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ORIGINAL RESEARCH PAPER

THE INFLUENCE OF BIOSYNTHESIZED EXOPOLYSACCHARIDES ON SOME CHARACTERISTICS OF FERMENTED DAIRY PRODUCTS

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In the current technology of fermented milk products, the ability to form exopolysaccharides (EPS) is a basic criterion to be used for the selection of starter cultures, together with their capacity to produce lactic acid, aroma substances, stability and texture. This paper aimed at screening lactic acid bacteria to be used as starter cultures for yogurt production based on their ability to biosynthesized exopolysaccharides. In case of the YF-L812 starter culture, the ability to synthesize EPS depended on the dry matter (DM) of the milk: a quantity of 93 mg EPS/l was recorded for the whole milk reconstituted to 14.5% DM and 80 mg EPS/l for the sample reconstituted to 11.5% DM. The investigation at the microstructure level revealed that yogurt obtained from reconstituted milk to 14.5% DM had a dense network gel with a high syneresis phenomenon, whereas yogurt made from reconstituted milk to 11.5% DM formed a network gel with large alveolar cavity enough to hold the entire amount of whey.

Keywords: exopolysaccharides, yogurt, syneresis, texture, microstructure

Introduction

Consistency and structure are very important characteristics in evaluating the quality of fermented dairy products. In order to stabilize the gel structure of these products and stop the syneresis, several hydrocolloids of vegetable origin (extracted from terrestrial plants and marine algae) and microbial polysaccharides were tested in the past years (Lamothe *et al*., 2002). Since many of the polymers usually used as additives are chemically modified, and their use is restricted in some countries, a viable alternative from technological and economical point of view is the use of exopolysaccharides-producing lactic acid bacteria for milk fermentation (Welman *et al*., 2006).

The success of the application of an EPS is determined by its ability to bind water, interact with proteins and increase milk serum phase viscosity. Therefore, the ability of lactic acid bacteria to form exopolysaccharides became a basic criterion for selecting the starter cultures, along with their ability to produce lactic acid and aroma substances, to stabilize and to ensure acceptable texture. Furthermore, since

they are non-digestible due to the glycosidic bonds, these polysaccharides are considered to be components with a functional role, suggesting that they are active prebiotics.

The aim of this study was to investigate the abilities of different strains of lactic acid bacteria to produce EPS and their impacts on the characteristics (viscosity, syneresis and gel microstructure) of the fermented dairy products.

Materials and methods

Materials

Four types of commercial starter cultures YC-X11, YF-L811, YF-L812 and XPL-1 produced by Chr. Hansen were tested. The first three starter cultures YC-X11, YF-L811, YF-L812 are part of Yo-Flex cultures (yogurt starter culture containing a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) while XPL-1 (eXact®Plus) is a mixed culture used for production of cream, or buttermilk, consisting of a combination of mesophilic strains - *Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis, Leuconostoc* species, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* and a *Streptococcus thermophilus* strain, added for texture improving (AD Chr-Hansen, 2006). Whole milk powder, produced by Euro Food Prod SRL SC Darling was used for carrying out the experiments.

The fermented milk products were prepared starting from whole milk powder. Milk was reconstituted at 8.5% DM, 11.5% DM and 14.5% DM, then was pasteurized for 20 minutes at 95°C. After cooling, they were distributed in the plastic dishes for inoculation with starter cultures of lactic acid bacteria. Fermentation was led according to the producer's recommendations for each culture and was stopped when the acidity and pH reached the titratable acidity of 80-90°T and *p*H 4.6. The fermentation was conducted as follows: in the case of YC-X11 culture, the fermentation was carried at 43°C for 4 hours 30 min., in the case of YF-L811 culture, at 43°C for 5 hours, in the case of YF-L812 culture, at 43°C for 6 hours 30 min, while in the case of XPL-1 culture, at 35°C for 10 hours.

After fermentation, the samples were cooled and stored at 4°C before analysis.

Methods

The lactic acid content was determined according to AOAC (1995) method (% lactic acid). The *p*H measurements were made using a Hanna digital *p*H meter.

The exopolysaccharides were extracted according to the adapted method of Garcia-Garibay and Marshall (1991). The samples were treated with 20% trichloroacetic acid to precipitate the proteins and were afterwards centrifuged at 2500×g at 4°C for 30 min. The supernatant collected after centrifugation was treated with absolute ethylic alcohol (1:3) and left overnight at 4°C for precipitating EPS, followed by a centrifugation at 2500×g at 4°C for 30 min. EPS precipitate was redissolved in distilled water and submitted to dialysis against distilled water for 24 h at 4°C. The quantitative determination of the EPS was made using the colorimetric phenol-sulphuric method of total sugar dosage (Vata et al., 2000).

The viscosity was measured with a Visco STAR viscosimeter type R.

