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# PRODUCTION OF CHOCOLATE PROBIOTIC DESSERT BASED ON CAMEL MILK USING *LACTOCASEIBACILLUS CASEI*

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**ABSTRACT**

A functional food is a food that contains nutrients, which has a positive effect on one or more functions in the human body. Probiotic foods are also a functional food that by consuming them can be benefited the nutritional properties of probiotic bacteria. Inoculation of lactic acid Proteolytic bacteria to milk leads to production of fermented dairy products rich in bioactive peptides. This research was conducted in two stages. At first, *Lactocaseibacillus* (*L.*) *casei* was inoculated into milk (cow and camel) and incubated for 12 h. The incubation time had a significant effect on the growth of *L. casei* ($p \leq 0.05$). The growth of *L. casei* in camel milk was significantly different from cow milk. ($p \leq 0.05$). Changes in pH and acidity during incubation were significant ($p \leq 0.05$) and there was a significant difference between cow and camel samples. During incubation of both milk increased proteolytic activity, antioxidant activity was observed while antioxidant activity in camel milk was more than cow milk. In sensory evaluation, no significant difference was observed between the two types of milk ($p > 0.05$). Four samples of chocolate dessert were prepared by inoculation of *L. casei* to cow and camel milk containing two types of sweeteners of sucrose and sucralose. During 28 days of storage, the survival of *L. casei* in all samples was more than 8 Log cfu/g. Two sweeteners and milk type showed significant impact on the survival of *L. casei* ($p \leq 0.05$). Survival of *L. casei* in desserts prepared with camel milk and sucrose sweetener was higher. Changes in acidity and pH in the samples were significant during 28 days of storage ($p \leq 0.05$) Desserts prepared with camel milk and sucrose sweetener had higher acidity and lower pH. The desserts prepared with cow's milk and sucrose sweetener have higher elasticity. The sensory evaluation test does not show any significant difference in odor, taste and overall acceptability with blank ($p > 0.05$).

## 1.Introduction

Milk and dairy products are an important part of human diet due to their nutritional and biological value. A group of dairy products that are world famous are dairy desserts (cream, puddings, cocktails, whipped cream) and the most important factor for this group of products is their rheological properties (viscosity and jelly). (Peter and Glyn, 2014). Creamy milk chocolate dessert as high accepted dairy products, could be alternative for incorporation by probiotics and prebiotics (Amna et al., 2015; Valencia et al., 2016)

Dairy dessert contains at least 50% of fresh milk or reconstituted milk and food additives (e.g. flavorings, sweeteners, thickeners and stabilizers), after passing thermal processes such as pasteurization, pasteurization with extended

shelf life, sterilization (Ibrahim et al., 2015; Kanmani et al., 2013; Kaur et al., 2015; Khaskheli et al., 2005; Konuspayeva et al., 2009; Kumar et al., 2016). Types of desserts including pudding, custard, mousse, flan, porridge and rice milk, and milk desserts are drinks. There are two common camel species, the Arabian dromedary (*Camelus drumderius*) and the Bactrian camel (*Camelus bactrianus*), the camel found in the mountains ((Bayarri et al., 2010; Beresford et al., 2001; Cardarelli et al., 2008; Ibrahem et al., 2016)). Food and Agriculture Organization (FAO) approximately estimates that more than 5.3 million tons of camel milk is produced worldwide (Ayyash et al., 2018). The dromedary camel is known for producing camel milk as a nutritious source in raw and fermented form. More than 60% of the dromedary population (totaling 23 million worldwide) is found in the arid and desert regions of Northeast Africa (Jilo, 2016). Camel milk which has been considered as an important ingredient in the diet in different continents, can been produced up to 3500 L for 18 months lactation of camel (Elagamy, 2000).

In the development of new probiotic products, the main goal is bacterial survival during the production and storage. A wide spectrum of variables has been reported as influencing factors on microbial survival including temperature, pH, acidity, the presence of other microorganisms, and probiotic strain (Valencia et al., 2016).

Chocolate milk desserts are one of suitable carriers for probiotic pH > 6 and humidity above 70% and there are no competing microorganisms (Valencia et al., 2016). Due to the lack production of chocolate dairy desserts, and according to the mentioned benefits for camel milk and probiotic bacteria in this study, using the probiotic bacterium *Lactocaseibacillus* (*L.*) *casei*, the possibility of producing chocolate dessert based on camel milk will be investigated.

The main purpose of this study is the simultaneous use of the properties of probiotic bacteria and camel milk for production of a functional food. So, by applying camel milk as carrier for probiotic *L. casei*, survival of microorganism during shelf life was investigated. The antioxidant, nutritional, rheological and sensory properties of produced dairy dessert was also characterized.

## 2. Material and methods

### 2.1. Production of probiotic chocolate dessert

Milk (camel and cow) was pasteurized at 80°C for 20 minutes in a water bath and then cooled to 43°C. Under the laminar hood, *L. casei* was weighed and 0.01% was added to the cooled pasteurized milk (camel and cow) mixed well and incubated at 37°C for 2 h. Changes in pH, acidity, bacterial growth, and antioxidant activity were examined during the 12 h incubation time. The final product was evaluated in terms of sensory properties. In order to produce chocolate dessert, milk (camels and cows) was first pasteurized and then gelatin was added to one third of this milk and placed in a water bath until complete solvation of gelatin in the milk.

Cocoa powder, sugar or sucralose and carrageenan were added to the rest of the formulation milk at 45°C and mix the ingredients thoroughly in the milk. Milk and gelatin were added to the rest of the ingredients and pasteurized in a water bath at a temperature of 80 to 85°C for 20 min. The ingredients were cooled in a cold-water bath to 43°C under sterile conditions. Then 0.05% of *L. casei* was added to samples. The chocolate dessert was produced with 4 different formulations according to Table 1.

Two different sweeteners, sucrose and sucralose, were used to supply equal sweetness of the product. Then, 40 g in each of the glass containers were packaged, cooled, and stored at 4°C for 28 days. (Argon Allegro, 2007). All 4 dessert samples were examined on the 1$^{st}$,7$^{th}$, 14$^{th}$, 21$^{st}$ and 28$^{th}$ days for microbial count, pH, and acidity. Rheology and sensory evaluation were carried out on the first day of production.

