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Rheological, textural, microstructural and sensory impact of exopolysaccharide-producing *Lactobacillus plantarum* isolated from camel milk on low-fat akawi cheese

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ABSTRACT

The aim of this study was to investigate the influence of EPS-producing *Lactobacillus plantarum* strains isolated from camel milk on chemical composition, rheological properties, texture profile, microstructure and sensory properties of low-fat akawi cheese. Low-fat akawi cheeses were made using three different cultures; traditional culture (non-EPS), commercial EPS culture (MEPS$^+$; ropy type) and *Lb. plantarum* isolated from camel milk (CEPS$^+$; ropy type). Moisture and salt contents of cheeses made with EPS$^+$ cultures were higher than those made with non-EPS$^-$ culture. The magnitude of storage modulus G′ was significantly higher than loss modulus for all cheeses during all storage periods. The magnitude of elastic G′ and complex G* moduli of non-EPS cheese were higher than MEPS$^+$ and CEPS$^+$ cheeses. Cheeses with non-EPS exhibited more hardness and adhesiveness than MEPS$^+$ and CEPS$^+$ at 0 and 7 days of storage. Hardness of cheeses at 0 day coincided with dynamic parameters, elastic G′ and complex G* moduli. At 0 day, microstructure of non-EPS showed large pores compared to cheeses made with EPS-producing cultures. Cheeses made with non-EPS$^-$ had lower scores in adhesiveness, aroma, saltiness and appearance but higher in sourness compared with those made with EPS$^+$ cultures at 0 day of storage.

1. Introduction

Exopolysaccharides (EPS) are long-chain polysaccharides composed of repeated units of mono-sugars or their derivatives. EPS produced by lactic acid bacteria (LAB) provide significant improvements on rheological, texture and mouth feel of fermented milk product especially cheeses (Welman & Maddox, 2003). EPS are either attached to the cell called capsular-EPS or found loose in the medium called ropy-EPS. Homopolysaccharides and heteropolysaccharides are main categories of EPS secreted by LAB (Ruas-Madiedo, Hugenholtz, & Zoon, 2002). EPS have novel physiological properties that lead to exceptional health benefits. It has been reported that EPS can contribute to human as a prebiotic or may have antitumor, antiulcer, immunomodulating and lowering cholesterol properties (Dilna et al., 2015; Ryan, Ross, Fitzgerald, Caplice, & Stanton, 2015). EPS contribute to technological functions including natural thickening agent and physical stabilizer (Duboc & Mollet, 2001). The technological impacts of emerged EPS-producing LABs have been investigated in cheeses and other fermented milk products (Duboc & Mollet, 2001).

Reduced-fat Cheddar cheeses made with ropy-EPS *Lactococcus lactic* ssp. *cremoris* (JFR1) have shown improved viscoelastic properties, microstructure, and texture profile (Awad, Hassan, & Muthukumarappan, 2005; Hassan & Awad, 2005; Hassan, Awad, & Muthukumarappan, 2005). Dabour, Kheadr, Benhamou, Fliss, and LaPointe (2006) have concluded that the ropy *L. lactis* ssp. *cremoris* JRF-1 strain increased moisture retention and enhanced structure of reduced-fat Cheddar cheese more than capsular *L. lactis* ssp. *cremoris* SMQ-461. Texture properties of low-fat and reduced-fat Turkish cheeses (Kasar) were improved using EPS-forming starter culture MYE92 (Şanli, Gursel, Şanli, Acar, & Benli, 2013). EPS-producing *Streptococcus thermophilus* SY-102 improved yield,

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structure and lowered syneresis of Mexican Panela cheese (Jiménez-Guzmán, Flores-Nájera, Cruz-Guerrero, & García-Garibay, 2009). Capsular EPS-producing *L. lactis* ssp. *lactis* or *L. lactis* ssp. *cremoris* (Lyofast MOS 062E) improved yield but not melting and texture properties of Prato cheese (Nepomuceno, Costa Junior, & Costa, 2016). Similarly, Zisu and Shah (2005) have reported that low-fat mozzarella cheese made with *S. thermophilus* 285 (capsular-EPS) had lowest moisture content and yield. Further studies of using of EPS in low-fat cheeses have been reviewed by Broadbent, McMahon, Oberg, and Welker (2001).

Technological functions, type, and structure of EPS is strain-dependent (Ruas-Madiedo et al., 2002; Welman & Maddox, 2003). Therefore, food manufacturers keep aim to screen various food products seeking for new LABs with EPS production ability (Angmo, Kumari, Savitri, & Bhalla, 2016; Das, Khowala, & Biswas, 2016; Zuo et al., 2016). Recently, Abushelaibi, Al-Mahadin, El-Tarabily, Shah, and Ayyash (2017) have isolated and characterized 9 LAB isolates from camel milk. These isolates showed promising probiotic characteristics and EPS production. Out of 9 stains, *Lactobacillus plantarum* KX881772 and KX881779 strains possessed the best EPS-production (ropy type) and probiotics characteristics hence were used in the current study.

