Fermented camel milk influenced by soy extract: Apparent viscosity, viscoelastic properties, thixotropic behavior, and biological activities

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ABSTRACT

During fermentation, camel milk forms a fragile, acid-induced gel, which is less stable compared with the gel formed by bovine milk. In this study, camel milk was supplemented with different levels of soy extract, and the obtained blends were fermented with 2 different starter culture strains (a high acidic culture and a low acidic culture). The camel milk-soy extract yogurt treatments were evaluated for pH value, acidity, total phenolic compounds, antioxidant capacities, degree of hydrolysis, α-amylase and α-glucosidase inhibition, angiotensin-converting enzyme inhibition, antiproliferative activities, and rheological properties after 1 and 21 d of storage at 4°C. The results revealed that some of the investigated parameters were significantly affected by the starter culture strain and storage period. For instance, the effect of starter cultures was evident for the degree of hydrolysis, antioxidant capacities, proliferation inhibition, and rheological properties because these treatments led to different responses. Furthermore, the characteristics of camel milk-soy extract yogurt were also influenced by the supplementation level of soy extract, particularly after 21 d of storage. This study could provide valuable knowledge to the dairy industry because it highlighted the characteristics of camel milk-soy yogurt prepared with 2 different starter culture strains.

Key words: camel milk, soy extract, antioxidant capacity, proliferation inhibition, rheology

INTRODUCTION

The worldwide camel dairy market represented a value of $6.9 billion in 2021 and is expected to represent about $18.3 billion by 2027, with a compound annual growth rate of 6.8% during the predicted period (Grand View Research, 2022). Fresh camel milk was only consumed by pastoralists for a long time and was considered as a gift for their hosts (Konuspayeva and Faye, 2021). It has been reported that camel milk is only appropriate for drinking in the fresh or sour form, restricting its consumption at a worldwide commercial scale (Bornaz et al., 2009; Al haj and Al Kanhal, 2010). Natural antimicrobial lactoferrins exist in camel milk, hindering the activity of starter cultures and therefore delaying curd formation. The physicochemical properties of camel milk differ from those of other milk species. For instance, the content of κ-casein in camel milk (less than 3.5% of the total casein) is lower than that of bovine milk by approximately 13%. In addition, camel milk lacks β-LG, which is considered a main protein in bovine milk that might cause allergic responses (Hinz et al., 2012). The particle size of camel milk casein micelles is larger than that of bovine milk (Hailu et al., 2016; Ho et al., 2022). Nevertheless, the heat treatment of camel milk at 100°C for 30 min before fermentation did not result in an acceptable texture, indicating that the firmness of yogurt is not associated with the natural antimicrobials present in camel milk (Hashim et al., 2009).

Fermented camel dairy products are characterized by a watery consistency and possess poor and fragile structures (Ibrahim et al., 2009; He et al., 2022). This structure may be attributed to the small size of camel milk fat globules. As yogurt texture is considered a crucial factor affecting its characteristics and
consumer acceptability, several efforts have been made to overcome the challenges associated with the poor texture of fermented camel milk products and to deliver a set-type yogurt. The enrichment of camel milk with skim milk powder (Abou-Soliman et al., 2017), stabilizers, and hydrocolloids (Sobti et al., 2020) has been reported. Camel milk products have gained growing attention due to their beneficial health aspects, such as antioxidant, antidiabetic, antimicrobial, and antihypertensive properties (Nongonierna et al., 2018). It has been reported that fermented products such as yogurt from camel milk could further increase the demand for products made from this type of milk (Muthukumaran et al., 2022).

The use of soy extract was first described in China about 2000 years ago. The initial plant-based milk aimed to deliver nutrients to consumers where the animal milk supply was insufficient (Singhal et al., 2017). Soy extract is regularly consumed by people with milk protein allergies and lactose intolerance. It is a good source of essential fatty acids, which play a beneficial role in cardiovascular diseases (Sethi et al., 2016). The global soy beverage market represented a value of about $5.97 billion in 2022 and is expected to grow at a compound annual growth rate of 8.4% between 2022 and 2032. Soy beverage sales are anticipated to meet a portion of the demand in the worldwide plant-based milk market, which is expected to be worth $14 billion in 2032 (Future Market Insights, 2022). The following crucial factors mainly drive the market growth of soy beverage: (1) being a good alternative to regular dairy milk because it contains similar amounts of protein with fewer calories, and (2) reducing blood cholesterol (Dong et al., 2016). Soy beverage is greatly beneficial for anemic and lactose-intolerant individuals as it is lactose-free and high in iron (Sekar et al., 2020). Its higher content of isoflavones has been associated with a reduced risk of some hormone-related cancers (Zhang et al., 2004). Additionally, the market growth of soy beverage may be attributed to the fact that food manufacturers use certain flavor substances, such as chocolate, vanilla, and strawberry, to mask the off-flavor of soy beverage. The main drawback of soy beverage intake is soy allergies, making it inappropriate for people allergic to soy proteins (Sethi et al., 2016).

