Food Research International 97 (2017) 170–177

Contents lists available at ScienceDirect

Food Research International

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

Particle formation induced by sonication during yogurt fermentation – Impact of exopolysaccharide-producing starter cultures on physical properties

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ARTICLE INFO

Keywords:
Fermented milk products
EPS
Graininess
Syneresis
Stirred yogurt
Sonication
Gelation

ABSTRACT

Two major quality defects of yogurt are syneresis and the presence of large particles, and several reasons have been extensively discussed. Vibrations during fermentation, particularly generated by pumps, must be considered as a further cause as latest research showed that both ultrasound and low frequencies induced visible particles. The aim of this study was to investigate the impact of sonication during fermentation with starter cultures differing in exopolysaccharide (EPS) synthesis on the physical properties of set (syneresis, firmness) and stirred yogurt (large particles, laser diffraction, rheology). Skim milk was fermented with starter cultures YC-471 (low EPS) or YF-L 901 (high EPS) (Chr. Hansen) and sonicated for 5 min at pH 5.2. Sonicated set gels exhibited syneresis and were softer than respective controls. The mechanical treatment was adjusted to quantify visible particles ($d \geq 0.9$ mm) in stirred yogurts properly. Sonication significantly increased particle numbers, however, the effect was less pronounced when YF-L 901 was used, indicating EPS as a tool to reduce syneresis and particle formation due to vibrations. Rheological parameters and size of microgel particles were rather influenced by starter cultures than by sonication.

1. Introduction

Texture properties of fermented milks like yogurt are a crucial criterion determining consumers' acceptance. Yogurt is made by fermenting milk with lactic acid bacteria, traditionally *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Basically, there are two different types of products: set-style yogurt and stirred yogurt (Tamime & Robinson, 2007). No matter which type is produced, milk is standardized, homogenized and heated to improve texture. Heating results in denaturation of whey proteins and an association with casein micelles (Kessler, 2002). After inoculation with starter culture, milk is fermented at 37–45 °C until pH 4.5–4.6. Casein micelles are destabilized with declining net charge and a continuous gel network is formed (Mokoonlall, Nöbel, & Hinrichs, 2016). In heated milk, aggregation occurs approximately at pH 5.3 as β-lactoglobulin has a higher isoelectric point than casein (Lee & Lucey, 2010). Set yogurt is directly fermented in cups and cooled after acidification has been completed. Its consistency should be smooth and semi-solid without surface whey (Lucey & Singh, 1998). Firmness of the set gel is related to the casein content that can be increased for example by adding skim milk powder or by ultrafiltration. Homogenizing and heat treatment also enhance firmness as volume fraction of casein is increased and the formed network is more branched (Walstra, Wouters, & Geurts, 2006, chap. 22). One major texture defect of set yogurt is syneresis, that is particularly promoted at high incubation temperatures. Whey separation can be counteracted by higher protein contents and intensive heating of the milk (Lucey, 2001; Lucey, 2004).

For stirred products the gelation is the same, however, larger batches are fermented in tanks. The gel is then broken up and cooled to approximately 20 °C, mixed with ingredients (fruit, color etc.), packed and finally cooled to 4–6 °C (Mokoonlall et al., 2016). The texture should be creamy, smooth and thick (Frøst & Janhøj, 2007; Nsabimana, Jiang, & Kossah, 2005). Stirred yogurt can be described as a suspension of microgel particles that are dispersed in the serum/acid whey (Van Vliet, Lakemond, & Visschers, 2004). Particles > 150 µm are considered to cause a rough mouthfeel (Cayot, Schenker, Houze, Sulmont-Rosse, & Colas, 2008). Additionally, large particles/lumps in the millimeter-scale can be present leading to a granular texture (Lucey, 2004). Several reasons have been discussed including protein composition, heat treatment of the milk (Remeuf, Mohammed, Sodini, & Tissier, 2003), fermentation temperature and starter culture (Küçükçetin, Weidendorfer, & Hinrichs, 2009). Some authors have also mentioned

