Effect of mono or co-culture of EPS-producing *Streptococcus thermophilus* strains on the formation of acid milk gel and the appearance of texture defects

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A B S T R A C T

One acidifying (ST1) and two texturing strains (ST2 and ST3) of *Streptococcus thermophilus* were used as pure or co-cultures to identify and understand their effects on the structuring of acid milk gels and on the appearance of texture defects, i.e., syneresis and graininess. Symbiosis between specific texturing and acidifying strains reduced acidification time and increased exocellular polysaccharide (EPS) content. The texturing strain could simultaneously produce low and high molar mass EPS and their distribution in mass and/or in number were influenced by the proportion of acidifying to texturing strain used. The results of this study suggest that the high molar mass EPS contributes to acid gel firmness, but less so compared with the acidification rate. The ability of strain ST3 to prevent texture defects, specifically graininess, did not depend on the acidification kinetics or final EPS content, but rather on the structural properties of EPS and/or the bacterial chain morphology.

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1. Introduction

Fermented milk such as yoghurt is one of the most popular dairy products in many countries. In the literature, set-type fermented milk is defined as a viscoelastic gel formed from the aggregation of casein micelles due to milk acidification (Tamime, Robinson, & Latrill, 2001). For manufacturers, a set-type acid milk gel must have a firm body, smooth texture, thick mouthfeel and structural stability. The most common texture defects in milk gels are spontaneous syneresis and graininess. Spontaneous syneresis, or wheying-off, indicates the separation of serum on the yoghurt surface, and graininess usually refers to lumpiness or granular texture due to the presence of large protein aggregates that range in size from 1 to 5 mm (Folkenberg, Dejmek, Skriver, & Ipsen, 2006; Lucey & Singh, 1997). The three main factors determining gel texture and the appearance of defects are the milk composition, the manufacturing process and the starter cultures (Lucey & Singh, 1997; Sodini, Remeuf, Haddad, & Corrieu, 2004). A positive correlation between graininess and high content of denatured whey proteins was reported in the literature (Lucey, 2004; Nguyen et al., 2018a; Remeuf, Mohammed, Sodini, & Tissier, 2003). However, the effect of the starter culture on the graininess has not been clarified.

Starter cultures can influence milk gel texture by their acidification rate and/or by their exocellular polysaccharide (EPS) production. EPS from "texturing" or "ropy" cultures are well known to improve the gel viscosity and reduce syneresis (Folkenberg et al., 2006; Marshall & Rawson, 1999; Mende, Mentner, Thomas, Rohm, & Jaros, 2012). Some authors have also observed a reduction in graininess when using a ropy strain or a high-level EPS-producing culture, but no further details on the mechanism of this phenomenon have been reported (Hassan, Ipsen, Janzen, & Qvist, 2003; Küçükçetin, Weidendorfer, & Hinrichs, 2009). In a previous study on the effects of heat treatment, milk composition and two EPS-producing starter cultures on the microstructure and the texture defects of acid milk gel, Nguyen et al. (2018a) have suggested that the presence of denatured whey proteins is critical for grain formation. Gel viscosity and texture defects appeared to be culture-dependent and were correlated with the amount, molar mass and intrinsic viscosity of the EPS produced. Furthermore, the

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gel microstructure obtained from the two cultures showed major differences in the location of bacterial cells in the gels, suggesting that simultaneously with the EPS effect, the length of bacterial cell chains could also interfere with the formation of the protein network during acidification due to steric hindrance. However, the different acidification kinetics of the two cultures used might influence gel structuring.

In industrial applications, commercial starter cultures for yoghurt fermentation usually consist of several strains of *Streptococcus thermophilus*, which are chosen for their specific properties such as fast initial acidification or texture promoting capacity. The interaction between different *S. thermophilus* strains could affect the total EPS production, but different trends were observed and no details on the acidification kinetics or EPS characterisation have been reported (Folkenberg et al., 2006). In addition, the influence of co-cultures of *S. thermophilus* strains on milk gelation has not yet been studied. The aim of this study was to identify the effects of mono or co-culture of EPS-producing *S. thermophilus* on the formation of acid milk gels and the appearance of texture defects. For this purpose, three *S. thermophilus* strains were used alone or in combination: one for its fast acidification characteristics and the other two for their texturing properties. Hence, seven starter cultures were formed with different ratios between acidifying and texturing strains to study the correlations between the gel texture characteristics (e.g., firmness, viscosity, syneresis, graininess), the kinetics of acidification and gelation, but also the quantity and structural features of EPS produced.

2. Materials and methods

2.1. Solvents and reagents

Low-heat skim milk powder (Spray 0) was purchased from Ingredia (France). The three *S. thermophilus* strains were provided by Chr. Hansen A/S, Arpajon (France). Lactose monohydrate and trichloroacetic acid (TCA) were from GPR Rectapur, VWR chemicals (Belgium). D(+)Glucose monohydrate and 1 N sodium hydroxide (NaOH) were from Merck (Germany). Phenol, $LiNO_3$ and NaN_3 were from Sigma (USA). Ethanol (95%, v/v) was from TechniSolv, VWR chemicals (France). Concentrated sulphuric acid (95%, w/w) was from Normapur, VWR chemicals (France).