The syneresis of the yogurt samples was monitored after 12 and 84 h of storage at 4°C by measuring the quantity of whey spontaneously separated on the surface of 100 g set yogurt (relationship 1). The syneresis was assessed using the relationship proposed by Folkenberg et al. (2006):

$$\text{Syneresis}\,(\%) = \frac{\text{Whey expelled}\,(\text{g})}{\text{Initial yogurt}\,(\text{g})} \times 100 \qquad (1)$$

The microstructure of the yogurt samples was studied using scanning electron microscope - Quanta 200, which allows obtaining images of the surface topography and composition of large samples, non-transparent to electronic fascicles. Yogurt samples were spread in a thin layer on a steel plate, perfectly smooth.

Results and discussion

The results obtained showed that, during fermentation with starter cultures of lactic acid bacteria, different biochemical changes occurred in milk composition, depending on the type of bacteria and milk composition. Figure 1. presents the lactic acid formed during fermentation, and in Figure 2. are shown the final pH values of the yogurts samples.

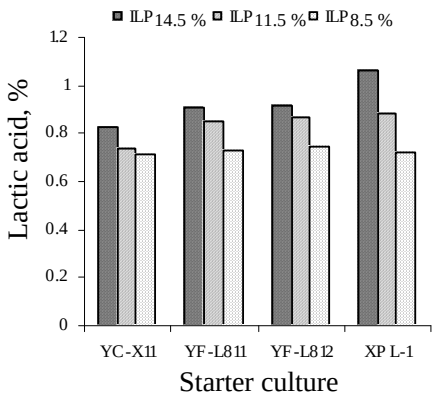

Figure 1. Lactic acid formed during fermentation

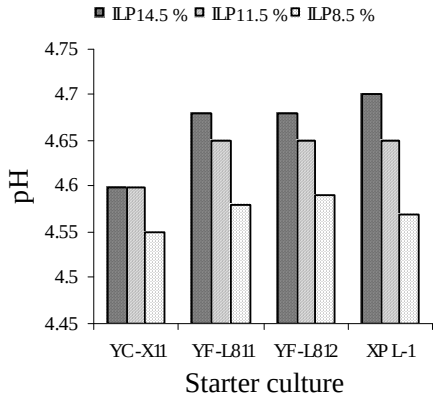

Figure 2. The final *p*H of yogurt samples

ILP 14.5% - Yogurt obtained from milk powder reconstituted to 14.5% DM
ILP 11.5% - Yogurt obtained from milk powder reconstituted to 11.5% DM
ILP 8.5% - Yogurt obtained from milk powder reconstituted to 8.5% DM

There are differences in the acidity values depending on the composition of the fermentative environment. The lactic acid content varied between 0.71% and 1.06% depending on milk composition. Figure 1 shows that a higher dry matter

content, consisting of higher amounts of protein and lactose, favoured the lactic acid production. Thus, in case of the yogurt sample obtained with XPL-1 starter culture from reconstituted milk with 14.5% DM, the amount of lactic acid was higher (49%) compared to the sample prepared from milk with 8.5% DM.

Instead, the final pH of yogurt samples containing higher dry matter (14.5%) was higher compared to the yogurt samples containing less dry matter (8.5%). Although the acidity increases, the pH does not decrease, probably due to the increased dry matter intake rich in proteins like lactoglobulin and lactoalbumin with buffering properties that do not allow the pH decrease (Simitaru and Segal, 2007).

In Figure 3. are shown the quantities of exopolysaccharide produced by each starter culture during milk fermentation.

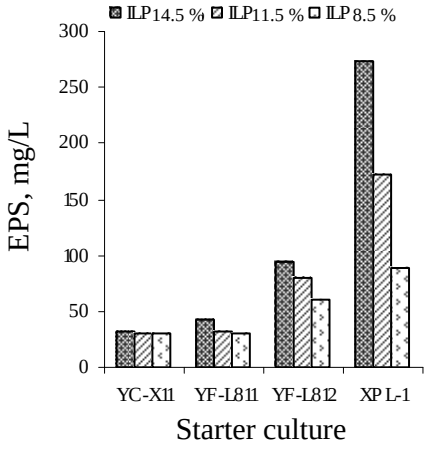

Figure 3. The amount of EPS produced by starter cultures tested

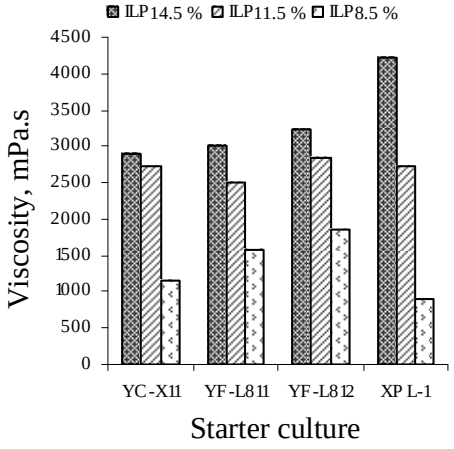

Figure 4. The viscosity of yogurt samples produced with different starter cultures

The quantity of exopolysaccharides secreted by lactic acid bacteria recorded high levels in yogurt samples obtained using the XPL-1 starter culture (273 mg/l for sample ILP 14.5%, 171 mg/l for sample ILP 11.5% and 88 mg/l for sample ILP 8.5%), followed by samples obtained with YF-L 812 (93 mg/l for sample ILP 14.5%, 80 mg/l for ILP 11.5% and 60 mg/l for ILP 8.5%). This dynamic content of EPS suggests that the presence of large amounts of protein and lactose in the fermentation medium favours the production of EPS by lactic acid bacteria.

