



# Manufacture of high-protein yogurt without generating acid whey – Impact of the final pH and the application of power ultrasound on texture properties

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## ABSTRACT

The fermentation of pre-concentrated milk is a challenging method to avoid acid whey during the manufacture of high-protein fermented milks like Greek yogurt. Milk concentrates (10% protein) were fermented to a final pH of 5.0, 4.8, or 4.6 and processed into stirred yogurt. Additionally, the potential of power ultrasound (US) as a post-processing tool was examined by sonicating the stirred yogurt with a sonotrode at 20 kHz. Set gels fermented to pH 4.8 and 5.0 were considerably softer than gels fermented to pH 4.6. Stirred yogurts fermented to pH 4.8 or 5.0 were less grainy and exhibited a reduced apparent viscosity and water-holding capacity. The application of US further decreased the visual graininess and product viscosity whereas the particle size was only slightly affected. The final pH and sonication are two powerful approaches to control the rheological properties of high-protein fermented milks, offering the potential for innovative processes and products.

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## 1. Introduction

High-protein fermented milk products are named according to their geographical origin and several technological aspects during manufacture. They mainly include concentrated yogurts labelled as Greek or Greek-style yogurt, strained yogurt, labneh (Middle East), or skyr (Iceland), but also fresh cheeses such as quarg (Tamime, Hickey, & Muir, 2014). The high popularity of these products is attributed to various health benefits that milk proteins have recently shown (Fekete, Givens, & Lovegrove, 2013; Pasiakos, 2015).

Conventionally, milk is acidified with lactic acid bacteria in fermentation tanks up to 100,000 m<sup>3</sup>. At approximately pH 4.6, the set gel is broken up by stirring and concentrated by ultrafiltration or centrifugation (Özer, 2006). However, this results in large amounts of acid whey that is unfavourable due to its limited applications (Konrad, Kleinschmidt, & Faber, 2012; Shon & Haque, 2007) and environmental concerns (Chen, Talebi, Gras, Weeks, & Kentish, 2018). Discharging acid whey into wastewater streams decreases the level of dissolved oxygen and can lead to fish kill and algal blooms (Erickson, 2017). Alternative manufacturing

procedures that involve a concentration step prior to the fermentation to reduce or avoid acid whey are of economic and ecological interest. In particular, ultrafiltration (UF) and microfiltration (MF) have been used (Schäfer et al., 2019; Uduwerella, Chandrapala, & Vasiljevic, 2018) as well as the addition of milk protein powders obtained from MF and UF retentates (Agarwal, Beausire, Patel, & Patel, 2015). The added value is increased by the functionality of the by-products, i.e., sweet permeates instead of acid whey (Heino, Uusi-Rauva, Rantamäki, & Tossavainen, 2007; Ratray & Jelen, 1996).

However, the industrial-scale fermentation of milk concentrates leads to both technical and sensory problems. Since there is a non-linear correlation between the protein content of the milk and the firmness of the acid-coagulated set gel, fermented concentrates, particularly ≥8% protein, exhibit an excessive firmness. Increasing the protein content by a factor of 2.5 from 4% to 10% resulted in a firmness increased by a factor of 8 (Körzendörfer, Schäfer, Hinrichs, & Nöbel, 2019). As a consequence, breaking up the set gel by the agitation system in the fermentation tank as well as drainage is impaired or not possible anymore. Moreover, further processing into a stirred product by breaking the gel into smaller particles is difficult due to the firm and dense network structure (Jørgensen et al., 2019). Particle size distribution in turn determines the smoothness and other sensory properties of the product

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(Cayot, Schenker, Houzé, Sulmont-Rossé, & Colas, 2008; Hahn et al., 2012; Krzeminski et al., 2013). Fermented milks from concentrates have often been reported to be grainy and coarse, associated with a high gel firmness of the set gel (Biliaderis, Khan, & Blank, 1992; Flüeler & Puhán, 1977; Jørgensen et al., 2015; Mistry & Hassan, 1992). The final properties of the stirred product could be beneficially adjusted by controlling the firmness of the initial gel and the mechanical post-processing step.

Apart from the total protein content, the firmness of an acid-coagulated milk gel is affected by the protein composition, heat treatment of milk, and fermentation temperature (Sodini, Remeuf, Haddad, & Corrieu, 2004). A decreased firmness of fermented milk concentrates (8% protein) was obtained by increasing the relative amount of native whey proteins (Jørgensen et al., 2015). Moreover, the firmness of the set gels was positively correlated to the mean particle size and perceived coarseness of the stirred yogurt. Another parameter that influences the gel firmness and viscosity of the stirred product is the final fermentation pH (Béal, Skokanova, Latrille, Martin, & Corrieu, 1999; Küçükçetin, 2008; Martin, Skokanova, Latrille, Béal, & Corrieu, 1999). With regards to fermented milk concentrates, the effect of the final fermentation pH on firmness and gel structure has not been researched yet.

In order to generate a smooth product from fermented concentrates, an intensified mechanical post-processing is essential. Rotor/stator devices like colloid mills may be used (Mokoonlall, Nöbel, & Hinrichs, 2016), however, almost no studies have been published that focus on fermented concentrates. Meletharayil, Metzger, and Patel (2016) reported a hydrodynamic cavitator to control the viscosity of high-protein yogurt from concentrate. Power ultrasound (US) is a further technology to induce acoustic cavitation offering versatile applications in food technology, e.g., homogenisation and emulsification (Chandrapala & Leong, 2015; Chemat, Zill-e-Huma, & Khan, 2011). Power US also decreased the viscosity of liquid milk concentrates (Zisu, Schleyer, & Chandrapala, 2013) and the gel firmness of fermented milk concentrates that were sonicated during acidification (Körzendörfer et al., 2019). In this study, high-protein yogurts from milk concentrates were manufactured and the effect of the final fermentation pH on the physical properties was investigated. Additionally, the potential of power US as a post-processing tool was examined.

## 2. Materials and methods

### 2.1. Milk standardization and pretreatment

Skim milk was provided weekly by the agricultural experiment station (Meiereihof, University of Hohenheim, Stuttgart, Germany) and pasteurized at 74 °C for 30 s. An FTIR spectrometer (LactoScope FTIR Advance; Delta Instruments, Drachten, the Netherlands) was used to analyze skim milk samples that averaged (in w/w) 3.5% protein, 0.1% fat, and 9.5% dry matter. The protein content was standardized to 10.0% ± 0.1 (w/w) using milk protein powder (TMP 80; Milei GmbH, Leutkirch, Germany) that was derived from ultrafiltered skim milk retentate and consisted of (in w/w) 79.9% protein, 1.1% fat, 7.3% lactose, and 4.4% moisture (manufacturer's specification). The nitrogen content of fortified skim milk concentrates was determined according to the method of Dumas (International Dairy Foundation, 185:2002) using a nitrogen analyzer (Dumatherm DT, Gerhardt GmbH & Co. KG, Königswinter, Germany). The total protein content was calculated by multiplying the nitrogen content by the conversion factor of 6.38. Standardized skim milks (2 kg per container) were heated to ≥ 85 °C for 30 min in a water bath (95 °C; AEJ 6-5; Aerne Analytic e. K., Weißenhorn, Germany) to denature whey proteins (>90%) and subsequently cooled to 6 °C in iced water.

