Health-promoting benefits of low-fat akawi cheese made by exopolysaccharide-producing probiotic *Lactobacillus plantarum* isolated from camel milk

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ABSTRACT

Lactic acid bacteria isolated from camel milk exhibit remarkable probiotic and exopolysaccharide (EPS)-producing characteristics. The health-promoting benefits of exopolysaccharide-producing probiotic *Lactobacillus plantarum* isolated from camel milk used for making low-fat akawi cheese were investigated. Three low-fat akawi cheeses were made using traditional culture (non-EPS-producing, EPS[−]), commercial EPS-producing (MEPS⁺), and camel milk EPS-producing (CEPS⁺) cultures. α -Amylase and α -glucosidase inhibitory activities, antioxidant activities, angiotensin-converting enzyme (ACE) inhibition, and antiproliferative activity were determined. Cheese made with CEPS⁺ culture exhibited comparable α -amylase inhibition to that of cheeses made with MEPS⁺. Scavenging rates of cheese made with EPS⁺ cultures were higher than those of cheese made with EPS[−] cultures. The percentage of α -glucosidase inhibition ranged from >45% at 0 d to ~55% at 21 d of storage. After 7 d of storage, the scavenging rate in CEPS⁺ cheese increased >60% by ABTS assay [2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid)] and >20% by DPPH assay (1,1-diphenyl-2-picrylhydrazyl). Throughout storage, cheese made with EPS⁺ cultures showed higher ACE-inhibition activity compared with EPS[−] cultures. Cheese made with CEPS⁺ showed ACE inhibition >70% after 7 d of storage. Antiproliferation activity of CEPS⁺ cheese increased from 38 to 48% during 7 d of storage and was maintained above 45% with prolonged storage. Low-fat akawi cheese produced with these cultures exhibited similar or greater health-promoting benefits compared

with cheese made using commercial starter cultures. Therefore, incorporation of these cultures in food is promising for commercial uses.

Key words: probiotic, exopolysaccharide (EPS), health-promoting benefit, antiproliferative

INTRODUCTION

Lactic acid bacteria (LAB) are extensively used to produce a wide range of dairy products such as fermented milk and cheeses. The proteolytic, lipolytic, and glycolytic activities of LAB (Ruas-Madiedo et al., 2002) contribute to flavor, texture, microstructure, and nutritious and health-promoting benefits of fermented dairy products, including cheese. Exopolysaccharides (EPS) produced by several LAB may significantly influence cheese attributes such as chemical composition, viscoelastic properties, sensory attributes, and health-promoting properties. These EPS are either attached on the bacterial surface (capsular EPS) or released to the growth environment (ropy EPS), where they can act as a natural thickening agent or stabilizer (Duboc and Mollet, 2001). Therefore, EPS may improve the rheological and textural properties of low-fat cheese. In addition to their technical properties, EPS are reported to have novel physiological properties leading to exceptional health-promoting benefits, including antitumor, antiulcer, immune-modulating, and cholesterol-lowering activities, as well as being considered prebiotic (Ruas-Madiedo et al., 2002; Caggianiello et al., 2016). Dilna et al. (2015) reported antioxidant activity, α -amylase inhibition, cholesterol lowering, and antiproliferative activities of EPS produced by *Lactobacillus plantarum* RJF4. The variation in health-promotion benefits of EPS depends on species, strain, and EPS type (Ryan et al., 2015b). Thus, new strains with novel EPS-producing properties will provide significant values to food industry.

