Effect of whey protein isolate addition on set-type camel milk yogurt: Rheological properties and biological activities of the bioaccessible fraction

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ABSTRACT

The manufacture of camel milk (CM) yogurt has been associated with several challenges, such as the weak structure and watery texture, thereby decreasing its acceptability. Therefore, this study aimed to investigate the effect of whey protein isolate (WPI) addition on the health-promoting benefits, texture profile, and rheological properties of CM yogurt after 1 and 15 d of storage. Yogurt was prepared from CM supplemented with 0, 3, and 5% of WPI and compared with bovine milk yogurt. The results show that the water holding capacity was affected by WPI addition representing 31.3%, 56.8%, 64.7%, and 45.1% for yogurt from CM containing 0, 3 or 5% WPI, and bovine milk yogurt, respectively, after 15 d. The addition of WPI increased yogurt hardness, adhesiveness, and decreased the resilience. CM yogurt without WPI showed lower apparent viscosity, storage modulus, and loss modulus values compared with other samples. The supplementation of CM with WPI improved the rheological properties of the obtained yogurt. Furthermore, the antioxidant activities of yogurt before and after in vitro digestion varied among yogurt treatments, which significantly increased after digestion except the superoxide anion scavenging and lipid oxidation inhibition. After in vitro digestion at d 1, the superoxide anion scavenging of the 4 yogurt treatments respectively decreased from 83.7%, 83.0%, 79.1%, and 87.4% to 36.7%, 38.3%, 44.6%, and 41.3%. The inhibition of α-amylase and α-glucosidase, angiotensin-converting enzyme inhibition, cholesterol removal, and degree of hydrolysis exhibited different values before and after in vitro digestion.

Key words: camel milk yogurt, whey protein isolate, in vitro digestion, health-promoting benefits, rheological properties

INTRODUCTION

Due to its unique nutritional aspects and digestibility compared with bovine milk (BM), camel milk (CM) has been applied to manufacture several products (Khalesi et al., 2017). Dairy products prepared from CM could have the possibility for progress in the dairy market because of the potential therapeutic attributes of CM, such as tuberculosis, asthma, diabetes, jaundice, dropsy, and visceral leishmaniasis treatments (Sulieman and Alayan, 2022). Yogurt is one of the most common fermented foods worldwide, with established beneficial health effects (Pei et al., 2017). The structural and rheological properties of BM, goat milk, or sheep milk yogurts have been widely investigated (Nguyen et al., 2018). Because of its different physicochemical properties, CM cannot form a firm gel after inoculating with starter cultures, resulting in a product with a weak and fragile structure and a watery texture (Hashim et al., 2009). CM contains more antibacterial substances, such as lactoferrin, lysozyme, and immunoglobulins, than BM (Pastuszka et al., 2016). Furthermore, CM yogurt showed higher antioxidant activities, antiproliferation action against cancer cell lines, inhibitions of α-amylase and α-glucosidase, and inhibition of angiotensin-converting enzyme (ACE) compared with BM yogurt (Ayyash et al., 2018). Due to the absence of β-lactoglobulin, CM is more digestible and induces fewer allergic reactions. Nevertheless, the nutritional and therapeutic features of CM make this kind of yogurt attractive to consumer, validating the higher price to some individuals (Bulca et al., 2022).

Utilizing a process to develop an acid coagulum from CM is a crucial issue because yogurt texture is a critical parameter affecting consumer acceptance and appa-
ent quality of the fermented product. The larger size of casein micelles, the low content of κ-CN, and the absence of β-lactoglobulin in CM are the key reasons for its inability to produce a desirable curd (Hailu et al., 2016; Khalesi et al., 2017). However, several attempts have been carried out to overcome the challenges of the fragile texture of fermented CM, including the supplementation with various stabilizers and hydrocolloids (Abou-Soliman et al., 2017). Nonetheless, limited information is available about employing whey protein isolate (WPI) or whey protein concentrate in fermented CM products.

Owing to their nutritional, physical, and economic benefits, whey protein powders have been used as a fashionable trend for increasing the protein content of foods (Tunick, 2008). The use of WPI has been reported to be associated with enhanced food system properties, such as water-binding (Kontopidis et al., 2004), gelling (Hashim et al., 2021), foaming (Leman et al., 2005), and emulsifying (Leman et al., 2005) properties. The supplementation of milk with whey protein powders to increase the content of protein and total solids increased the viscosity and firmness of yogurt and reduced the syneresis (Abou-Soliman et al., 2017). Water holding capacity (WHC) is negatively correlated with hardness (Lu et al., 2020). However, yogurt supplemented with WPI displayed a more compact structure with more hardness and lower syneresis (Delikanli and Ozcan, 2014). Bioaccessibility implies the release of nutrients from a food matrix in the intestinal cavity. The Food and Drug Administration has defined bioaccessibility as the rate and extent to which bioactive ingredients or moieties are absorbed from a product and became accessible at the target site (Fang, 2014). Therefore, the present study aimed to investigate the biological activities of the bioaccessible fractions of set-type CM yogurt supplemented with 2 levels (3% and 5%) of WPI compared with BM yogurt after 1 and 15 d of storage at 4°C. Furthermore, texture profile analysis and rheological properties of the experimental yogurt were also evaluated. Our study’s primary objective did not entail the creation of a new food product; hence, conducting sensory evaluations was beyond the scope of our research.

**MATERIALS AND METHODS**

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

**Materials**

Skim CM powder, skim BM powder, and WPI (90 g/100 g dry base) were purchased from a local market (Al Ain, UAE). The composition of CM, BM, and WPI powders employed in this study is presented in Supplemental Table S1 (https://doi.org/10.17632/28jy32m4sy1; Ayyash, 2023). A direct-in-vat “YoFlex Express” culture composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* was obtained from Chr-Hansen, Dubai (UAE). High purity analytical grade chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, Missouri), unless otherwise indicated.