### 2.2. Yogurt manufacture

A quantity of 250 g of the frozen starter culture (YC-471; Chr. Hansen A/S, Hørsholm, Denmark) was diluted with 1000 g of skim milk (6 °C) and thawed before use. Preheated milk (3 kg) was filled in stainless steel containers ( $d = 175$  mm,  $l = 230$  mm) and put in a tempered water bath (ED; Julabo Labortechnik GmbH, Seelbach, Germany). After temperatures had equilibrated, milk was inoculated with 7.5 mL of the starter culture stock solution and fermented at 43.5 °C. Acidification was continuously monitored with pH sensors (SE555X/2-NMSN; Knick Elektronische Messgeräte GmbH & Co. KG, Berlin, Germany; software: DAQ Factory Express; Azo Inc., Memphis, TN, USA). Fermentations were stopped at pH 5.0, 4.8, and 4.6, respectively. For this purpose, set gels were manually broken by moving a perforated disc 20 times up and down and then filled in polypropylene containers (900 g,  $d = 102$  mm,  $l = 155$  mm). After cooling in iced water for 60 min, samples were stored overnight at 10 °C until further analysis and processing. The degree of post-acidification during cold storage was recorded by measuring the pH 1, 3, and 9 d after fermentation. The number of independent repetitions of the experiments was  $i \geq 3$ .

### 2.3. Agitation test

The development of the gelation during acidification was monitored with a rheometer (AR2000ex; TA Instruments, New Castle, DE, USA). Agitation tests served to describe the fracture behavior during stirring up the set milk gel and estimate its processability in a large-scale fermentation tank. Separate samples were prepared in smaller glass jars (140 g,  $d = 50$  mm,  $l = 70$  mm) and fermented at 43.5 °C (K25; Huber Kältemaschinenbau GmbH, Offenburg, Germany). Every 0.1 pH, a vane geometry (four paddles,  $d = 15$  mm,  $l = 28$  mm) was immersed into the warm milk/gel and rotated by linearly increasing the rotational velocity for 90 s. In total, four complete revolutions were done and the required torque  $M$  was recorded. Agitation tests were directly carried out in the glass jars and a new jar was used for every measurement. The mechanical power  $P$  was calculated according to

$$P = 2\pi Mn \quad (1)$$

where  $n$  is the rotational speed. Additionally, the work done  $W$  for half the initial rotation ( $t = 33$  s) of the vane geometry was calculated.

$$W = \int_0^t P dt \quad (2)$$

### 2.4. Penetration test

Penetration tests of the cooled samples (10 °C) were accomplished one day after fermentation with a universal testing machine (5944; Instron, Norwood, MA, USA; load cell: 50 N; software: Bluehill 3). Six measurements were done per container using a cylindrical probe ( $d = 10$  mm). The test speed was set to 0.5 mm s<sup>-1</sup> for 30 s and the force was recorded. Gel firmness was defined as the first maximum force.

### 2.5. Mechanical post-processing and sonication

Pre-sheared samples were processed into stirred yogurt according to a modified method described by Körzendörfer, Nöbel, and Hinrichs (2017). This technique is comparable to a needle valve that is commonly used in industrial processes to smooth

the product. Yogurts were filled in a large syringe ( $d_i = 58$  mm,  $l = 290$  mm; Hausamann & Co AG, Zürich, Switzerland) and pumped 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 flow rate of  $40 \text{ mL s}^{-1}$  that corresponds to a representative shear rate  $\dot{\gamma}_{rep}$  of  $15,100 \text{ s}^{-1}$  within the nozzle

$$\dot{\gamma}_{rep} = (4 \dot{V}) / (\pi r^3) \quad (3)$$

where  $\dot{V}$  is the volume flow ( $\text{m}^3 \text{ s}^{-1}$ ) and  $r$  the radius of the nozzle (m).

Half of the stirred yogurts underwent a second mechanical post-processing step by means of a sonication treatment. Power US was generated by an ultrasonic device (Sonopuls HD 2200; Bandelin electronic GmbH & Co. KG, Berlin, Germany) with a maximum ultrasonic nominal output of 200 W. The ultrasonic generator (GM 2200) is used for controlling and transforms low-frequency voltage (50 Hz) into high-frequency voltage (20 kHz). Electrical voltage from the generator is transformed into mechanical vibrations of 20 kHz (ultrasonic converter: UW 2200). A booster horn (SH 213 G) amplifies the vibrations that are finally transmitted into the sample by the probe equipped with a titanium flat tip (TT13;  $d = 13$  mm,  $l = 5$  mm). The amplitude was set to 10% that corresponds to a displacement of  $16.5 \mu\text{m}$  (peak-to-peak). Yogurt samples ( $20^\circ \text{C}$ ) were filled in small plastic cups (85 g per cup) and sonicated for  $t = 5$  s. The cups were moved during the treatment to ensure a homogeneous distribution of the sound waves.

Additionally, ultrasonic intensity was determined using a calorimetric method described by Tiwari, Muthukumarappan, O'Donnell, and Cullen (2008). The ultrasonic power  $P_{US}$  was calculated with demineralized water ( $m = 0.085$  kg) according to

$$P_{US} = mc_p \left( \frac{dT}{dt} \right)_{t=0} \quad (4)$$

where  $m$  is the mass,  $c_p$  is the specific heat of water ( $4.18 \text{ kJ kg}^{-1} \text{ K}^{-1}$ ), and  $(dT/dt^{-1})$  is the change in temperature over time ( $\text{K s}^{-1}$ ). Afterward, ultrasonic intensity  $UI$  was determined according to

$$UI = \frac{4 P_{US}}{\pi d^2} \quad (5)$$

where  $d$  is the diameter of the probe. Sonication parameters above resulted in an ultrasonic power of  $P_{US} = 30$  W and  $UI = 22.5 \text{ W cm}^{-2}$ . The mechanical energy input  $E$  was calculated to be  $1765 \text{ J/kg}^{-1}$  yogurt due to the US treatment.

$$E = \frac{P_{US} t}{m} \quad (6)$$

All stirred yogurt samples were stored in containers (500 mL) at  $10^\circ \text{C}$  until further analyses (WHC, transmission images, laser diffraction). For rheological measurements, separate samples in glass jars (100 mL, 3 jars per sample) were stored for 7 d at  $10^\circ \text{C}$  and not disturbed until analyses to allow rebodding.