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Several studies have reported technological functions and physiological properties of EPS in low-fat cheeses including Cheddar (Costa et al., 2010; Ryan et al., 2015a), Kasar (Şanlı et al., 2013), mozzarella (Perry et al., 1998; Zisu and Shah, 2005), Prato (Nepomuceno et al., 2016), Mexican Panela (Jiménez-Guzmán et al., 2009), and Swiss-type (Ryan et al., 2015a) cheeses. Akawi cheese is a well-known Middle Eastern cheese that is extensively consumed by people in North Africa, Middle Eastern, and Gulf Cooperation Council countries (Abd El-Salam and Alichanidis, 2004). Low-fat akawi cheese is used as a major ingredient to prepare various types of sweets, especially Kunafah, which is very popular among people in the above-mentioned regions (Ayyash et al., 2012). To the best of our knowledge, no information is available about low-fat akawi cheese made with an EPS-producing culture. Nine LAB have been isolated from camel milk and identified as having robust probiotic characteristics and EPS production capabilities (Abushelaibi et al., 2017). Out of 9 LAB isolates, *Lactobacillus plantarum* KX881772 and *Lb. plantarum* KX881779 were found to be very promising and hence were used in the current study. The tests of *o*-phthalaldehyde (OPA); 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS); α -amylase and α -glucosidase inhibition; angiotensin-converting enzyme (ACE) inhibition; and cytotoxicity against cell lines are recognized in vitro indicators for proteolytic activity (McSweeney and Fox, 1997); antioxidant (Power et al., 2013); anti-diabetic (Lauritano and Ianora, 2016); antihypertensive (Gobbetti et al., 2004); and antiproliferative (Elfahri et al., 2016) activities, respectively. These indicators are used to evaluate in vitro health-promoting benefits of functional food products (Mohanty et al., 2016; Marccone et al., 2017). The objectives of this study were to investigate the health-promoting benefits (α -amylase and α -glucosidase inhibitory activities, ACE-inhibitory activity, and antiproliferative activity) of low-fat akawi cheese made with EPS-producing (EPS⁺) probiotic *Lb. plantarum* KX881772 and KX881779 strains isolated from camel milk (CEPS⁺) compared with a commercial EPS-producing culture (MEPS⁺).

MATERIALS AND METHODS

Culture Propagation

Two white cheese cultures, White-Classic (non-EPS-producing, EPS⁻; *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and White-Daily commercial EPS-producing (MEPS⁺; *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactococcus lactis* ssp.

lactis), were provided by Chr. Hansen Holding A/S (Hørsholm, Denmark) and 2 EPS-producing bacterial strains *Lactobacillus plantarum* KX881772 and KX881779 isolated from camel milk (CEPS⁺; Abushelaibi et al., 2017) were used in the current study. The *Lb. plantarum* KX881772 and KX881779 strains were stored in de Man, Rogosa, and Sharpe (MRS) broth (Oxoid, Basingstoke, UK) with 50% glycerol at -80°C. To activate the *Lb. plantarum* KX881772 and KX881779 (CEPS⁺) cultures, a 100- μ L aliquot of each culture was individually transferred into MRS broth and incubated at 37°C for 24 h. A weekly culture transfer was carried out to maintain bacterial activity. For all cultures (EPS⁻, MEPS⁺, and CEPS⁺) and before the experiments, 2 successive culture transfers were carried out in MRS broth, and a third transfer was in sterilized reconstituted skim milk (13% wt/vol) and incubated at 37°C for 24 h.

Cheese Making

Akawi cheese was manufactured according to the procedure described by Ayyash et al. (2012). Briefly, homogenized, pasteurized, low-fat (1%) bovine milk was tempered to 40°C. Bacterial culture was added at 1% (vol/vol) and mixed for 2 min. Three batches of cheese were made using EPS⁻, MEPS⁺, and CEPS⁺ (*Lb. plantarum* KX881772 and KX881779 mixed at a ratio of 1:1) cultures. After 45 min of ripening, the milk was coagulated using double-strength chymosin per the manufacturer instructions (Chy-Max; Chr. Hansen). The milk coagulated in 40 min, and the curd was cut into 1-cm³ cubes using cheese knives and allowed to settle for 5 min. The curd was stirred for 20 min at 40°C. The whey was drained and curd cubes were transferred to a cheesecloth. Curd pieces were wrapped in cheesecloth in small portions (~250 g) and pressed for 90 min. Young cheese pieces were brined in 10% (wt/vol) brine solution overnight at 4°C. Then, the cheese pieces were vacuum-packaged and stored at 4°C. Cheeses were sampled at 0, 7, 14, and 21 d of storage at 4°C.