**Set-Type Yogurt Making**

CM and BM powders were reconstituted in deionized-distilled water at 12.5% total solids. The reconstituted CM was divided into 3 portions; CM only (control), CM supplemented with 3 g of WPI/100 mL (CMW3), and CM supplemented with 5 g of WPI/100 mL (CMW5). These levels were selected after a preliminary work was carried out by using different levels (0%, 1%, 3%, 5%, and 7%) of WPI to select the maximum level that could be added to CM to prepare a set-type yogurt comparable to that of BM. After mixing, all the prepared mixtures (CM, CMW3, CMW5, and BM) were kept overnight at 4°C for full hydration. In the next day, the samples were heated at 85°C for 30 min, followed by rapid cooling to 45°C, and tempered for 10 min. The mixtures were inoculated with the starter culture according to manufacturer instructions (0.1 g/100 g). The inoculated mixtures were distributed into cups with 100-mL volume and the filled cups were incubated at 43°C until a pH of 4.5 was reached (3–4 h), after which fermented milk samples were placed at 4°C overnight, and samples were taken for analysis after 1 and 15 d. To prepare the water-soluble extract, the pH value 50 g of yogurt sample was adjusted to 4.6 by using 1.0 M HCl or NaOH, followed by centrifugation at 4°C and 8,000 × g for 15 min. The supernatant was filtered via a syringe filter (0.45 μm) and kept at −20°C for subsequent analysis. Yogurt samples were prepared in triplicate.

**Titratable Acidity, pH Value, and Water Holding Capacity**

The titratable acidity of yogurt samples was determined by mixing 10 g of the sample with 20 mL of distilled water and 3 to 4 drops of phenolphthalein and titrating the mixture with sodium hydroxide (0.1 N; IDF, 2012). The pH value was measured with a digital pH meter (Thermo Orion pH meter, model 420, Waltham). Furthermore, the WHC of yogurt treatments was evaluated according to the method reported by Delikanli and Ozcan (2017). Briefly, yogurt samples
The apparent viscosity, viscoelastic properties, and thixotropic behavior of yogurt treatments were evaluated by using a Discovery Hybrid Rheometer HR-2 (TA Instruments, New Castle, DE) according to the method described by Ayyash et al. (2020). The bob-cup geometry system comprises of a smart swap concentric cylinder Peltier steel jacket fitted with an aluminum cylinder cup (44 mm diameter). The bob dimensions were 31.09 mm in diameter and 37.25 mm in length, and a gap size of 100 µm was applied. Yogurt samples were kept overnight at 4°C. In the next day, samples were permitted to reach room temperature (23 ± 0.1°C) for the rheological analysis. The dependence of apparent viscosity (in mPa s) on shear rate in the range 10 to 1,000 s⁻¹ 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 (mPa s⁻¹), \( \dot{\gamma} \) indicates the shear rate (s⁻¹), \( m \) refers to the consistency coefficient (mPa s⁻¹), and \( n \) is the flow behavior index (Supplemental Table S2; https://doi.org/10.17632/28jy32m4sy.1; Ayyash, 2023). All analyses were performed in duplicate.

For viscoelastic properties, the frequency sweep test was used to evaluate the viscoelastic behavior of yogurt samples at various frequencies ranging from 0.1 to 20 Hz at a constant strain within the linear viscoelastic region (<1%). The viscoelastic parameters storage (\( G' \)) and loss (\( G'' \)) moduli were recorded using oscillation-time test at a frequency of 1.0 Hz. Three time segments were applied with the following conditions: (1) segment 1:200 s, stress 0.2 Pa; (2) segment 2:60 s, stress 50 Pa; and (3) segment 3:400 s, stress 0.2 Pa.

**In Vitro Digestion by INFOGEST2.0**

The in vitro digestion of yogurt samples was performed following the procedure previously established by INFOGEST (Brodkorb et al., 2019). Briefly, yogurt samples (3 g) were subjected to in vitro oral (0.5 mL amylase 75 U/mL, salivary fluid, and 25 µL of 0.3 M calcium chloride for 2 min), gastric (1.6 mL pepsin 2,000 U/mL, gastric lipase 60 U/mL, gastric juice pH 3.0, and 5 µL of 0.3 M calcium chloride for 2 h), and intestinal (5 mL pancreatin 100 U/mL, 2.5 mL bile 10 mmol/L, duodenal juice pH 7.0, and 40 µL of 0.3 M calcium chloride for 2 h) digestion. A dialysis membrane (10 kDa) containing 25 mL of sodium bicarbonate solution (0.5 M) was immersed in the digesta at the beginning of the intestinal digestion stage (Rodríguez-Roque et al., 2014). Finally, the internal fraction (10 kDa) inside the dialysis membrane, referred to as the bioaccessible fraction, was kept at −20°C for further analysis. The previously prepared water-soluble extract of each treatment was analyzed as the undigested sample.

**Antioxidant Activities**

The following antioxidant activities of yogurt samples before (undigested) and after (bioaccessible) in vitro digestion were determined as reported by Arise et al. (2016), unless otherwise was mentioned.

**2,2-Diphenyl-1-picrylhydrazyl and 2,2’-Azino-Bis (3-Ethylbenzothiazoline-6-Sulfonic Acid)**

The radical scavenging activities of the undigested and bioaccessible fractions were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid, ABTS•⁺) using the following equation:
Scavenging activity(%) = \left[1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of blank}}\right] \times 100. \quad [3]

**Superoxide Anion Scavenging.** The assay of SAS was performed by mixing Tris-HCl buffer (4.5 mL, 50 mM, at pH 8.2) and deionized water (4.2 mL) and incubating the mixture at 25°C for 20 min. Next, 0.5 mL of the preheated samples (undigested and bioaccessible fractions) and pyrogallol (0.3 mL; 3 mM) dissolved in 10 mM HCl (control) were added and thoroughly mixed. Ascorbic acid (0.1 mg/mL) was used as a positive control. The absorbance of the mixtures was then measured at 320 nm, and the SAS activities were determined based on the absorbance readings and the following established equation:

\[
\text{SAS(\%)} = \frac{\Delta A/\text{min}_{\text{blank}} - \Delta A/\text{min}_{\text{sample}}}{\Delta A/\text{min}_{\text{blank}}} \times 100, \quad [4]
\]

where \(\Delta A\) refers to the change in absorbance.