## 2.6. Syneresis

To evaluate the storage stability of the stirred yogurts, the measuring setup shown in Supplementary material Fig. S1 was developed. After the sonication treatment, approximately 80 g yogurt ( $m_y$ ) were filled in the planar area ( $60 \times 60 \times 30$  mm) that was separated by a perforated metal ( $d = 1.5$  mm) from the sloping area. Samples were covered with plastic wrap and stored at  $8^\circ \text{C}$ . Spontaneously expelled whey was quantified ( $m_t$ ) after  $t = 1, 4$ , and 7 d. Syneresis  $S_t$  was expressed as a percentage (w/w).

$$S_t(\%) = \frac{\sum_0^t m_t}{m_y} \times 100 \quad (7)$$

## 2.7. Water-holding capacity (WHC)

The WHC was determined as described in our previous study (Körzendörfer et al., 2019). Stirred yogurt samples ( $m_y = 40$  g) were centrifuged (Biofuge primo R; Heraeus Holding GmbH, Hanau, Germany) at  $1000 \times g$  and  $10^\circ \text{C}$  for 10 min. After removing the supernatant, the pellet weight  $m_p$  was recorded. Measurements were performed 7 d after fermentation in triplicate. The WHC was calculated according to

$$WHC(\%) = \frac{m_p}{m_y} \times 100 \quad (8)$$

## 2.8. Laser diffraction spectroscopy

Particle size distributions of small microgel particles ( $d < 0.5$  mm) were determined by static light scattering (LS 13320, Beckman-Coulter Inc., Miami, FL, USA) based on Mie theory as described by Mookoolall et al. (2015). Yogurt samples were diluted with demineralized water (6%, w/w) and stirred for 15 min at 150 rpm. Approximately 1 mL of the diluted sample was added to the dispersion unit (obscurator: 14–16%). Three successive runs were performed of each sample. Measurements were repeated three times and the volume-weighted 10th, 50th, and 90th percentiles ( $d_{10}$ ,  $d_{50}$ ,  $d_{90}$ ) were calculated.

## 2.9. Transmission images: texture and large particles

Transmission images served to evaluate the texture of stirred yogurts with regards to visual graininess and large particles (Küçükçetin, 2008; Nöbel et al., 2016). A thin layer (1.2 mm) of the yogurt sample was scratched out with a scraper and metal bar spacers on a glass plate from which transmission images (source of light: Comic Master Tracer LED-A4, Too Marker Products Inc., Tokyo, Japan) were taken using a digital camera (8-bit grayscale; MicroPublisher 3.3 RTV, QImaging, Surrey, Canada). One image ( $120 \times 90$  mm) represents approximately 13 g yogurt. Data was collected from at least 8 independent images per sample. Finally, the most representative images were selected.

## 2.10. Rheological characterization

Stirred yogurt samples were analyzed 7 d after the mechanical treatment at  $10^\circ \text{C}$  using a rheometer (MCR 502; Anton Paar GmbH, Graz, Austria) with a coaxial cylinder geometry (CC27). After an equilibration time of 10 min, storage moduli ( $G'$ ), loss moduli ( $G''$ ), and phase angles ( $\delta$ ) were determined by means of oscillating measurements in the linear viscoelastic region ( $\gamma = 0.0025$ ) by performing a 30-s time sweep at a constant frequency of  $10 \text{ rad s}^{-1}$ . Flow curves were measured in successive rotational measurements using the same sample. The shear rate was linearly increased from 0 to  $500 \text{ s}^{-1}$  within 3 min. After a hold step (3 min,  $500 \text{ s}^{-1}$ ), the shear rate was linearly decreased to  $0 \text{ s}^{-1}$  within 3 min. Measurements were repeated three times and the maximum shear stress was calculated (Körzendörfer, Temme, Schlücker, Hinrichs, & Nöbel, 2018). Additionally, the apparent viscosity  $\eta_{100}$  was calculated at a shear rate of  $100 \text{ s}^{-1}$  as this parameter was associated with the perceived oral viscosity (Skriver, Holstborg, & Qvist, 1999).

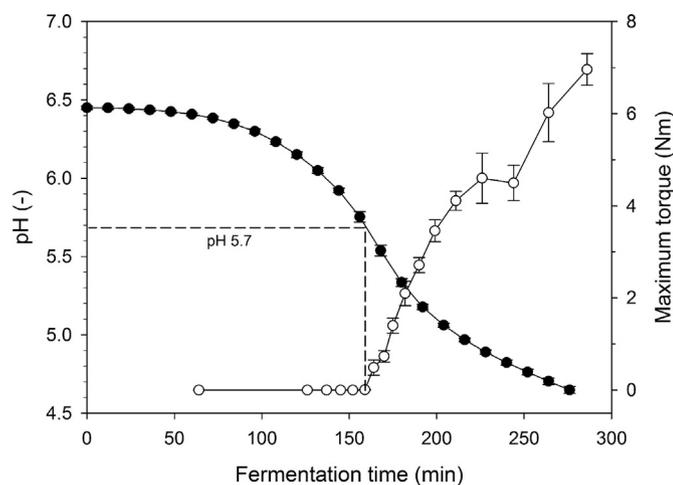
### 2.11. Statistical analyses

Statistical analyses were conducted with SigmaPlot (v. 12.5; Systat Software Inc., San Jose, CA, USA). A one-way ANOVA on ranks (Tukey test) served to compare multiple groups. The effect of the two factors “final fermentation pH” and “sonication” on the structural properties of the stirred yogurts was evaluated by performing a two-way ANOVA (Tukey test). To take account of weekly variations the factor “fermentation experiment” was added. A significance level of  $\alpha = 0.05$  was chosen in all cases. Results are given as arithmetic means and standard errors.

## 3. Results and discussion

### 3.1. Acidification and gelation

Agitation tests during fermentation were done to study the gelation behavior of the milk concentrate. Fig. 1 shows the acidification profile of milk protein concentrates fermented at 43.5 °C and corresponding data from agitation tests. During the beginning of the fermentation, the milk is still in the liquid state and the maximum torque remains unchanged. At pH 5.7 (159 min), the maximum torque increases indicating the onset of the gelation. The sharp, almost linear increase is followed by a plateau phase from pH 5.0 to 4.8. Afterwards, the maximum torque increases again with continuing acidification. Detailed data from agitation tests performed at pH 5.0, 4.8, and 4.6 are presented in Fig. 2(a). Torques increase linearly with the angle of rotation until a maximum ( $M_{\max}$ ) is reached after a rotation by approximately 70° and the gel breaks. The vane then keeps rotating in the disrupted gel so that the torque decreases to a relatively constant lower value. The maximum torque required to break the gel at pH 4.6 was  $6.96 \pm 0.34$  Nm. Gels agitated at pH 4.8 and 5.0 only reached  $4.50 \pm 0.38$  Nm and  $4.11 \pm 0.21$  Nm (Table 1). Softer gels also led to a reduced mechanical power and energy needs. This is relevant when considering the fermentation of concentrates on an industrial scale and the design of the agitation system. The steadily increasing stiffness of acid-coagulated milk gels during acidification is attributed to the decreasing zeta potential of the casein micelles. Several authors have monitored the increase of the storage modulus during fermentation, revealing a maximum



**Fig. 1.** pH (●) during acidification and maximum torques (○) from agitation tests as a function of fermentation time. Skim milk concentrates (10%, w/w, protein) were fermented at 43.5 °C and the maximum torques were determined using a rheometer equipped with a vane geometry. The sudden increase at pH 5.7 indicates the onset of gelation.

gel strength around pH 4.6, which is the isoelectric point of caseins (Lucey, 2017).