Bacterial Enumeration

The LAB in cheeses were enumerated according to Ayyash and Shah (2011). An aliquot of grated cheese (10 g) was taken from the shredded cheese block, placed in a stomacher bag, and blended with 90 mL of sterile distilled water using a Stomacher-400 laboratory blender (Seward Medical, London, UK). Appropriate serial dilutions were made using 0.1% (wt/vol) peptone, and LAB populations were counted using MRS agar (Oxoid). Inoculated plates in duplicate were in-

cubated anaerobically at 37°C for 48 h using anaerobic jar system (Don Whitley Scientific Ltd., Shipley, UK).

Proteolysis Assessment

Water-soluble extracts (**WSE**) were prepared according to Kuchroo and Fox (1982) by homogenizing a 50-g grated subsample of cheese with 100 mL of deionized-distilled (**dd**) water. The slurries were centrifuged at $8,000 \times g$ for 15 min at 4°C.

OPA. The OPA of WSE was determined as described by Ayyash et al. (2012). Briefly, 50 μ L of WSE was placed into a 1.5-mL cuvette and mixed with 1 mL of OPA reagent prepared in a 50-mL volumetric flask as follows: 25 mL of 100 mM disodium tetraborate (Merck Pty Ltd., Kenilworth, NJ), 2.5 mL of 20% (wt/wt) SDS (Merck Pty Ltd.), 40 mg of OPA dissolved in 1 mL of methanol (Sigma-Aldrich, St. Louis, MO), and 100 μ L of β -mercaptoethanol (Sigma-Aldrich). The volume was completed to 50 mL with addition of Milli-Q water. The cuvette was inverted twice and then incubated for 2 min at room temperature. Absorbance was measured at 340 nm using a UV-Vis spectrophotometer (Jenway 6300, Jenway, Stone, UK).

TFAA. The TFAA of WSE was assayed as described by Ayyash et al. (2012). Briefly, an aliquot (100 μ L) was placed in a glass tube and diluted with 1 mL of Milli-Q water. Two milliliters of Cd-ninhydrin reagent (0.8 g of ninhydrin was dissolved in a mixture of 10 mL of glacial acetic acid (100%) and 80 mL of ethanol (99.5%) followed addition of 1 g of CdCl₂ dissolved in 1 mL of Milli-Q water. The mixture was heated at 84°C for 5 min and then cooled at room temperature; then, absorbance was measured at 507 nm using a UV-Vis spectrophotometer (Jenway 6300, Jenway). All chemicals were purchased from Sigma-Aldrich.

α -Amylase Inhibition

Before the assay, stored WSE was vortexed for 1 min followed by centrifugation at $10,000 \times g$ for 5 min. The α -amylase inhibition assay was carried out according to the method described by Kim et al. (2004) with minor modifications. Briefly, 100 μ L of α -amylase from human salivary (1.0 unit/mL, Sigma-Aldrich) was premixed with 100 μ L of WSE. After incubation at 37°C for 5 min, 250 μ L of 1% starch was added as a substrate in PBS (pH 6.8) to start the reaction. The reaction was performed at 37°C for 5 min and terminated by the addition of 200 μ L of DNS reagent (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH). The reaction mixture was heated for 15 min at 100°C and diluted with 2 mL of distilled water in an ice

bath. α -Amylase activity was determined by measuring absorbance at 540 nm.

