cell counts were performed by the standard pour-plate method after serial dilutions in saline solution (0.85% w/v) according to American Public Health Association (APHA, 1978). The results were expressed as Log cfu/g when fresh and after 5, 10, 15 and 21 day of storage for fermented milk and after 1, 5, 10 and 15 days for heated camel milk.

Statistical analysis:
Experimental data was analyzed using analysis of variance (ANOVA) and significant differences among means from a triplicate analysis at (P≤0.05) were analyzed by Duncan’s multiple range test (DMRT) using the SPSS (2012) software.

RESULTS AND DISCUSSION

Orotic acid content in various milk species:
The orotic acid content of various milk species samples presented in Table 1. It could be noticed that, camel’s milk recorded the highest value for orotic acid content (98.357±0.9 μg/ml), followed by cow’s milk, sheep’s milk and goat’s milk (83.456±0.7, 37.285±1.2 and 19.548±1.4); respectively. These results agreement with previous researcher (Larson and Hegarty, 1979; Akalin and Gonc 1996), who reported that mid lactation cow, sheep and goat’s milk contained 82.43±9.64; 30.59±1.71 and 23.87±1.36mg/l orotic acid, respectively. Variations in orotic acid content of the studied milk samples are due to several factors account for this variation including breed of animal, nutrition’s stage of lactation, milking times, method of determination and finally could be due to the process of milk secretion (Ahmed, et al., 1978; Motyl et al., 1991).

Table 1. The average of orotic acid content in milk of various species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Orotic acid (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>camel</td>
<td>98.357±0.9</td>
</tr>
<tr>
<td>goat</td>
<td>19.548±1.4</td>
</tr>
<tr>
<td>sheep</td>
<td>37.285±1.2</td>
</tr>
<tr>
<td>cows</td>
<td>83.456±0.7</td>
</tr>
</tbody>
</table>

Table 2. Effect of freezing raw camel milk on pH, acidity and orotic acid during storage.

<table>
<thead>
<tr>
<th>Component</th>
<th>fresh</th>
<th>1-month</th>
<th>2-month</th>
<th>3-month</th>
</tr>
</thead>
<tbody>
<tr>
<td>orotic acid(μg/ml)</td>
<td>98.357±0.008</td>
<td>98.341±0.01*</td>
<td>98.293±0.013*</td>
<td>98.376±0.007*</td>
</tr>
<tr>
<td>pH</td>
<td>6.66±0.01*</td>
<td>6.65±0.04*</td>
<td>6.66±0.005*</td>
<td>6.64±0.014*</td>
</tr>
<tr>
<td>Acidity (%)</td>
<td>0.15±0.024</td>
<td>0.15±0.048*</td>
<td>0.15±0.034*</td>
<td>0.15±0.011*</td>
</tr>
</tbody>
</table>

*Means followed by different letters in the same row are significantly different (P<0.05).

Effect of processing camel’s milk on orotic acid stability:
1. Effect of freezing:
Average concentrations of orotic acids during fresh and after 1, 2 and 3 months of frozen storage in Table 2. Concentrations of orotic acids during storage were not statistically significant (P≤0.05, pH and acidity) for acids, indicating that frozen storage significantly changed the levels of these acids. Mean differences for other organic acids did not differ by large standard deviations. Freezing also caused a significant (P ≤ 0.05) increase in formic and uric acid and reduction of tartaric acid in soft cheese (Izzo et al., 2002). However, significant effects that freezing can have on the organic acid content of cow’s milk Mozzarella cheese due to differences in milk source (cow versus goat) or cheese type and composition (Califano and Bevilacqua, 1999).
at different treatments (65°C/30 min, 72°C/15 s and 80°C/15 s) with different significant (p≤0.05) degrees depending on the heat temperature. This is explained by the development of acidity and the microbial load that consumes orotic acid during storage, leading to a significant increase in acidity and a decrease in orotic acid content. The maximum reduction of orotic acid showed in Fig 1 during storage (15 days) at (65°C/30 min, 72°C/15 s and 80°C/20 s) were (27.59, 20.71 and 16.74%); respectively.

### Table 3. Effect of heat treatments on pH, acidity and orotic acid content in camel’s milk during storage:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage period</th>
<th>Orotic acid (µg/ml)</th>
<th>pH</th>
<th>Acidity (%)</th>
<th>TVBC (log cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(63°C)-30min</td>
<td>1-day</td>
<td>96.97±0.07</td>
<td>6.69±0.02</td>
<td>0.15±0.01</td>
<td>7.97±0.011</td>
</tr>
<tr>
<td></td>
<td>5-days</td>
<td>89.75±0.01</td>
<td>6.62±0.05</td>
<td>0.17±0.01</td>
<td>8.34±0.21</td>
</tr>
<tr>
<td></td>
<td>10-days</td>
<td>80.90±0.08</td>
<td>6.56±0.01</td>
<td>0.18±0.02</td>
<td>8.56±0.007</td>
</tr>
<tr>
<td></td>
<td>15-days</td>
<td>69.59±0.07</td>
<td>6.19±0.12</td>
<td>0.20±0.04</td>
<td>8.62±0.006</td>
</tr>
<tr>
<td>(72°C)-15sec</td>
<td>1-day</td>
<td>96.69±0.01</td>
<td>6.68±0.02</td>
<td>0.16±0.03</td>
<td>7.88±0.009</td>
</tr>
<tr>
<td></td>
<td>5-days</td>
<td>94.27±0.01</td>
<td>6.65±0.03</td>
<td>0.17±0.02</td>
<td>8.08±0.021</td>
</tr>
<tr>
<td></td>
<td>10-days</td>
<td>87.13±0.03</td>
<td>6.60±0.16</td>
<td>0.17±0.01</td>
<td>8.15±0.060</td>
</tr>
<tr>
<td></td>
<td>15-days</td>
<td>76.35±0.08</td>
<td>6.25±0.07</td>
<td>0.19±0.03</td>
<td>8.30±0.049</td>
</tr>
<tr>
<td>(80°C)-20 sec</td>
<td>1-day</td>
<td>97.10±0.04</td>
<td>6.68±0.01</td>
<td>0.16±0.02</td>
<td>7.74±0.122</td>
</tr>
<tr>
<td></td>
<td>5-days</td>
<td>96.03±0.01</td>
<td>6.59±0.01</td>
<td>0.17±0.02</td>
<td>7.85±0.164</td>
</tr>
<tr>
<td></td>
<td>10-days</td>
<td>91.86±0.70</td>
<td>6.47±0.01</td>
<td>0.18±0.05</td>
<td>8.08±0.088</td>
</tr>
<tr>
<td></td>
<td>15-days</td>
<td>80.26±0.22</td>
<td>6.36±0.02</td>
<td>0.18±0.03</td>
<td>8.15±0.154</td>
</tr>
</tbody>
</table>

