THE INFLUENCE OF FERMENTATION TEMPERATURE ON THE DEVELOPMENT OF EXOPOLYSACCHARIDES IN YOGHURT PRODUCTION

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Abstract

Exopolysaccharides (EPS) have potential for development and exploitation as functional ingredients with health and economic benefits in dairy industry. The information on the biosynthesis, molecular organisation and fermentation conditions of EPS is rather scarce and the kinetics of EPS formation are poorly described. This study was designed to evaluate the effect of fermentation temperature on the development of lactic acid bacteria (LAB) starters EPS production potential. The commercial starters (Harmony 1.0, TWIST 1.0 and YF-L902, Chr.Hansen, Denmark) are used for yoghurt production. Milk samples were incubated at 38° C, 40° C and 43° C for 7, 6 and 5 hours, respectively, reaching pH 4.5. EPS and lactic acid concentration, LAB colony forming units (CFU) were measured in yoghurt samples using an appropriate analytical technique or standard procedures.

The production of intracellularly synthesized EPS varies roughly from 25.28 to 440.81 mg L^{-1} during fermentation. The fermentation temperature significantly contributes to EPS concentration because the increased rate of fermentation temperature is attributed to increased metabolic activity of LAB. Thermophilic strains produce maximal amounts of EPS under conditions optimal for growth. There isn't established the correlation between the amount of EPS and CFU of LAB in samples fermented at different temperatures, but there is found stable CFU concentration in samples with higher initial EPS concentration during the shelf-life of yoghurt. EPS in their natural environment are thought to play a role in the protection of the microbial cell against desiccation to phagocytosis and phage's attack, osmotic stress, adhesion to solid surfaces and biofilm formation. The fermentation temperature has a crucial role for the development of yoghurt quality and functional properties.

Keywords: exopolysaccharides, lactic acid bacteria, starters, yoghurt, fermentation.

Introduction

Lactic acid bacteria (LAB) have been extensively studied for their economic importance in food fermentation. LAB secrete exopolysaccharides, which play a significant role in the protection of microbial cell against phagocytosis, phage attacks, antibiotics, toxic compounds, osmotic stress and bacteriocins (De Vuyst, Degeest, 1999; Ruas-Madiedo et al., 2001; O'Connor et al., 2007), and in assuring the proper consistency and texture of fermented dairy products and cheese (Petry et al., 2000; Folkenberg et al., 2006). Additionally, some of EPS have prebiotic properties that could find important applications in functional foods (Ruas-Madiedo et al., 2010; Hidalgo-Cantabrana et al., 2012).

EPS are long-chain polysaccharides consisting of branched, repeating units of sugars or sugar derivatives. These sugar units are mainly glucose, galactose and rhamnose in different ratios. EPS are secreted into cells surroundings during growth and are not attached permanently to the surface of the microbial cell. This distinguishes them from the structurally similar capsular polysaccharides, which do remain permanently attached to the surface of the cell (Cerning, 1990; De Vuyst et al., 2001).