**Table 1.** The amount of used raw ingredients to produce chocolate dessert in terms of weight percentage (per 100 grams of dessert).

| Treatments | Camel milk | Cow milk | Water | Cocoa powder | Gelatin | Carrageenan | Sucrose | Sucralose+ Maltodextrin | *L. casei* |
|---|---|---|---|---|---|---|---|---|---|
| Dessert 1 | 80.45 | - | - | 5 | 1.3 | 0.2 | 13 | - | 0.05 |
| Dessert 2 | 80.45 | - | 3 | 5 | 1.3 | 0.2 | - | 10 | 0.05 |
| Dessert 3 | - | 80.45 | - | 5 | 1.3 | 0.2 | 13 | - | 0.05 |
| Dessert 4 | - | 80.45 | 3 | 5 | 1.3 | 0.2 | - | 10 | 0.05 |

### 2.2. Microbial test

A serial dilution was prepared for microbial count, $10^{-5}$, $10^{-6}$ and $10^{-7}$ dilutions. Triplicated culture of probiotic in MRS agar medium was cultured as a pour plate incubated for 72 h at 37°C and number of colonies per gram was reported (Argon Allegro 2007).

### 2.3. Physicochemical Analysis

The pH, and acidity of milk samples were determined according to AOAC methods (AOAC, 2005). The pH value of milk samples was evaluated with a pH meter (Metrom, Switzerland) at room temperature. The titratable acidity was determined in milk by titration method and in dessert samples by potentiometric method.

### 2.4. Antioxidant activity using the 1, 2-diphenyl 1-picrylhydrazyl radical scavenging method (DPPH)

To measure the antioxidant activity of probiotic milk (camel and cow), first aqueous extract solution was prepared, for this purpose, using hydrochloric acid or 1M sodium hydroxide, the pH of milk samples was increased to 4.6 and it was centrifuged at 4 ° C for 15 minutes at 9000 g. The luminaire was smoothed using Whatman paper with a pore size of 0.45 µm; then 3.8 ml of methanol solution containing 0.1 mmol of DPPH radical was added to 0.2 ml of the prepared extract. The mixture was shaken evenly for one minute and placed in a dark place at room temperature for 30 minutes. Then the absorbance of the tested samples was measured by using a spectrophotometer at 517 nm against the control sample. The percentage of free radical scavenging effect was obtained by Eq. 1 (Ayash et al., 2018).

DPPH percentage of inhibition effect (%)

$$= (Ac\text{-}As)/Ac \times 100 \quad (1)$$

where, $A_S$ and $A_c$ are the sample and control absorption.

In order to investigate the rheological behavior of dessert samples, a Physica MRC 301 Rheometer (Anton-par Company of Austria) was used. The oscillation test was performed on the first day. The device was equipped with a water circulator to control the temperature and all tests were performed at a temperature of 10±1 °C. The probe of the device was in the form of a plate and the distance between the plates was 1 mm. Strain oscillation test was performed in the strain range of 0.01-100 and a constant frequency of 1 Hz. Frequency scan oscillation test was performed on a fixed strain of 0.5 in the frequency range of 0.01-100 Hz and the storage modulus (G'), dissipation modulus (G"), drop tangent or tan δ, complex modulus and complex viscosity were measured by Eqs 2 and 3 (Bayarri et al., 2010).

$$\tan \delta = G''/G' \quad (2)$$

$$G^* = \sqrt{(G')^2 + (G'')^2} \quad (3)$$

### 2.5. Sensory evaluation

Two samples of milk (camel and cow) were coded with random three-digit codes and according to the 8-point hedonic method by 20 male and female evaluators, different properties of the sample including taste, aroma and general

acceptance were scored (A larger number indicated greater utility). This test was performed at the sixth hour of incubation (Abolfazli et al., 2014). Sensory evaluation of dessert samples was performed on the second day. All four dessert samples were coded with random three-digit codes and according to the 8-point hedonic method by 28 male and female evaluators, different properties of the sample such as taste, aroma, texture and general acceptance were scored.

### 2.6. Statistical analysis

To investigate the effect of two samples of milk (camel and cow) on changes in *L. casei* growth, acidity and pH, antioxidant changes and sensory evaluation, a completely randomized design in the form of factorial test. In order to investigate the effect of two variables of dessert base type (cow milk and camel milk) and two dessert formulations with sugar and sucralose on a total of four samples, a completely randomized plan was used as a factorial test. The experiments were performed in three replications. Analysis of variance and mean comparison were performed using LSD test at 95 percent confidence level. In all stages, statistical analysis of data was performed using SPSS software. Excel software was used to draw the graphs.

## 3. Results

### 3.1 Acidity and pH

The initial pH of camel milk before starter inoculation was 6.4 and that of cow milk was 6.5; during 12 h of incubation in both types of probiotic milk (camel and cow), the pH decreased to 3.5 in camel milk and 5.4 in cow milk, respectively. The results showed that camel milk had lower pH and higher acidity than cow milk during incubation. Comparison of the mean of the main effects showed that the type of milk (camel and cow) and incubation time had a significant effect on pH changes ($p \leq 0.05$) Interactions of milk type (camel and cow) and incubation time had no significant impact on pH changes (Figure 1) ($p > 0.05$).

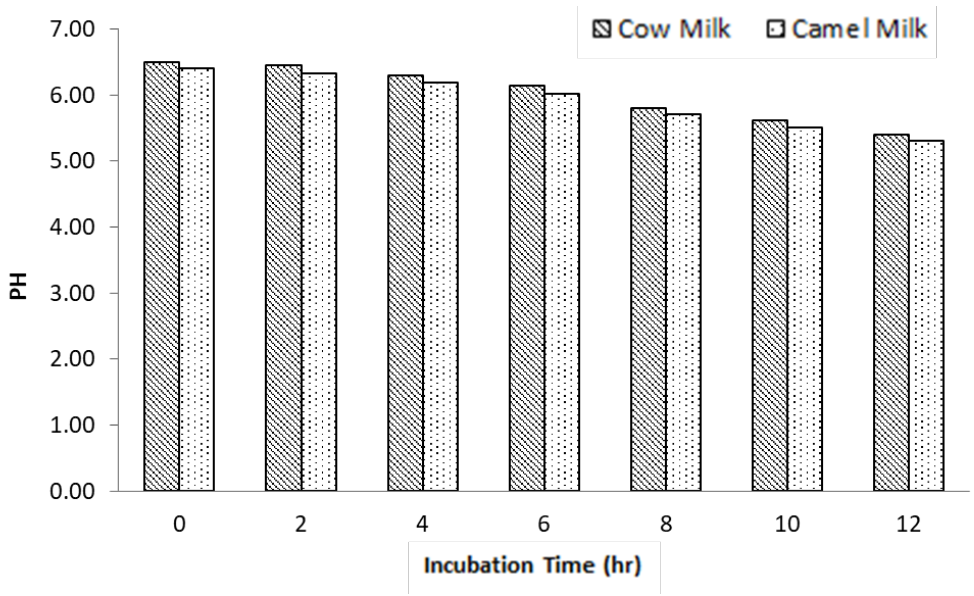


**Figure 1.** Comparison of the interaction effects of milk factors and incubation time on the pH response variable.

Camel milk acidity before starter inoculation was 0.22% lactic acid and the acidity of cow milk was 0.17 percent lactic acid and the difference between them was significant ($p \leq 0.05$). As the incubation time increased, the acidity of both types of probiotic milk (camel