α -Glucosidase Inhibition

Before the assay, stored WSE were vortexed for 1 min followed by centrifugation at $10,000 \times g$ for 5 min. The α -glucosidase inhibition assay was carried out according to the method detailed in Kim et al. (2004) with some modifications. α -Glucosidase (1 unit/mL) was dissolved in 100 μ L of 0.1 M potassium phosphate buffer (pH 6.8) and mixed with 50 μ L of WSE. After incubation at 37°C for 10 min, 5 mM *p*-nitrophenol from *p*-nitrophenyl α -D-glucopyranoside (pNPG; 50 μ L) was added as substrate. The enzymatic reaction was performed at 37°C for 30 min and stopped by the addition of 1 mL of 0.1 M Na₂CO₃. α -Glucosidase activity was determined by measuring the release of *p*-nitrophenol from pNPG at 400 nm. Solution without sample was used as a control, and solution without substrate was used as a blank. The inhibition percentage was calculated as follows (where Abs = absorbance):

$$\text{Inhibition \%} = \left(1 - \frac{\text{Abs sample} - \text{Abs blank}}{\text{Abs control}} \right) \times 100.$$

Antioxidant Activity

Before the antioxidant assays, stored WSE were vortexed for 1 min followed by centrifugation at $10,000 \times g$ for 5 min.

Radical-Scavenging Rate by DPPH. Radical-scavenging activity by DPPH was assayed according to Elfahri et al. (2016). Briefly, 800 μ L of DPPH reagent (0.1 mM DPPH dissolved in 95% methanol) was added to 200 μ L of WSE in glass test tubes. The samples were shaken vigorously and incubated in the dark at room temperature for 30 min. Methanol was used as a blank. The absorbance of the incubated samples was measured at 517 nm. The radical-scavenging activity (%) was expressed as follows:

$$\text{Scavenging rate (\%)} = \left(1 - \frac{\text{Abs sample}}{\text{Abs blank}} \right) \times 100.$$

Radical-Scavenging Rate by ABTS. Radical-scavenging rate by ABTS radical (**ABTS^{•+}**) was assayed according to Sah et al. (2014). A stock solution of ABTS was prepared by mixing stock solutions of 7.4 mM ABTS aqueous solution and 2.6 mM potassium persulfate aqueous solution in equal quantities (molar ratio = 1:0.35) and allowing them to react for 12 h in

the dark at room temperature. Fresh ABTS reagent was prepared by mixing 1 mL of ABTS^{•+} solution with 50 to 60 mL of buffered methanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm after equilibration at 30°C. Twenty microliters of properly diluted WSE in dd-water was added to 2 mL of ABTS reagent and incubated at 30°C for 6 min. The absorbance of the mixture was measured at 734 nm. Similarly, 20 μ L of dd-water was used instead of the sample for the blank. Radical-scavenging activity was calculated as follows:

$$\text{Scavenging rate (\%)} = \left(\frac{\text{Abs blank} - \text{Abs sample}}{\text{Abs blank}} \right) \times 100.$$

ACE Inhibition

Before the ACE inhibition assays, stored WSE was vortexed for 1 min followed by centrifugation at $10,000 \times g$ for 5 min. The ACE inhibition of WSE was assayed according to methods detailed in Ayyash and Shah (2011). Briefly, 5 mL of WSE was freeze-dried and dissolved in 1 mL of Tris buffer (50 mM, pH 8.3) containing 300 mM NaCl. An ACE enzyme (from rabbit lung) and hippuryl-histidyl-leucine (HHL) purchased from Sigma-Aldrich was prepared in Tris buffer. The assay consisted of 100 μ L of 3.0 mM HHL, 100 μ L of ACE enzyme (1.25 mU/mL), and 100 μ L of dissolved WSE sample. The mixture was placed in a glass tube and then incubated for 30 min at 37°C in a water bath without mixing and then for an additional 30 min after mixing. Glacial acetic acid (200 μ L) was added to stop ACE enzyme activity. The mixture was kept at -20°C to be analyzed later using HPLC. The resulting hippuric acid (HA) from the previous reaction was determined using HPLC. An external standard curve was prepared to quantify the HA in assay samples. An aliquot (20 μ L) of the mixture was injected into the HPLC system (ThermoFisher Scientific, Waltham, MA). The system was fitted with a reverse-phase column (C18, 250 mm long $\times$ 4.6 mm in diameter, 5- μ m diameter of the HPLC column particles inside with a guard column (C18, 4 $\times$ 3.0 mm). The separation was conducted at room temperature ($\sim 22^\circ\text{C}$) at a flow rate of 0.8 mL/min. The mobile phase was an isocratic system consisting of 12.5% (vol/vol) acetonitrile (Sigma-Aldrich) in Milli-Q water, and the pH was adjusted to pH 3.0 using glacial acetic acid (Sigma-Aldrich). The detection device was a UV-Vis detector set at 228 nm. The control reaction mixture contained 100 μ L of buffer instead of the assay sample; the control was expected to liberate the maximum amount of HA from the substrate due to uninhibited ACE activity. The percentage inhibition of enzyme activity was calculated as follows:

$$\text{ACE inhibition (\%)} = \left(\frac{\text{HA control} - \text{HA sample}}{\text{HA control}} \right) \times 100.$$

Antiproliferative Activities

Before the assay, WSE were filtered through Macrosep Advance Spin Filter 3 kD (Pall Corporation, Port Washington, NY). Filtrates were assayed against Caco-2 and MCF-7 carcinoma cell lines according to method details by Elfahri et al. (2016). Cell lines used for the cell cytotoxicity assay included a colon cancer cell line (Caco-2; provided by Dr. Carine Platat, Nutrition and Health Department, United Arab Emirates University) and a breast cancer cell line MCF-7 (Faculty of Medicine and Health Sciences, United Arab Emirates University). Cells were grown in humidified incubator (37°C and 5% CO₂) in Dulbecco's modified Eagle's medium (Gibco, Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated bovine serum (FBS) and 1% penicillin and streptomycin (Invitrogen). Cell lines were initially passed and counted in hemocytometer before the seeding. Cells were seeded at 1×10^3 concentration in the 96-well plate and incubated in appropriate medium overnight. For the treatment, 25 μ L of filtered WSE was added to each well and incubated for 72 h at 37°C and 5% CO₂. Cells without WSE were used as a control. Afterward, 20 μ L of warmed Abcam Cell Cytotoxicity Assay Kit (ab112118, Abcam, Cambridge, MA) was added and incubated at 37°C for at least 5 h. The absorbance was measured at 570 and 605 nm according to the kit manufacturer's protocol. The ratio of optical density (OD) at 570 nm to OD at 605 nm was used to determine the inhibition of the cell growth in each well. The proliferative inhibition was calculated as follows:

$$\text{Proliferative inhibition (\%)} = [1 - R_{\text{sample}} - R_0] / (R_{\text{ctrl}} - R_0) \times 100,$$

where R_{sample} is the absorbance ratio of OD₅₇₀/OD₆₀₅ in the presence of the WSE; R_{ctrl} is the absorbance ratio of OD₅₇₀/OD₆₀₅ in the absence of the WSE (vehicle control); and R_0 is the averaged background (non-cell control) absorbance ratio of OD₅₇₀/OD₆₀₅.

Statistical Analysis

Cheese making was carried out in duplicate, each experimental unit was subsampled in duplicate, and each subsample was assayed in duplicate. Thus, result values are mean $\pm$ standard deviation (SD) of $n = 8$. Two-way ANOVA was carried out to investigate the effect of cul-

ture type and storage period on cheese parameters ($P < 0.05$). Means comparisons at same storage period was performed using Tukey's test ($P < 0.05$). All statistical analyses were carried out using Minitab 17.0 software (Minitab Inc., State College, PA).