Akawi cheese is well-known Middle Eastern cheese that is extensively consumed by people in North Africa, Middle Eastern and Gulf Cooperation Council (GCC) countries (Abd El-Salam & Alichanidis, 2004). Akawi cheese is also used as an ingredient in various type of sweets especially Kunafah which is popular among people in the above countries (Ayyash, Sherkat, & Shah, 2012). Due to health promotion, there is rising awareness about reduced fat food as well as free fat products especially low-fat cheeses. Low-fat cheese represents a good choice for the development of new products with functional properties (Banks, 2004). To the best of our knowledge, no information available related to functionality of these new EPS-producing probiotics and low-fat akawi cheese. Therefore, low-fat akawi cheese is selected to examine the characteristics of the new *Lb. plantarum* KX881772 and KX881779 strains. The aim of this study was to investigate the influence of EPS-producing *Lb. plantarum* strains isolated from camel milk on chemical composition, rheological properties, texture profile, microstructure and sensory properties of low-fat akawi cheese.

2. Materials and methods

2.1. Culture propagation and storage

Two white cheese cultures namely White-Classic™ (non-EPS; *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and White-Daily™ commercial EPS-producing ropy type (MEPS+; *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Lactococcus lactis* subsp. *lactis*) were kindly provided by Chr-Hansen Holding A/S (Horsholm, Denmark) and two bacterial strains *Lactobacillus plantarum* KX881772 and KX881779 EPS-producing (ropy) isolated from camel milk (CEPS+) (Abushelaibi et al., 2017) were used in the current study. *Lb. plantarum* KX881772 and KX881779 were stored in de Man, Rogosa, and Sharpe (MRS) broth (Oxoid, Hampshire, UK) with 50% glycerol at −80 °C. To activate the *Lb. plantarum* KX881772 and KX881779 (CEPS+) cultures, a 100-μL aliquot of each culture was individually transferred into MRS broth and incubated at 37 °C for 24 h. A weekly culture transfer was performed to maintain the bacterial activity. For all cultures (non-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 (RSM 13% w/v) and incubated at 37 °C for 24 h.

2.2. Cheese making

Akawi cheese was manufactured according the procedure described by Ayyash et al. (2012). Briefly, low-fat (1%), homogenized, and pasteurized bovine milk was tempered at 40 °C. Bacterial culture was added at 1% (vol/vol) and mixed for 2 min. Three batches were made using non-EPS$^-$ producing culture (non-EPS), commercial EPS-producing (MEPS+) and camel isolated EPS-producing (CEPS+, *Lb. plantarum* KX881772 and KX881779 mixed a ratio 1:1) cultures. After 45 min, the milk was coagulated using double-strength chymosin per the manufacturer instructions (CHY-MAX; Chr. Hansen) for 40 min. The curd was cut into 1-cm^3 cubes using cheese knives and settled for 5 min. Cut curd was stirred for 20 min at 40 °C. The whey was drained, and curd cubes were transferred to cheese cloth. The curd pieces were wrapped in a cheese cloth in small portions (~250 g) and pressed for 90 min. The cheese pieces were brined in 10% (w/v) brine solution overnight at 4 °C and vacuum-packaged (2.4 oxygen transfer rate cc/100in^2/24 h at 73 F°, 0.36 moisture vapor transfer g/100in^2/24 h at 100 F°) and stored at 4 °C. Cheeses were sampled at 0, 7, 14 and 21 days of storage at 4 °C.

2.3. Gross composition, water activity (a_w) and pH values

The moisture content was determined by the oven-drying method at 105 °C, ash content by muffle furnace method, fat content by Gerber method, and protein content by the Kjeldahl method, according to AOAC. (2005). For pH measurement, grated cheese (25 g) was homogenized with 25 mL of distilled water, and the pH was measured using a digital pH meter. The water activity (a_w) was measured by HygroLab-C1 (Rotronic, NY, USA).

2.4. Proteolysis assessment (water soluble nitrogen WSN)

Water soluble extract (WSE) was prepared according to Kuchroo and Fox (1982) by homogenizing 50 g grated cheese sample with 100 mL deionized-distilled water (dd-water). The slurries were centrifuged at 8000 × g for 15 min at 4 °C. WSN was assessed using the Kjeldahl method (AOAC., 2005). The WSN expressed as a percentage of total nitrogen.

2.5. Rheological analysis

Rheological analyses were carried out according to Hassan et al. (2005) with minor modifications. Briefly, samples were cut at least 3 mm deep of the cheese blocks. These samples were immediately placed in small airtight plastic containers and equilibrated at room temperature (25 ± 1 °C) for at least 20 min. Small oscillatory amplitude measurements were performed with a Discovery Hybrid Rheometer HR-2 (TA Instruments, New Castle, DE, USA). The measuring geometry consisted of 2 parallel plates with a diameter of 40 mm and 3-mm gap size (sample thickness). Excessive cheese was trimmed carefully and the sample was allowed to rest for 1 min on the rheometer to allow the stresses induced during sample handling to relax. The linear viscoelastic range was determined by performing a strain sweep. All oscillation amplitude results during 21 day of storage are presented in Fig. S1. Frequency, set at 1.5 Hz, as the percentage of strain values varied from 0.01 to 2%, resulting in a strain sweep. A strain in the linear region (0.01%–1%) was then selected and a frequency sweep was performed. To select the linear range, the complex modulus and oscillation stress as functioned to strain were plotted. For frequency sweep test, strain was set at 0.1% as the frequency varying from 0.01 to 10 Hz, resulting in a frequency sweep at 25 °C. The dynamic parameters storage (elastic; G′), loss (viscous; G″), complex modulus (G*), complex viscosity (η),