and cow) increased significantly. After 12 hours of incubation, the acidity of camel milk reached 0.46 % lactic acid and in cow milk it reached 0.43 % lactic acid. Comparison of the mean of main and interaction effects of milk type and incubation time showed that these two levels of camel milk and cow milk and incubation time have a significant effect on acidity changes ($p \leq 0.05$) (Figure 2).

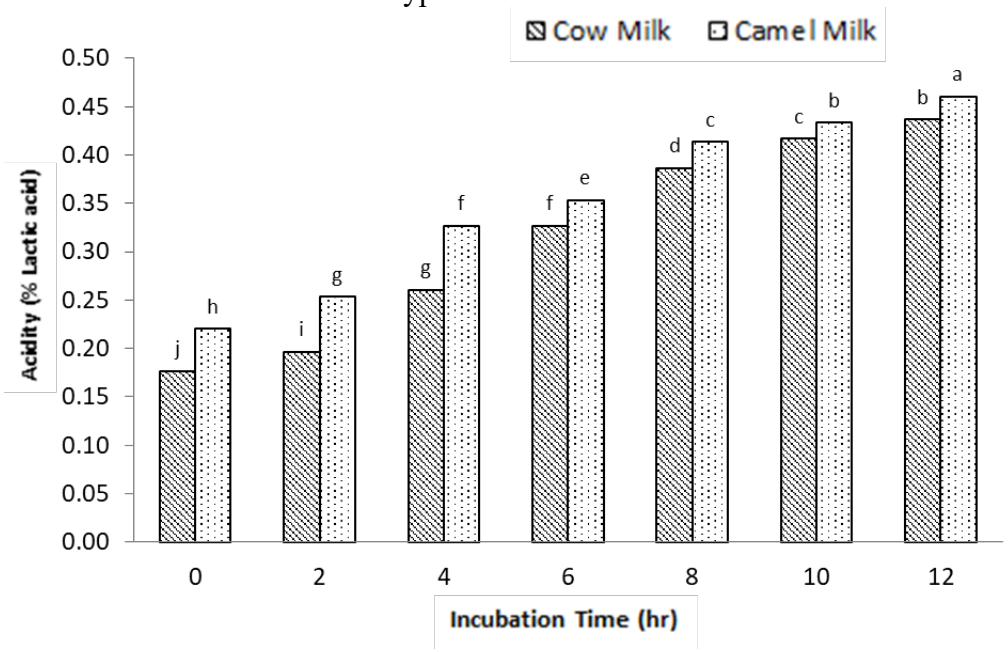


**Figure 2.** Comparison of the interaction effects of milk factors and incubation time on the acidity response variable.

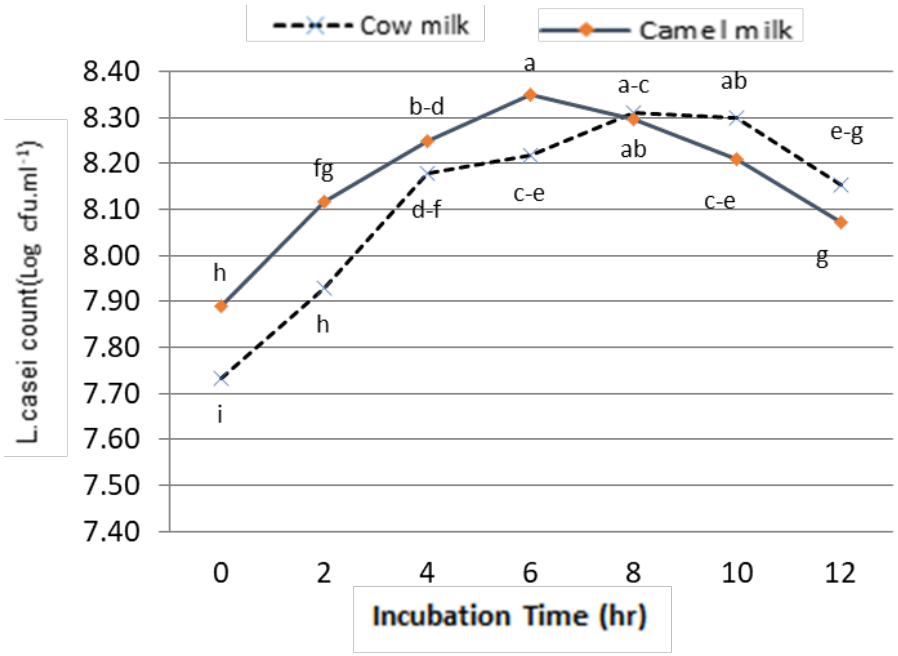


**Figure 3.** *L. casei* growth curve during incubation time.

### 3.2. Growth of *L. casei*

Bacterial growth changes of *L. casei* were examined during 12 hours of incubation. During 12 hours of incubation, the growth of *L. casei* in both types of milk (camel and cow) was higher than 7 Log cfu/ml. Microbial growth and count in camel milk was higher than cow milk and the difference between them was significant ($p \leq 0.05$). Microbial count in camel milk at zero moment 7.89 Log cfu/ml after 6 hours of incubation reached its maximum level (8.31 Log cfu/ml) and in cow milk at zero moment 7.73 Log cfu/ml and the maximum microbial count (8.31 Log cfu/ml) was observed after 8 hours of incubation, after which the growth of *L. casei* in both types of milk decreased with a slight slope. Comparison of the mean of the main effects of milk type (camel and cow) and incubation time showed that these two factors have a significant effect on microbial growth and count ($p \leq 0.05$). Comparison of the average interaction effects of milk (camel and cow) and incubation time on the growth variable of *L. case*i showed that their effect is significant ($p < 0.05$) Figure 3.