The total yield of EPS produced by LAB depends on the composition of the medium and the conditions in which the strains grow, i.e. temperature, pH and incubation time. The production of intracellularly synthesized EPS varies roughly from 0.045 to 0.350 g L^{-1} when the bacteria are grown under non-optimized culture conditions. Optimal culture conditions result in EPS yields from 0.150 to 0.600 g L^{-1}, depending on the strain (Cerning, 1990; Ruas-Madiedo et al., 2010). Optimal conditions of temperature, pH and incubation time result in improved EPS yields. Several reports show that low temperatures markedly induce slime production of LAB (De Vuyst, Degeest, 1999; Cerning et al., 1992). This effect has been explained, based on information for EPS production from Gram-negative bacteria, by the fact that slowly growing cells exhibit much slower cell wall polymers biosynthesis, making more isoprenoid lipid carrier molecules available for EPS biosynthesis (Petry et al., 2000; De Vuyst et al., 2001). However, several investigators find higher EPS production by LAB strains at higher cultivation temperature (De Vuyst, Degeest, 1999; Degeest et al., 2001; Grobben et al., 1995) and under conditions optimal for growth, for instance, with respect to pH (De Vuyst and Degeest, 1999; Degeest et al., 2001; Mozzi et al., 1994; Mozzi et al., 1996; Degeest, De Vuyst, 1998; Grobben et al., 1998) and oxygen tension (Degeest et al., 2001; Degeest, De Vuyst, 1998; Grobben et al., 1998; Behare et al., 2009). Whereas mesophilic strains seem to produce maximal amounts of EPS under conditions not optimal for growth at low temperatures; EPS production from thermophilic lactic acid bacteria appears to be growth-associated, i.e. maximal production under conditions optimal for growth is observed (De Vuyst et al., 1998). In the case of growth-associated production, EPS biosynthesis generally starts almost simultaneously with growth of LAB, shows a maximum rate when the culture is in its exponential growth phase and reaches a maximum towards at the end of the active growth (De Vuyst et al., 1998). It means that during storage time of fermented dairy products EPS biosynthesis would be

continued with the different speed due to stationary growth phase of LAB.

EPS degradation often takes place upon prolonged incubation (De Vuyst and Degeest, 1999; Mozzi et al., 1996; De Vuyst et al., 1998; Gassem et al., 1997) and further biochemical reactions during the shelf-life of fermented dairy products. This may be due to glycohydrolase activity of LAB (De Vuyst, Degeest, 1999; Cerning et al., 1992; Gancel, Novel, 1994; Cerning et al., 1990). However, a marked reduction in the EPS yield upon prolonged fermentation seems to be dependent on the strain used and both chemical and physical conditions (temperature, pH, etc.) (De Vuyst, Degeest, 1999; De Vuyst et al., 1998; Gancel, Novel, 1994).

This study was designed to evaluate the effect of fermentation temperature on the development of lactic acid bacteria starters EPS production potential.

Materials and Methods

Preparation of samples

Pasteurized and cooled milk samples were inoculated with different starters (given in Table 1) and incubated at 38 °C, 40 °C and 43 °C for 7, 6 and 5 hours, respectively, until pH of coagulum reached 4.5. The amount of starters was added based on the recommendations of starters' manufacturer Chr. Hansen (Denmark).

Table 1

The characteristic of commercial starters for yoghurt production used in the study

Starter code	Composition	Producer
Harmony 1.0	*Streptococcus thermophilus, Lactobacillus delbrueckii subsp.bulgaricus, Lactobacillus fermentum*	
TWIST 1.0	*Streptococcus thermophilus, Lactobacillus johnsonii, Lactobacillus delbrueckii subsp. bulgaricus*	Chr. Hansen, Denmark
YF-L902	*Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus*	

Fermented milk samples were stirred and maturated at 4–6 °C for 10-12 hours and stored at 4–6 °C for 7 days.

Determination of pH, CFU of LAB and EPS

An amount of EPS synthesized by starters' cultures, pH and colony forming units (CFU) of lactic acid bacteria were detected in yoghurt samples after maturation and at the end of the shelf-life of yoghurts. MRS medium (de Man Rogosa & Sharpe; Scharlau, Spain) was used for analysing CFU of LAB. pH of samples was measured by pH-meter Jenway 3520.

EPS were determined in yoghurt samples according to below given methodology (Ruas-Madiedo, de los Reyes-Gavilán, 2005).

Isolation of EPS

300–500 mL of fermented sample were put into the laboratory flask and boiled in water bath at 100 °C for 30 minutes. After cooling samples were centrifuged at 8000 min^{-1} for 10 minutes and 17 mL of 85% trichloracetic acid were added to 100 mL of sample. Samples were cooled up to 4 °C and again centrifuged at 8000 rpm for 10 minutes. Precipitation of EPS from samples was provided using cold ethanol (-20 °C, 1:3). Samples were stood in the fridge for 48 h and late centrifuged (4 °C, 8000 min^{-1}, for 10 minutes), dissolved precipitation in dH_2O and defined EPS.