Yogurt samples for comprehensive structural analyses were prepared from concentrates fermented to pH 4.6, 4.8, and 5.0, respectively. Fermentations were stopped by breaking the gel and cooling in iced water. Results from penetration tests performed the next day are shown in Fig. 2(b). Samples fermented to pH 4.6 still exhibited the firmest structure. The maximum force was reduced by 42% (pH 4.8,  $P < 0.05$ ) and 73% (pH 5.0,  $P < 0.05$ ) due to the higher pH (Table 1). When penetration tests were performed, the actual pH of all samples was below the final fermentation pH as the acid formation continued during cooling. Nevertheless, with regards to the gel strength, the order of the samples from agitation (intact gel) and penetration test (broken gel) was equal. Softer (set) gels are generally formed when the fermentation temperature is lowered (Haque, Richardson, & Morris, 2001; Körzendörfer et al., 2019). Moreover, both increasing and decreasing the native casein to whey protein ratio have been reported to increase gel firmness (Chua, Deeth, Oh, & Bansal, 2017; Puvanenthiran, Williams, & Augustin, 2002; Tamime, Kalab, & Davies, 1984; Zhao, Wang, Tian, & Mao, 2016). In contrast, weaker gels were obtained when the whey proteins stayed in the undenatured form (Guggisberg, Eberhard, & Albrecht, 2007). Our results show that the final fermentation pH is another parameter to affect the gel strength.

### 3.2. Physical properties of stirred yogurts

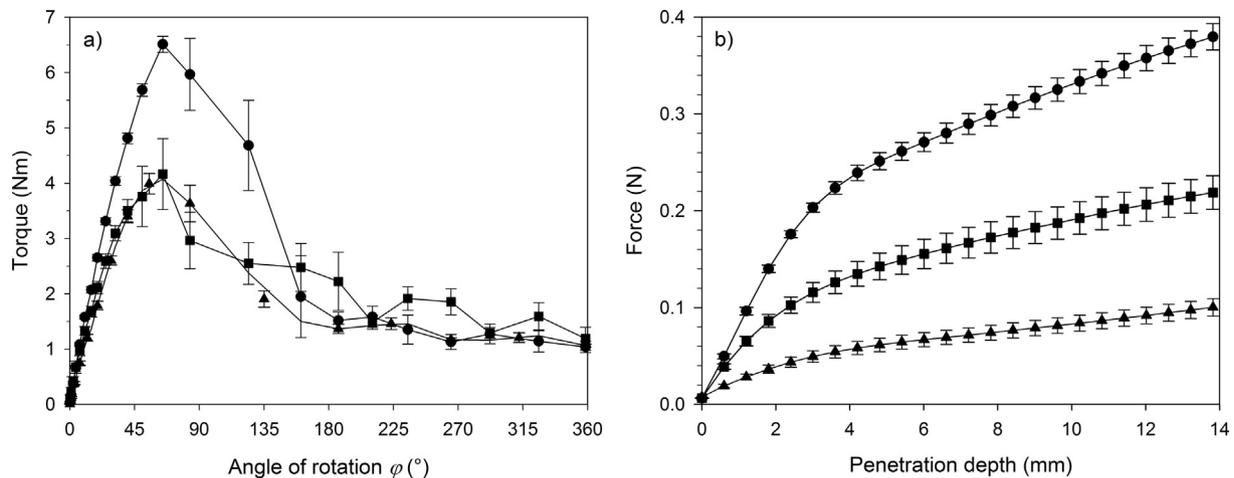
Broken gels differing in the final fermentation pH were processed into stirred yogurt and half of the sample set was additionally sonicated for 5 s. Several physical properties of stirred yogurts were then analysed.

#### 3.2.1. Post-acidification and syneresis during storage

Fig. 3(a) shows the pH of stirred yogurts during cold storage. Regardless of the final fermentation pH, all samples show a steady decrease of the pH over time. The sharp decrease after the first day of storage can be explained by the high metabolism activity of bacteria when fermentations were stopped by the relatively slow cooling process. Afterwards, the pH decrease is moderate and pH values are arranged according to the final fermentation pH. After 9 days, all controls differed significantly (Table 2). Sonicated yogurts exhibited a lower pH compared with the corresponding control. US was often reported to increase the metabolic activity of lactic acid bacteria. Cavitation effects can improve the membrane permeability (sonoporation) by opening the cell membrane so that enzymes such as  $\beta$ -galactosidase are released from the cell (Abesinghe et al., 2019; Nguyen, Lee, & Zhou, 2009). The increased enzyme activity can likewise be attributed to a reduced activation energy or an altered enzyme structure (Delgado-Povedano & de Castro, 2015; Ewe, Abdullah, Bhat, Karim, & Liong, 2012; Huang et al., 2017). However, it is also possible that the metabolism of bacteria was slightly increased/reactivated in the sonicated yogurts since samples were temporarily warmed up to 20 °C before the sonication treatment.

Despite the high buffering capacity, yogurts from concentrates exhibited considerable post-acidification that is strongly dependent on the starter culture (Béal et al., 1999; Damin, Minowa, Alcantara, & Oliveira, 2008). According to the manufacturer, the starter culture used in this study forms relatively high amounts of acid during storage. Thus, even if a higher fermentation pH is chosen, the pH of the final product and its acidic taste can still be controlled by selecting an appropriate starter culture that shows a specific ability to produce acid during cold storage.

Syneresis was determined by a gravitational method and results are shown in Fig. 3(b). An increase over time was observed for all samples, but yogurts fermented to pH 4.6 exhibited the lowest



**Fig. 2.** Required torque (a) to stir up and break the set gel directly after fermentation at 43.5 °C until a final pH of pH 4.6 (●), 4.8 (■), and 5.0 (▲), respectively; Texture profile analysis (b) of the corresponding broken up gels measured one day after fermentation by penetration tests with a cylindric plunger ( $d = 10$  mm) at 10 °C.