2.2. Starter cultures

Three different frozen lyophilised strains of *S. thermophilus* ST1, ST2, ST3 from the Chr Hansen culture collection (Chr Hansen A/S) were used alone (pure culture) or in combination (co-culture) in seven different starter cultures. From manufacturer information, strain ST1 possess a cell wall protease (PrtS+) and could play a role as acidifying strain. ST2 or ST3 were texturing strains without cell wall protease (PrtS-) and were chosen to provide different textural types of milk gels: firm or viscous, respectively.

Cultures ST1, ST2 and ST3 were composed of a single *S. thermophilus* strain corresponding to the name of the culture. The four co-cultures ST1+2 (1/1.7), ST1+2 (5/1), ST1+3 (1/1.7) and ST1+3 (5/1) consisted of the combination of acidifying strain (ST1) with a texturing strain (ST2 or ST3) with the cfu ratios of acidifying strain/texturing strain corresponding to the name of the culture. All strains were stored at −80 °C. Strains were thawed and mixed, then 2 g of the final culture were added to 100 mL of UHT skim milk. Ten millilitres of this pre-inoculated milk were immediately added to 1 L of pasteurised milk to start the lactic fermentation. The final inoculation rate for the seven starter cultures was 10^6 cfu mL^{-1} pasteurised milk. Sampling, thawing and formulation were performed in the laboratory under sterile conditions.

2.3. Acid milk gel manufacture

The milk-based preparation was carried out as described by Nguyen et al. (2018b). The milk formulation contained 11.8% (w/w) dry matter, 4.0% (w/w) proteins and 6.7% (w/w) lactose. The skim milk powder was dissolved in ultra-pure water during 1 h at room temperature and then kept for 12 h at 4 °C before the pasteurisation at 95 °C for 6 min. After the heating step, milk suspensions were cooled to 43 °C and immediately inoculated with the starter cultures. Fermentation procedures, pH monitoring and gel structuring have previously been reported by Nguyen et al. (2018b). In brief, the fermentation was carried out at 43 °C with pH control until a final pH of 4.65 was reached. In parallel, gel formation was monitored using a Physica MCR 300 rheometer (Anton Paar, St Albans, UK) equipped with concentric cylinder geometry (CC27) that operated in continuous oscillation mode at a frequency of 1 Hz and 1% strain. The gel point was defined as the moment when the storage modulus (G′) first exceeds the loss modulus (G″) (Winter, 1987). After acidification, the gels were stored undisturbed in plastic cups at 4 °C for 7 days before determination of texture properties.

2.4. Gel texture properties

The textural parameters of set-type acid milk gel at day 7 including the firmness, the viscosity, the spontaneous syneresis and the grain defect were characterised as described by Nguyen et al. (2018a).

The firmness of acid milk gel was determined immediately after removing out of the cold room (4 °C) using a texture analyser equipped with an acrylic cylinder compression probe (diameter = 40 mm, thickness = 5 mm). The compression test was carried out with a speed of 5 mm s^{-1}, the trigger force of 5 g and a penetration depth of 15 mm. The gel firmness (N) corresponding to the maximum compression force, i.e., the rupture point of the gel, was calculated with the Exponent software (Stable Micro Systems, Godalming, UK).

For the viscosity measurement, the set-type acid milk gels were stirred 20 times with a spoon and 20 g of sample was placed in the rheometer cup. The viscosity of the stirred gel (η, Pa s) was determined using a Physica MCR 300 rheometer equipped with concentric cylinder geometry at a shear rate of 300 s^{-1} (13 °C) obtained after a linear ramp from 0.271 s^{-1}–300 s^{-1} in 210 s.

The spontaneous syneresis (% w/w) was the percentage of whey on the surface of the undisturbed gel to the total gel weight and was measured according to the siphon method (Amatayakul, Sherkat, & Shah, 2006).

The grain defect was determined by image analysis. After stirring 5 times with a spoon, 1 g of acid gel was dispersed in 100 mL of distilled water by stirring at 400 rpm for 2 min. 10 mL of diluted sample was poured into a plastic Petri dish and visualised with a digital camera in black and white mode. The image analysis was performed using ImageJ 1.49v software (National Institutes of Health, USA) with Fiji plug-in. Grains with a diameter >1 mm were enumerated (Remeuf et al., 2003). The grains were classified into different size groups by their diameter: 1–1.5 mm, 1.5–2.0 mm, 2.0–2.5 mm, 2.5–3 mm or >3 mm. The results were expressed as the number of grains of each size per gram of gel.

2.5. EPS quantification

EPS quantification was performed using the protocol 1 described by Nguyen et al. (2018b). The EPS were extracted in hot-acidic medium and dialysed to obtain a high extraction yield. The total sugar content was determined by the phenol-sulfuric acid method using glucose as the standard (range of concentration from

0 to 200 mg L^{-1}). The acidified milk with glucono-δ-lactone (GDL) was used as blank and the residual milk sugars after dialysis were subtracted from all data.

2.6. EPS characterisation

EPS were extracted without a heating step using the protocol 3 described by Nguyen et al. (2018b) to avoid EPS denaturation. The extraction process included a step of pH correction to 7, the protein precipitation in an acidic medium, followed by the precipitation of EPS by ethanol before dialysis and lyophilisation. The structural properties of extracted EPS were characterised using high-performance size exclusion chromatography coupled with multi-angle laser light scattering detector (HPSEC-MALS). The material and operating conditions were reported in Nguyen et al. (2018b). The weight-average molar mass (M_w) and the intrinsic viscosity ($[\eta]$) were determined using the software ASTRA 6.1.2 (Wyatt Technologies, Santa Barbara, CA). The M_w was calculated using the Berry's model (2nd order). The data were analysed using a refractive index increment (dn/dc) of 0.145 mL g^{-1} according to the literature (Lambo-Fodje et al., 2007).