The characteristics and applications of soy beverages in different dairy products such as yogurt, cheese, and ice cream have previously been investigated (Le et al., 2021; Kumari et al., 2022). Therefore, this study aims to evaluate the characteristics of camel milk yogurt supplemented with different levels (0, 154, 308, and 462 mL) of soy extract, fermented by 2 different starter cultures (a high acidic culture [CH] and a low acidic culture [YF]), and stored at 4°C for 21 d.

**MATERIALS AND METHODS**

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

**Materials**

Skim camel milk powder (Camelicious, Dubai, UAE), pasteurized at 72°C for 16s followed by drying at 180–200°C, and soybean seeds were purchased from local markets (Al Ain, UAE). Two different starter cultures, the first one (CH-1) composed of Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus, and the second one (YF-L812), also composed of L. bulgaricus and S. thermophilus, were donated by Chr. Hansen (Dubai, UAE). All chemicals and reagents used in this study were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated.

**Preparation of Soy Extract**

Soybean seeds (500 g) were washed several times with distilled water and soaked in deionized-distilled (dd) water (1:3, wt/vol) overnight at room temperature. Then, the mixture was heat-treated at 100°C for 20 min. After that, the water was decanted, and the drained soybeans were hand-washed thoroughly. Afterward, the soybeans were placed in a mixer-grinder and blended with dd-water (1,200 mL) at room temperature for 10 min at the highest speed. The resulting slurry was filtered through a double-layer cheesecloth, and the final volume of the filtrate was adjusted to 2,000 mL with dd-water. The chemical composition of the resultant soy extract was as follows: moisture (83.2 g/100 g), protein (7.1 g/100 g), lipids (3.2 g/100 g), carbohydrates (5.6 g/100 g), and ash (0.94 g/100 g). The chemical composition of the soy extract was determined according to AOAC International (2007).

**Preparation of Camel Milk-Soy Extract Yogurt**

The formulations of camel milk and soy extract were prepared as shown in Table 1. The treatments were differentiated based on the soy protein content in each formula. Therefore, camel milk-soy extract mixtures were respectively 0, 2, 4, and 6 g/100 g of soy protein representing SL0 (camel milk only as control), SL2, SL4, and SL6 of treatments. The total solids content of camel milk was maintained at 12.5 g/100 mL in the final volume (500 mL) for all blends. The blends were prepared according to Table 1, and the obtained
blends were stored at 4°C overnight to allow complete hydration of camel milk. In the next day, the blends were heated to 85°C for 30 min in a shaker-water bath, followed by cooling to 43°C and then equilibrated for 10 min. Afterward, the tempered blends and control were inoculated with 0.1 g of direct-in-vat starter cultures CH or YF and thoroughly mixed for 2 min. The inoculated blends were dispensed into 100-mL cups and incubated at 43°C until a pH of 4.5 was reached. Yogurt treatments were directly stored at 4°C, and samples were taken for analysis in the next day (d 1) and after 21 d (d 21) of storage. The whole experiment was repeated on 3 different occasions. Supplemental Figure S1 (https://data.mendeley.com/datasets/h7fdb6225t/1; Ayyash, 2023) shows a flowchart of experimental yogurt production.

**Characteristics of Camel Milk-Soy Extract Yogurt**

**pH Value and Titratable Acidity.** The pH values of camel milk-soy extract yogurt samples were determined using a calibrated digital Start-3100 pH meter (Ohaus Corporation, NJ). In addition, the titratable acidity, expressed as lactic acid (%), was measured by mixing yogurt samples (10 g) with distilled water (20 mL) and titrating with NaOH (0.1 N) using 3 to 4 drops of phenolphthalein indicator to an endpoint of faint pink color (ISO/TS 11869:2012/IDF 150:2012).

**Total Phenolic Compounds and Degree of Hydrolysis.** To determine the total phenolic compounds (TPC) of yogurt samples, the water-soluble extract (WSE) of each sample was prepared by adjusting the pH value to pH 4.6 using sodium hydroxide (1.0 M) or hydrochloric acid (1.0 M) and centrifugation at 10,000 × g for 15 min at 4°C. Then, the supernatant was filtered over a 0.45-µm syringe filter (Mixed Cellulose Esters, EMD Millipore Co.) and kept at -20°C for further analysis (Ayyash et al., 2018). The TPC content of yogurt samples was determined using the Folin-Ciocalteu procedure (Cho et al., 2013), and the results of TPC were expressed as milligrams of gallic acid equivalents per gram of the dry extract.

To determine the degree of hydrolysis (DH) of yogurt treatments, the previously prepared extract was mixed for 1 min with a vortex mixer and centrifuged at 10,000 × g for 5 min at 4°C. O-phtaldialdehyde (OPA) method was used to estimate the DH of yogurt samples. O-phtaldialdehyde of the WSE was estimated according to the method of Sah et al. (2014).