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http://dx.doi.org/10.1016/j.foodres.2017.04.006
Received 9 February 2017; Received in revised form 6 April 2017; Accepted 6 April 2017
Available online 07 April 2017

that mechanical damage during gelation, for example due to shaking, negatively affects the formation of the protein network. This will result in a preliminary set gel with increased syneresis and in an inhomogeneous mixture of lumps and whey after stirring (Lucey, 2001; Walstra et al., 2006, chap. 22). Particles can be eliminated by further mechanical treatment with filters or valves but the product's viscosity will become low (Mokoonlall et al., 2016; Weidendorfer, Bienias, & Hinrichs, 2008). In order to realize a quasi-continuous operation regarding packaging, multiple fermentations are carried out simultaneously in several fermentation tanks up to 100 m^3. In this way, the process steps filling, fermentation, draining, cleaning and sterilization can be performed time-shifted. In our previous study (Körzendörfer, Temme, Nöbel, Schlücker, & Hinrichs, 2016), we have shown that machinery, in particular running pumps and CIP operations (high flow velocities, water/steam hammer etc.), generate unavoidable vibrations of a broad frequency band. These are transferred via pipes to the fermentation tanks. Type and speed of the pump as well as constructional circumstances affect spectra and amplitudes. The effect of vibrations cannot be studied in industry as process parameters like milk composition vary and occurring vibrations are random and individual. Thus, several lab-scale experiments with ultrasound were done. Short-time sonication during fermentation for only 5 min in the pH range 5.4–5.1 induced numerous large particles (Nöbel, Ross, et al., 2016), therefore this period must be considered critical. As a result, an experimental procedure was established to simulate fermentations with reproducible particle formation due to sonication. Moreover, an experimental setup was developed that enables generating individual frequencies and complex excitations. In pilot-scale experiments, low-frequency vibrations during gelation (25–1005 Hz) increased the particle number by a factor of 2.6 (Körzendörfer et al., 2016).

It has been reported that too low levels of exopolysaccharides (EPS) may cause granular textures (Tamime & Robinson, 2007). Mende, Rohm, and Jaros (2016) recently reviewed the effects of EPS in yogurt and related products. EPS are often discussed to yield benefits in yogurt since they improve viscosity and prevent syneresis of both set and stirred yogurt (Abbasi et al., 2009; Broadbent, McMahon, Welker, Oberg, & Moineau, 2003; Buldo et al., 2016; Folkenberg, Dejmek, Skriver, & Ipsen, 2005; Güler-Akin, Serdar Akin, & Korkmaz, 2009). Hence, costs for stabilizers can be saved and lower dry matter contents can be chosen (Duboc & Mollet, 2001). However, EPS only increases the serum viscosity slightly and the way they work is not clarified completely (Walstra et al., 2006, chap. 22). Buldo et al. (2016) highlighted that EPS could reduce the amount of large particles in stirred yogurt. We hypothesize that EPS further offers the potential to prevent textural defects such as syneresis and lumpiness due to sonication. The aim of this study was to investigate the correlation between starter cultures and the effect of sonication during fermentation. Two starter cultures differing in EPS synthesis were applied and physical properties of set and stirred yogurts were evaluated.

2. Materials and methods

2.1. Milk standardization and pretreatment

Fresh bovine raw milk was provided by the agricultural experiment station (Meiereihof, University of Hohenheim, Germany). The milk was skimmed at 55 °C (SA 10; Frautech S.r.l., Schio, Italy) and pasteurized (74 °C, 30 s). The protein content was standardized to 3.40 ± 0.01% (w/w) using reconstituted ultrafiltered skim milk permeate, which consisted of 5.2% (w/w) permeate powder (Bayolan PT; BMI e. G., Landshut, Germany) and demineralized water. Protein and fat contents were analyzed with an FTIR spectrometer (LactoScope FTIR Advance; Delta Instruments, Drachten, the Netherlands). Milk was heated to 95 °C for 256 s and subsequently cooled to 6 °C using a pilot plant (150 L/h; Asepto GmbH, Dinkelscherben, Germany).