2.7. Statistical analysis

All analyses were conducted at least in triplicate and the values were expressed as the mean ± standard deviation. The experimental data were analysed statistically using the Fischer's and Student's tests with 95% confidence level and using Pearson's correlation coefficient functions for the correlation between means.

3. Results and discussion

3.1. Characterisation of starter cultures

3.1.1. Acidification kinetics

The kinetics of milk acidification induced by lactic fermentation were monitored by pH evolution between pH 6.5 and 4.6 (Fig. 1). The parameters evaluated were the total acidification time and the maximum acidification rate (V_{max}) (Table 1). As an acidifying strain, ST1 presented the highest V_{max} value of all pure cultures and the shortest acidification time (~300 min) compared with ST2 and ST3. The acidification curve profiles of strains ST2 and ST3 (Fig. 1) were very different from that of strain ST1. ST2 and ST3 had significantly slower acidification rates during the first 160 min and also a stronger slowdown in acidification after the shoulder observed around pH 6.25. This specific profile corresponded to the strains'
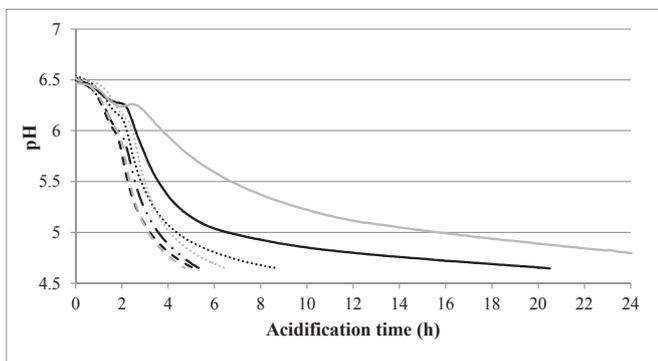

Fig. 1. Kinetics of acidification monitored during skim milk fermentation by cultures ST1 (— · · —), ST2 (——), ST1+2 (1/1.7) (•••••), ST1+2 (5/1) (— —), ST3 (——), ST1+3 (1/1.7) (••••••), ST1+3 (5/1) (— —). Skim milk suspensions were pasteurised at 95 °C for 6 min.

urease activity, which produced ammonia from milk urea and induced a slight alkalisation of the medium. Furthermore, the final count of bacterial cells at the end of the fermentation was ~10^9, 10^7 or 10^6 cfu g^{-1} for ST1, ST2 or ST3, respectively, and was consistent with the kinetics observed. These differences in acidification kinetics and cell growth were related to the proteolytic activity of these 3 strains. Indeed, strain ST1 has a cell envelope-associated proteinase (PrtS+) that hydrolyses caseins into peptides and essential amino acids to ensure high cell density growth and thereby generating high acidification activity (Galia, Perrin, Genay, & Dary, 2009). When this enzymatic activity is lacking, such as for ST2 and ST3 (PrtS−), the growth of *S. thermophilus* strains in milk is less optimal depending on the specific intracellular peptidases and amino acids requirements of the strain (Galia et al., 2009).

The addition of ST1 to ST2 or ST3 in four co-cultures resulted in a considerable decrease in acidification time and a significant increase in V_{max} (Fig. 1, Table 1). For example, the total acidification time for cultures of ST2 was reduced from 1351 min (pure culture) to 528 min or 304 min when ST1 was added with a ST1/ST2 ratio of 1/1.7 or 5/1, respectively. Furthermore, while pure culture of ST3 had the slowest acidification kinetics of all cultures tested, the ST1+3 co-cultures had better acidification performance as compared with ST1+2 co-cultures for the same ST1 ratio. These results suggested that the proteinase activity of ST1 produced peptides and essential amino acids that stimulated growth of both texturing strains. However, the amino acid requirements seemed to be different between ST2 and ST3, as indicated by their differing rates of acidification. ST1 metabolites may be more adapted to the needs of ST3 than to those of ST2, which allowed for a better symbiotic relation between ST1 and ST3 than between ST1 and ST2.

3.1.2. EPS production and characterisation

The EPS produced by the seven starter cultures were extracted for quantification (Fig. 2) and for macromolecular characterisation (molar mass distribution (M_w) and intrinsic viscosity ($[\eta]$)) (Fig. 3). Although the strain ST1 was not considered as textural strain, it was able to produce a substantial amount of EPS of ~55 ± 4 mg glucose kg^{-1} of gel in a short time of acidification (~300 min) (Fig. 2). However, the EPS produced have low M_w of ~9.2 × 10^4 g mol^{-1} and low $[\eta]$ of ~42 mL g^{-1}, which could explain the poor texturing property of this strain. These results also confirmed that macromolecular properties are a more critical characteristic for the functional properties of EPS than the total amount of polymers (Mende, Rohm, & Jaros, 2016). Even after an acidification time that was 4—5 times longer than for culture ST1, cultures ST2 and ST3 did not produce a greater quantity of EPS (~30 mg glucose kg^{-1} and ~52 mg glucose kg^{-1}, respectively), particularly for ST2 (Fig. 2). However, the EPS produced by these two texturing strains had very different macromolecular properties from each other and from those of the ST1 culture. Based on elution profiles of HPSEC-MALS analysis, EPS produced by ST2 or ST3, could be divided into two groups of low and high M_w as also observed in the literature (Petry et al., 2003; Vaningelgem et al., 2004). The EPS produced by the strain ST3 have a M_w and intrinsic viscosity significantly higher than those produced by cultures ST2 or ST1 (Fig. 3). Furthermore, EPS from ST3 consisted of 80% high M_w polymers in number, in contrast to EPS from ST2, which contained only 35% high M_w molecules.