RESULTS AND DISCUSSION

LAB Enumeration and Proteolysis Assessment

Bacterial count and OPA and TFAA assay results (as assessment of proteolysis) are presented in Figure 1 (panels A, B, and C, respectively). The populations of LAB did not differ significantly between the cheeses produced by different starter cultures in the same storage period. The number of LAB remained $>7.0 \log_{10}$ cfu/g in all cheeses during 21 d of storage at 4°C (Figure 1A). The proteolysis parameters OPA and TFAA increased significantly ($P < 0.05$) with prolonged storage of all cheeses (Figure 1B and 1C). Levels of OPA and TFAA in all cheeses produced with MEPS⁺ and CEPS⁺ were significantly higher ($P < 0.05$) than those of cheeses produced with EPS⁻ cultures. At the end of storage, the OPA level in CEPS⁺ cheeses were the same as that in MEPS⁺ cheeses, and TFAA values in CEPS⁺ cheeses were close to those in MEPS⁺ cheeses. The OD_{340nm} of OPA in CEPS⁺ cheese increased by 1.430 compared with 1.230 in MEPS⁺ cheeses at the end of storage period.

The greater values of OPA and TFAA, as indicators for secondary and advanced proteolysis (McSweeney and Fox, 1997), respectively, in cheeses made with EPS⁺ cultures may be due to accelerated primary proteolysis along with high proteolytic activities of EPS⁺ cultures. Similarly, Oluk et al. (2014) reported that TFAA were higher in Tulum cheese made with an EPS⁺ culture. Furthermore, proteolytic activities of CEPS⁺ cultures isolated from camel milk were comparable to that produced with MEPS⁺ culture. Therefore, these cultures may be used in the production of different dairy products such as cheese or yogurt produced from camel milk to enhance their texture.

α -Glucosidase and α -Amylase Inhibition

The inhibitory effects of all cheeses on α -glucosidase or α -amylase activity during 21 d of storage are presented in Figure 2A and B, respectively. The percentage of α -glucosidase inhibition ranged from $>45\%$ at 0 d to $\sim 55\%$ at 21 d of storage. Although α -glucosidase inhibition increased ($P < 0.05$) during storage (Figure 2A), ANOVA showed that α -glucosidase inhibition differed numerically but not significantly ($P > 0.05$) between experimental cheeses. On the other hand, α -amylase

inhibition was significantly higher ($P < 0.05$) in cheeses made with EPS⁺ cultures than with EPS⁻ cultures during storage. It was interesting to note that cheeses made with CEPS⁺ culture showed higher α -amylase inhibition compared with MEPS⁺ at d 21 of storage (Figure 2B). The α -amylase inhibition in CEPS⁺ cheese remained $>30\%$ during 14 d of storage followed by a rapid increase to $>50\%$ at 21 d of storage (Figure 2B).

The inhibition of α -amylase and α -glucosidase is considered an effective approach to control diabetes via diminishing carbohydrate hydrolysis (Donkor et al., 2012). The general inhibition of both α -amylase and

Figure 1. (A) Lactic acid bacteria count, (B) o-phthalaldehyde (OPA), and (C) total free AA (TFAA) of 3 experimental cheeses during 21 d of storage. EPS⁻ = non-exopolysaccharide (EPS)-producing culture; MEPS⁺ = commercial EPS-producing culture; CEPS⁺ = EPS-producing *Lactobacillus plantarum* isolated from camel milk. Values are mean $\pm$ SD (n = 8). Color version available online.

Figure 2. (A) α -Glucosidase inhibition and (B) α -amylase inhibition of 3 experimental cheeses during 21 d of storage. EPS⁻ = non-exopolysaccharide (EPS)-producing culture; MEPS⁺ = commercial EPS-producing culture; CEPS⁺ = EPS-producing *Lactobacillus plantarum* isolated from camel milk. Values are mean $\pm$ SD (n = 8). Color version available online.

α -glucosidase enzymes may be attributed to (1) bioactive peptides, particularly small peptides produced by the action of proteolytic enzymes secreted by cheese cultures (Gomes da Cruz et al., 2009), and (2) the bioactivity of EPS produced by cheese cultures, especially EPS⁺ cultures (Aluko, 2012). Several researchers have reported that small peptides have shown antidiabetic activity in egg white, soybean, legumes, and fish (Vadivel et al., 2011; Yu et al., 2011, 2012). Nehir El et al. (2015) reported that small peptides in goat milk and kefir products exhibited inhibition against α -amylase and α -glucosidase. The proteolysis assessment (OPA and TFAA assays) in the present study showed that the experimental cheeses produced bioactive peptides. This reveals the consistency between proteolytic activities (Figure 1) and α -amylase and α -glucosidase inhibition results. In the current study, we assumed that the EPS produced by EPS⁺ cultures contributed to α -amylase and α -glucosidase inhibition, which in turn may explain the higher percentages of α -amylase inhibition in cheeses made with EPS⁺ cultures compared with EPS⁻ culture. Our result demonstrated that a culture isolated