**Antioxidant Capacities by DPPH and ABTS.** The radical scavenging activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was carried out as described by Elfahri et al. (2016). The radical scavenging activity (%) was calculated as follows:

\[
\text{scavenging rate} = 1 - \frac{\text{absorbance of sample}}{\text{absorbance of blank}} \times 100.
\]

The radical scavenging rate by the 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulphonic acid) (ABTS•+) method was determined following the procedure reported by Sah et al. (2014). A stock ABTS solution was prepared by mixing stock solutions of ABTS aqueous solution (7.4 mM) with potassium persulfate aqueous solution (2.6 mM) in equal amounts (a molar ratio of 1:0.35) and permitting them to interact for 12 h at room temperature in the dark. To obtain an absorbance of 0.70 ± 0.02 at 734 nm after equilibration at 30°C, ABTS•+ solution (1 mL was mixed with 50–60 mL of the buffered methanol) to prepare the fresh ABTS reagent. Twenty microliters of an adequately diluted WSE in dd-water was added to 2 mL of ABTS reagent and kept at 30°C for 6 min. Also, 20 µL of dd-water was used as a blank. The absorbance was measured at 734 nm, and the radical scavenging activity (%) was calculated as follows:

\[
\text{scavenging rate} = \left(1 - \frac{\text{absorbance of sample}}{\text{absorbance of blank}}\right) \times 100.
\]

**α-Amylase and α-Glucosidase Inhibition.** α-Amylase and α-glucosidase inhibition of yogurt treatments was performed following the procedure reported by Kim et al. (2004). The percentage of α-amylase and α-glucosidase inhibition was determined as follows:

\[
\text{inhibition rate} = \left(1 - \frac{\text{activity of sample}}{\text{activity of blank}}\right) \times 100.
\]

### Table 1. Formulation of camel milk-soy extract blends

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soy extract (mL)</th>
<th>Deionized-distilled water (mL)</th>
<th>Skim camel milk powder (g)</th>
<th>Final volume (mL)</th>
<th>Soy protein content (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL0</td>
<td>0</td>
<td>500</td>
<td>62.5</td>
<td>500</td>
<td>0.0</td>
</tr>
<tr>
<td>SL2</td>
<td>154</td>
<td>346</td>
<td>62.5</td>
<td>500</td>
<td>2.0</td>
</tr>
<tr>
<td>SL4</td>
<td>308</td>
<td>192</td>
<td>62.5</td>
<td>500</td>
<td>4.0</td>
</tr>
<tr>
<td>SL6</td>
<td>462</td>
<td>38</td>
<td>62.5</td>
<td>500</td>
<td>6.0</td>
</tr>
</tbody>
</table>

1The calculations were carried out based on a total protein content in the soy extract of 7.1 g/100 g.
inhibition(%) = \left(1 - \frac{\text{absorbance of sample} - \text{absorbance of blank}}{\text{absorbance of control}}\right) \times 100.

**Angiotensin-Converting Enzyme Inhibition.**

Angiotensin-converting enzyme (ACE) inhibition activity of yogurt treatments was analyzed according to Ayyash et al. (2018). The inhibition of enzyme activity (%) was calculated as follows:

\[
\text{ACE - inhibition(%) = } \left(1 - \frac{\text{HA control} - \text{HA sample}}{\text{HA control}}\right) \times 100,
\]

where HA = hippuric acid.

**Antiproliferative Activities.** To determine the antiproliferative activity of yogurt treatments, the WSE were filtered via Macrosep Advance Spin Filter 3 kDa (Pall Corporation, NY). The filtrates were assessed against colon cancer cell line Caco-2 (Nutrition and Health Department, UAEU) and breast cancer cell line MCF-7 (Faculty of Medicine and Health Sciences, UAEU), according to Elfahri et al. (2016) and modified by Ayyash et al. (2018). The viability of cells in each well was determined from the ratio between optical density at 570 nm (\(\text{OD}_{570}\)) and optical density at 605 nm (\(\text{OD}_{605}\)), and the following equation was used to calculate the proliferative inhibition activity:

\[
\text{proliferative inhibition(%) = } \left(1 - \frac{\text{R}_{\text{sample}} - \text{R}_o}{\text{R}_{\text{ctrl}} - \text{R}_o}\right) \times 100,
\]

where \(\text{R}_{\text{sample}}\) refers to \(\text{OD}_{570}/\text{OD}_{605}\) absorbance in the presence of WSE, \(\text{R}_{\text{ctrl}}\) refers to \(\text{OD}_{570}/\text{OD}_{605}\) absorbance in the absence of WSE (vehicle control), and \(\text{R}_o\) refers to the average background (noncell control) \(\text{OD}_{570}/\text{OD}_{605}\) absorbance.