Regarding the strain ST2 and its co-cultures with ST1, the increase in the proportion of ST1 from 0 to 37% [culture ST1+2 (1/1.7)] and to 83% [culture ST1+2 (5/1)] induced a significant increase in the amount of EPS produced from 30 to ~52 and to ~67 mg glucose kg^{-1}, respectively (Fig. 2), while the time of acidification was significantly decreased (Table 1). The same trend was also observed when ST1 was added to strain ST3. Furthermore, the

Table 1
Acidification parameters of skim acid milk gel obtained using 3 pure strain cultures (ST1, ST2, ST3) and 4 co-cultures [ST1+2 (1/1.7), ST1+2 (5/1), ST1+3 (1/1.7), ST1+3 (5/1)].[a]

Parameters	ST1	ST2	ST1+ST2		ST3	ST1+ST3	
			1/1.7	5/1		1/1.7	5/1
Acidification							
Acidification time (min)	348 ± 49^{ad}	1351 ± 109^{b}	528 ± 33^{c}	304 ± 24^{a}	ND	395 ± 27^{d}	287 ± 11^{a}
V_{max} (10^{-3} pH unit min^{-1})	17.1 ± 1.7^{a}	14.0 ± 0.3^{b}	16.9 ± 1.3^{a}	20.0 ± 0.3^{c}	6.5 ± 0.1^{d}	18.1 ± 1.7^{ac}	20.0 ± 0.1^{c}
Gelation							
Time of gel point (min)	206 ± 13^{a}	388 ± 36^{c}	208 ± 10^{a}	157 ± 11^{b}	371 ± 18^{c}	179 ± 11^{b}	164 ± 10^{b}
pH of gel point	5.16 ± 0.05^{a}	5.15 ± 0.05^{a}	5.23 ± 0.01^{a}	5.21 ± 0.05^{a}	5.57 ± 0.01^{c}	5.39 ± 0.06^{b}	5.22 ± 0.01^{a}

[a] Reconstituted skim milk was pasteurised at 95 °C, 6 min. V_{max} is the maximum acidification rate. Data were obtained by monitoring the pH evolution (acidification) and rheological parameters (gelation) during fermentation at 43 °C; values of each characteristic affected with different letters were significantly different for $p = 0.05$ (ND, not determined).

macromolecular characteristics of the EPS produced by ST2 and its co-cultures were broadly similar (Fig. 3A,D). For the strain ST3 and its co-cultures ST1+3, a significant decrease of EPS M_w was observed when the acidification time decreased (Pearson's $r = 0.951$) due to the increase in ST1/ST3 ratio. These results suggested a chain length extension of EPS from ST3 during longer acidification time. Laws, Leivers, Chacon-Romero, and Chadha (2009) reported a similar trend in which EPS M_w produced by *Lactobacillus acidophilus* increased 3.7 times from 6 h to 24 h of fermentation in skim milk supplemented with glucose at 42 °C and at a controlled pH of 5.8. In association with cell counting, the authors observed that EPS synthesis and chain length extension continued after the end of the exponential phase, using sugar nucleotides already produced during in the latter phase.

When looking in more details at the results of EPS M_w distribution (in mass or in number, Fig. 3B, C), different trends could be observed. Firstly, the presence of the acidifying strain ST1 at a level of 37% in culture ST1+2 (1/1.7) and ST1+3 (1/1.7) did not change the M_w distribution characteristics as compared with pure strains (Fig. 3B, C), suggesting that in these conditions ST1 produced a negligible quantity of EPS, but had an enhancing effect on the EPS production of texture strains. In contrast, a significant change in the M_w distribution of EPS was observed with 83% ST1 in cultures ST1+2 (5/1) and ST1+3 (5/1), in that the low M_w EPS then became predominant. These findings could be related to the short acidification time observed with these co-cultures and/or to the prevalence of the acidifying strain over the texturing strain. Furthermore, we observed that the high M_w EPS group from culture ST1+3 (5/1) presented a lower average M_w, but a higher $[\eta]$ than for ST1+2 (5/1).

These findings suggested that the structure of EPS produced by ST2 could be more flexible and/or branched than those produced by ST3. It has been shown that for two EPS with identical structure (i.e. repeating units branching, stiffness of side chain), a higher M_w could lead to a higher $[\eta]$ and therefore to a higher gel viscosity (Faber, Zoon, Kamerling, & Vliegenthart, 1998; Looijesteijn, van Casteren, Tuinier, Doeswijk-Voragen, & Hugenholtz, 2000; Mende et al., 2016). However, other structural factors such as side chain stiffness and branching also made a considerable contribution to the $[\eta]$ of EPS. Generally, for a given M_w, a rigid linear polysaccharide will tend to extend under shearing, as opposed to a flexible branched molecules which will tend to compact, and will then contribute to increase the viscosity of the solution (Whistler & BeMiller, 1997).