### 3.3. Antioxidant activity

During 12 hours of incubation, the antioxidant activity of both types of milk (camel and cow) was examined. At first hour after inoculation of *L. casei*, the level of antioxidant activity of camel milk and cow milk was not different ($p > 0.05$). During incubation, the antioxidant activity of both types of milk (camel and cow) increased with a higher percentage of antioxidant activity in camel milk than cow milk. Before incubation, the percentage of antioxidant activity in camel milk was 1.04± 0.1 and after 6 h of incubation reached 24.84 ± 0.28, in cow milk, the antioxidant activity before incubation was 0.79 ± 0.21% which reached 16.7±0.28% after 6 h incubation; The maximum amount of antioxidant activity was observed in camel milk after 6 hours and in cow in the eighth hour of incubation. The percentage of antioxidant activity in camel milk decreased after 6hours and in cow milk after 8 hours of incubation. Comparison of the mean of the main effects showed that two factors, milk type and incubation time have a significant effect on changes in antioxidant activity ($p \leq 0.05$) (Figure 4)

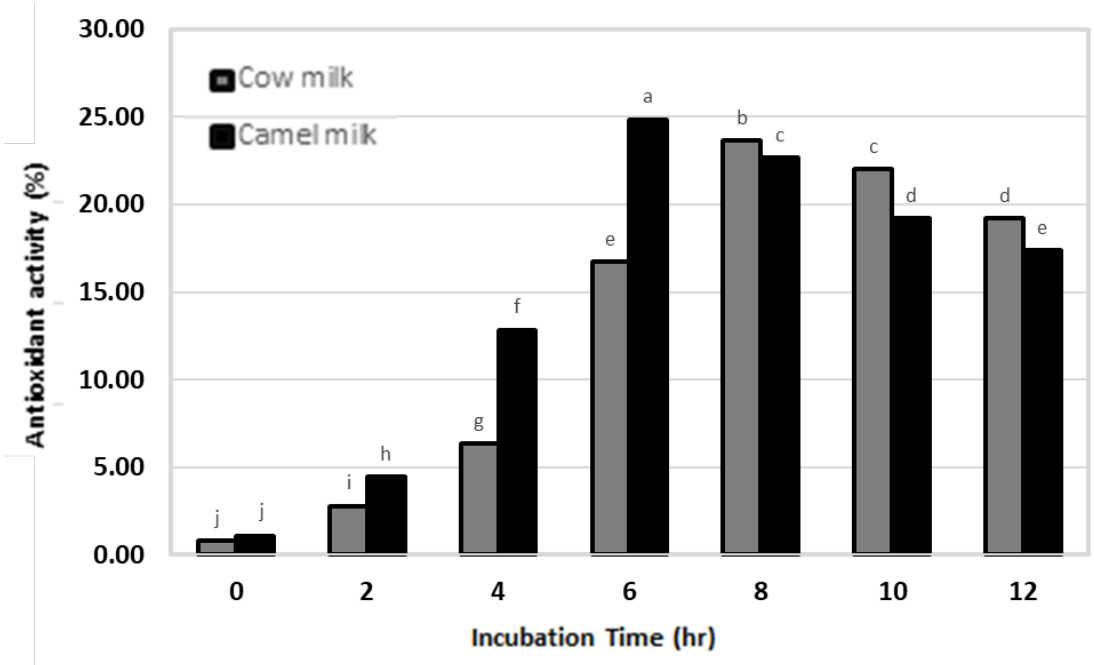


**Figure 4.** Comparison of the interaction effects of milk factors and incubation time in the response.

### 3.4. Sensory properties

After 6 hours of incubation, the sensory properties of milk (cow and camel) were examined. Mean comparison showed that the type of milk (cow and camel) has no significant effect on the variables of taste, aroma and overall evaluation (p>0.05), and both types of fermented milk (cow and camel) in terms of taste parameters, aroma and overall rating received low scores. Generally, in the sensory evaluation, fermented cow milk received a higher score than fermented camel milk. (Table 2).

**Table 2.** Comparison of the average type of milk on the variables of taste, aroma and overall evaluation.

| Milk | Variable traits | | |
|---|---|---|---|
| | Flavor | Aroma | overall evaluation |
| camel milk | 2.65 ± 0.67 [a] | 2.45 ± 0.6 [a] | 2.60 ± 0.50 [a] |
| cow milk | 3.00 ± 0.79 [a] | 2.35 ± 0.74 [a] | 2.65± 0.74 [a] |

Means that have common letters don't have significant difference (p>0.05).

### 3.5. pH and acidity

During 28 days of storage, the PH decreased in all dessert samples from 6.2 on the first day to 5.26 on the 28th day. During 28 days of storage at refrigerator temperature, the acidity of the samples increased from 0.24 percent lactic acid on the first day to 0.46 percent lactic acid on the 28th day. The results showed that time factor had a significant effect on changes in acidity and PH of all dessert samples (P<0.05) (Tables 3).

**Table 3.** Comparison of the average effect of day on pH and acidity.

| Factor | Levels | Variable traits | |
|---|---|---|---|
| | | pH | Acidity (% lactic acid) |
| | First | 6.20 ± 0.07 [a] | 0.24 ± 0.02[e] |
| | Seventh | 6.08 ± 0.11[b] | 0.28 ± 0.03[d] |
| Day | fourteenth | 5.74 ± 0.35[c] | 0.36 ± 0.08[c] |
| | twenty-first | 5.49 ± 0.23[d] | 0.41 ± 0.05[b] |
| | twenty-eight | 5.26 ± 0.24[e] | 0.46 ± 0.05[a] |

Means that have common letters don't have significant difference (p>0.05).

Both levels of camel milk and cow milk had a significant effect on increasing acidity and decreasing pH (P<0.05) the rate of increase in acidity in chocolate probiotic dessert based on camel milk was higher than chocolate probiotic dessert based on cow milk and the rate of decrease in PH in camel milk based on probiotic dessert samples was significantly different compared to cow milk- based dessert samples (p<0.05) (Table 4).

**Table 4.** Comparison of the mean effect of milk type on pH and acidity.

| Factor | levels | Variable traits | |
|---|---|---|---|
| | | pH | Acidity (% lactic acid) |
| Milk | cow | 5.94±0.30[a] | 0.30 ± 0.07 [b] |
| | camel | 5.56±0.43[b] | 0.39±0.09 [a] |

Means that have common letters don't have significant difference (p>0.05).

The comparison of the mean interactions of day and type of milk (camel and cow) on the pH and acidity variables was significant (P<0.05). The sample of probiotic desserts prepared with camel's milk on the 28th day had higher acidity and lower PH, respectively, compared to the samples of probiotic desserts prepared with cow's milk.