**Rheological Properties**

The apparent viscosity, viscoelastic properties, and thixotropic behavior of yogurt treatments after 1 and 21 d of storage were evaluated using Discovery Hybrid Rheometer HR-2 (TA Instruments, New Castle, DE) at 25°C. The bob-cup geometry system comprises a smart swap concentric cylinder Peltier steel jacket fitted with an aluminum cylinder cup (44 mm diameter). The bob dimensions were 31.09 in diameter and 37.25 mm in length, and a gap size of 100 μm was applied. The apparent viscosity (mPa·s) dependence on a shear rate of 10–1,000 1/s for all yogurt treatments was fitted to the Power law model:

\[
\eta(\dot{\gamma}) = m(\dot{\gamma})^{n-1},
\]

where \(\eta\) refers to the apparent viscosity, \(\dot{\gamma}\) indicates the shear rate (1/s), \(m\) refers to the consistency coefficient, and \(n\) is the flow behavior index (Supplemental Table S1, https://data.mendeley.com/datasets/h7fd6225/1; Ayyash, 2023). All analyses were performed in duplicate. The squared error was calculated to assess the rheological modeling uncertainty as follows:

\[
\text{Squared error} = \frac{(\text{measured viscosity} - \text{predicted viscosity})^2}{(\text{measured viscosity})^2}.
\]

A strain sweep was performed to conclude the linear viscoelastic range for small deformation rheology. Frequency, adjusted at 1.0 Hz, as strain values percentage differed from 0.1 to 100%, causing a strain sweep. As a function of strain, both complex modulus and oscillation stress were plotted to select the linear range. Then, a strain in the linear region of 0.01% to 1% was chosen and a frequency sweep was accomplished at 25°C. Furthermore, the thixotropic behavior of yogurt samples was investigated by 2 methods. In the first method, the flow curves were measured by increasing the shear rate (forward measurement) and decreasing the shear rate (backward measurement), while the second method was the oscillatory 3 interval thixotropy test. The test was performed as an oscillatory test with the following 3 test intervals: 1) very low shear to simulate behavior at rest at low strain within the LVE region \((2 \times 10^{-7} \text{ MPa})\), 2) strong shear to simulate structural breakdown of the sample during application \((2 \times 10^{-5} \text{ MPa})\), and 3) very low shear to simulate structural regeneration at rest using the same low strain value as in the first test interval \((2 \times 10^{-7} \text{ MPa})\).

**Statistical Analysis**

One-way ANOVA was performed to investigate the effect of the blended ratio at the same storage period and the effect of storage at the same blended ratio on the measured parameters \((P < 0.05)\). Result values are mean ± standard deviation. Mean comparisons at same storage period were performed using Tukey’s test \((P < 0.05)\). Furthermore, the principal component analysis (PCA) was performed to assess the structural correlation of the variables (loadings) and observations (scores) to visualize the relationship between different experimental yogurts. The statistical analyses were carried out by XLSTAT software (Addinsoft, New York, NY).
RESULTS AND DISCUSSION

**pH Value and Titratable Acidity**

Figure 1a displays that the pH values of yogurt samples were in the range of 4.3 and 4.6 for the different treatments after 1 d of storage. The lowest pH value was recorded for SL0 prepared with YF strain. After 21 d of storage, it was observed that the pH values of all treatments significantly decreased with values ranging from 3.9 to 4.2. Similarly, Shori (2013) stated that yogurt prepared from a mixture of soybean and camel milk exhibited a steady decline in pH throughout 21 d of storage (4.05–4.04) with no significant differences compared with plain camel milk yogurt except after 7 d. The titratable acidity of yogurt treatments after 1 d of storage ranged between 0.49 and 0.97% as shown in Figure 1b. After 21 d of storage, the titratable acidity increased in all yogurt treatments, and SL0 treatment prepared with CH strain showed the highest value (0.92%), while SL6 prepared with CH strain exhibited the lowest acidity of 0.62%. Postacidification of yogurt occurred during cooling, and this could be explained by the remaining metabolic activity of yogurt starter cultures. The action of β-galactosidase released by lactic acid bacteria to hydrolyze lactose remained active even during cooled storage (Kailasapathy and Sultana, 2003). This promoted the increase of lactic acid, citric acid, acetic acid, formic acid, butyric acid, and acetaldehyde formed by yogurt bacteria as metabolic byproducts (Östlie et al., 2005).

It was reported that the constant decline in the pH value of camel milk yogurt could be attributed to the ability of camel milk to resist changes in pH during fermentation, even in the presence of acidic conditions due to the intrinsic high buffering potential of milk.

Figure 1. pH values (a), titratable acidity (b), total phenolic compounds (c), and degree of hydrolysis (DH; d) of camel milk-soy extract yogurt treatments. Values are means ± SE (n = 3). Red and blue bars represent d 1 and 21, respectively. GAE = gallic acid equivalents. a–e = means with different lowercase letters at the same storage time differed significantly (P < 0.05). A–D = means with different uppercase letters at the same storage time differed significantly (P < 0.05). SL0 = camel milk only as a control; SL2 = camel milk + 2% soy protein; SL4 = camel milk + 4% soy protein; SL6 = camel milk + 6% soy protein; CH = high acidic culture; YF: low acidic culture.
Total Phenolic Compounds and Degree of Hydrolysis