Overall, the results presented in this section showed that the acidification kinetics, the production and the macromolecular characteristics of EPS depended not only on the strains but also on the interaction between textural and acidifying strains. The combination between ST1 and ST3 seemed to be more efficient than the combination between ST1 and ST2. Furthermore, all pure cultures could produce EPS, but these EPS differed in terms of quantity, distribution in mass or in number and intrinsic viscosity. In the same way, the overall distribution of low and high M_w EPS is strongly correlated with the interaction between acidifying and texturing strains. It is quite difficult to compare these findings with other studies because very few data are reported on the EPS production of mixtures of *S. thermophilus*. Folkenberg et al. (2006) observed different trends of EPS production in fermented milk by 5 *S. thermophilus* strains in pure or in mix cultures of two strains. Their results suggested that the interaction between two *S. thermophilus* strains could be compatible or not, depending on the strain used. However, they did not report acidification time or molecular characteristics of EPS, which made difficult the comparison with our results.

3.2. Influence of starter cultures on milk gelation and textural properties of gels

3.2.1. Gelation kinetics

The kinetics of gelation were monitored by the evolution of the storage modulus (G') and the loss modulus (G''), characterised by time and pH of the gel point. As presented in Table 1, the time and pH of gelation depended on the culture used. The pH of the gel point was approximately 5.2 for the cultures ST1, ST2, ST1+2 (1/1.7) and ST1+2 (5/1), and was not influenced by the rate of acidification. The same pH value was reported for the gel point of heated milk acidified chemically using glucono-delta-lactone (Lucey, Tamehana, Singh, & Munro, 1998; Tamime, Robinson, & Latrille, 2001). In the case of ST3, the pH of the gel point was found to be significantly higher at 5.57 and decreased to 5.4 and 5.2 when
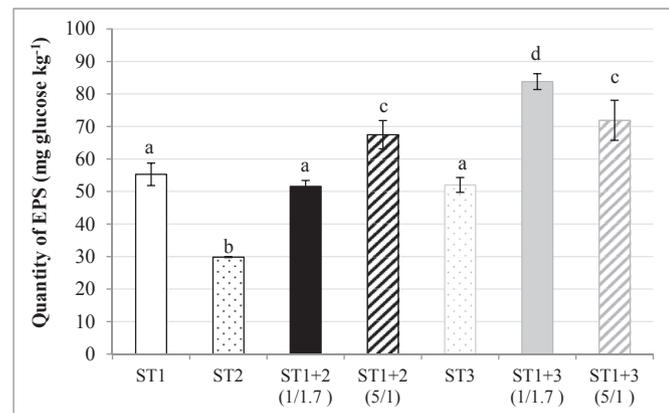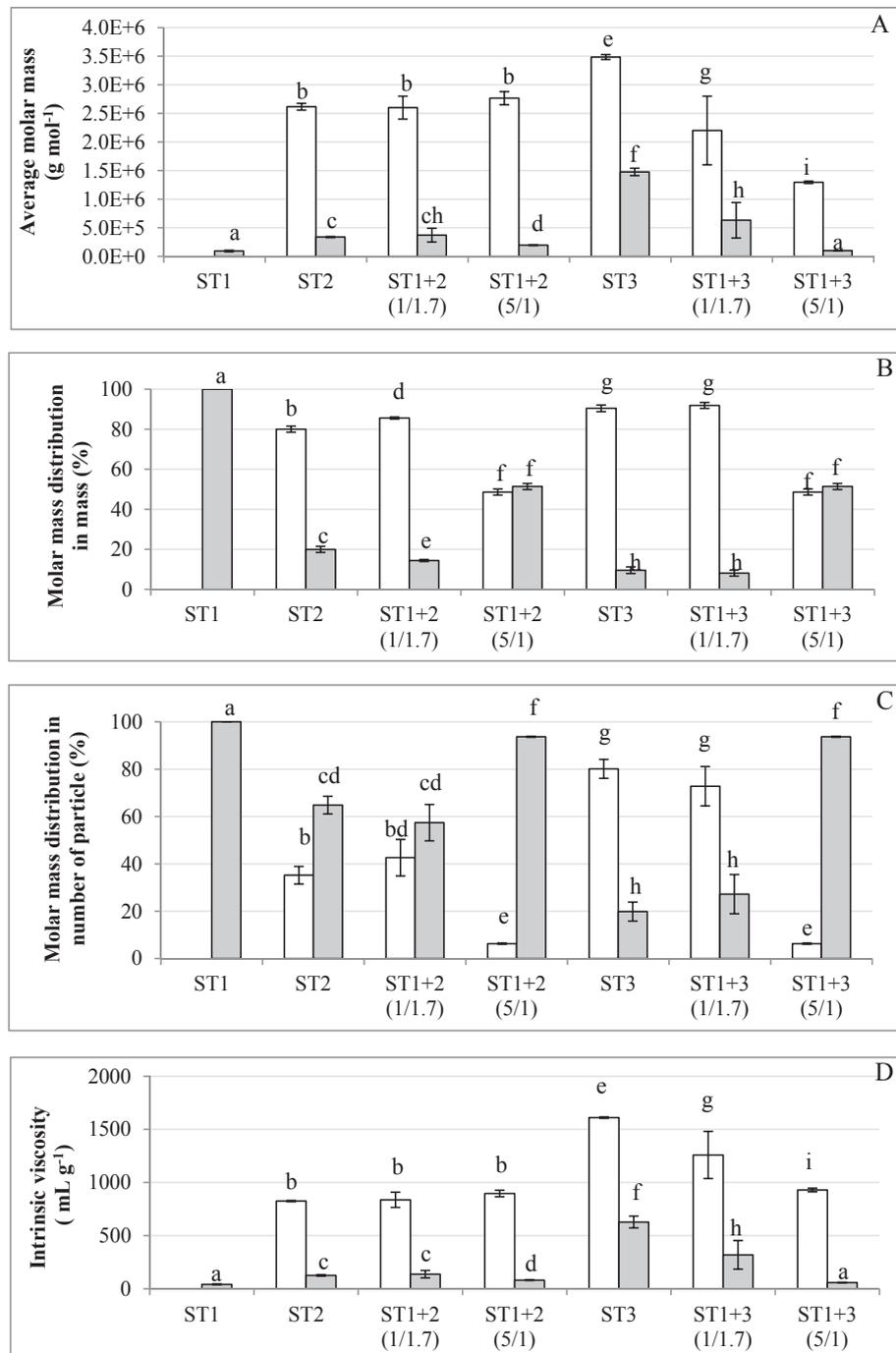
Fig. 2. Quantity of EPS (mg glucose kg^{-1}) produced by the 7 cultures/co-cultures (ST1, ST2, ST1+2 (1/1.7), ST1+2 (5/1), ST3, ST1+3 (1/1.7), ST1+3 (5/1)) during fermentation of skim milk pasteurised at 95 °C for 6 min. Mean values ± standard deviation (n = 3). Values affected with different letters were significantly different for $p = 0.05$.