Comparison of the mean of two type of sweeteners, sucrose and sucralose-maltodextrin, showed that these 2 factors have a significant effect on the acidity and pH of the product (p≤0.05). The increase in acidity and decrease in pH in the sample of desserts prepared with sucrose sweetener was more than the sample of desserts prepared with sucralose-maltodextrin.

### 3.6. Survival of the *L. casei*

The survival of *L. casei* was examined in a sample of probiotic desserts during 28 days off refrigeration. Data showed the survival of *L. casei in* cow and camel milk 8.76±0.02 and 8.83±0.21. Comparison of the mean effect of milk type on the survival of *L. casei* demonstrated that two levels of camel milk and cow milk had a significant effect on the survival of *L. casei* (p≤0.05) and *L. casei* survival in probiotic desserts prepared with camel milk was higher than probiotic desserts prepared with cow milk.

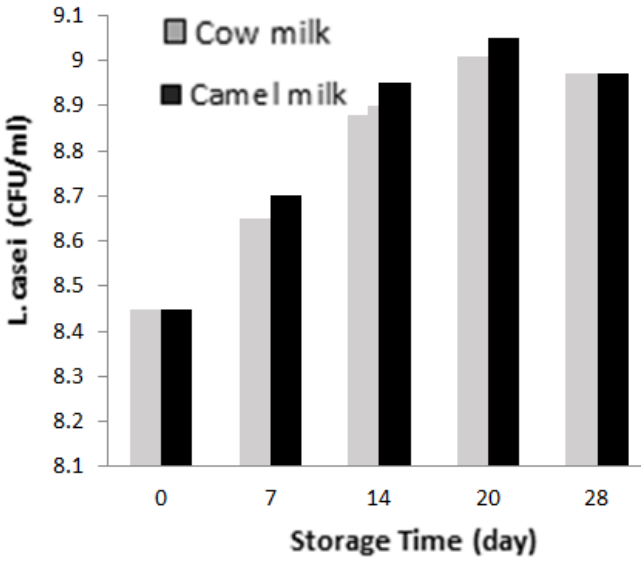


**Figure 5.** Interactions of milk type and time (day) on the survival of *L. casei*.

Comparison of the mean time factor on the survival of *L. casei* showed that its effect was significant (p<0.05) and survival of *L. casei* increased during 21 days of storage; the survival rate of *L. casei* in chocolate desserts samples was 8.45 Log cfu/g on the first day and reached 9.01 Log cfu/g on the 21st day and then decreased. Survival of *L. casei* during 28 days

of storage at 4C$^{o}$ in the sample of probiotic desserts prepared with camel milk was higher compared to the sample of probiotic desserts prepared with cow's milk. On the 21$^{st}$ day, the survival of *L. casei* in probiotic desserts based on camel milk was 9.05 Log cfu/g and in probiotic desserts based on cow milk in the 21$^{st}$ day was 8.97 Log cfu/g. Comparison of the mean interactions of time (day) and type of milk (cow and camel) on the survival of *L. casei* was not significant ($p>0.05$) (Figure 5).

Survival of *L. casei* in milk containing sucrose and sucralose maltodextrin were 8.82±0.21and 8.76±0.20. These results showed that two sweetener levels (sucrose and sucralose maltodextrin) had a significant effect on survival of *L. casei* which was higher in the sample of probiotic desserts prepared with sucrose sweetener than probiotic desserts prepared with sucralose maltodextrin sweetener.

### 3.7. Rheological feature

The two most important parameters obtained from the oscillation tests are the storage modulus or elastic modulus (G') and the viscosity modulus or loss modulus (G"). In this test, at a constant frequency of 1 Hz, the effect of strain changes on the storage and loss modulus is investigated. The amount of dissipation and storage modulus at the point of intersection in 4 different dessert samples. The results showed that G' and G" at point of intersection (Pa) of the samples 1, 2, 3, and 4 were 91.57, 65.00, 100.02, and 78.82. According to these data, dessert No. 3, which is actually a probiotic dessert prepared with cow milk and sucrose sweetener, has the highest amount of G' and G". Adding the sucralose maltodextrin sweetener instead of sucrose in both probiotic dessert formulations prepared with camel milk and cow milk, Reduced G 'and G" at the point of intersection (Table 6).

### 3.8. Frequency scanning oscillation test

In this test, at a constant strain of 0.5%, the frequency is changed from 0.01 to 100 Hz and the amount of storage modulus, loss modulus, complex modulus, complex viscosity and drop tangent are determined. In three selected frequencies of 0.25, 2.5 and 25 Hz, the values of the parameters obtained from this test are compared. The results of comparing the mean showed that the two levels of camel milk and cow milk have no significant effect on the rheological characteristics of probiotic dessert samples ($p>0.05$). The two sweetening levels of sucrose and maltodextrin sucralose had a significant effect on the rheological properties of probiotic dessert samples ($p\leq0.05$) and the measured parameters for the sample of probiotic desserts that used sucrose sweetener in their formulation were higher than the sample of probiotic desserts with maltodextrin sucralose sweetener. Comparison of the mean interactions of milk (camels and cows) and sweeteners (sucrose and sucrose maltodextrin) had no significant effect on the rheological characteristics of probiotic desserts (Table 6).

### 3.9. Sensory characteristics

4 dessert samples were examined in terms of aroma, texture and overall evaluation. The effect of milk type (camel and cow) and sweetener (sucrose and maltodextrin sucralose) on the variable of aroma response was significant, the samples of desserts prepared with cow milk had a higher score in terms of aroma compared to the samples of desserts prepared with camel milk, Also, desserts that used sucralose sweetener in their formulation scored higher in terms of aroma parameter compared to sucrose sweetener. The mean of the main effects of milk (camels and cows) and sweeteners (sucrose and sucrose maltodextrin) on the variables of texture properties and overall product evaluation did not make a significant difference and all dessert samples received a score above 5 in terms of texture and overall evaluation (Table 7).

