International Dairy Journal 53 (2016) 10–19

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Effect of exopolysaccharide-producing starter cultures and post-fermentation mechanical treatment on textural properties and microstructure of low fat yoghurt

Lanjun Zhang [a, *], Ditte Marie Folkenberg [b], José Manuel Amigo [a], Richard Ipsen [a]

[a] Department of Food Science, Faulty of Science, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark
[b] Chr. Hansen A/S, Bøge Allé 10-12, DK-2970 Hørsholm, Denmark

ARTICLE INFO

Article history:
Received 12 June 2015
Received in revised form
25 September 2015
Accepted 26 September 2015
Available online 24 October 2015

ABSTRACT

The microstructure and texture of yoghurts produced by four different exopolysaccharide (EPS)-producing starter cultures and mechanically treated post-fermentation at four levels of intensity (applied back-pressure) were studied. Two *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB) strains were used in combination with two *Streptococcus thermophilus* (ST) strains and yoghurts were formulated by pairwise combining one LB and one ST strain. The choice of ST strain was the major determinant for the rheological properties of the yoghurts, since one of the ST strains conferred a ropy texture and resulted in yoghurts with decreased water holding capacity and an open microstructure. In addition, one of the LB strains used produced both aggregated and threadlike EPS and improved water holding. When combined with an ST strain that produced neglible amounts of EPS this LB strain resulted in yoghurt where a moderate mechanical treatment post-fermentation was able to further improve the water holding capacity.

1. Introduction

In the past three decades, the physicochemical properties of yoghurts have been extensively studied (Lucey, 2004; Sodini, Remeuf, Haddad, & Corrieu, 2004). It has been shown that EPS and capsular polysaccharides (CPS), primarily synthesised in situ by lactic acid bacteria (LAB), play an important role in contributing to textural attributes such as thickness and creaminess in low fat stirred yoghurts (Folkenberg, Dejmek, Skriver, & Ipsen, 2005; 2006b). The textural properties are, in turn, affected by the chemical composition and structural characteristics of the EPS/CPS (Cerning, 1990; De Vuyst, De Vin, Vaningelgem, & Degeest, 2001; Laws & Marshall, 2001; Ruas-Madiedo, Hugenholtz, & Zoon, 2002). It has been proposed that, rather than EPS concentration, the monosaccharide composition (e.g., proportion of glucose), the molecular mass, and the radius of gyration affect the intrinsic viscosity and the flow behaviour of yoghurts (Faber, Zoon, Kamerling, & Vliegenthart, 1998; Petry et al., 2003; Tuinier et al., 1999). However, yoghurts with varied strain combinations of LAB, even when sharing a common strain, have shown distinctively different properties in terms of apparent viscosity, gel firmness and water retention capacity, etc.

Different results have been reported regarding the effect of EPS on the textural properties of yoghurts due to the diverse characteristics of the EPS produced by different starter cultures or strains. For example, Hassan, Frank, Schmidt, and Shalabi (1996a, 1996b) reported that a ropy *Lactobacillus bulgaricus* (LB) culture induced higher yield stress in yoghurts than a culture producing non-ropy encapsulated EPS. In another study, a yield stress of zero was reported for yoghurts containing EPS (Hassan, Ipsen, Janzen, & Qvist, 2003). Concerning the water holding capacity, Folkenberg, Dejmek, Skriver, Skov Guldager, and Ipsen (2006a) reported that severe syneresis occurred when a starter culture was formulated from EPS producing strains of both LB and *Streptococcus thermophilus* (ST). In contrast, GüLer-Akin, Serdar Akin, and Korkmaz (2009) stated that the syneresis was significantly decreased in yoghurts made by EPS-producing starter cultures compared with those produced by non-EPS-producing strains. Moreover, Rawson and Marshall (1997) found that the storage conditions after yoghurt production are also important, since the texture of yoghurt recovered after destructive shearing upon subsequent storage for 24 h at 4 °C when

* Corresponding author. Tel.: +45 35332964.
E-mail address: lanjun@life.ku.dk (L. Zhang).

http://dx.doi.org/10.1016/j.idairyj.2015.09.008
0958-6946/

the yoghurts were produced using a starter combination of ropy LB and non-ropy ST.

To date, scarce information exists regarding the effect of post-fermentation mechanical treatment on the physiochemical properties and microstructure of different yoghurts. Hence, the present study was undertaken, aiming to systematically investigate the microstructure and texture of low fat yoghurts influenced by different strain combinations of EPS-producing starter cultures and variations of post-fermentation mechanical treatment.

2. Materials and methods

2.1. Starter cultures

Four mixed-strain starter cultures and four single-strain starter cultures were provided by Chr. Hansen A/S (Hørsholm, Denmark) in the form of frozen pellets as direct vat set (DVS) cultures. The four mixed-strain starter cultures were used to produce yoghurts for studying the influence of EPS types and post-fermentation mechanical treatment on the microstructure and texture of the yoghurts. The four single-strain starter cultures were only used for initial microscopy to check the morphology of each single strain and the EPS synthesised by the strain.

The four single-strain starter cultures were *Lb. bulgaricus* CHCC-10935 and CHCC-5213, and *S. thermophilus* CHCC-13140 and CHCC-3048. As reported by the manufacturer both CHCC-10935 (LB) and CHCC-13140 (ST) produce high amounts of EPS, whereas CHCC-5213 (LB) produces low amounts of EPS, and CHCC-3048 (ST) produces negligible amounts. Each of the four mixed-strain starter cultures was formulated with one LB strain and one ST strain in pairwise combinations from the four single strains. Accordingly, Yog-1 was produced by a starter culture in strain combination of CHCC-10935 (LB) and CHCC-13140 (ST); Yog-2 by the combination of CHCC-10935 (LB) and CHCC-3048 (ST); Yog-3 by CHCC-5213 (LB) and CHCC-13140 (ST); and Yog-4 by CHCC-5213 (LB) and CHCC-3048 (ST). For all combinations a ratio of 75% ST to 25% LB was used, as this is in the range normally used in commercial cultures in order to limit post-acidification.