**Superoxide Dismutase.** The experiments were performed at 25°C, and the absorbance was recorded every 30 s for a period of 5 min. The superoxide dismutase (SDM) activity rate was determined by mixing 0.1 M Tris-HCl buffer, deionized water, and the sample with pyrogallol. The SDM activity was expressed as the amount of enzyme that inhibited 50% of pyrogallol oxidation per minute in each mL of the reaction solution (Marklund and Marklund, 1974).

**Hydroxyl Radicals Scavenging.** 1,10-phenanthroline (12.5 mM) was dissolved in sodium phosphate buffer (20 mM, pH 7.4) while FeSO_4 (2.5 mM) and hydrogen peroxide (20 mM) were each separately dissolved in distilled water. An aliquot (50 µL) of undigested, bioaccessible fraction, or buffer (control) was added to 50 µL of 1, 10-phenanthroline and 50 µL of FeSO_4. To initiate reaction, 50 µL of hydrogen peroxide solution was added to the mixture, which was then covered and incubated at 37°C for 1 h with constant shaking. Thereafter, the absorbance of the mixtures was measured at 536 nm every 10 min for a period of 1 h. The absorbance was also determined for a blank (does not contain sample or H_2O_2) and a control (does not contain sample). The hydroxyl radicals scavenging (HRS) activity of undigested and bioaccessible fractions was calculated using the following equation:

\[
\text{HRS(\%)} = \frac{\Delta A/\text{min}_{\text{blank}} - \Delta A/\text{min}_{\text{sample}}}{\Delta A/\text{min}_{\text{blank}}} \times 100, \quad [5]
\]

where \(\Delta A\) is change in absorbance.

**Metal Chelating.** The metal chelating (MC) was determined by adding 0.5 mL of a ferrous chloride solution (0.2 mM) to 0.1 mL of undigested and bioaccessible fractions, and the reaction was then initiated by the addition of 0.2 mL of ferrozine (5 mM). The mixture was incubated for 10 min at room temperature, and the absorbance was measured at 562 nm.

\[
\text{MC(\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100. \quad [6]
\]

**Lipid Oxidation Inhibition.** The inhibitory activity of lip oxidation inhibition (LO) was determined according to Shabbir et al. (2013). Briefly, 50 mM phosphate buffer (pH 7.0) were mixed with the undigested or bioaccessible fractions, and the mixture was centrifuged for 60 min at 12,000 × g at 4°C to obtain the supernatant (100 µL). Then, the supernatant was mixed with 2 mL of TBA–TCA–HCl reagent and boiled for 30 min, followed by cooling, and measuring the absorbance at 535 nm compared with a control (without sample). LO activity was calculated using the following equation:

\[
\text{LO inhibition (\% )} = \left[1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right] \times 100. \quad [7]
\]

**Ferric Reducing-Antioxidant Power.** Briefly, 250 µL of undigested and bioaccessible fractions were mixed with 250 µL of 0.2 M sodium phosphate buffer (pH 6.6) and 250 µL of potassium ferricyanide solution (1%) prepared in distilled water. A control reaction was also prepared containing only buffer and ferricyanide. The resulting mixture was incubated at 50°C for 20 min, after which 250 µL of 10% aqueous trichloroacetic acid was added. Then, 250 µL aliquot of the reaction mixture was combined with 50 µL of aqueous ferric chloride solution (0.1%), followed by adding 200 µL of distilled water. The resulting mixture was allowed to stand at room temperature for 10 min and centrifuged at 1,000 × g for 10 min. The supernatant was then analyzed for absorbance at 700 nm.

**Total Antioxidant Capacity.** The total antioxidant capacity (TAC) was determined according to the procedure reported by Shabbir et al. (2013). In brief, 100 µg of the samples were mixed with 1 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate), covered and incubated in a boiling water bath at 95°C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution was measured at 765 nm against a blank. The
TAC (%) was calculated according to the following equation:

\[
TAC(\%) = \left( \frac{\text{Absorbance of sample} - \text{Absorbance of control}}{\text{Absorbance of control}} \right) \times 100.
\]

[8]

Reducing Power. In brief, a volume of 1 mL of the samples was mixed with 2.5 mL of ultrapure water and 2.5 mL of potassium ferricyanide (10 mg/mL) and incubated at 50°C for 20 min. The resulting reaction solution (2.5 mL) was then mixed with 2.5 mL of ultrapure water and 0.5 mL of ferric chloride (1 mg/mL). After 10 min of incubation in the dark, the absorbance of the mixtures was measured at 700 nm. A blank sample consisting of only water was also used for comparison (Cui et al., 2022).