2.2. Starter cultures

Two commercial frozen starter cultures YC-471 and YF-L 901 (Chr. Hansen, Hørsholm, Denmark) were used. Both cultures consist of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* strains but differ in the production of EPS. According to the manufacturer's specifications YC-471 results in yogurt with medium viscosity and strong flavor. In contrast, YF-L 901 yields a mild yogurt with extra high viscosity. Before every experiment, a quantity of 250 g of each frozen starter culture was diluted with 1000 g skim milk (6 °C) to ensure a homogeneous distribution of the two species. The stock solutions (20% w/w) were thawed at room temperature with occasional stirring.

2.3. Fermentation and ultrasound treatment

Milk was preheated to 42 °C and 2.5% starter culture stock solution (i.e. 0.05% in total) were added, respectively. Inoculated milks were filled in 100 mL glass jars (d = 45 mm, l = 65 mm) and fermented in a water bath (K25; Huber Kältemaschinenbau GmbH, Offenburg, Germany) at 42 °C. Acidifications were monitored with pH sensors (SE555X/2-NMSN; Knick Elektronische Messgeräte GmbH & Co. KG, Berlin, Germany) continuously (software: DAQ Factory Express; Azo Inc., Memphis, USA). At pH 5.2 half of the samples were temporarily transferred into an ultrasonic water bath (300 W, V = 19 L; RK 1028/H; Bandelin electronic GmbH & Co. KG, Berlin, Germany) and sonicated at 35 kHz for 5 min. The temperature was maintained at 42 °C using a second water bath (RE212; Lauda Dr. R. Wobser GmbH & Co. KG, Lauda-Königshofen, Germany) to avoid increase in temperature due to energy dissipation. In order to neglect any effects of moving, controls were also removed at the same time and subsequently put back. Fermentations were stopped at pH 4.6 by immersing the glass jars in iced water for 20 min. Samples were stored overnight at 10 °C until further analysis. The whole yogurt production was performed three times.

2.4. Syneresis and firmness of set yogurts

Spontaneously expelled whey was removed and quantified as suggested by Lucey (2004) before the firmness measurements and poured back afterwards. The quantity of the whey is expressed as

$$\text{Syneresis } (\%) = \frac{\text{expelled whey (g)}}{\text{initial milk (g)}} \times 100 \tag{1}$$

Additionally, the protein content of the remaining set gel P_{eff} was calculated according to Eq. (2). The protein content of expelled whey was supposed to be 0.8%.

$$P_{\text{eff}}\,(\%) = \left(\frac{3.4}{\frac{100 - \text{syneresis } (\%)}{100}} - \text{syneresis } (\%) \times 0.008 \right) \tag{2}$$

Penetration tests of set yogurts were done with a universal testing machine (5944; Instron, Norwood, USA; load cell: 50 N; software Bluehill 3). Sample temperature was 10 °C and only one measurement was performed in the middle of the glass jar using a cylindrical probe (d = 5 mm). The test speed was set to 30 mm/min for 30 s. The first maximum force (F_{max}), the distance where the break took place (s_{Fmax}), the initial slope from 0 to 2 mm (m_{init}) and the force at the maximum penetration depth of 15 mm ($F_{15\text{ mm}}$) were calculated.

2.5. Stirred yogurt production

Set yogurt gels were slightly broken with a spoon and sheared at approximately 10 °C with a large syringe/piston pump through a nozzle (d_i = 3 mm, l = 25 mm). The piston was moved by the universal testing machine (5944; Instron, load cell: 2 kN) to ensure a constant