EFFECT OF EXOPOLYSACCHARIDE STARTER CULTURE AND SOLIDS ON SYNERESIS OF YOGHURT

Cartașev A.

IP "Institutul Ştiinţifico-Practic de Horticultură şi Tehnologii Alimentare"

Cartașev Anatoli: toleo407@gmail.com

Abstract: The aim of this study was to compare whey separation in yogurts with solid contents 9,5% and 12,5% and exopolysaccharide (EPS) producing starter cultures using siphon and centrifugation methods of determination. The level of whey separation determined by this two methods decreased as the total solids increased. Was observed decrease in whey separation in yogurts made using EPS-producing starter cultures as determined by the siphon method at both solid contents as compared with those made with non-EPS starter cultures. A higher level of whey separation was observed in the product made using non-EPS starter cultures as determined by centrifugation method than that with EPS starter cultures at 9,5% solids. At 12,5% solids level, the values were lower in the product made by EPS starter cultures than that made with non-EPS starter cultures.

Key words: yoghurt, exopolysaccharide, starter cultures, solid content, syneresis

Introduction

Syneresis or spontaneous whey separation on the surface of yogurt is regarded as a defect. This problem can be reduced or eliminated by use of stabilizers (starch, gelatine and vegetable gum) or exopolysaccharide (EPS)-producing starter cultures. Due to consumer awareness of natural products, the use of stabilizers is restricted in some countries.

The use of EPS-producing starter cultures helps decrease the level of whey separation in yogurt [1,2].

To determine the best strategy for preventing syneresis in set yogurts, a correct method for the determination of whey syneresis is essential. The breakage of the yogurt gel as well as the presence of EPS may influence the result. This study compared the syneresis as determined by two methods centrifugation and siphon in yogurts made using non-EPS and ropy EPS starter cultures.

The use of cultures producing EPS increases resistance of yogurt coagulum to thermal and physical shock [3]. The presence of viscous extracellular materials produced by some culture bacteria were also demonstrated to play an important role in achieving satisfactory firmness and apparent viscosity of yoghurt [1,4].

Materials and methods

Micro-organisms

Ropy EPS-producing strain of *Streptococcus thermophilus* CNMN LB-51 and *Lactobacillus delbrueckii ssp. bulgaricus* CNMN LB-42 and non-EPS-producing *Streptococcus thermophilus* CNMN LB-52 were used in this study.

The *Streptococcus thermophilus* strains were previously selected and identified from the fermented milk products of spontaneous fermentation and was deposited in the Laboratory of Food Biotechnology of ISPHTA (Rep. of Moldova) in the Branch Collection of lactic acid bacteria and National Collection of Nonpathogenic

Microorganisms and characterized for their EPS production. The stock cultures were maintained at −18°C in 12% (w/w) sterile reconstituted skim milk (RSM) containing 20% (v/v) sterile glycerol. The microorganisms were activated by growing separately in sterile RSM for 18 h. The process was repeated three times.

Yogurt manufacture

Skim milk powder was used to prepare 4 samples of nonfat set yogurts at 9,5% and 12,5% (w/w) total solids with non-EPS and ropy EPS starter cultures. The SMP was reconstituted, followed by heat treatment at 65°C for 20 min, cooling to 40°C and inoculation with 2% (v/v) of starter cultures. A combination of 1% (v/v) of *L. delbrueckii ssp. bulgaricus* CNMN LB-42 with either 1% (v/v) of *S. thermophilus* CNMN LB-51 ropy EPS and non-EPS *S. thermophilus* CNMN LB-52. The inoculated mix was aseptically transferred into separate glass bottles at 100 mL quantities. The incubation was carried out at 40°C until pH 4.7 was reached, then the products were transferred to fridge (4°C).

EPS purification and quantification

The method of separation and quantification of EPS in yogurts was next. The proteins in 50 mL of diluted yogurt sample (1:1 yogurt : distilled water) were precipitated with 4 mL of 20% (w/v) trichloroacetic acid and separated by centrifugation (Hettich® ROTINA 38/38R, Germany) at 10000 × g for 30 min (4°C). The pH of the supernatant was adjusted to 6.8 with 40% (w/v) NaOH followed by boiling in a sealed container at 100°C for 30 min to denature the whey proteins, which were separated by centrifugation (10000× g, 30 min, 4°C). An equal volume of cold absolute ethanol was mixed with the supernatant and kept 12h at 4°C to precipitate the carbohydrates, which were then separated by centrifugation (10000 × g, 30 min, 4°C). The resultant carbohydrate pellet was re-suspended in 10 mL of distilled water. Total EPS (expressed as mg/L) was estimated in each sample by phenol–sulphuric method.