Biological Activities

\(\alpha\)-Amylase and \(\alpha\)-Glucosidase Inhibitions.\n
The inhibitions of \(\alpha\)-amylase and \(\alpha\)-glucosidase in the undigested and bioaccessible fractions were determined as described by Kim et al. (2004). The percentage of inhibition was determined as follows:

\[
\text{Inhibition(\%)} = \left( 1 - \frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{Absorbance of control}} \right) \times 100.
\]

[9]

Angiotensin-Converting Enzyme Inhibition. The ACE inhibition (%) of the undigested and bioaccessible fractions was performed following the procedure reported by Liu et al. (2018). Briefly, 10 \(\mu\)L of the sample was mixed with 45 \(\mu\)L of hippuryl-histidyl-leucinesolution, preincubated for 5 min at 37°C, and then incubated with 10 \(\mu\)L of ACE (0.1 U/mL) in borate buffer (0.1 M) containing 0.3 M NaCl for 30 min at 37°C. Then, 85 \(\mu\)L of 1 M HCl was added to all samples to stop the reaction, except the control blank in which 85 \(\mu\)L of 1 M HCl was added before preincubation. Ethyl acetate (1 mL) was used to extract the formulated hippuric acid. Afterward, the ethyl acetate layer (800 \(\mu\)L) was collected and evaporated at 100°C for 30 min. The residue was dissolved in distilled water (800 \(\mu\)L), and the absorbance was recorded at 228 nm. The percentage of ACE-inhibition activity was determined as follows:

\[
\text{ACE-inhibition(\%)} = \left( 1 - \frac{A_c - A_t}{A_{bc} - A_t} \right) \times 100,
\]

where \(A_t\) refers to absorbance without sample solution (sample replaced by buffer solution), \(A_c\) indicates the absorbance in the presence of ACE and sample solution, and \(A_{bc}\) refers to the absorbance of the blank (HCl was added before ACE addition).

Cholesterol Removal. The cholesterol removal (CR\%) of the undigested and bioaccessible fractions was determined according to the method reported by Miremadi et al. (2014), and calculated as follows:

\[
\text{CR(\%)} = \left( \frac{100 - \text{residual cholesterol at each incubation interval}}{100} \right) \times 100.
\]

[11]

Degree of Hydrolysis. The degree of hydrolysis (DH\%) of the undigested and bioaccessible fractions was determined according to the method of Sah et al. (2014).

Statistical Analysis

The obtained data were expressed as mean values ± standard deviations (SD). Two-factor ANOVA test was used to study the effect of WPI addition level at the same storage period and to evaluate the effect of storage period at the same addition level of each parameter \((P < 0.05)\). Moreover, Tukey’s test \((P < 0.05)\) was performed to compare means at similar storage periods. In addition, principal component analysis (PCA) was applied to evaluate the structural correlation of variables and observations to understand the relationship between different yogurt treatments. The statistical analysis was performed using JMP v16.2 (SAS Institute Inc.).

RESULTS AND DISCUSSION

Titratable Acidity, pH Value, and Water Holding Capacity

Table 1 shows the titratable acidity, pH values, and WHC of yogurt treatments. CM yogurt samples with and without WPI exhibited similar acidity values (~1.0%) at the first day of storage, while BM yogurt exhibited a slightly lower acidity (0.9%). After 15 d, the acidity of all yogurt samples significantly increased, and BM yogurt showed the lowest acidity value (1.3%). Shori and Baba (2014) reported minor differences in the acidity of BM and CM yogurts. The results show that the acidity of CM yogurt increased with the addition level of WPI at d 15. Furthermore, Lee and Lucey (2010) reported that the use of whey proteins increased...
Whey protein isolate (WPI) is known to enhance the structural characteristics of camel milk yogurt (CM) by increasing the buffering capacity of its constituents, leading to a more stable pH level. This results in reduced syneresis (Amatayakul et al., 2006). The supplementation of CM with WPI has been reported to reduce the decrease in the pH value and to enhance the structural characteristics of CM yogurt (Ali et al., 2021a). It has been observed that the pH of yogurt is influenced by the buffering capacity of its constituents, such as lactic acid, caseins, and inorganic phosphate. The higher the buffering capacity, the more limited the changes in pH due to acid production during storage. As reported by Salaïn et al. (2005), substituting skim milk powder with whey proteins can increase the buffering capacity of yogurt at pH 4.0. This may account for the limited changes in pH values despite higher values in titratable acidity.

The WHC of yogurt is a marker of its ability to retain whey in the curd (Lee and Lucey, 2010). Despite being a natural phenomenon, syneresis is adversely observed by consumers, who usually relate it to undesirable variations in yogurt quality and as a sign of deterioration. Therefore, consumers favored yogurt with a low degree of syneresis. The WHC of yogurt treatments during storage, specifying the syneresis amount, is presented in Table 1. The results showed significant differences in the WHC of yogurt samples, and the control yogurt exhibited the lowest values making up 31.7% and 31.3% after 1 and 15 d of storage, respectively. WPI obviously affected the WHC of yogurt, because CMW5 treatment had higher WHC values (68.8% and 64.7%) than the treatment CMW3 (46.9% and 56.8%) after 1 and 15 d, respectively. The fortification of CM with WPI reduced yogurt syneresis, indicating that increased levels of whey proteins in proportion to casein caused less syneresis (Amatayakul et al., 2006). Furthermore, as depicted in Table 1, the incorporation of WPI in CM yogurt resulted in a similar WHC as that of BM yogurt. This suggests that the addition of WPI has the potential to enhance the structural characteristics of CM yogurt. The supplementation of milk with whey proteins and subsequent heat treatment had a positive influence on the WHC of yogurt, signifying an increase in the firmness of protein network, causing immobilization of a great quantity of free water and fewer vulnerability to liberating water (Mahomud et al., 2017a). These findings may be of great interest to manufacturers of CM products.

**Texture Profile Analysis**

After the heat treatment and acidification of milk, the protein aggregates develop a 3-dimensional network (Guyomarc’h et al., 2009). The failure of CM to develop a yogurt structure has been reported (Mbye et al., 2022). This was attributed to the compositional properties of CM, such as the large micelle size, low ratio of κ-CN-to-β-CN (≈0.33 in BM vs. ≈0.05 in CM), various whey protein components, and the higher proteolytic activity compared with BM. Although the required decline in the pH value was not influenced by CM and was achieved after 3 to 4 h, CM gelation needed a longer time because 95% of κ-CN needed to be hydrolyzed to initiate gelation (Kamal-Eldin et al., 2020). The different texture properties, including hardness, adhesiveness, and resilience of yogurt treatments after 1 and 15 d are summarized in Table 2. At d 1, there were significant differences in the hardness of yogurt treatments. The control sample had the lowest hardness.