The content of TPC in yogurt samples is presented in Figure 1c. It was shown that after 1 d of storage, the TPC content was in the range of 8.9 to 12.8 mg of gallic acid equivalents/g, and the lowest value was recorded for SL0 prepared with the bacterial strain YF. However, the treatment SL6 prepared with CH strain showed the highest value. The TPC content increased after 21 d of storage in all yogurt samples. Additionally, TPC content significantly increased with the increase in soy extract addition level because SL6 treatments showed the highest values. The TPC content increased after 21 d of storage in all yogurt samples. Additionally, TPC content significantly increased with the increase in soy extract addition level because SL6 treatments showed the highest values. The variations in TPC between yogurt samples may be due to the hydrolysis of proteins by the starter cultures, which might produce bioactive compounds, such as amino acids with phenolic chains, through the fermentation and storage of yogurt (Joung et al., 2016). In addition, some bacterial metabolites, such as free amino acids, certain peptides, and organic acids, could affect phenolics analysis (Bastola et al., 2017). Furthermore, this increase might be associated with the metabolic activity of yogurt bacteria, which release free phenolics in milk by breaking down complex phenolic compounds (Blum, 1998). It was reported that the TPC of yogurt prepared with commercial starter and L. casei significantly increased after one week of storage; nevertheless, this value drastically decreased after 2 wk of storage. This reduction was attributed to phenolic compounds’ degradation by the enzymatic activity of starter cultures through storage (McCue and Shetty, 2005). Moreover, Shori (2013) showed that TPC in plain camel yogurt declined after one week and increased after 2 wk of storage. Several studies observed an increase in the TPC of yogurt during storage (Joung et al., 2016), while others reported a decline (Madhu et al., 2012).

Figure 1d displays the DH of yogurt treatments after 1 and 21 d of storage. Proteolytic activity is a tentative marker of the health-promoting benefits. O-Phthalaldehyde evaluates small peptides and free amino acids in fermented foods (McSweeney and Fox, 1997). Moreover, oligopeptides are one of the main bioactive compounds in fermented milk (Park, 2009). It was shown that there were significant differences in the DH values of yogurt treatments. After 1 d of storage, SL0 treatment prepared with CH strain showed the highest value (18.5%) compared with other yogurt treatments, and the lowest value (4.7%) was recorded for SL0 treatment prepared with YF strain. The DH increased with the progress in the storage period in all yogurt samples, and DH was also affected by the addition level of soy extract. The effect of yogurt strains on the DH was evident in this parameter because significantly different values were observed, particularly for SL0 and SL4 treatments. It was reported that the proteolytic activities of camel milk and bovine milk yogurts varied with the use of different Lactobacillus strains (Ayyash et al., 2018) because lactobacilli produce numerous proteolytic enzymes, such as peptidases, proteinases, and aminopeptidases (Park, 2009).

Antioxidant Capacities by DPPH and ABTS

The scavenging rates of yogurt treatments prepared with CH and YF strains are presented in Figure 2a for DPPH and Figure 2b for ABTS. The DPPH values of yogurt treatments ranged from 11.6% to 30.0%. SL0, prepared with CH strain, and SL6, prepared with YF strain, showed the lowest values, and the highest value was recorded for SL0 prepared with YF. The DPPH values increased with the progress of the storage period in the different treatments. Additionally, the DPPH values increased with the additional level of soy extract in the treatments prepared with CH strain. A diverse trend was shown for treatments prepared with YF strain, as SL6 exhibited the lowest value. The effect of starter culture strains (CH or YF) on the DPPH was noticeable, as SL0 and SL6 treatments showed significantly different values after 1 and 21 d of storage.

Similarly, the ABTS values of yogurt samples increased with the progress in the storage period, except for SL0 prepared with CH strain (Figure 2b). After 21 d of storage, ABTS values ranged between 52.7% and 88.8% in the different treatments. (Shori, 2013) revealed that the presence of soybean during the production of yogurt increased the antioxidant capacities in both bovine milk yogurt and camel milk yogurt, mak-
ing up 61.76% and 53.16%, respectively, as compared with plain yogurt representing 26.41% and 15.44% for plain-bovine milk yogurt and plain camel milk yogurt, respectively. Phenolic compounds are classified as one of the most important categories of natural antioxidants. These compounds are considered crucial antioxidant constituents responsible for the deactivation of free radicals due to their capability to offer hydrogen atoms to free radicals. The antiradical and antioxidant activities of phenolic compounds are certainly associated with the numbers of hydroxyl groups attached to the aromatic ring (Sroka and Cisowski, 2003). The antioxidant capacity of soybean has been linked to its content of polyphenolic compounds and isoflavones (Slavin et al., 2009). Furthermore, proteolysis of milk proteins and production of organic acids (Correia et al., 2005) due to the metabolic activities of starter cultures during yogurt fermentation and storage could be other reasons for improved antioxidant capacities.