Fig. 3. Macromolecular properties of extracted EPS from the 7 cultures/co-cultures (ST1, ST2, ST1+2 (1/1.7), ST1+2 (5/1), ST3, ST1+3 (1/1.7), ST1+3 (5/1): A, average molar mass; B, molar mass distribution in mass; C, molar mass distribution in number of particles; D, intrinsic viscosity. The EPS were classified in high molar mass (☐) or low molar mass (▨).

strain ST1 was added at 37 and 83%, respectively (Table 1). The occurrence of the gel point at a higher pH could be related to the presence of EPS and their interactions with milk proteins (Mende, Peter, Bartels, Rohm, & Jaros, 2013b).

In the case of strain ST3 and its co-cultures, a positive correlation (Pearson's r = 0.992) was observed between the pH of gelation and EPS macromolecular properties (M_w, $[\eta]$), but not with the EPS quantity. The decrease in the pH of the gel point could be related to a decrease of the average M_w of EPS and/or of the proportion of high M_w EPS (Fig. 3). On the other hand, for all

cultures, a predominant proportion in number of low M_w EPS corresponded to a gel point occurring at a lower pH of ~5.2 (Fig. 3C). Mende et al. (2013a,b) also reported a similar trend. The authors observed early gelation due to the depletion effect between casein micelles and added bacterial EPS or commercial dextran (Tuinier, Grotenhuis, Holt, Timmins, & de Kruif, 1999). These two neutral polysaccharides, bacterial EPS and dextran had different M_w of 2.6 × 10^6 Da and 5 × 10^5 Da, respectively. Compared with bacterial EPS, addition of 15−80 times more dextran was required to achieve a similar effect on gelation point.

3.2.2. Gel texture

Gel texture was characterised by four parameters: (i) firmness, (ii) viscosity, (iii) spontaneous syneresis (Table 2) and (iv) graininess (Fig. 4). Gels from the ST3 culture are not mentioned in this section because after 24 h of acidification, the gels obtained at a final pH of ~4.8 were heterogeneous with an upper gel-like part and a lower liquid-like part.

3.2.2.1. Firmness.

Gel firmness is the main texture property of set-type acid milk gel and this parameter is mainly governed by the protein network and not by bacterial EPS (Folkenberg et al., 2006; van Marle & Zoon, 1995). For the monocultures used in the present study, ST1 provided the lowest gel firmness while ST2 produced the highest gel firmness, which could be related to its long acidification time of nearly 24 h. Moreover, it was observed for strain ST2 and its co-cultures ST1+2 that the higher the proportion of ST1, the faster the acidification and the lower the gel firmness (Pearson's r = 0.998). According to the literature, slow protein aggregation could result in continuous network formation with more connected proteins, reducing the rearrangement of particles during gel formation and contributing to an increase in gel rigidity during fermentation at low temperatures (Lucey & Singh, 1997; Sodini et al., 2004).

In the present study, it is interesting to note that although the cultures ST1, ST1+2 (5/1) and ST1+3 (5/1) induced similar acidification times (Table 1), the resulting gel firmness was significantly different in the order: ST1+2 (5/1) > ST1+3 (5/1) > ST1, consistent with the order of the average M_w of EPS produced. In other words, the M_w of EPS from ST1+2 (5/1) was higher than from those of ST1+3 (5/1) and ST1 (Pearson's r = 0.987). These findings suggested that the presence of high M_w EPS was also an important determining parameter for gel firmness, though not as much as the acidification rate.