**Table 6 a.** Comparison of mean milk and sweetening factors on the rheological properties of dessert texture.

| Factor | levels | mean traits | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | G'(Pa) | | | G"(Pa) | | | G*(Pa) | | |
| | | 0.25 | 2.5 | 25 | 0.25 | 2.5 | 25 | 0.25 | 2.5 | 0.25 |
| Milk | cow | 337.25±59.41[a] | 379±64.38[a] | 447.75±82.12[a] | 38.02±6.11[a] | 41.75±6.51[a] | 59.97±8.92[a] | 339.33±59.70[a] | 381.27 ± 64.77[a] | 451.17 ± 82.42[a] |
| | camel | 316.25±67.14[a] | 358.50±74.17[a] | 419.75±83.59[a] | 35.2 ±6.96[a] | 38.55±7.76[a] | 56.75±11.07[a] | 318.17±67.52[a] | 360.58 ± 74.58[a] | 422.85 ± 84.17[a] |
| Sweetener | sucrose | 381± 14.67[a] | 427.75±15.90[a] | 501.75±38.35[a] | 41.97±2.91[a] | 46.17±2.39[a] | 66.75±3.17[a] | 383.28±14.82[a] | 430.26 ± 16.06[a] | 505.37 ± 38.48 |
| | sucrose maltod extrin | 272.5±16.42[b] | 309.75±19.80[b] | 365.75±21.31[b] | 31.25±2.47[b] | 34.12±2.66[b] | 49.97±3.42[b] | 274.22±16.57[b] | 311.59 ± 19.93[b] | 368.65 ± 21.7[b] |

**Table 6 b.** Comparison of mean milk and sweetening factors on the rheological properties of dessert texture.

| Factor | levels | mean traits | | | | | |
|---|---|---|---|---|---|---|---|
| | | Complex Viscosity (Pa.S) | | | Damping factor | | |
| | | 0.25 | 2.5 | 25 | 0.25 | 2.5 | 25 |
| Milk | cow | 1357 ± 245.13 [a] | 152.25 ± 27.35 [a] | 18.05 ± 3.15 [a] | 0.11 ± 0.005 [a] | 0.11 ± 0.002[a] | 0.13 ± 0.005 [a] |
| | camel | 1307.5 ± 326.94 [a] | 136 ± 21.83 [a] | 16.62 ± 3.05 [a] | 0.11 ± 0.002 [a] | 0.1 ± 0.001 [a] | 0.13 ± 0.004 [a] |
| Sweetener | sucrose | 1567.5 ± 126.06 [a] | 164 ± 17.18 [a] | 19.92 ± 1.38 [a] | 0.1 ± 0.004 [b] | 0.1 ± 0.002 [b] | 0.13 ± 0.005 [a] |
| | sucrose maltod extrin | 1097.5 ± 74.1 [b] | 124.25 ± 7.22 [b] | 14.75 ± 0.96 [a] | 0.11 ± 0.000 [aa] | 0.1 ± 0.002 [a] | 0.13 ± 0.003 [a] |

**Table 7.** Comparison of the mean effect of the main effects of milk type and sweetener on the variables of aroma response, texture and overall evaluation.

| | | | mean traits | |
|---|---|---|---|---|
| Factor | levels | Aroma | Texture | overall evaluation |
| | | | | |
| milks | cow | 5.60 ± 1.03 [b] | 6.26 ± 1.31 [a] | 5.87 ± 1.36 [a] |
| | camel | 6.57 ± 1.10 [a] | 6.28 ± 1.28 [a] | 6.17 ± 1.56 [a] |
| sweetener | sucrose | 5.78 ±1.00 [b] | 6.17± 1.25 [a] | 5.76 ± 1.36 [a] |
| | sucrose maltodextrin | 6.39± 1.26 [a] | 6.37 ± 1.34 [a] | 6.28± 1.53 [a] |

Means that have common letters don't have significant difference ($p>0.05$).

## 4. Discussions

### 4.1. pH and acidity

Before fermentation, the pH of camel milk was lower than that of cow milk, which may be due to the high content of vitamin C and organic acids in camel milk (Farah et al., 2007). During incubation, for 12 hours, the pH of both types of milk (camel and cow) decreased and the acidity increased due to the activity of the beta-galactosidase enzyme released by lactic acid bacteria during fermentation, which breaks down lactose and produces lactic acid, acetic acid, citric acid, butyric acid, etc. The mentioned acids lead to an increase in acidity and decrease in pH in the fermented product (Ayash et al., 2018). Camel milk had a lower pH and higher acidity than cow milk during incubation; the results of this study are consistent with the report of Ayash et al. (2018). They stated that camel milk fermented by *Lactoplantibacillus plantarum* extracted from camel milk had higher acidity and lower pH, respectively, than cow fermented milk, because antimicrobial compounds in camel milk are higher than in cow milk and some probiotic species are more compatible with camel milk, which as a result leads to their growth and production of more organic acids. Abu-Tarbush reports (1996) showed that the difference in pH between camel milk and fermented cow milk was significantly different from four species of Bifidobacterium and decrease in pH in camel milk was more than cow milk, which could be due to low buffering capacity of camel milk compared with cow milk and the difference in buffering capacity between camel milk and cow milk is related to the difference in the ratio of specific proteins and salts in each type of milk. Monteagudo-Mera studies (2011) showed that there is a significant difference in the acidity of camel milk and cow milk fermented for 6 hours with the same probiotic. The results of Felfoul et al. (2017) research on fermented milk (camel and cow) by *Enterococcus faecium* and *Streptococcus macedonicus* showed that the acidity of fermented camel milk for 20 hours at 42°C was higher than cow milk. In another study by Ayash et al. (2018) on camel milk fermented by *Lactococcus lactis* extracted from camel milk and compared with cow milk, the results showed that camel milk had higher acidity than cow milk during 21 days of storage. The results of this study are consistent with similar studies.

According to Bresford et al. (2001), the best pH for most bacteria to grow is close to neutral and pH below 5 stops them from growth. During storage of the product for 28 days, the pH of the product decreased significantly. This decrease in pH is consistent with the report of Irkin and Goldaz (2011) who stated that because the

growth of *L. casei* and its ability to produce acid is high, it reduces the pH during the storage period of the crop.

Valencia et al. (2016) also reported an increase in acidity of desserts increased during the storage period of 28 days. This increase in acidity is expected from *L. paracasei* as an arbitrary heterofermentative bacterium and producers of acetic and lactic acid as well as $CO_2$. Other factors such as 5°C and the addition of sugar, which can be broken down into glucose and fructose. Then glucose can be converted to lactic acid, which affects the metabolism of this strain.

Argon Allegro et al. (2007) stated that the acidity of probiotic desserts decreases during 28 days of storage time which is due to the presence of the probiotic bacterium *L. paracasei*.

The results of Patel et al. (2008) study on probiotic and synbiotic chocolate mousse showed that during 28 days of storage in probiotic samples containing *L. paracasei* and synbiotic samples containing *L. paracasei* and inulin compared to the control sample, the increase in acidity was significantly higher due to the presence of *L. paracasei* and the production of lactic acid by this bacterium.