**α-Amylase and α-Glucosidase Inhibition**

The inhibition of α-amylase and α-glucosidase for camel milk-soy extract yogurt fermented by CH and YF strains is illustrated in Figures 2c and 2d, respectively. It was shown that there were significant differences in α-amylase inhibition (%) among yogurt treatments after 1 and 21 d of storage. After 1 d of storage, SL2 prepared with CH strain showed the lowest inhibition value (25.2%), while SL4 prepared with YF strain showed the highest inhibition value (73.2%). After 21 d of storage, the lowest inhibition value (36.6%) was recorded for SL0 = camel milk only as a control; SL2 = camel milk + 2% soy protein; SL4 = camel milk + 4% soy protein; SL6 = camel milk + 6% soy protein; CH = high acidic culture; YF: low acidic culture.
SL0 prepared with CH strain, and the highest inhibition value (66.9%) was recorded for SL4 prepared with YF strain. As shown in Figure 2d, α-glucosidase inhibition (%) varied among yogurt treatments. α-Glucosidase inhibition increased in all yogurt treatments with the increase in storage period, except for SL2 prepared with YF strain which decreased from 49.6% after 1 d to 41.7% after 21 d. The inhibition activities of α-amylase and α-glucosidase are considered effective in managing diabetes through weakening the hydrolysis of carbohydrates (Donkor et al., 2012). The overall inhibition of α-amylase and α-glucosidase enzymes might be ascribed to bioactive peptides, mainly smaller peptides (Gomes da Cruz et al., 2009), formed by proteolytic enzymes produced by yogurt strains. The different potential antiabetic peptides of camel milk proteins have been reported by (Redha et al., 2022; Shahein et al., 2022). Peptides with branched amino acids, such as phenylalanine, tyrosine, lysine, tryptophan, and cationic residues, can inhibit α-amylase (Rivero-Pino, 2023). In addition, the presence of leucine or phenylalanine at the C-terminus position, and phenylalanine or glycine at the N terminus of α-amylase inhibitor peptides, has been reported (Ngoh and Gan, 2016). Conversely, the incidence of hydroxyl or basic side chains at the N terminus, methionine or alanine at their C-terminus, and proline close to their C-terminus, are more probable to exhibit α-glucosidase inhibitory action because of their hydrophobic interaction with the active sites (Di Stefano et al., 2018). It was stated that camel milk peptides LPVP and MPVQA exhibited a higher antiabetic potential, with a dipeptidyl peptidase IV IC50 of 87.0 and 93.3 μM, respectively (Nongonierma et al., 2018). Shori and Baba (2014) reported that α-amylase and α-glucosidase inhibition was higher in camel milk yogurt than in bovine milk yogurt. Also, the variations in lactic acid bacterial species and strains might cause qualitative and quantitative differences in proteolytic activity (El-Salam and El-Shibiny, 2013).

**Angiotensin-Converting Enzyme Inhibition and Antiproliferative Activities**

Figure 3a presents the ACE inhibition of yogurt samples. The treatment SL0, prepared with CH strain, showed a significantly lower ACE-inhibition value (31.1%) compared with other treatments after 1 d of storage. However, it significantly increased to 66.2% after 21 d of storage. However, ACE inhibition decreased with the progress in the storage period in the other treatments. ACE inhibition is considered an in vitro index for the antihypertensive characteristics of fermented foods (Gobbetti et al., 2004). The higher ACE inhibition in camel milk may be attributed to the high proteolytic activity, which supports the assumption that camel milk proteins may be more vulnerable to hydrolysis by the proteolytic enzymes released by yogurt strains.

Furthermore, it has been reported that naturally occurring peptides in camel milk yogurt prepared with different Lactobacillus strains might be responsible for the higher ACE inhibition compared with bovine milk yogurt (Ayyash et al., 2018). Tagliazucchi et al. (2016) identified 17 bioactive peptides with ACE inhibition activities in camel milk. The antihypertensive tripeptide isoleucine-proline-proline was determined in digested camel milk, and the quantity of the released peptide represented 2.56 mg/L of milk. Moreover, Soleymanzadeh et al. (2019) identified a new ACE inhibitory peptide (MVPYPQR) in fermented camel milk with an IC50 value of 1.61 mg/mL. The high ACE inhibition in fermented camel milk may be attributed to the higher content of proline in camel milk caseins (El-Salam and El-Shibiny, 2013). Additionally, ACE inhibitory peptides have been reported in several soybean products, such as soy beverage, fermented soy beverage, and soy-based infant formulas (Alauddin et al., 2015; Wongsa et al., 2022).