Quantification of EPS

5% phenol solution in water (dissolve 5 g fresh phenol in dH_2O and fill up to 100 mL into the flask) was prepared. Also 1 mg mL^{-1} glucose solution (dissolve 250 mg glucose in dH_2O and fill up to 250 mL into the flask) was prepared. For obtaining of calibration line, glucose solutions prepared in different proportions in 6 eppendorfs were used.

400 µL of sample was put into a glass tube and added 400 µL of 5% phenol solution in water. For controlling, 400 µL dH_2O + 400 µL of 5% phenol solution in water was used.

After that, 2 mL of concentrated sulphuric acid were added sharply into the solution in tube. Let the samples stood for 10 minutes, then stirred and let them stood for 10 minutes at 30 °C. Samples at 490 nm in quartz cuvettes were measured and compared with the control sample. The amount of EPS (mg) was calculated using glucose calibration line.

Statistical analysis

Descriptive statistics was carried out to determine the differences of produced EPS concentration in different samples by Microsoft Windows for SPSS 14.0 software. Correlation analysis was used for determination of the differences between EPS concentration and CFU of LAB in yoghurt samples.

Results and Discussion

Exopolysaccharides production of LAB is an important attribute for the fermented dairy products (Jolly et al., 2002; Welman, Maddox, 2003; Ruas-Madiedo et al., 2010). Many of the thermophilic microorganisms produce exopolysaccharides and are of great technological importance. Thermophilic lactic acid bacteria starters show an optimum temperature ranging around 40–43 °C. By providing the microorganisms in their optimum temperature range, incubation time of approximately 5 hours was achieved. It was generally accepted that the lower fermentation temperature the longer it takes to reach a certain pH and the firmness of the final products. Milk acidification was carried out for all yoghurts until pH 4.5. Then, they were rapidly cooled and stirred before storage at 4–6 °C. For better understanding the influence of different fermentation temperatures on the functional properties of yoghurt samples, especially EPS concentration and colony

forming units of LAB, pH dynamics of samples during fermentation was studied.

pH dynamics is quite similar during fermentation of samples in the same temperature. At the end of fermentation the pH of yoghurt samples ranged from 4.50 to 4.57 in the analysed samples using different starters and fermentation temperatures. The residual acidification activity was observed for all samples during the shelf-life with stable pH values ranging from 4.25 to 4.28 (Harmony 1.0), from 4.16 to 4.20 (YF-L902) and from 4.30 to 4.35 (TWIST 1.0) depending on conditions for fermentation. However no significant effect of EPS concentration was observed on yoghurt postacidification during the self-life and according to the findings of Doleyres and co-authors (2005) no significant effect of EPS was observed on yoghurt postacidification.

The production of intracellularly synthesized EPS in yoghurt samples varies roughly from 25.28 to 440.81 mg L^{-1} during fermentation. Our results showed that starters TWIST 1.0 and YF-L902 produced greater quantities of EPS, while Harmony 1.0 starter lower amounts of EPS (Table 2).

Table 2

EPS concentration in yoghurt samples depending on chosen commercial starters and fermentation temperature, mg L^{-1}

Starter	Fermentation temperature, °C		
	38 °C	40 °C	43 °C
Harmony 1.0	32.10±6.33	63.55±3.13	152.79±9.80
YF-L902	137.07±8.61	175.34±23.82	304.19±15.15
TWIST 1.0	228.78±6.33	302.11±31.09	395.52±45.77

The fermentation temperature significantly contributes to EPS concentration because the increased rate of fermentation temperature is attributed to increased metabolic activity of LAB (Figure 1, a, b, c).