<table>
<thead>
<tr>
<th>Yogurt1</th>
<th>Titratable acidity (% of lactic acid)</th>
<th>pH</th>
<th>WHC (%)</th>
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<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 15</td>
<td>Day 1</td>
</tr>
<tr>
<td>CM</td>
<td>1.0 ± 0.0&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.9 ± 0.5&lt;sup&gt;A&lt;/sup&gt;</td>
<td>4.4 ± 0.2&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>CMW3</td>
<td>1.0 ± 0.0&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.1 ± 0.4&lt;sup&gt;A&lt;/sup&gt;</td>
<td>4.3 ± 0.0&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>CMW5</td>
<td>1.0 ± 0.0&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.3 ± 0.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>4.4 ± 0.0&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>BM</td>
<td>0.9 ± 0.1&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.3 ± 0.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>4.3 ± 0.1&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
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</table>

<sup>a–c</sup>Means with different lowercase superscripts in the same column are significantly different for each parameter, and <sup>A–B</sup> values with different uppercase superscripts in the same row are significantly different for each parameter.

<sup>1</sup>CM = camel milk yogurt (control); CMW3 = camel milk yogurt containing 3% of whey protein isolate; CMW5 = camel milk yogurt containing 5% of whey protein isolate; BM = bovine milk yogurt.
(27.2 g) compared with BM yogurt (174 g), and the addition of WPI significantly affected yogurt hardness. Yogurt samples containing 5% of WPI exhibited the highest hardness values representing 399 g and 291.7 g after 1 and 15 d, respectively. After 15 d, the hardness of all yogurt samples significantly (P < 0.05) decreased except for CM control sample. Yogurt hardness tends to increase over time during storage, and this can be explained by post-acidification. Post-acidification leads to larger casein particles due to increased hydrophobic and electrostatic interactions among proteins, which can ultimately result in a firmer texture (Deshwal et al., 2021). Nastaj et al. (2019) reported that the use of whey proteins increased the hardness of yogurt. Similarly, Yildiz-Akgül (2018) revealed that the addition of WPI increased yogurt hardness and decreased the level of syneresis. Moreover, Matumoto-Pintro et al. (2011) reported that the increased curd strength of yogurt prepared with WPI was attributed to the high content of thiol groups.

A similar trend was seen for the adhesiveness because the control CM yogurt showed lower values (0.19 mJ and 0.20 mJ after 1 and 15 d, respectively) than BM yogurt (2.70 mJ and 0.50 mJ after 1 and 15 d, respectively). CM supplementation with WPI increased yogurt adhesiveness. After 15 d, the adhesiveness of all yogurt samples had significantly decreased except the control sample. Similar findings were reported by Nastaj et al. (2019), who found that the adhesiveness of yogurt increased with increasing supplementation level of milk with WPI. Regarding resilience, the results show that CM yogurt samples had significantly higher values than BM yogurt. The addition of WPI decreased yogurt resilience. After 15 d, the resilience of all yogurt treatments increased. The variations in the textural properties of CM and BM yogurt curd may be ascribed to the lack of β-lactoglobulin and the very low content of κ-CN in CM (Lajnaf et al., 2018). Also, the weak curd structure in CM yogurt may be affected by the relative content of casein fractions (Kamal et al., 2017), the weak interaction between serum proteins and casein (Meena et al., 2014). CM also varies from BM in its total protein content and the relative abundances of proteins component (Hailu et al., 2016). The structure of low-fat yogurt was improved by using undenatured whey proteins (Jørgensen et al., 2015).

### Rheological Properties

**Apparent Viscosity.** Flow sweep and shear tests of the fermented experimental yogurts stored at 4°C after 1 and 15 d are presented in Figure 1a and Figure 1b, respectively. The Power law fit depicted the predicted model verified the experimental data (Supplemental Figures S1a and S1b; https://doi.org/10.17632/28jy32m4sy.1; Ayyash, 2023; Supplemental Table S2;). In general, the apparent viscosity decreased by increasing the shear rate observed in all samples (Bridges and Robinson, 2020). However, the viscosity of the control CM yogurt was lower than other yogurts for all values of the applied shear rates during d 1 and d 15. Moreover, the control CM yogurt showed a shear thickening behavior. The addition of WPI increased the viscosity of CM yogurt. The viscosities of the experimental yogurts had the following order CMW5 > CMW3 > BM > CM. The increased viscosity by increasing the level of whey proteins can be due to the protein contents of the product and the heat treatment of the milk-base.
supplemented with WPI (Remeuf et al., 2003). The increase in yogurt viscosity by adding WPI may be attributed to protein aggregates caused by the heat-induced interactions between the denatured whey proteins and casein micelles through disulfide bonds (Lucey et al., 1999). The effect of milk supplementation with dairy proteins on the increase in viscosity has been previously reported (Akalın et al., 2012). Also, it was shown that all experimental yogurts exhibited a shear thinning behavior as the viscosity decreased by increasing the shear rate except the control yogurt which showed a shear thickening (Figures 1a and 1b). CM yogurts exhibited a Newtonian behavior at low shear rate (<10 1/s) and some kind of shear thickening at high shear rate due to the increase in the particle-particle interactions that where the solid particles cannot readily flow past each other and begin to clog causing a rise in viscosity. The critical shear rate was relatively the same at d 1 and d 15.