When comparing the gels of co-cultures with the same ST1 ratio, the gels containing ST2 had a higher firmness than those containing ST3 (Table 2). Other studies have also reported a decreased gel firmness when using a ropy culture which is defined as a high EPS producing culture with high [η] such as the culture ST3 (Hassan et al., 2003; Hess, Roberts, & Ziegler, 1997; Mende et al., 2012). Using microscopic analyses, Hassan et al. (2003) observed that the EPS of ropy cultures were mainly located and segregated in the pores of the gel rather than attached to the protein network. Hence, the gel structure from a ropy culture was less dense than that of non-ropy culture. Mende et al. (2012) and Rohm and Kovac (1994) also suggested that the large amount of uncharged EPS produced by ropy culture could have partially prevented the development of protein–protein bonds during gel formation and thus reduced the rigidity of acid milk gel.

3.2.2.2. Stirred gel viscosity.

Stirred gel viscosity is correlated with a thick mouthfeel and with the smoothness of fermented milk products (Folkenberg et al., 2006). In this study, the stirred gel viscosity varied depending on the culture and the macromolecular properties of the produced EPS. ST1 produced only low M_w EPS

with low [η] and formed gels presenting the lowest viscosity. When ST2 was used, even if a low quantity of EPS was produced, the viscosity of the stirred gels formed was higher than for strain ST1. This result could be explained by the high percentage (80%) in mass of EPS with high M_w produced by culture ST2.

Concerning the co-cultures of ST2 or ST3, increasing the ratio of the acidifying strain decreased the stirred gel viscosity from 193 to 164 mPa s for ST1+2 (1/1.7) and (5/1), respectively, or from 301 to 239 mPa s for ST1+3 (1/1.7) and (5/1), respectively (Table 2). These results could be directly linked to the macromolecular properties of the EPS produced (Fig. 3) and suggested a positive correlation between the quantity of high M_w EPS and the stirred gel viscosity (Pearson's r = 0.890). Petry et al. (2003) also reported a striking correlation between an elevated proportion of the high M_w EPS fraction and viscosity of acid milk gel. In the present study, we also observed that even though the amount and molar mass distribution of EPS were similar, gels from culture ST1+3 (1/1.7) were more viscous than those from culture ST1+2 (1/1.7). This result could be partially explained by the higher [η] of the EPS produced by ST3 and probably by a different molecular rigidity as already mentioned in section 3.1.2. Also when comparing ST1+2 (5/1) and ST1+3 (5/1), despite both co-cultures exhibiting a low proportion of high M_w EPS and similar [η] of EPS, the viscosity of stirred gels was significantly different, suggesting that EPS were not the only factor contributing to this textural property. In a previous study based on gel microstructure observations, Nguyen et al. (2018a) suggested that in parallel to EPS production, the morphology of bacterial cell chain length should also be taken into account to understand the texture results.

3.2.2.3. Spontaneous syneresis defect.

Spontaneous syneresis is the contraction of a gel without exerting external forces and is related to an instability of the gel network that results in the loss of ability to entrap the entire serum phase (Lucey & Singh, 1997). Gels obtained with pure culture of ST1 presented a medium level of syneresis while those from ST2 had the highest syneresis, possibly related to the long acidification time. For co-cultures of ST2, this texture defect decreased from 2.7 to 1.4% when the ratio of ST1/ST2 changed from 1/1.7 to 5/1. In this case, a positive correlation was observed between syneresis, gel firmness and time of acidification (Pearson's r = 0.999) suggesting that gel contraction could occur during the slow fermentation, thereby hardening the gel and expelling the serum. In contrast, low syneresis levels were observed for co-culture of ST1+3. Decreased gel syneresis has been reported with the use of ropy strains that produce a high quantity of high M_w EPS (Amatayakul et al., 2006; Kristo, Miao, & Corredig, 2011). It can be assumed that the ropy EPS located in the gel pores could bind the serum and thus reduce the syneresis.

3.2.2.4. Graininess defect.

Graininess or lumpiness is considered as an undesirable defect because consumers usually expect a smooth product (Lucey, 2004). Remeuf et al. (2003) measured graininess by the number of grains with a diameter above 1 mm in 1 g of acid milk gel. In the present study, the graininess defect was determined

Table 2
Textural parameters of pasteurised acid milk gel using 6 different cultures[a].

Cultures	ST1	ST2	ST1+ST2		ST1+ST3	
			1/1.7	5/1	1/1.7	5/1
Firmness (N)	3.36 ± 0.18[a]	6.44 ± 0.15[d]	4.78 ± 0.08[c]	4.14 ± 0.13[b]	4.43 ± 0.25[b]	3.85 ± 0.11[e]
Viscosity (mPa s) at γ = 300 s^{-1}	138 ± 12[a]	186 ± 4[c]	193 ± 3[c]	164 ± 2[b]	301 ± 10[e]	239 ± 15[d]
Spontaneous syneresis (%, w/w)	1.8 ± 0.3[a]	6.3 ± 0.3[c]	2.7 ± 0.4[b]	1.4 ± 0.4[a]	0.4 ± 0.2[e]	0[d]