In Figure 4 is shown the viscosity of yogurt samples fermented with different types of lactic acid bacteria. In case of all tested starter culture, the highest values of viscosity were recorded for the samples obtained from milk with 14.5% DM. The viscosity of yogurt samples was directly proportional to the amount of EPS recorded. The high viscosity of the yogurt samples directly correlated with the amount of EPS biosynthesised by lactic acid bacteria, as well as with the protein content.

A particular behaviour was observed for the yogurt sample obtained with starter culture YF-L 812. In the case of this starter culture, the yogurt sample obtained from milk with 14.5% DM that is characterised by the highest amount of EPS and highest viscosity, presented a pronounced syneresis phenomenon. The syneresis values of the yogurt samples after the 12 and 24 hours of storage at 4ºC are presented in Table 1.

Table 1. The amount of whey separated from yogurt samples surface obtained using YF-L 812 culture

Sample	Syneresis %	
	12 hours	84 hours
ILP 14.5%	2.58	2.21
ILP 11.5%	0.05	0
ILP 8.5%	0.83	0.46

In order to explain this phenomenon, the texture of the yogurt samples fermented with YF-L 812 was examined by means of the scanning electron microscope Quanta 200 (Fig. 5). In figure 5A and B are displayed the microscope images (4000 × mag.) of the yogurt samples ILP 14.5% and ILP11.5% DM.

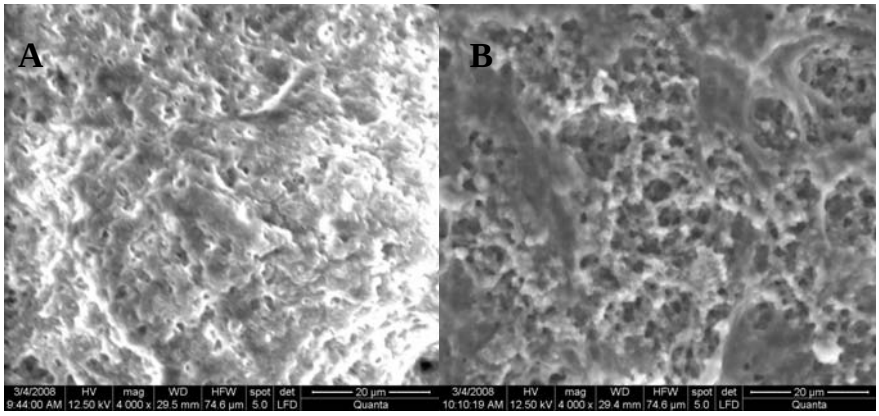

Figure 5. Microstructure of yogurt samples of the culture YF-l812
A - ILP14.5% (yogurt made from milk with 14.5% DM)
B - ILP11.5% (yogurt made from milk with 11.5% DM)

In the case of the sample ILP 14.5% (Figure 5a), one can see a dense microstructure with very small alveolar-capillary cavities of gel network formed. Instead, the sample ILP 11.5% (Figure 5b) had an airy microstructure of the gel network, with larger cavities of capillaries.

Therefore, it can be assumed that a high amount of dry matter (increased intake of protein) increases the number of protein-protein links, resulting in a denser and

more rigid gel network, that expels whey from the coagulum surface. A smaller amount of dry matter allows a gel matrix with large capillary, enough to retain the whey, thereby avoiding the phenomenon of syneresis.

Conclusions

All tested starter cultures are EPS producers. The largest amount of EPS was recorded for XPL-1 starter culture, followed by YF-L 812.

High dry matter content of the fermentation medium favoured the production of EPS, but their interaction with environmental components led to a dense structure.

Microstructure of yogurt, studied with scanning electron microscopy, revealed that the yogurt made from reconstituted milk powder at 14.5% DM using YF-L 812 starter culture, showed a dense gel network with high syneresis phenomenon, due to interaction of EPS with components of the fermentative environment, while yogurt made from milk powder reconstituted to 11.5% DM formed a gel network with alveolar-capillary cavities large enough to retain the entire amount of whey.

To obtain high quality fermented dairy products it is not necessary to use a large amount of EPS, because it is not necessarily directly proportional to viscosity. The thickening effect produced by EPS is dependent on the molecule structure and their interaction with other components of the fermentation medium.

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