**Viscoelastic Properties (Frequency Sweep).** The viscoelastic characteristics of yogurt samples at various frequencies tested by frequency sweep after 1 and 15 d are presented in Figure 1 (c, d). All samples had storage modulus (G') higher than loss modulus (G'″) within the investigated frequency range (1−100 rad/s). This implies that all experimental yogurts had higher elastic than viscous properties. On d 1, the G' of the treatment CMW5 was similar to BM yogurt, followed by CMW3, while the control yogurt had the lowest G' values. The higher G' for CM containing 5% of WPI indicates the maximum gelation capability. Similarly, G'″ values of CMW5 and BM were higher, followed by CMW3. These results imply that the addition of WPI enhanced the elastic properties of CM yogurt. This may be attributed to the fact that casein micelles were mainly linked by particle-to-particle attachment in large chains with relatively small interspaced cavities instead of by particle fusion into aggregates (Sandoval-Castilla et al., 2004). Complexes of soluble proteins increased yogurt rheological properties more than micelle-bound complexes. It was reported that yogurt prepared with the addition of 2% WPI and heated skim milk had significantly higher G' and firmness than skim milk yogurt (Mahomud et al., 2017b).

On d 15, G' and G'″ of CMW3, CMW5, and BM yogurt treatments had a similar trend but slightly lower values than d 1 (Figure 1d). CM yogurt displayed a shift from a watery phase to a more viscous and gel-like phase as frequency values increased and by increasing the concentration of WPI. The addition of WPI may result in the development of the gel network due to the interaction with milk caseins. The tan delta (δ, loss factor) is displayed in Supplemental Figures S1c and S1d (supplementary material). It was shown that BM yogurt and CM treatments supplemented with WPI exhibited similar behavior at d 1 and 15. The tan δ of BM and CMW5 treatments remained constant with the increase in frequency. While CMW3 showed a strong peak around a frequency of 85 rad/s, then decreased. It was shown that at d 1, tan δ of the control yogurt gradually decreased with the increase in frequency. While at d 15, the tan δ increased in the beginning, then decreased at a frequency higher than 10 rad/s. Marafon et al. (2011a) reported that the G' values of control yogurt decreased during storage, which elucidated the increase in tan δ reaching a peak on d 28. The slight decline in tan δ only on d 14 of storage of yogurt samples fortified with dairy proteins was explained by the stable increase in the G' value during the same time.

**Thixotropic Behavior.** An oscillatory time sweep is a quick test to see if a system possesses time-dependent rheological characteristics. This test keeps track of specific viscoelastic characteristics over time to see whether and how material qualities change after it has been loaded. Evaluation of material's behavior over time can be directly observed by choosing proper parameters for control variable (a value of either oscillatory stress or strain found within the linear viscoelastic area), frequency, and temperature of interest. When thixotropic materials are sheared at constant shear rates, the viscosity will decline over time, indicating a progressive structural breakdown (Abu-Jdayil, 2003). Thixotropy is observed in specifically weak structures such as yogurt, in which the 3-dimensional network is destroyed by shear. Because the energy needed to break down the yogurt structure is relative to the hysteresis area, the difference between the areas under shear stress-shear rate curves can be used to assess thixotropy (Cruz et al., 2013).

An oscillatory time sweep of yogurt treatments at d 1 and d 15 is shown in Figure 2, where G' and G'″ are displayed. It was shown that the control yogurt had the lowest G' and G'″ values during storage. G' and G'″ values of other yogurt treatments were different because the G' values of CMW3, CMW5, and BM yogurt treatments were higher than G'″ values at d 1 and d 15. The lower G'″ values mean that yogurt gels comprising added dairy proteins exhibited solid-like properties (Purwandiari et al., 2007). In general, G' and G'″ decreased after 200 s, and then increased and remained constant until 600 s. The addition of WPI increased G' and G'″ values, and BM yogurt had the highest values. BM yogurt and CMW5 exhibited similar G' and G'″ values at d 1 and d 15. Marafon et al. (2011b) reported that milk-base fortification with dairy proteins increased G' and G'″ moduli compared with control yogurt. This trend demonstrates that the material’s properties constantly increase until a steady value is obtained at 600 s. This is illustrated...
by the increased elastic modulus, indicating that the material’s structure has reached an equilibrium state. Once this time is determined, any subsequent tests on this material should be set up with the same pre-shear conditions, followed by the required equilibration time (i.e., 600 s). It was seen that yogurt treatments showed a thixotropic behavior, as previously reported for other dairy products (Debon et al., 2010).

**Antioxidant Activities**

Table 3 shows the radical scavenging activities (%) as measured by the DPPH and ABTS tests of yogurt treatments before and after in vitro digestion during storage. At d 1, both the undigested and bioaccessible samples from CM yogurt displayed higher DPPH values than that of BM yogurt. The DPPH of yogurt treatments significantly increased after digestion, because the bioaccessible samples showed higher DPPH activity than undigested samples. The addition of WPI decreased the DPPH value in yogurt samples. It was shown that the DPPH value of yogurt treatment CMW3 was lower than that of CMW5 treatment before digestion. After 15 d of storage, the DPPH values of the undigested samples decreased, while a diverse trend was seen for bioaccessible samples, in which the DPPH increased during storage except the control sample which decreased from 86.6% to 76.3% after 15 d. This indicates that the antioxidant activity of yogurt was affected by the hydrolysis of peptide and protein by digestive enzymes.

Regarding the ABTS, the results show that the bioaccessible samples had a significantly \( (P < 0.05) \) higher ABTS scavenging than the undigested samples for all treatments after 1 and 15 d of storage (Table 3). The ABTS was affected by the addition of WPI particu-
larly in the undigested samples, while the bioaccessible samples exhibited comparable values during storage for all yogurt treatments. The higher DPPH and ABTS scavenging levels of the undigested and bioaccessible samples from CM yogurt relative to BM yogurt could be attributed to CM proteins susceptibility to proteolysis by the digestive enzymes (Ayyash et al., 2021a). Casein peptides with high contents of tryptophan, histidine, tyrosine, and methionine are released during in vitro digestion of dairy products, revealing remarkable antioxidant activities (Lamothe et al., 2019; Simonetti et al., 2021).