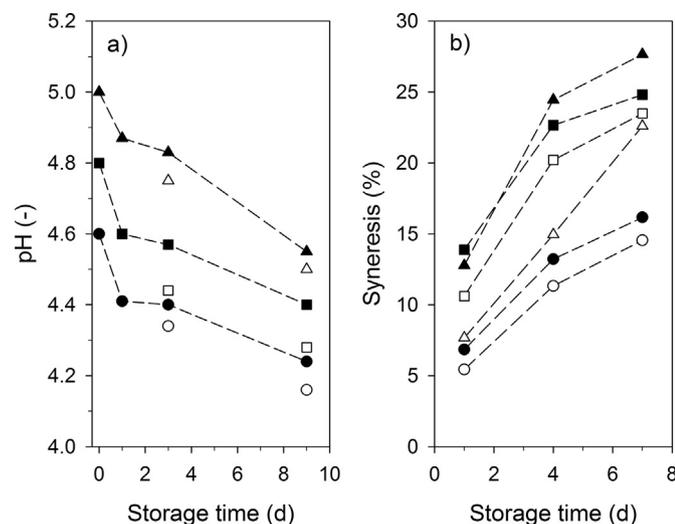
**Table 1**

Fermentation times and physical characteristics of fermented milk concentrates acidified until different pH values.<sup>a</sup>

Final fermentation pH (–)	Fermentation time (min)	Agitation test			Penetration test	Post-acidification
		$M_{\max}$ (Nm)	$P_{\max}$ (W)	$W$ (J)	$F_{\max}$ (mN)	pH (–)
5.0	212 ± 2 <sup>a</sup>	4.11 ± 0.21 <sup>a</sup>	1.17 ± 0.12 <sup>a</sup>	7.6 ± 0.4 <sup>a</sup>	106 ± 9 <sup>a</sup>	4.87 ± 0.03 <sup>a</sup>
4.8	245 ± 4 <sup>b</sup>	4.50 ± 0.38 <sup>a</sup>	1.20 ± 0.16 <sup>a</sup>	8.3 ± 1.0 <sup>a</sup>	227 ± 18 <sup>b</sup>	4.60 ± 0.02 <sup>b</sup>
4.6	286 ± 5 <sup>c</sup>	6.96 ± 0.34 <sup>b</sup>	2.10 ± 0.27 <sup>b</sup>	12.0 ± 1.3 <sup>b</sup>	393 ± 14 <sup>c</sup>	4.41 ± 0.02 <sup>c</sup>

<sup>a</sup> Abbreviations are:  $M_{\max}$ , maximum torque;  $P_{\max}$ , mechanical power;  $W$ , work done for half the initial rotation;  $F_{\max}$ , maximum force. Agitation tests were performed directly after fermentation at approximately 40 °C; penetration tests were performed of the broken up gel at 10 °C after one day of storage; pH was measured one day after fermentation at 10 °C. A one-way ANOVA on ranks (Tukey test) was performed to compare multiple groups; values in a column with different superscript letters differ significantly ( $P < 0.05$ ).

values. Syneresis of non-sonicated controls differed after 7 days of storage ( $P < 0.05$ , Table 2). Sonicated samples showed less syneresis compared to the corresponding controls, and the effect was most



**Fig. 3.** Post-acidification (a) and syneresis (b) of control and sonicated stirred yogurt as a function of storage time. Yogurts were fermented until a final pH of 4.6 (●, control; ○, sonicated), 4.8 (■, control; □, sonicated), and 5.0 (▲, control; △, sonicated), respectively. Samples were stored at 8 °C.

pronounced for yogurt fermented to pH 5.0. A higher final fermentation pH during manufacture may give products with increased whey separation, but this could be counteracted by an exopolysaccharide-producing strain (Gyawali & Ibrahim, 2016). The measuring setup is an appropriate method to evaluate wheying-off during the storage of high-protein yogurts. However, the mesh size of the perforated screen should be decreased as samples with a low viscosity may flow through the pores.

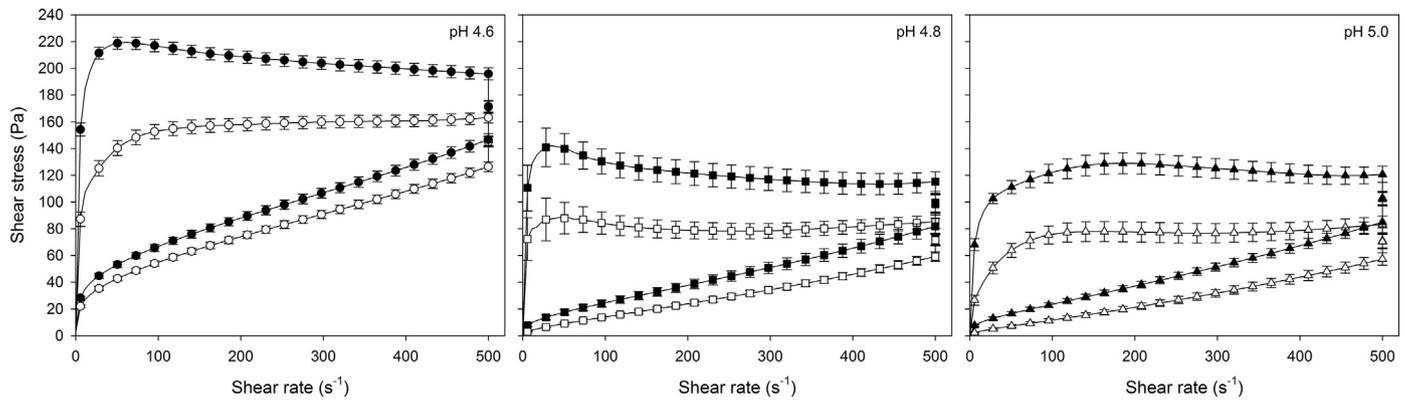
### 3.2.2. Rheological properties

Flow curves from rotational measurements of stirred yogurts are shown in Fig. 4. The control fermented to pH 4.6 exhibited the highest shear stresses. Flow curves of controls fermented to pH 4.8 and 5.0 are on a similar level but deviate at the beginning of the measurement up to approximately 200  $s^{-1}$ . Shear stresses of all sonicated yogurts were considerably decreased indicating structural degradation due to the US treatment. Characteristic rheological parameters from oscillation and rotational measurements are listed in Table 3. The final fermentation pH had a significant effect ( $P < 0.05$ ) on the apparent viscosities and maximum shear stresses of both controls and sonicated yogurts, respectively. The apparent viscosity of controls was reduced by 40% (pH 4.8) and 43% (pH 5.0) compared to the control fermented to pH 4.6. The results demonstrate that a large part of the yogurt structure is formed at the very end of the fermentation, i.e., from pH 4.8 to 4.6. In relation to a specific pH, sonication decreased ( $P < 0.001$ ) all parameters from rotational rheology. The apparent viscosity of yogurt fermented to pH 4.6 was decreased from  $2.14 \pm 0.05$  to  $1.52 \pm 0.05$  Pa (–29%). At a

**Table 2**  
Post-acidification and syneresis of stirred yogurts fermented until different final fermentation pH values and treated with power US after fermentation.<sup>a</sup>

Final fermentation pH (–)	Post-acidification pH (–)		Syneresis (%)	
	Control	Sonicated	Control	Sonicated
4.6	4.24 ± 0.02 <sup>A</sup>	4.16 ± 0.03 <sup>a*</sup>	16.2 ± 0.2 <sup>A</sup>	14.6 ± 0.3 <sup>a***</sup>
4.8	4.40 ± 0.01 <sup>B</sup>	4.28 ± 0.02 <sup>b**</sup>	24.8 ± 0.6 <sup>B</sup>	23.5 ± 2.1 <sup>b</sup>
5.0	4.55 ± 0.03 <sup>C</sup>	4.50 ± 0.03 <sup>c</sup>	27.7 ± 1.5 <sup>C</sup>	22.6 ± 1.4 <sup>b***</sup>

<sup>a</sup> pH was measured 9 days after fermentation and a one-way ANOVA was performed; syneresis was determined after 7 days and a two-way ANOVA was performed. Control and sonicated samples fermented until different pH values with different uppercase and lowercase superscript letters, respectively, differ significantly ( $P < 0.05$ ); asterisks indicate that sonicated samples differ significantly from the control (\* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ ).