Our results are comparable with the findings of several authors (De Vuyst, Degeest, 1999; Laws et al., 2001; De Vuyst et al., 2001) that the higher EPS production of LAB was observed at the higher cultivation temperature, because of this grown-associated EPS production with thermophilic starter cultures and because of the limited number of catabolic pathways that provide energy in LAB (substrate level phosphorylation and secondary metabolic energy generation), cell synthesis is limited, and so is the energy-demanding EPS biosynthesis.

We also observed EPS degradation during the self-life of yoghurt. The reduction of EPS yield seems to be dependent on the starter used and biochemical reactions due to LAB metabolism during the storage of samples. The highest degradation was observed in yoghurt samples where after fermentation and maturation were determined the higher yield of EPS (in samples fermented with starters TWIST 1.0 and YF-L902 at 40 °C and 43 °C).

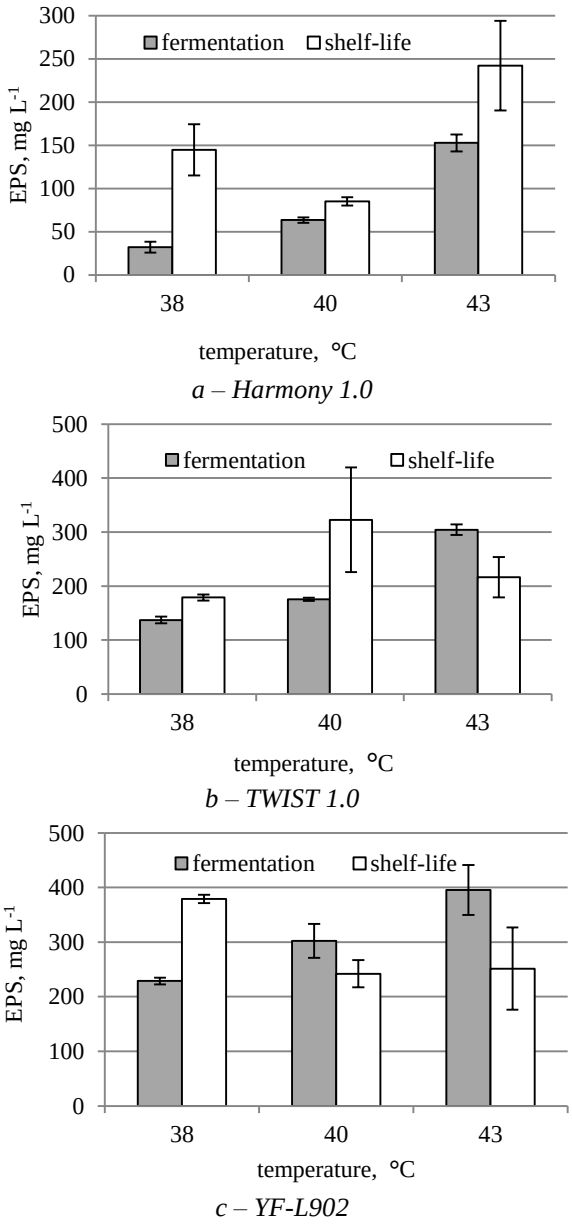


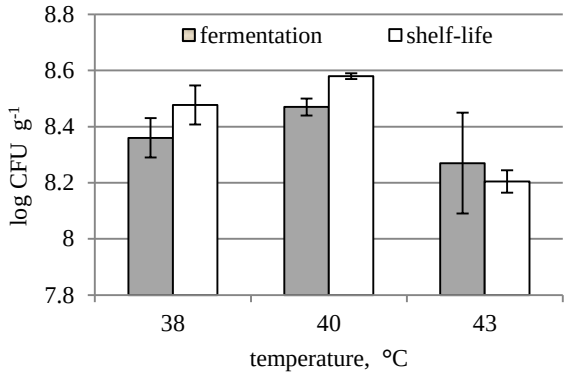
Figure 1. The comparison of EPS concentration in yoghurt samples after fermentation and at the end of shelf-life

We also noticed the tendency to increase EPS concentration during the self-life of product (in samples fermented with starters Harmony 1.0 at 38 °C, 40 °C and 43 °C; TWIST 1.0 at 38 °C and 40 °C; YF-L902 at 38 °C). A possible interpretation of our results in thermophilic starters is that isoprenoid phosphate carriers are primarily needed for cell wall synthesis growth (Petry et al., 2000). This may explain our finding that EPS production is increased in stationary phase cells.