The WSE (<3 kDa) of yogurt treatments’ proliferation inhibition against Caco-2 and MCF-7 carcinoma cell lines are displayed in Figure 3b and 3c, respectively. Yogurt treatments prepared with YF strain exhibited higher proliferation inhibition against Caco-2 cells than those prepared with CH strain, and SL0 prepared with YF strain showed the highest inhibition value (85.3%) after 1 d of storage. Figure 3c shows significant differences in the proliferation inhibition against MCF-7 cells. SL0 prepared with CH strain showed the lowest inhibition value (26.5%), while SL6 prepared with CH strain showed the highest value (66.4%) after 1 d of storage. After 21 d of storage, SL0 prepared with YF strain showed the lowest inhibition value (34.1%), and SL2 prepared with YF strain exhibited the highest value (96.5%). The antiproliferation activity of camel milk and soy beverage was reported in several studies (Park, 2009; Tan et al., 2016). The antiproliferative activity mechanisms of milk peptides have been explained by various hypotheses (Sahna et al., 2022), such as the competition between peptides and cancer growth factors for cancer cell membrane receptors and the specific cytotoxicity of released peptides on cancer cells, stimulating programmed cell death (Pessione and Cirrincione, 2016). High antiproliferation activity of camel milk yogurt prepared with different lactobacilli strains was ascribed to the more prominent competition ability of peptides resulting from camel milk yogurt than those from bovine milk yogurt. Also, the specific cytotoxicity of camel milk yogurt peptides stimulating apoptosis might contribute to this action (Ayyash et al., 2018).
Principal Component Analysis

PCA summarizes similarities and variations between treatments and associations among their chemical constituents. PCA models provide a simple description of similarities among treatments based on analytical results (Santiago-García et al., 2021). The differences between the types of yogurt treatments (antidiabetic, antihypertensive, antioxidant, cytotoxicity, and fermentation) were analyzed using multivariate analysis to generate a PCA score plot (Figure 4). The first principal component (F1) and second principal component (F2) accounted for 30.09% and 23.89% of the variability of the original variables, respectively. The data presented in F1 and F2 indicate the general data attributes of yogurt samples (Liu et al., 2012). pH value, titratable acidity, and α-glucosidase (after 1 and 21 d), as well as DH and TPC (after 1 d of storage), were positively correlated with PC1 and related to SL0 prepared with CH, SL2 prepared with CH, SL4 prepared with CH, and SL6 prepared with YF. The rest of the variables were negatively correlated with PC1 and related to SL4 prepared with CH, SL6 prepared with CH, SL0 prepared with YF, SL2 prepared with YF, and SL4 prepared with YF. As seen in Figure 4b, SL0 treatments were grouped separately from other yogurt treatments.

Rheological Properties

Apparent Viscosity. Figure 5 displays the apparent viscosity of yogurt treatments prepared with CH and YF strains using different soy extract levels measured after 1 and 21 d of storage. The results show that for both starter culture strains, the apparent viscosity
Figure 4. Principal component analysis map (A) and confidence ellipses (B) of camel milk-soy extract yogurt treatments. TA = titrable acidity; DH = degree of hydrolysis; ABTS = antioxidant reagent; DPPH = antioxidant reagent; GLU = glucosidase inhibition; AMY = amylase inhibition; Caco-2 = antiproliferative cell line; MCF-7 = antiproliferative cell line; TPC = total phenolic compound; D1 = storage d 1; D21 = storage d 21; SL0 = camel milk only as a control; SL2 = camel milk + 2% soy protein; SL4 = camel milk + 4% soy protein; SL6 = camel milk + 6% soy protein; CH = high acidic culture; YF: low acidic culture.
increased with increasing soy extract addition level, and decreased with progress in the storage period for treatments with and without soy extracts. After 1 d of storage, the viscosity of yogurt treatments prepared with CH or YF was approximately the same for the treatments SL0 and SL2, while the viscosity of yogurt made with YF was higher for SL4 and SL6. After 21 d of storage, the viscosity of YF yogurt was higher than CH yogurt with different soy extract levels. Shear thinning behavior was observed in all yogurt samples. Additionally, the Power law model fit the data well (solid lines in the figures). The consistency coefficient value (representing the viscosity) increased with soy extract addition level and decreased with storage time, as shown in the supplementary materials (Supplemental Table S1). In general, YF yogurt treatments showed higher consistency coefficient values. The deviation from the Newtonian behavior increased with the soy extract addition level. Yogurt samples exhibited higher shear thinning behavior at 1 d compared with those after 21 d. For 1-d treatments, YF yogurt showed a higher deviation from Newtonian behavior, while at 21 d, both strains had approximately the same deviation. Previous studies have reported that the apparent viscosity and viscoelastic properties of camel milk yogurt were noticeably lower than bovine milk yogurt prepared.
with the same starter cultures, which was ascribed to the weak protein-protein network of camel milk yogurt compared with bovine milk yogurt. Furthermore, the variation in α, β, and κ casein ratios in camel and bovine milks could contribute to the weak network of camel milk yogurt (Al haj and Al Kanhal, 2010; Ayyash et al., 2020).