**Fig. 4.** Flow curves of control and sonicated stirred yogurts measured at 10 °C by rotational rheology after 7 days of storage. Yogurts were fermented until a final pH of 4.6 (●, control; ○, sonicated), 4.8 (■, control; □, sonicated), and 5.0 (▲, control; △, sonicated), respectively.

final pH of 4.8 and 5.0 the decrease amounted to 35% and 38%, respectively. Fig. 4 also reveals differences in the courses of the (upwards) curves, e.g., in regard to the shear rate where the maximum shear stress occurs. Furthermore, a sharp increase of the shear stress at the beginning of the rotational measurement implies a yield point. The shear rate which correlates best with the oral perception depends on the complex flow properties of a material and may vary between the samples (Shama & Sherman, 1973). Therefore, future studies should include sensory analysis to evaluate the oral perception.

Storage moduli of stirred yogurts are shown in Fig. 5(a). Controls fermented to pH 4.6 (1978 ± 85 Pa) and 4.8 (1894 ± 186 Pa) did not differ significantly. Stopping the acidification at pH 5.0 resulted in a considerable decrease. Sonication of stirred yogurts further

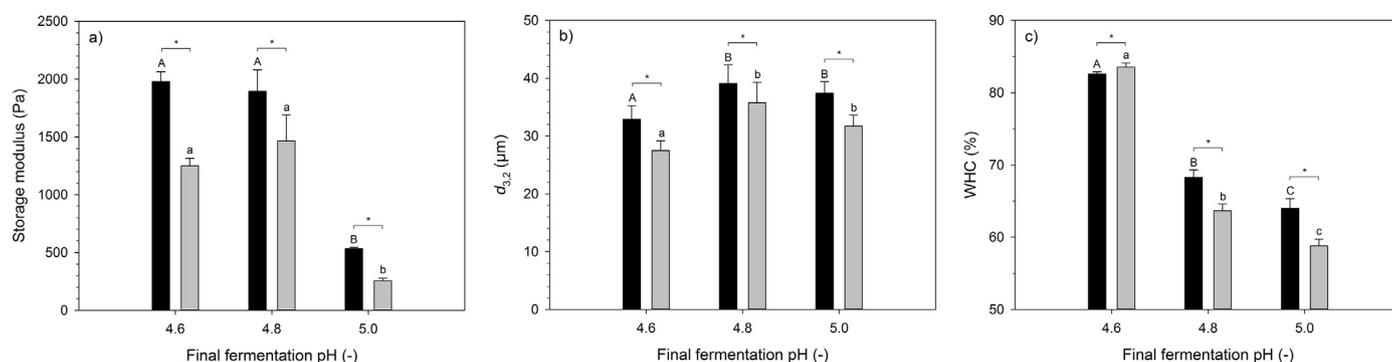
diminished storage moduli by 37% (pH 4.6), 23% (pH 4.8), and 52% (pH 5.0). Similar effects of the final fermentation pH and sonication were observed for loss moduli and phase angles (Table 3). Storage moduli of all samples exceeded the loss modulus ( $G' > G''$ ). Thus, the elastic (solid) component was higher than the viscous component in any case so that all yogurts are still classified as viscoelastic solids. An increase of the phase angle due to a higher pH and/or sonication indicates a shift to a more viscous-like flow behavior.

Rheological properties of the final stirred product are basically determined by (i) the structure of the preliminary set gel and (ii) the post-processing. Since altering the final fermentation pH and the sonication treatment as a post-processing step resulted in distinctly different rheological properties, both approaches should be considered to develop high-protein yogurts with specific flow

**Table 3**  
Rheological properties of stirred yogurts from oscillatory and rotational measurements.<sup>a</sup>

Oscillatory measurements						
Final fermentation pH (–)	$G'$ (Pa)		$G''$ (Pa)		$\delta$ (°)	
	Control	Sonicated	Control	Sonicated	Control	Sonicated
4.6	1978 ± 85 <sup>A</sup>	1251 ± 62 <sup>a***</sup>	522 ± 27 <sup>A</sup>	348 ± 18 <sup>a***</sup>	14.73 ± 0.15 <sup>A</sup>	15.53 ± 0.22 <sup>a***</sup>
4.8	1894 ± 186 <sup>A</sup>	1465 ± 225 <sup>a***</sup>	516 ± 90 <sup>A</sup>	401 ± 60 <sup>a***</sup>	15.23 ± 0.04 <sup>A</sup>	15.37 ± 0.10 <sup>a</sup>
5.0	533 ± 11 <sup>B</sup>	256 ± 22 <sup>b***</sup>	145 ± 3 <sup>B</sup>	72 ± 6 <sup>b***</sup>	15.27 ± 0.08 <sup>B</sup>	15.83 ± 0.10 <sup>a***</sup>
Rotational measurements						
Final fermentation pH (–)	$\eta_{100}$ (Pa s)		$\tau_{max}$ (Pa)		$\tau_{500}$ (Pa)	
	Control	Sonicated	Control	Sonicated	Control	Sonicated
4.6	2.14 ± 0.05 <sup>A</sup>	1.52 ± 0.05 <sup>a***</sup>	220 ± 5 <sup>A</sup>	165 ± 4 <sup>a***</sup>	147 ± 4 <sup>A</sup>	127 ± 4 <sup>a***</sup>
4.8	1.28 ± 0.09 <sup>B</sup>	0.83 ± 0.08 <sup>b***</sup>	147 ± 13 <sup>B</sup>	103 ± 11 <sup>b***</sup>	82 ± 6 <sup>B</sup>	59 ± 3 <sup>b***</sup>
5.0	1.21 ± 0.07 <sup>C</sup>	0.75 ± 0.07 <sup>c***</sup>	129 ± 8 <sup>C</sup>	84 ± 7 <sup>c***</sup>	85 ± 4 <sup>B</sup>	57 ± 5 <sup>b***</sup>

<sup>a</sup> Milk concentrates were fermented until a final fermentation pH of 4.6, 4.8, or 5.0 and treated with power US after fermentation. Abbreviations are:  $G'$ , storage modulus;  $G''$ , loss modulus;  $\delta$ , phase angle;  $\eta_{100}$ , apparent viscosity;  $\tau_{max}$ , maximum shear stress;  $\tau_{500}$ , shear stress at 500 s<sup>-1</sup>. A two-way ANOVA (Tukey test) was performed to compare multiple groups. Control samples and sonicated samples fermented until different pH values with different uppercase and lowercase superscript letters, respectively, differ significantly ( $P < 0.05$ ); asterisks indicate that sonicated samples differ significantly from the control (\*\*\* $P < 0.001$ ).