*Means followed by different letters in the same column are significantly different (P≤0.05). TVBC=Total viable bacterial Counts.

**Fig. 1.** The effect of heat treatments of camel milk during cold storage on the reduction of orotic acid.

**Fig. 2.** The effect of fermentation of camel milk and cold storage on pH and acidity.

**Table 3: Effect of heat treatments on pH, acidity and orotic acid content in camel's milk during storage:**

- **Treatments:** (63°C)-30min, (72°C)-15sec, (80°C)-20 sec.
- **Storage period:** 1-day, 5-days, 10-days, 15-days.
- **Orotic acid (µg/ml):** 96.97±0.07, 89.75±0.01, 80.90±0.08, 69.59±0.07, 96.69±0.01, 94.27±0.01, 87.13±0.03, 76.35±0.08, 97.10±0.04, 96.03±0.01, 91.86±0.70, 80.26±0.22.
- **pH:** 6.69±0.02, 6.62±0.05, 6.56±0.01, 6.19±0.12, 6.68±0.02, 6.65±0.03, 6.60±0.16, 6.25±0.07, 6.68±0.01, 6.59±0.01, 6.47±0.01, 6.36±0.02.
- **Acidity (%):** 0.15±0.01, 0.17±0.01, 0.18±0.02, 0.20±0.04, 0.16±0.03, 0.17±0.02, 0.17±0.01, 0.19±0.03, 0.16±0.02, 0.17±0.02, 0.18±0.05, 0.18±0.03.
- **TVBC (log cfu/ml):** 7.97±0.011, 8.34±0.21, 8.56±0.007, 8.62±0.006, 7.88±0.009, 8.08±0.021, 8.15±0.060, 8.30±0.049, 7.74±0.122, 7.85±0.164, 8.08±0.088, 8.15±0.154.

**3. Effect of fermentation time and cold storage:**

Acidification profiles, acid production capacity has a significant impact on processing and shelf life (Zare et al., 2012; Nguyen et al., 2014). As shown in Figs 2 & 3, the acid production capacity of probiotic fermented camel milk (PFCM) and traditional fermented camel milk (TFCM), pH and acidity were monitored every 2 h during fermentation and after 1, 7, 15 and 21 days in a refrigerator temperature (5°C). Acidification profiles could provide insight into the effects of probiotics and traditional strains on metabolism, similar trends in pH decrease were observed for all samples. The rate of pH (4.60) decrease occurred 8-10 h for TFCM and PFCM; respectively (Fig 2). Fermented camel milk has a longer clotting time compared to other milks; this is due to differences in the size and properties of casein particles (Shuiep et al., 2013). Compared to cow milk coagulation, camel milk coagulation consistently contains larger casein micelles (Farah and Ruegg, 1989). Thus, the addition of probiotic strains (PFCM) can slightly reduce the fermentation time. Clearly, the pH of the PFCM-fermented samples were significantly lower than that of the TFCM samples during the storage period (P≤0.05), the results agree with (Masud et al. 1991; Kneifel et al., 1992; Eissa et al. 2010; Eissa et al. 2011).

In Fig 4, showed the second hour of fermentation, lactic acid was actively produced, and orotic acids were consumed. In Fig 3, orotic acid showed a maximum reduction at 10 h of fermentation, approximately 33.42 & 44.16% for TFCM & PFCM, and from 54.99 & 58.53% for TFCM & PFCM during 21 days of cold storage; respectively, because orotic acid is an intermediate in the synthesis of nucleotides and free pyrimidine and forms oroditylate in the presence of orotate phosphoribosyl transferase and growth factor for probiotic bacteria (Güzel-Seydim et al., 2000). This reduction was also reported for buttermilk (Marsili et al., 1981) and yogurt (Saidi and Warthesen, 1989; Fernandez-Garcia and McGregor, 1994). (Alm, 1982) reported a 45-48% reduction of orotic acid in products made from fresh milk, however, orotic acid and citric acid were moderately consumed during fermentation, and the slight decrease in orotic acid continued until the 7 day of cold storage. On the other hand, the obtained results showed that, the fermented camel milk during fermentation contained a total bacterial count of 6.214 and 6.738 log cfu/g viable bacterial cells in TFCM and PFCM; respectively. The initial bacterial count increased significantly (P≤0.05) during storage of both types of yogurt, and peaked on day 10 and then decreased significantly (P<0.05) to 7.924 and 8.394 log cfu/g for TFCM and PFCM; respectively, and then began to decline.


SPSS (2012). Statistical Package for Social Science, Release 11.5, SPSS INC, Chicago, USA.