**Viscoelastic Properties.** Regarding the amplitude sweep (linearity test), Supplemental Figure S2 (https://data.mendeley.com/datasets/h7fdb6225t/1; Ayyash, 2023) shows examples of the linearity behavior of storage ($G'$) and loss ($G''$) moduli in a specific range of oscillation strain. If $G'$ is higher than $G''$, the material behaves similar to a solid, and destructions will be recoverable or elastic. If $G'$ is lower than $G''$, the energy applied to deform the materials will be dissipated viscously, and the material will behave more similar to a liquid (Tabilo-Munizaga and Barbosa-Cánovas, 2005). It was found that at an oscillation strain of 1%, both moduli for both strains were linear during the frequency sweep tests. Storage modulus and loss modulus for yogurt treatments are shown in Supplemental Figures S3 and S4 (https://data.mendeley.com/datasets/h7fdb6225t/1; Ayyash,
For CH yogurt treatments at 1 d of storage, the effect of soy extract addition on storage and loss moduli was unclear. $G'$ was higher than $G''$ for yogurt prepared from camel milk and bovine milk at different frequencies, which is a characteristic of weak viscoelastic gels characteristic of yogurt (Meyer et al., 2011; Kamal et al., 2017). Tan δ was greater than 1 until a frequency of approximately 4 Hz, and the effect of soy extract was unclear. For CH yogurt treatments at 21 d of storage, both moduli increased with soy extract addition level, with the increase in storage modulus being more evident in the presence of soy extract. For YF treatments at 1 d of storage, both moduli increased with the addition level of soy extract, except for the SL6 treatment, where its storage modulus was very low until a frequency of 3.4, which suddenly increased with frequency. Tan δ was less than 1, except for the SL6 exception at low frequency, and decreased with soy extract addition level. The storage modulus dominated over the loss modulus. For YF yogurt samples at 21 d of storage, both moduli increased with the soy extract addition level. Furthermore, tan δ was less than 1 and slightly decreased with soy extract addition level. The storage time decreased both moduli, and its effect was more evident on the loss modulus.

Figure 7. Flow behavior of yogurt treatments: yogurt prepared with high acidic culture after 1 d (a), yogurt prepared with high acidic culture after 21 d (b), yogurt prepared with low acidic culture after 1 d (c), and yogurt prepared with low acidic culture after 21 d (d) of storage. Blue, red, green, and purple lines represent SL0 (camel milk only), SL2, SL4, and SL6, respectively.
Thixotropic Behavior. The presence of hysteresis indicates thixotropic behavior, and the area between the forward and backward flow curves measures the thixotropic degree (Cruz et al., 2013). Most samples showed a clear hysteresis loop with varying areas (i.e., thixotropic behavior; Figure 7). The area between the forward and backward curves was calculated and reported in Supplemental Table S2 (https://data.mendeley.com/datasets/h7fdb6225t/1; Ayyash, 2023). The degree of thixotropy increased with soy extract addition and decreased with storage time. After one day of storage, both bacterial strains exhibited similar thixotropy. Generally, YF yogurt treatments showed a higher degree of thixotropy.

In the second method, a step test with preset oscillation for all 3 test intervals was used: (a) behavior at rest at very low stress, (b) behavior during structural breakdown when shearing is stronger, high shear stress, and (c) behavior during structural recovery at rest with very low shear stress again. The results are presented in the form of $G'$ and $G''$ in Figure 8. In all yogurt samples except SL0 prepared with the CH strain, the storage modulus was greater than the loss modulus. To evaluate structural regeneration, regeneration in

![Figure 8. Thixotropic properties of yogurt treatments: yogurt prepared with high acidic culture after 1 d (a), yogurt prepared with high acidic culture after 21 d (b), yogurt prepared with low acidic culture after 1 d (c), and yogurt prepared with low acidic culture after 21 d (d) of storage. Blue, red, green, and black represent SL0 (camel milk only), SL2, SL4, and SL6, respectively. $G'$ = storage modulus; $G''$ = loss modulus.](image-url)
the third test interval was determined at a previously defined point in time (t3: 651 s) and was quoted as a percentage of G’ value at rest at the end of the first interval (t1: 204 s) as shown in Supplemental Table S3 and Supplemental Figure S5 (https://data.mendeley.com/datasets/h7f6d6225t/1; Ayyash, 2023). Based on the structural regeneration parameter, the highest thixotropic behavior was detected in SL2 treatments, while the lowest thixotropic behavior was exhibited by SL0 prepared with the CH strain after 21 d of storage, which agrees with the results of the hysteresis area method. It was clear that at high levels of soy extract (LS6), the structural regeneration was around 50%.

CONCLUSIONS

In this study, we evaluated the characteristics of camel milk yogurt prepared with 2 different starter culture strains and soy extract at varying levels. The effect of the starter culture strain was evident for certain parameters, such as DH, antioxidant capacities, antiproliferation activities, and rheological properties, resulting in diverse values for yogurt treatments. Furthermore, the addition of soy extract, especially after 21 d of storage, affected the properties of yogurt. The degree of thixotropy of yogurt samples increased with soy extract addition level and decreased with storage time. The higher bioactivity observed in camel milk yogurt prepared with different strains may be attributed to the stronger competition ability of peptides derived from camel milk yogurt compared with those from bovine milk yogurt. This study could serve as a foundation for further investigations into the nutritional and functional properties of camel milk-soy extract mixtures. Additionally, the addition of soy extract improved the texture and rheological properties of fermented camel milk, and future studies are needed to understand the interactions between soy extract components and camel milk.

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