### 4.2. Growth changes of *L. casei*

Bacteria need strong proteolytic and glycolytic systems to provide the necessary nutrients for their growth to function properly in milk. While glucose is essential to meet the basic needs of bacterial growth, the supply of amino acids needed to sustain bacterial growth is provided by complex proteolytic systems that lead to bacterial growth in milk (Elfahri et al., 2016). In this research, the growth of *L. casei* during the fermentation process increased with increasing incubation time and the growth rate of this bacterium in camel milk was significantly different from cow milk and its growth in camel milk was higher than cow milk, this is consistent with the results of a study by Varga et al. (2013); they reported that the microbial count of Lactobacillus acidophilus in fermented camel milk was higher than that of fermented cow milk. *L. casei* growth decreased after 6 hours of incubation in camel milk and after 8 hours of fermentation in cow milk; this is consistent with the results of the research by Leclerc et al. (2002). They stated that the growth of *Lactobacillus helveticus* decreased slightly after 10 hours of milk fermentation due to the increase in lactic acid concentration. Type of selected probiotic species, the presence of hydrogen peroxide in the environment due to bacterial metabolism, inoculation temperature, the concentration of organic acids produced by the bacterium, inoculation level, also fermentation time affects the viability of lactic acid bacteria during fermentation (Rybka & Kailasapathy, 1996).

Abu-Tarbush (1996) showed that the growth of different species of Bifidobacterium in two types of camel and cow milk during incubation for 36 hours at 37°C is significantly different. Some of these species have higher growth in camel milk and some in cow milk.

Ayash et al. reported that in camel milk and cow milk fermented by two species of probiotic bacteria, *Lactococcus lactis* K782 extracted from camel milk and *Lactobacillus acidophilus*, *Lactococcus lactis* k782 count during 21 days of storage was higher in camel milk, and *Lactobacillus acidophilus* count was higher in cow milk. They suggested that this may be due to the presence of antimicrobial compounds in camel milk and the greater compatibility of *Lactobacillus lactis* k782 with camel milk compared to *L. acidophilus*.

Research by Ayash et al. (2018) on the growth of four species of probiotic bacteria in camel and cow milk showed that some bacteria grow more in camel milk and some in cow milk and one of the for this is the higher antimicrobial compounds in camel milk compared to cow milk and its effect on the growth of some bacteria. The probiotic food should include $10^6$ cfu/g at the time of consumption (Boylston et al., 2004). The viability of *L. casei* in chocolate dessert was evaluated in this study, and the results showed that during 28 days of refrigeration the viability of *L. casei* was higher than Log 8 cfu/g and during 21 days of storage at 4°C. This is consistent with research by Patel et al. (2008)

they reported that the growth of *L. paracasei* in chocolate mousse increased during 28 days of refrigerated storage temperature.

Argon Allegro et al. (2007), in a study on probiotic and synbiotic chocolate mousse stated that lowering the pH during 28 days of refrigerated chocolate mousse was not sufficient to reduce the viability of *L. paracasei*. Its viability in all chocolate mousse was > 7 Log CFU/g after 28 days storage. Viability of *L. paracasei* increased during 21 days of storage at 5°C.

Valencia et al. (2016) in a study on chocolate milk dessert containing *L. paracasei* stated that the viability of this bacterium during 28 days of storage was higher than 8 Log CFU/g, which is more than recommended for probiotic products.

Heenan et al. (2004) reported that with the addition of probiotic bacteria to frozen herbal desserts, the bacterial population remains at about $10^7$ CFU/g during six months of storage. The authors stated that dessert is accepted as a suitable food with sensory properties to transmission of probiotic bacteria. Helland et al. (2004) evaluated the growth and metabolism of four probiotic species of *Bifidobacterium animal*, *Lactobacillus acidophilus* La5 and *Lactobacillus rhamnosus* in pudding. They concluded that probiotics survival was between 8Log to 9.1 CFU/g for 21 days. The buffering capacity of food is an important factor for the viability of probiotic bacteria. So, milk is a suitable carrier with a stable pH (Silva et al., 2012).

### 4.3. Evaluation the changes in antioxidant activity

Bioactive compounds in foods, especially dairy-fermented products, may reduce the effect of superoxidase, hydroxyl, peroxyl, and radicals formed by cell oxidation. These bioactive peptides, especially peptide-derived proteins, neutralize free radicals by donating electrons (Ayash et al. 2018; Gadhiya et al., 2015). Bioactive peptides as antioxidants in fermented milk may inhibit peroxidation of essential fatty acids (El-Salam & El-Shibiny, 2013). According to the results of this test, the level of antioxidant activity of both types of milk (camel and cow) increased during incubation, which is consistent with the results of the study of Elfahri et al. (2016); They reported that the antioxidant activity of milk containing *Lactobacillus helveticus* increased during incubation due to increased bacterial proteolytic activity, which led to the production of bioactive peptides that have antioxidant properties.

The antioxidant activity of camel milk during fermentation was higher than that of cow milk. This is consistent with the results of the research of Ayash et al. (2018); they stated that the reason for the high antioxidant activity of fermented camel milk compared to fermented cow milk is the higher proteolysis rate in camel milk and the nature of the bioactive peptides in camel milk.

Similarly, Moslehi Shad et al. (2013) stated that fermentation of milk (camel and cow) by *L. rhamnosus* increases antioxidant activity, which may be due to the hydrolysis of s1α and β-casein by proteolytic and peptidolytic enzymes of *Lactobacillus rhamnosus*. In fact, peptide fragments extracted from camel milk fermented by *Lactobacillus rhamnosus* showed higher antioxidant activity than cow milk. They stated that these findings indicate that the nature and composition of peptides are not same in camel milk and fermented cow milk, and these peptides play an important role in neutralizing ABTS radicals and antioxidation activity.

Felfoul et al. (2017) by studying the antioxidant activity of fermented camel milk and comparing it with fermented cow milk stated that the free radical scavenging activity of camel milk is higher than cow milk, which may be due to the richness of camel milk with vitamin C comparing to cow milk. Another reason is the high antioxidant activity of peptides derived from camel milk caseins, especially β-casein. The results of Amal and Salinity (2013) research showed that yogurt made from soy and camel milk has higher antioxidant activity than yogurt made from soy and cow milk. The maximum level of antioxidant activity was observed in camel milk at sixth hour of incubation and in

cow milk at the eighth hour after which it decreased in both types of milk (camel and cow). Elfaheri et al. (2016) stated that the antioxidant activity of milk containing *Lactobacillus helveticus* increased from zero time to 12 during incubation and then decreased slightly, which could be due to the hydrolysis of some antioxidant components by *Lactobacillus helveticus* which leads to decreased antioxidant activity. They also reported that antioxidant activity in fermented milk depends on the metabolic activity of lactic acid bacteria, which varies between different bacterial species, in addition to bacterial resistance and growth at low pH conditions.