2.2. Yoghurt production

All yoghurts were formulated to contain 4.5% protein and 0.1% fat. The yoghurt milk was reconstituted from 491 g of low heat skim milk powder (Arla Foods, Viby, Denmark) dissolved in 3 L demineralized water using a Silverson L5M-A Laboratory Mixer (Silverson Machines Ltd., Waterside, UK) and hydrated overnight at 4 °C in stainless steel buckets. The hydrated yoghurt milk was pasteurized on the next day at 90 °C for 20 min in a water bath and cooled to 43 °C before starter culture inoculation (0.02%) and yoghurt fermentation. The fermentation was terminated when the pH reached 4.55 and the yoghurt gel was initially broken using a stirrer fitted with a perforated disk (Ø = 8 cm, L = 38 cm) at 43 °C. The yoghurt was then immediately transferred into a funnel and passed through a plate heat exchanger and subsequently mechanically treated at 20 °C by applying a back-pressure of 0, 1, 2 or 4 bar using a specially designed post-fermentation treatment unit (PTU) (FH Scandinox A/S, Galten, Denmark) as shown in Fig. 1. The temperature was controlled by use of ice water. The yoghurts were packed in 250 mL plastic cups, and stored at 4 °C for one week. Each type of yoghurt was produced at least three times.

2.3. Visualisation of single strains

The four single-strains as well as the EPS synthesised were visualised using confocal laser scanning microscopy (CLSM, Leica TCS SP5, Leica, Microsystems, Heidelberg, Germany) in combination with Alexa Fluor® wheat germ agglutinin 488 (WGA 488) (Molecular Probes, Eugene, OR, USA). A water objective of 63 × (N.A. 1.2) was used. The cells of LAB were investigated in transmission mode (hence appear black in images), whereas EPS were visualised following excitation at 488 nm and the emission was taken between 505 and 540 nm. Prior to preparing the microscope slides for image visualisation, each frozen strain in the original sealed package was thawed using running tap water. Subsequently, the thawed strain within its original medium was diluted using cold, pasteurized skimmed milk (1:4, v/v) in a sterile hood and kept on ice until image acquisition. The sample (100 μL) was examined immediately following addition of WGA 488 (10 μL) at a working solution of 45.5 μg mL^{-1} in chambered cover-glass for inverted microscopes (155383 German borosilicate Sterile, Thermo Scientific, Waltham, MA, USA).

2.4. Characterisation of yoghurts

2.4.1. Microstructure of yoghurts

After one week storage, all yoghurt samples were observed using a CLSM Leica TCS SP5, equipped with an inverted DMI 6000 microscope and multiple laser lines. A water objective of 63 × (N.A. 1.2) was used. Three fluorescent dyes were combined in each yoghurt sample including the two lectin conjugates of Alexa Fluor® concanavalin A 633 (ConA 633; Molecular Probes, Eugene, OR, USA) and WGA 488, and rhodamine B (Sigma–Aldrich, Munich, Germany). All yoghurt samples were sequentially scanned by three laser lines (Argon 488, He–Ne 543, and He–Ne 633). The lectin probes were used to visualise EPS, and rhodamine B was used to stain the protein gels of the yoghurts. The method essentially followed the procedure described by Zhang, Folkenberg, Qvist, and Ipsen (2015). All samples contained an equal concentration of dyes in the same volume of yoghurt, and the EPS visualised by ConA was shown in red, the EPS visualised by WGA in green, and the protein network in blue. The full size of all obtained images was 246 μm × 246 μm with a resolution of 1024 × 1024 pixels. At least five images were taken from each yoghurt sample and a total of 286 images were acquired.

2.4.2. Rheological properties

The rheological properties of the four types of yoghurts were characterised at 13 °C using an AR-G2 rheometer (TA Instruments, New Castle, DE, USA) fitted with a cone-plate (40 mm 2° steel cone) geometry. An equilibration time of 10 s was applied before measurement. Flow curves were obtained using upward as well as downward shear rate sweeps in the interval 0.001 to 1000 s^{-1} in 25 steps. An oscillatory strain sweep was applied at a constant frequency of 1 Hz in the interval 9.0×10^{-5} to 1.0 in 21 steps on the sample after a resting time of 1 min, to further characterise mechanical breakdown of the yoghurts. Prior to the loading of the sample, the yoghurt was stirred gently in the cup with a plastic spoon five times clockwise and then five times anticlockwise. Duplicate measurements were performed for each yoghurt sample.

The following parameters were used to characterize the flow properties of yoghurts: η_{1_max} denotes the maximum apparent viscosity in the initial shear rate interval of 9.9×10^{-4} to 3.18×10^{-3} s^{-1}; η_2 is the apparent viscosity at 1000 s^{-1}; σ_o denotes the yield stress found from fitting (SigmaPlot 13.0, Systat Software, San Jose, CA, USA) to the Herschel-Bulkley model (Hassan et al., 2003; Steffe, 1996; Sun & Gunasekaran, 2009):

$$\sigma = \sigma o + K(\dot{\gamma})^n$$

where σ is the shear stress, K the consistency index, n the flow behaviour index, and $\dot{\gamma}$ the shear rate.