[a] Reconstituted skim milk was pasteurised at 95 °C for 6 min; values of each characteristic affected with different letters were significantly different at $p = 0.05$.
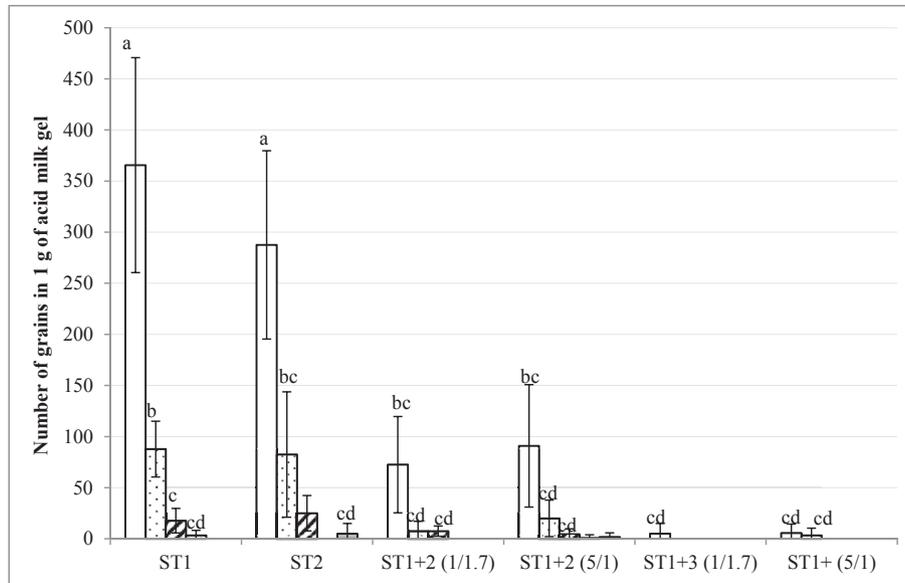
Fig. 4. Graininess defect of acid milk gels made by 6 different cultures [ST1, ST2, ST1+2 (1/1.7), ST1+2 (5/1), ST1+3 (1/1.7), ST1+3 (5/1)]. Grains were classified by their diameters: 1—1.5 mm (▢), 1.5—2 mm (▢), 2—2.5 mm (▨), 2.5—3 mm (▦) and >3 mm (▪). Mean values ± standard deviation (n = 9).

by the number of grains, but also by their diameter from 1 mm to above 3 mm. Larger grains were more damaging for texture perception even in low number (Fig. 4). The graininess was high when using pure cultures ST1 and ST2. In the mixtures, regardless of the strain ratio, co-cultures of ST3 presented a low graininess while the co-cultures of ST2 showed a medium granular texture (Fig. 4).

According to the literature, grains are large aggregates of proteins that appear under certain conditions such as a high fermentation temperature, excessive whey protein to casein ratio or with the use of certain types of starter culture (Lucey, 2004). However, only few studies have focused on the impact of starter culture on the appearance of grain. In agreement to our result, Küçükçetin et al. (2009) reported that the number of grains and their size were significantly reduced when using a high-level EPS-producing starter culture, rather than a medium- or low-level EPS-producing culture. Results from the present study provided additional data on this phenomenon. Indeed, it is worth noting that cultures ST1+2 (5/1) and ST1+3 (5/1) had a similar kinetics of acidification and gelation (Table 1) and similar production of EPS (Fig. 2) that presented identical intrinsic viscosity and EPS molar mass distribution (Fig. 3B, C,D). However, despite these similarities, the texture of the resulting gels from ST1+2 (5/1) presented more major defects (syneresis and graininess) as compared with gels from culture ST1+3 (5/1). These new data support the inhibitory effect of strain ST3 against appearance of texture defects, specifically graininess, which has been observed in a previous study (Nguyen et al., 2018a). Therefore, we suggested that this interesting technological property of culture ST3 could be related to the bacterial chains morphology and/or the structural properties of the EPS produced and that it did not depend on the acidification kinetics or final EPS content.

4. Conclusion

It is known that the symbiosis between texturing and acidifying strains of *S. thermophilus* reduces the acidification time and has an impact on the final acid milk gel structure. In this work, we selected one acidifying strain (ST1) and two texturing strains (ST2, ST3) strains, and used them singly or in co-cultures (ST1+2 or ST1+3) at two different ratios (1/1.7 or 5/1) in pasteurised skim milk to assess and understand the influence of their interaction on the acidification kinetics, EPS production, gel structure and appearance of texture defects.

In pure culture, due to the presence of proteinases in the cell wall, the acidifying strain provided rapid acidification and produced a medium amount of EPS. However, these EPS had a low M_w and intrinsic viscosity $[\eta]$ hence the gel obtained had the lowest firmness and viscosity, and the most graininess defect. Both texturing strains did not have the proteinase activity and showed a slow acidification. They both produced EPS with high molar mass but with different macromolecular properties.

In co-culture, the strains ST2 and ST3 had positive symbiotic relations with ST1, as showed by the reduction in acidification time and the increase in EPS production. Furthermore, the viscosity of gels increased significantly, probably due to the production of high M_w EPS by the texturing strains. Texture defects such as syneresis and graininess disappeared with use of the co-cultures of ST1+3. Moreover, co-cultures ST1+3 (5/1) and ST1+2 (5/1) had some similarities in terms of (i) acidification, (ii) gelation kinetics, (iii) EPS production and molar mass distribution and (iv) gel firmness. However, despite these similarities, gels from co-culture ST1+2 (5/1) showed worse texture defects as compared with ST1+3 (5/1).

These results highlight the fact that the inhibitory effect of a strain against the appearance of texture defects is not correlated to the acidification rate or EPS content, but that it could instead be explained by differences in the structural properties of EPS and/or in the morphology of bacterial chains.

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