Regarding the SAS of yogurt treatments before and after in vitro digestion, the undigested samples exhibited significantly ($P < 0.05$) higher values compared with the bioaccessible fractions in all yogurt treatments. Undigested samples from BM yogurt showed the highest SAS value (87.4%) at d 1. After 15 d of storage, the SAS increased in all treatments except BM yogurt sample which decreased to 80.8% (Table 3). The bioaccessible fractions showed lower SAS values, and BM yogurt samples exhibited the lowest value (8.9%) after 15 d. Superoxide anions are free radicals including unpaired electrons on oxygen that indirectly initiate the oxidation of lipids because of hydrogen peroxide and superoxide, acting as ancestors of hydroxyl radical and singlet oxygen (Karadag et al., 2009). Table 3 also shows the SDM activity of yogurt treatments before and after in vitro digestion. The results revealed significant ($P < 0.05$) differences in the SDM activity for undigested and bioaccessible yogurt samples after 1 and 15 d of storage. The bioaccessible showed higher SDM activity values than undigested samples of all yogurt treatments. Furthermore, it was shown that the

![Figure 2. Thixotropic behavior of yogurt treatments after 1 and 15 d of storage. CM = yogurt camel milk only (red), CMW3 = yogurt camel milk+3% WPI (black), CMW5 = yogurt camel milk+5% WPI (green), and BM = yogurt bovine milk only (blue). G’ (a and b) and G” (c and d). Three time segments were applied with the following conditions: first time (200 s, stress 0.2 Pa), second time (60 s, stress 50 Pa), and third time (400 s, stress 0.2 Pa).]
SDM value of yogurt treatments before and after in vitro digestion was influenced by the addition of WPI.

The HRS activity of yogurt treatments before and after in vitro digestion during storage is shown in Table 3. We detected significant ($P < 0.05$) differences between the different treatments. The undigested sample from BM yogurt had the lowest HRS value making up 5.6% and 4.9% after 1 and 15 d, respectively. After in vitro digestion, the HRS significantly increased because bioaccessible samples showed higher HRS activity values.
than the undigested samples for all yogurt treatments. Similarly, the MC significantly ($P < 0.05$) varied among yogurt treatments before and after digestion, and BM yogurt exhibited the lowest values representing 9.2% and 20.1% after 1 and 15 d, respectively. We observed no significant differences in the MC values of the bioaccessible samples after 1 and 15 d of storage. However, LO inhibition decreased after in vitro digestion of yogurt because undigested samples showed higher LO values as compared with the bioaccessible samples, and BM yogurt showed the lowest values (7.9% and 23.5% after 1 and 15 d, respectively). Also, it was shown that LO inhibition varied by the addition of WPI.

The FRAP of yogurt treatments before and after in vitro digestion is shown in Table 3. This parameter is a helpful marker of the antioxidant level to reduce oxidative stress caused by reactive oxygen groups (Küçük et al., 2007). The undigested samples exhibited significantly ($P < 0.05$) lower FRAP values compared with the bioaccessible fractions. The addition of WPI significantly affected this parameter particularly in the undigested samples after 15 d of storage. BM yogurt undigested sample had the lowest FRAP values accounting for 9.0 µg/mL and 10.2 µg/mL after 1 and 15 d, respectively. CMW3 and CMW5 of the undigested samples had higher FRAP activity than CM and BM yogurt treatments. This indicates that WPI addition increased the FRAP activities of the fermented CM. This may be attributed to the antioxidant activity of whey protein-derived peptides specifically in iron-catalyzed food matrix, indicating their action in slowing or preventing the peroxidation of lipids and fatty acids (Iskandar et al., 2015). Furthermore, lactoferrin was reported as a key factor in the scavenging activity of whey proteins (Ünal and Akalın, 2012).

Regarding the TAC, it was shown that there were significant ($P < 0.05$) differences in the TAC values before and after in vitro digestion of yogurt treatments (Table 3). The TAC of bioaccessible fractions was significantly ($P < 0.05$) higher than that of undigested samples after 1 and 15 d of storage. The results show that the TAC of yogurt samples varied with WPI addition to CM. The degradation of antioxidants usually reduce the antioxidant capacity of foods (Yuksel et al., 2010). The TAC of fermented dairy products depends on peptides content, in addition to certain amino acids and metalloproteins (Li et al., 2020). The ferric RP of yogurt treatments before and after in vitro digestion is also shown in Table 3. The control treatment exhibited significantly ($P < 0.05$) higher RP values as compared with BM yogurt. Furthermore, the RP significantly increased after in vitro digestion, because undigested samples showed significantly lower values than bioaccessible samples after 1 and 15 d. In addition, the effect of WPI on this parameter was noticeable, because CM yogurt samples containing WPI showed lower values than the control CM yogurt treatment.

**Inhibitions of α-Amylase and α-Glucosidase.** The activity of α-amylase and α-glucosidase inhibitions is an indirect parameter to evaluate the antidiabetic aspects of fermented milk by reducing the hydrolysis of carbohydrates (Tundis et al., 2010; Donkor et al., 2012). Figures 3a and 3b respectively show the inhibitions of α-amylase and α-glucosidase of yogurt treatments before and after in vitro digestion. At d 1, the undigested CM yogurt treatments exhibited significant differences in their α-amylase inhibitions. The control treatment showed the highest α-amylase inhibition (44.1%), and the inhibition value decreased with the addition of WPI. The results show that α-amylase inhibition increased in undigested yogurt treatments containing WPI after 15 d, and BM yogurt had the lowest value. In addition, bioaccessible fractions showed lower α-amylase inhibition values as compared with the undigested samples. Also, it was shown that there were no significant differences between the bioaccessible samples from different yogurt treatments after 1 and 15 d of storage (Figure 3a).