**Fig. 5.** Storage modulus (a), Sauter mean diameter (b), and water-holding capacity (c) of control (black bars) and sonicated (grey bars) stirred yogurts fermented until a final fermentation pH of 4.6, 4.8, and 5.0, respectively. An asterisk indicates that the sonicated sample differs significantly from the control ( $P < 0.05$ ).

properties. Several authors reported a decreased viscosity when the milk was acidified to pH 4.8 compared with 4.4 (Béal et al., 1999; Küçükçetin, 2008; Martin et al., 1999); however, these studies were focused on stirred yogurt with a lower protein content. US technology, in contrast, was commonly applied to treat the milk before or during the fermentation, and various effects on yogurt structure were attributed to fat homogenisation and whey protein denaturation. The application of power US as a post-processing step for fermented milk has not been reported yet. In our previous work, US was applied during the fermentation of milk concentrates, also resulting in a reduced viscosity of the stirred yogurt (Körzendörfer et al., 2019). Confocal laser scanning microscopy indicated that the US-induced shear forces decreased the volume fraction of microgel particles which in turn heavily influences the apparent viscosity of the product. Therefore, the strong viscosity reduction due to power US in the present study is as well supposed to be attributed to a decreased volume fraction.

### 3.2.3. Microstructure

Particle size measurements from stirred yogurts were performed to determine microstructural differences. The Sauter mean diameters are shown in Fig. 5(b). An increase was observed for both control and sonicated yogurts when the final pH was increased. Controls exhibited a Sauter mean diameter of  $32.9 \pm 2.3 \mu\text{m}$  (pH 4.6),  $39.1 \pm 3.2 \mu\text{m}$  (pH 4.8), and  $37.4 \pm 2.0 \mu\text{m}$  (pH 5.0). Pairwise comparisons revealed that the  $d_{3,2}$  was reduced at all final pH values due to the sonication treatment ( $P < 0.05$ ). Volume-weighted particle diameters are listed in Table 4. Increasing the final pH from 4.6 to 4.8 or 5.0 resulted in an increased  $d_{10}$  of controls and sonicated yogurts. Medium-sized particles ( $d_{50}$ ) also increased by trend at higher final pH values. With regards to larger particles ( $d_{90}$ ), the yogurts fermented to pH 4.8 exhibited the highest values. Sonication decreased ( $P < 0.001$ ) the  $d_{10}$  by 12–24%, depending on the final pH. A decrease in the  $d_{50}$  was only noticed at

pH 4.6. No effect of sonication could be found on the  $d_{90}$ . Both sonication and the final fermentation pH altered the particle size distribution of the stirred yogurts, whereas an increase of the pH rather led to larger particles. As a higher pH gave softer set gels, a facilitated particle size reduction, and hence, smaller particles were expected. The microgel particle size of (already stirred) concentrated fermented milks was reported to increase at higher ( $>30 \text{ }^\circ\text{C}$ ) temperatures (Hahn et al., 2014). Thus, we suppose that all yogurts were subjected to post-aggregation during cooling, especially when the final pH was higher than 4.6. The isoelectric point of caseins where the molecules carry no net electrical charge is 4.6. At higher pH values, caseins are still negatively charged and the final network has not been formed yet. If the gel is then broken and the acidification is not instantly stopped, the net charge of caseins will further decrease resulting in interparticular and intraparticular rearrangements.

Sonication primarily altered the particle size distributions by reducing the size of smaller microgel particles ( $d_{10}$ ). No distinct effect was observed for particles larger than  $20 \mu\text{m}$ . Disagglomeration is one application of power US that has been reported. Sonication decreased the particle size of agglomerated sugar crystals (Guo, Jones, Li, & Germana, 2007) and gelled whey proteins (Ashokkumar et al., 2009; Gordon & Pilosof, 2010). Furthermore, US was used to accelerate the solubilization of milk protein powders by breaking powder particles apart (Chandrapala, Martin, Kentish, & Ashokkumar, 2014; McCarthy, Kelly, Maher, & Fenelon, 2014) whereas the structure of single casein micelles stayed unchanged (Chandrapala, Martin, Zisu, Kentish, & Ashokkumar, 2012). The sonotrode used in our study had a relative low power and was only operated at an amplitude of 10% for 5 s. According to the cited literature, an enhanced reduction of the particle size is expected when the sonication is intensified by increasing the amplitude or sonication time. Preliminary tests showed that higher amplitudes result in an excessive reduction of

**Table 4**  
Particle sizes of stirred yogurts calculated by laser diffraction spectroscopy.<sup>a</sup>

Final fermentation pH (–)	$d_{3,2}$ ( $\mu\text{m}$ )		$d_{10}$ ( $\mu\text{m}$ )		$d_{50}$ ( $\mu\text{m}$ )		$d_{90}$ ( $\mu\text{m}$ )	
	Control	Sonicated	Control	Sonicated	Control	Sonicated	Control	Sonicated
4.6	$32.9 \pm 2.3^A$	$27.5 \pm 1.7^{a***}$	$12.0 \pm 0.8^A$	$9.8 \pm 0.5^{a***}$	$83.7 \pm 10^A$	$71.2 \pm 7.3^{a*}$	$367.9 \pm 59.8^{AB}$	$351.3 \pm 56.9^a$
4.8	$39.1 \pm 3.2^B$	$35.7 \pm 3.6^{b***}$	$14.3 \pm 1.3^B$	$12.6 \pm 1.3^{b***}$	$111.6 \pm 13.8^B$	$122.7 \pm 17.8^b$	$475.4 \pm 75.8^B$	$520.5 \pm 85.4^b$
5.0	$37.4 \pm 2.0^B$	$31.7 \pm 1.9^{b***}$	$14.8 \pm 1.0^B$	$11.3 \pm 0.7^{b***}$	$91.3 \pm 6.2^{AB}$	$85.1 \pm 7.2^a$	$323.1 \pm 31.8^A$	$315.4 \pm 35.7^a$

<sup>a</sup> Milk concentrates were fermented until a final fermentation pH of 4.6, 4.8, or 5.0 and treated with power US after fermentation. Abbreviations are:  $d_{3,2}$ , Sauter mean diameter;  $d_{10}$ , volume-weighted 10th percentile;  $d_{50}$ , volume-weighted 50th percentile;  $d_{90}$ , volume-weighted 90th percentile. A two-way ANOVA (Tukey test) was performed to compare multiple groups. Particle sizes of control samples and sonicated samples fermented until different pH values with different uppercase and lowercase superscript letters, respectively, differ significantly ( $P < 0.05$ ); asterisks indicate that sonicated samples differ significantly from the control ( $*P < 0.05$ ,  $**P < 0.01$ , and  $***P < 0.001$ ).

viscosity (data not shown). Hence, power US is supposed to be an effective technology to modify the size and shape of microgel particles.