from camel milk (CEPS⁺) had comparable proteolytic activity to the MEPS⁺ culture, which may produce antidiabetic peptides (Figure 2). The mechanisms of physiological activities of EPS remain unclear and this requires further investigation (Badel et al., 2011). To the best of our knowledge, EPS as prebiotics can promote human health (Ruas-Madiedo et al., 2002).

Antioxidant Activity (DPPH and ABTS)

The radical-scavenging rates (%), measured by DPPH and ABTS assays, of cheeses during 21 d of storage are presented in Figure 3A and 3B). Scavenging rates of experimental cheeses made with EPS⁺ cultures were higher ($P < 0.05$) than that of EPS⁻ cultures (Figure 3A and 3B). Cheese made with CEPS⁺ culture exhibited comparable antioxidant activity to that made with MEPS⁺ culture. Scavenging rates increased significantly ($P < 0.05$) with prolonged storage (Figure 3A and 3B). After 14 d of storage, the scavenging rate by ABTS in CEPS⁺ cheese increased $>60\%$ (Figure 3B) and $>20\%$ by DPPH (Figure 3A). This implies that antioxidant activities in CEPS⁺ increased during storage.

Bioactive compounds in foods, especially fermented dairy products, play a crucial role in elevating the effect of reactive oxygen species such as superoxide ($O_2^{\bullet-}$, $\bullet OOH$), hydroxyl ($\bullet OH$), and peroxy ($ROO^{\bullet}$) radicals formed by oxidatively stressed cells (Benbrook, 2005). These bioactive compounds, especially protein-derived peptides, possess the ability to donate electrons to neutralize free radicals. Therefore, the presence of several amino acid residues in the peptide chain can enhance antioxidant properties (Aluko, 2012). The DPPH and ABTS results of the present study may be attributed primarily to bioactive peptides resulting from the proteolytic action of cheese cultures. The higher DPPH and ABTS of cheeses made with EPS⁺ cultures may also be attributed to bioactive EPS produced by these cultures. It is noteworthy that scavenging rates of DPPH were lower than those of ABTS. This result may be attributed to the nature of the antioxidant test. Floegel et al. (2011) reported lower DPPH scavenging rates than ABTS for several US food products.

ACE Inhibition

Figure 4 displays the ACE inhibitory activities (%) of the 3 experimental cheeses during 21 d of storage. Throughout storage, the experimental cheeses made with EPS⁺ cultures exhibited higher ($P < 0.05$) ACE inhibitory activity compared with those made with EPS⁻. Low-fat akawi cheese made with CEPS⁺ demonstrated comparable ACE inhibitory activity to that of

Figure 3. Antioxidant activity by (A) 1,1-diphenyl-2-picrylhydrazyl (DPPH) and (B) 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) of 3 experimental cheeses during 21 d of storage. EPS⁻ = non-exopolysaccharide (EPS)-producing culture; MEPS⁺ = commercial EPS-producing culture; CEPS⁺ = EPS-producing *Lactobacillus plantarum* isolated from camel milk. Values are mean $\pm$ SD (n = 8). Color version available online.

MEPS⁺. The CEPS⁺ cheese had ACE inhibitory activity >70% after 7 d of storage (Figure 4A).