In sensory evaluation of milk (camel and cow) after 6 hours of incubation, both types of milk received a low average score in terms of smell, taste, and overall evaluation. In terms of taste and overall evaluation, camel milk received a lower score than cow milk. Due to the increased acidic taste in both types of milk (cow and camel) during the incubation period, the product was not accepted. The results of this study are consistent with the results reported by Felfoul et al. (2017). They stated that cow milk fermented by *Enterococcus faecium* received a higher score than camel milk fermented by this bacterium; It may be due to the differences in the structure and composition of both types of milk (camel and cow); such as differences in the amount of lactose in camel milk compared to cow milk, high salt content in camel milk, camel milk is richer in vitamin C compared to cow milk.

Ranadheera et al. (2016), in a study on fermented cow and goat milk, stated that both types of milk received low scores in terms of sensory evaluation due to the development of an unpleasant acidic taste that is produced during fermentation in the product. The results of Gomes et al. (2013) evaluation of fermented dairy beverages made with cow milk and goat milk and a mixture of both types of milk showed that the fermented beverages had a highly acidic taste during 28 days of storage. They stated that taste and aroma are important factors in the acceptance of the product by the consumer and the decision to buy dairy products and the addition of fruit and flavorings and sugar largely obscures the sour taste of the product.

The higher the strain point of the intersection, the greater the tolerance of the sample to mechanical stress and transport, in other words, the more stable it is (Tarrega and Costell, 2006). According to the above mentioned information, the amount of G' and G'' at the intersection point also indicates the structural cohesion and intermolecular connections of the sample; the higher the value of these two parameters, the stronger the intermolecular connections and the more cohesive the structure. The maximum amount of G' and G" at the intersection of the dessert was based on cow milk and sucrose sweetener.

### 4.4. Frequency scanning oscillation test

The information shows that in all samples of the storage modulus either G' is above the dissipation modulus or G''; therefore, all dessert samples show solid viscoelastic behavior. The maximum amount of storage modulus or elastic and viscosity of the complex was related to a dessert prepared with cow milk and sucrose sweetener. Arcia et al. (2010) reported that desserts with higher inulin concentration have higher viscoelastic properties and storage modulus curve or G' was above the loss modulus or G''. Tarrega and Costell (2006) reported on starch-based low-fat-dairy desserts at frequency of 1 Hz in different dessert with increasing starch concentration (2.5, 3.25, and 4 percent), the storage modulus and complex viscosity increases. The tangent decreases, indicating a relative increase in the elasticity of the sample to viscoelasticity. Bavarri et al. (2010) stated that the binding of casein micelles to kappa carrageenan through electrostatic bonding stabilizes the gel and increases the viscoelastic behavior. Thomas et al. (2008) stated that desserts that use kappa carrageenan in their formulation require more energy to break down their structure than samples that do not use kappa carrageenan in their formulation. These results indicate the suitability of kappa carrageenan as a structural hydrocolloid for

dairy products. Considering that gelatin and kappa carrageenan were used in the formulation of all probiotic dessert samples and according to the properties mentioned for kappa carrageenan, between dessert samples prepared with two types of milk (camel and cow) in terms of rheological features no significant differences were observed.

Adding sucralose instead of sucrose did not have a significant effect on sensory properties, which is consistent with a report by Demorais et al. (2015). They evaluated the sensory properties of dietary probiotic chocolate desserts and found that sucralose is the best sucrose substitute for probiotic chocolate milk desserts compared to other sweeteners such as aspartame, neotam, and stevia, because it makes the least changes in sensory properties.

Irkin and Guldas (2011) stated that chocolate pudding containing *L. casei* received the lowest score in sensory evaluation of texture. They stated this is probably due to the high proteolytic activity of *L. casei*. Also, the pudding prepared with *L. casei* had the lowest taste and smell score compared to the puddings prepared with Lactobacillus acidophilus and *Bifidobacterium lactis*. In sensory evaluation in terms of texture, all four dessert samples had a score above 6, considering that in the formulation of all four dessert samples, texture factors were used, this issue helped to improve the oral texture of the samples. According to Lethuaut et al. (2003), the composition and structure of food are involved in understanding mouth feel and the use of gelling and thickening agents changes the texture of food. However, textural factors may alter taste perception and vice versa. In terms of average scores related to sensory evaluation in all parameters of aroma, texture, and overall evaluation, each four dessert samples had a score above 5and this is consistent with the results of research by Kardley et al. (2008). They showed suitability of chocolate mousse dessert as probiotic carrier with acceptable viability and sensory properties. Their results showed no significant impact on taste and aroma of chocolate mousse containing *L. paracasei* during 7 days of storage. Argon Allegro et al. (2007) investigated on chocolate mousse containing *L. paracasei* and inulin reported that there was no significant difference in the results of sensory evaluation between control, probiotic and synbiotic samples. They stated that the addition of probiotics and prebiotics does not change the sensory evaluation of products by the consumer. Similarly, the results of the study by Patel et al. (2008) showed that there was no significant difference in terms of sensory evaluation between the control sample and the probiotic and synbiotic samples.

## 5. Conclusions

Investigating of the growth of Lactobacillus casei in milk (camel and cow) during 12 hours of incubation led to increased acidity and decreased the pH of both types of milk (camel and cow). Camel milk had significantly higher acidity and lower pH compared to cow milk ($p<0.05$). The growth of this bacterium during incubation in camel milk was significantly higher than cow milk ($p<0.05$). The fermentation of both types of milk (camel and cow) with *L. casei* increased their antioxidant activity during incubation time and the level of antioxidant activity in camel milk was higher than cow milk. In sensory evaluation, fermented camel milk received a lower score compared to fermented cow milk. The results of examining the properties of probiotic chocolate dessert based on camel milk in this study showed that this product has suitable conditions for the growth of *L. casei*. The growth and viability of this bacterium in dessert after 28 days of storage were higher than recommended for probiotic products. Due to the use of Kappa carrageenan and gelatin in the formulation of probiotic dessert, no significant difference was observed between different samples of probiotic dessert based on camel milk and cow milk ($p>0.05$). In addition, the chocolate dairy dessert evaluated in this study can be an example of functional foods with sensory properties accepted by the consumer.

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