As shown in Fig. 5(c), the WHC of yogurts decreased ( $P < 0.05$ ) when the final fermentation pH was increased. Controls exhibited a WHC of  $82.6 \pm 0.3\%$  (pH 4.6),  $68.3 \pm 1.1\%$  (pH 4.8), and  $64.0 \pm 1.4\%$  (pH 5.0). These results indicate structural differences of the microgel particles. Sonication slightly increased the WHC of yogurts fermented to pH 4.6. In contrast, a decreased WHC was observed at a final pH of 4.8 and 5.0. Optimal water binding of yogurt occurs at pH 4.4–4.6 (Lucey, 2017), and a lower WHC of stirred yogurt was reported when the final fermentation pH was increased from 4.4 to 4.8 (Küçükçetin, 2008). Both parameters, WHC and syneresis (determined by the gravitational method), were similarly altered by the final fermentation pH whereas the effect of sonication was contrary. The centrifugal method rather describes the particular water binding of the individual microgel particles. We assume that the reduced WHC caused by US is due to a decreased volume fraction of microgel particles. In contrast, the gravitational method is more suitable to evaluate the water binding of the microgel suspension as a whole cohesive system, particularly with regards to the storage stability of the product. The decreased syneresis of the sonicated yogurts may be explained by the decreased size of microgel particles and higher homogeneity of the product (see section 3.2.4). This indicates a generally finer structure with more branched channels and smaller pores, so that the draining of the whey (= continuous phase) was slowed down.

#### 3.2.4. Visual appearance

Transmission images of stirred yogurts were prepared to evaluate the visual appearance (Fig. 6). Black spots represent leftover fragments of the set gel that were not broken apart into micrometer-scale particles. The upper line shows the controls differing in the final fermentation pH. Controls fermented to pH 4.6 and 4.8 are grainy and exhibit numerous large particles up to 4 mm. White areas indicate a pasty consistency and originate from large particles that carried yogurt away during scratching out the sample. Fermenting the milk to pH 5.0 resulted in a thinner and more

homogeneous product. Transmission images of the corresponding sonicated yogurts are presented in the line below. The US treatment altered the visual appearance of all samples. At a final pH of 4.6, the sonicated yogurt has a smoother background but still contains large particles. At higher final pH values, a similar, more pronounced effect of US could be observed so that large particles are more exposed due to the smoother background. Yogurt that was fermented to pH 5.0 and sonicated has a uniform background in which only few particles are distributed. As a result, the final fermentation pH had a crucial effect on the visual appearance and graininess of the yogurt. Smoother products can be obtained by raising the final pH. Sonication is an efficient technique to increase the homogeneity of high-protein yogurt. As sonication rather led to a smoother background and large particles remained, an additional post-processing step where large lumps are eliminated (e.g., by a sieve) may improve the final texture.

#### 4. Conclusions

The large-scale fermentation of pre-concentrated milk is challenging since gels exhibit a high firmness so that several processing steps are aggravated. This makes it difficult to obtain products with good texture properties. The final pH at which the milk gel is broken by agitation is a crucial factor that determines its firmness. Increasing the final pH is an effective strategy to decrease the firmness that can be implemented quickly in the industry. Processing of a softer gel will result in a lower viscosity and more homogeneous visual appearance. We conclude that the optimal final pH for fermented concentrates is in the range of 4.7–4.9, that is, higher than for conventional yogurt (pH 4.4–4.6). Further experiments are useful to study the effect of the cooling process with regards to post-acidification and post-aggregation. Stopping the fermentation at higher pH values may require a starter culture with a favorable acidification profile. Yogurts from fermented concentrates differing in the pH should be evaluated in sensory tests to rate the acidic taste and aroma profile.

The application of power US as a post-processing step is a highly effective tool to improve the visual appearance and modify the flow

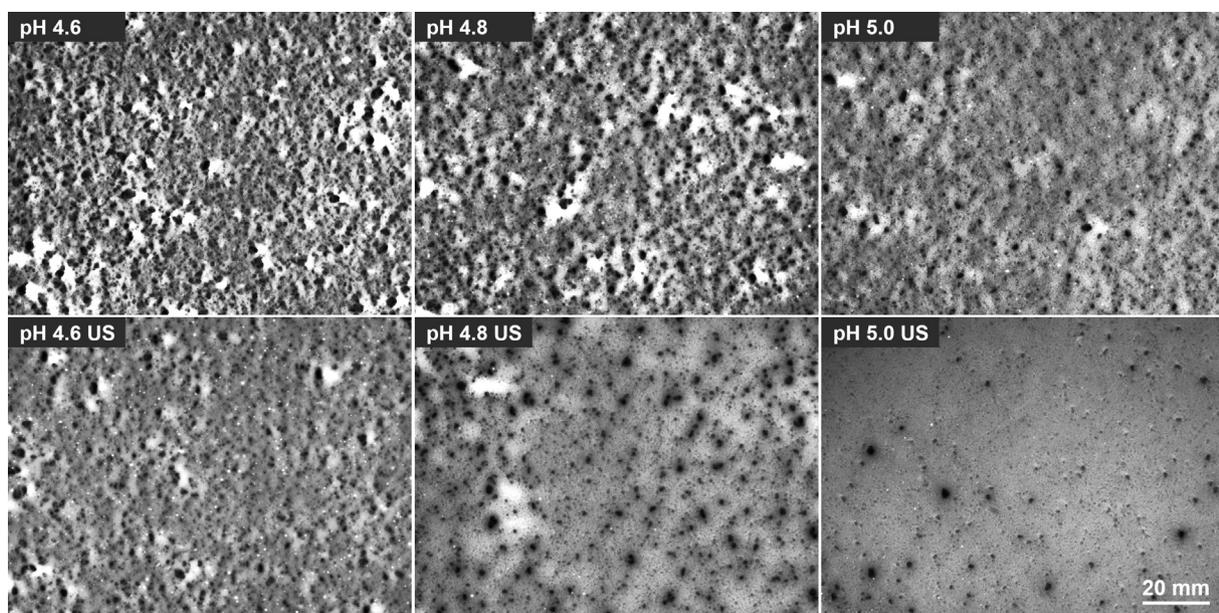


Fig. 6. Transmission images (120 mm × 90 mm, layer thickness 1.2 mm) of control (above) and sonicated (below) stirred yogurts. Yogurts were fermented until a final pH of 4.6, 4.8, and 5.0, respectively. Each image shows approximately 13 g yogurt; black spots represent large, visible particles.

properties by considerably decreasing the product viscosity. As only slight effects on the particle size distribution were observed, determining the volume fraction or microscopy would be useful to clarify microstructural alterations. The impact of US seems to be more efficient when the sonicated medium has a lower viscosity. Various sonication parameters have to be studied including ultrasonic power (amplitude), energy input (sonication time), and product temperature. Our study reveals that the final pH and power US are two powerful approaches to control the rheological properties of high-protein fermented milks. Finally, this offers the potential to develop innovative products with tailored texture properties and will help to establish sustainable processes without the accumulation of acid whey.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.idairyj.2019.104541>.

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