Inhibition of ACE activity is an *in vitro* indicator of the antihypertensive properties of fermented dairy products (Gobbetti et al., 2004). The higher ACE inhibitory activity of the cheese made with EPS⁺ cultures may be attributed to the presence of EPS in these cheeses. In general, the results of ACE inhibition in the current study are consistent with results of Ayyash et al. (2012). It has been reported that ACE inhibition may be primarily attributed to the bioactivity of casein-derived peptides that may be produced by proteolytic enzymes from added cultures (Ayyash et al., 2012). Ruas-Madiedo et al. (2002) reported that EPS promotes human health as a prebiotic or due to antitumor, antiulcer, immunomodulating, or cholesterol-lowering activities. Therefore, we postulate that EPS in low-fat akawi cheeses may have contributed to the ACE inhibitory activities. Further work is needed to investigate the mechanism of ACE inhibition by EPS. The antiproliferative activity of bioactive peptides,

especially small peptides, in dairy products has been extensively reported (Clare and Swaisgood, 2000; Hoerr and Bostwick, 2000).

Antiproliferative Activities

The antiproliferative activities of 3 cheeses against human epithelial carcinoma (Caco-2) and breast cancer (MCF-7) cell lines are shown in Figures 5A and 5B, respectively. All cheeses exhibited antiproliferative activity against Caco-2 and MCF-7 cell lines. The antiproliferative activities against Caco-2 (Figure 5A) increased significantly ($P < 0.05$) throughout the storage period. Antiproliferation activity of CEPS⁺ cheese increased from 38 to 48% during 7 d of storage and maintained antiproliferation above 45% with prolonged storage (Figure 5A). It is noteworthy that proliferation inhibition against MCF-7 was higher than that against Caco-2 (Figure 5A and 5B). Figures 5A and 5B demonstrate that antiproliferative activities of WSE extracted from cheeses made with CEPS⁺ cultures were comparable to those in cheese from MEPS⁺. The EPS⁻ culture had similar antiproliferative activities to MEPS⁺ and CEPS⁺ cultures (Figure 5A and 5B).

The antiproliferation activities of milk peptides have been reported by Park (2009). Several hypotheses have been proposed explaining the mechanism(s) of the antiproliferative activities of milk peptides. Competition between peptides and cancer growth factors for cancer cell-membrane receptors is one such hypothesis. Another hypothesis claims that released peptides have specific cytotoxicity on cancer cells, which induces apoptosis (Picot et al., 2006; Pessione and Cirrincione, 2016). In the current study, the maximum molecular size in

Figure 4. Angiotensin-converting enzyme (ACE) inhibition of 3 experimental cheeses during 21 d of storage. EPS⁻ = non-exopolysaccharide (EPS)-producing culture; MEPS⁺ = commercial EPS-producing culture; CEPS⁺ = EPS-producing *Lactobacillus plantarum* isolated from camel milk. Values are mean $\pm$ SD (n = 8). Color version available online.

Figure 5. Antiproliferative activity against (A) Caco-2 and (B) MCF-7 cell lines of 3 experimental cheeses during 21 d of storage. EPS⁻ = non-exopolysaccharide (EPS)-producing culture; MEPS⁺ = commercial EPS-producing culture; CEPS⁺ = EPS-producing *Lactobacillus plantarum* isolated from camel milk. Values are mean $\pm$ SD (n = 8). Color version available online.

the filtered WSE was $\leq 3,000$ Da. Gandhi and Shah (2016) reported antiproliferative activities for akawi cheese against Caco-2 cells. Although those authors did not filter the WSE to control the molecular size of the extract, the antiproliferative percentages reported by Gandhi and Shah (2016) agree with the results from the current study.

CONCLUSIONS

Newly isolated *Lb. plantarum* KX881772 and KX881779 strains from camel milk demonstrated promising probiotic characteristics in low-fat cheese compared with commercial cultures. Moreover, low-fat akawi cheese produced with CEPS⁺ cultures exhibited similar and, in some instances, more pronounced (e.g., α -amylase inhibition) health-promoting benefits than the commercial starter culture (MEPS⁺). *Lactobacillus plantarum* cultures showed similar performance to commercially available cultures and deserve further consideration.

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