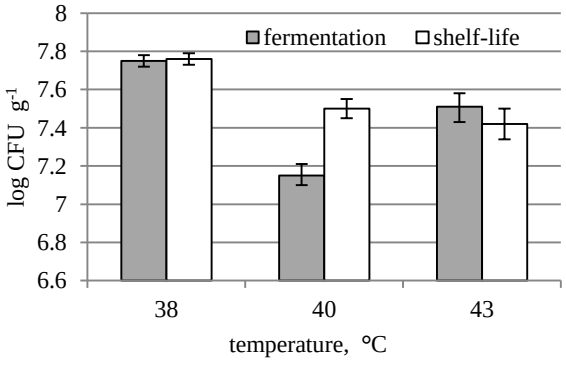
EPS production influences the amount of CFU of LAB in yoghurt samples (Figure 2, a, b, c).

There isn't established the correlation between the amount of EPS and CFU of LAB in samples fermented at different temperatures, but there is found stable CFU concentration in samples with higher EPS

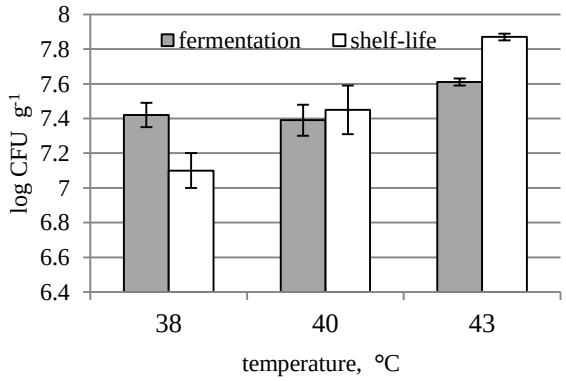
concentration during the shelf-life of yoghurt (Figure 2, b and c). We could explain it with EPS functional properties that EPS in their natural environment are thought to play a role in the protection of the microbial cell.



a – Harmony 1.0



b – TWIST 1.0



c – YF-L902

Figure 2. The changes of CFU of LAB in yoghurt samples after fermentation and during the shelf-life

We also observed that yoghurt samples with the highest initial EPS concentration and the highest degradation level of EPS showed stable CFU of LAB during the shelf-life of samples. We found that the higher reduction of CFU of LAB was noted in yoghurt samples with the increasing yield of EPS during the shelf-life (Harmony 1.0 at 43 °C, YF-L902 at 38 °C). We explain it with the energy-demanding EPS biosynthesis instead of the growing activity of LAB.

A lot of work has been done in the field of isolating and characterising the composition of EPS produced by various strains of lactic acid bacteria (Faber et al., 2002; Grobben et al., 2000; Petry et al., 2000; Van Calsteren et al., 2002; Ruas-Madiedo et al., 2005) but the functionality of EPS in fermented milk is still not completely clear. The fermentation temperature has a crucial role for the development of yoghurt quality and functional properties, too.

Conclusions

Analysing starters' producing EPS showed large variations in concentration from 25.28 to 440.81 mg L^{-1} in samples fermented in different temperatures.

The fermentation temperature significantly contributes to EPS concentration because the increased rate of fermentation temperature is attributed to increased metabolic activity of LAB.

The correlation between the amount of EPS and CFU of LAB in samples fermented at different temperatures is not established, but there is found stable CFU concentration in samples with higher EPS concentration during the shelf-life of yoghurt.

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