The α-glucosidase inhibition of the undigested and bioaccessible samples is shown in Figure 3b. The results show that there were no significant differences in the inhibition values of the undigested samples at d 1, and the control sample had the lowest value (9.6%). The bioaccessible samples exhibited significantly ($P < 0.05$) higher α-glucosidase inhibition values after 1 and 15 d, and BM yogurt treatments had the lowest inhibition value. The variations in the inhibitions of α-amylase and α-glucosidase in the bioaccessible fractions from all yogurt treatments reduced after in vitro digestion. This indicates that similar quantities of bioactive compounds possessing comparable α-amylase and α-glucosidase inhibitions were released by the hydrolytic actions caused by digestive enzymes (Ayyash et al., 2021b). The overall inhibitions of α-amylase and α-glucosidase may be ascribed to bioactive peptides formed by the proteolytic enzymes released by yogurt strains (Gomes da Cruz et al., 2009).

**ACE Inhibition and CR Rate.** The ACE inhibitory is an in vitro index of the antihypertensive characteristics of fermented milk (Gobbetti et al., 2004). Figure 3c displays the ACE inhibitions of the undigested and bioaccessible samples after 1 and 15 d of storage. We detected no significant differences in
the ACE inhibitions of the undigested samples at d 1, which increased after 15 d with no significant differences between CM yogurt treatments. BM yogurt exhibited the lowest inhibition value before digestion after 15 d. It was shown that the ACE inhibition significantly (\( P < 0.05 \)) increased after in vitro digestion of yogurt samples. The different yogurt treatments showed similar inhibition values after 1 and 15 d, and BM yogurt had the lowest inhibition value. The higher ACE inhibition of CM may be due to its higher proteolytic activities supporting the assumption that CM proteins are more vulnerable to hydrolysis by the proteolytic enzymes released by yogurt starter cultures (Alhaj, 2017; Ayyash et al., 2018). Moreover, the high ACE inhibition in CM yogurt was attributed to the higher content of proline in CM caseins (El-Salam and El-Shibiny, 2013; Moslehishad et al., 2013).

The CR activities of the undigested and bioaccessible fractions after 1 and 15 d of storage are shown in Figure 3d. The results show that the undigested samples of all yogurt treatments exhibited similar CR activities after 1 and 15 d with no significant differences. CR activity decreased in the bioaccessible fractions, and BM yogurt showed the highest CR value compared with other yogurt treatments. The bioactive peptides in CM yogurt might have a positive influence on reducing cholesterol levels. Also, CM contains orotic acid, which assumed to reduce the level of cholesterol in the blood (Kumar et al., 2016). Fermented CM might reduce the levels of triglycerides and cholesterol by several mechanisms, such as the potential of intestinal flora to inhibit cholesterol absorption, and the ability of lactic acid bacteria to inhibit the enzymes of cholesterol synthesis, and thereby reducing the level of cholesterol (Zhang et al., 2008; Yahya et al., 2018).

**Degree of Hydrolysis.** The DH of yogurt treatments before and after in vitro digestion is presented in Supplemental Figure S2 (https://doi.org/10.17632/28jy32m4sy.1; Ayyash, 2023). It was shown that there were no significant differences in yogurt samples before and after digestion.
Figure 4. Principal component analysis (PCA) of the variables (loading plot, A) and observations (score plot, B) of yogurt treatments.
in vitro digestion during storage, and BM yogurt had significantly lower DH value (27.3% and 27.2% after 1 and 15 d, respectively) compared with CM yogurt samples. The DH was not significantly affected by the addition of WPI. After in vitro digestion, the DH of yogurt treatments decreased (in the range between 22.5% and 24.2%) with no significant differences among the different treatments. CM yogurt had a weak acid-induced curd causing a weak gel in the stomach, where proteins are more vulnerable to gastric proteases. It was reported that the higher DH of CM and BM yogurts might be ascribed to the hydrolysis of casein by proteolytic activity of the starter culture enzymes (Ayyash et al., 2021a). Moreover, the DH can be affected by the type of yogurt starter cultures which produce various proteolytic enzymes (Park, 2009; Ayyash et al., 2018).

**Principal Component Analysis**

PCA was presented to investigate the relation between antioxidant activity parameters (DPPH, ABTS, SAS, SDM, HRS, MC, LO, FRAP, TAC, and RP), DH, inhibitions of α-amylase and α-glucosidase, ACE inhibition, and CR. Two principal components explained about 50.5% of the total variation in the results (PC1 33.8% and PC2 16.7%). The loading plots presented in Figure 4A reveal positive connections between antioxidant activity parameters, α-amylase and α-glucosidase inhibitions, and DH, indicating that the compounds responsible for these factors could share similar structures and attributes. In addition, the score plots shown in Figure 4B indicate obvious differences between the experimental yogurt (CM, CMW3, CMW5, and BM) and between the storage times (d 1 and d 15). At d 1, the control sample and yogurt prepared with 3% of WPI (CMW3) were obviously distinguished from yogurt prepared with 5% of WPI (CMW5) and BM yogurt, but after 15 d of storage, the situation noticeably changed, and CMW3 and CMW5 samples became closer to each other and the control sample and distinguished from BM yogurt. This suggests that adding WPI up to 5% resulted in yogurt with distinct characteristics compared with yogurt prepared with BM.

**CONCLUSIONS**

The characteristics of CM yogurt supplemented with WPI (3% and 5%) compared with BM yogurt after 1 and 15 d of storage were evaluated. The CM yogurt containing WPI showed higher hardness, representing 107.7 and 399.9 g at d 1 and 81.7 and 291.7 g at d 15 for CMW3 and CMW5, respectively. Besides, CM yogurt supplemented with WPI can improve or reduce antioxidant and biological activities through in vitro digestion. At d 1, DPPH values increased from 19.0%, 16.7%, 17.9%, and 15.8% before digestion to 86.6%, 65.2%, 67.9%, and 59.2% after in vitro digestion of CM, CMW3, CMW5, and BM, respectively. The differences in the strength of CM and BM yogurt curd may be ascribed to the lack of β-LG and the very low content of κ-CN in CM. Control yogurt displayed lower apparent viscosity, G' and G" values than other treatments. Further studies are recommended to evaluate the microbiological quality and microstructure of CM yogurt as affected by WPI.

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