Determination of spontaneous syneresis by the siphon method

The level of spontaneous whey separation in yogurts was determined using a siphon method. In our study, a cup of set yogurt was taken from the cold room (4°C), weighed and kept at an angle of approximately 45°to allow whey collection at the side of the cup. A needle connected to a syringe was used to siphon the whey from the surface of the sample, and the cup of yogurt was weighed again. The siphon was carried out within 10 s to prevent further leakage of the whey from the gel. The syneresis was expressed as the percent weight of the whey over the initial weight of the yogurt sample.

Determination of syneresis by the centrifugation method

A cup of set yogurt removed from the cold room was stirred 20 times clockwise and anticlockwise with a glass rod. Approximately 30 g of the stirred yogurt was transferred into polypropylene conical tube. The stirred samples were then centrifuged(Hettich® ROTINA 38R, Germany) at 10000 × g for 15 min at 10°C. The separated whey was weighed. The syneresis was expressed as the percentage weight of the whey separated from the gel over the initial weight of the gel.

Results and discussion

The EPS concentration in yogurts made at 9,5% and 12,5% total solids using non-EPS and ropy EPS starter cultures is shown in Table 1. The 12,5% products had higher EPS concentration (~92 mg/l) than those at 9,5% products (~90 mg/l). The level of EPS detected was at 17,4 and 20,3 mg/l in non-EPS yogurts made at 9,5% and 12,5% solids, respectively

Table 1. Some chemical and physical properties of yoghurt samples

Properties	Total solids (%, w/w)	Yoghurt made using EPS starter	Yoghurt made using non-EPS starter
Titratable Acidity (%lactic acid)	9,5 12,5	0.765±0.009 0.784±0.0005	0.771±0.005 0.791±0.0005
EPS (mg/l)	9,5 12,5	90.4±0,5 92.1±1	10.6±1 12.4±0.3
Kinematic viscosity, cm·s·g^{-1}	9,5 12,5	67.0±4.3 69.6±0.1	17.4±1.1 20.3±0.6
Total LAB, (log cfu/ml)	9,5 12,5	8.3 9.3	8.1 9.0

The increase in the concentration of EPS in 12,5% yogurts compared with those at 9,5% suggested the influence of milk solids content on the activities of bacteria. Other workers have also shown that the increase in the concentration of available nutrients affected the EPS and lactic acid production by lactic acid bacteria [5,6,7,8].

Figure 1 shows the levels of syneresis as determined by the siphon method in yogurts made at 9,5% and 12,5% solids using non-EPS and ropy EPS starter cultures. At 9,5% solids, the yogurt made using EPS starter cultures showed a significantly lower level of syneresis. The level of whey separation in the product made at 12,5% solids using non-EPS starter cultures decreased significantly as compared to that at 9,5% solids.

The level of syneresis as determined by the centrifugation method in yogurts made at 9,5% and 12,5% solids using non-EPS and ropy EPS starter cultures is shown in Figure 2. The yogurts made at 9,5% solids using non-EPS starter cultures had the higer level of whey separation. The low level of whey separation was detected in the product made using ropy EPS starter cultures. In yogurts made at 12,5% solids, the results showed the the same trend. The products made by using ropy EPS starter cultures had the lowest level of whey separation, whereas those made using non-EPS starter cultures had the highest level of whey separation.

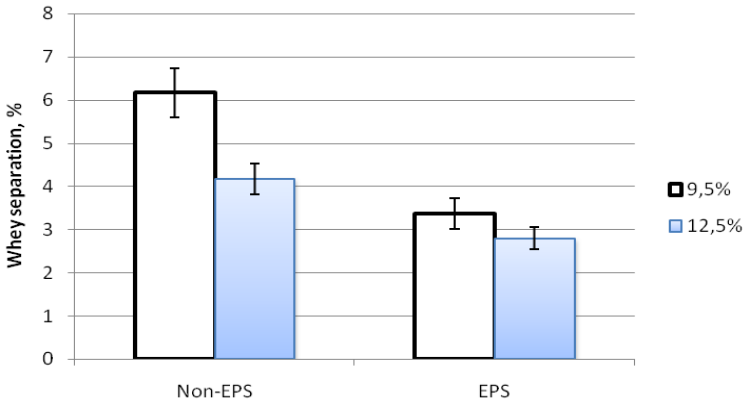

Fig. 1. The level of spontaneous whey separation determined by the siphon method in set yougurt made at 9,5% and 12,5% total solid using non-EPS and ropy EPS-producing starter cultures; error bars represent standard deviation

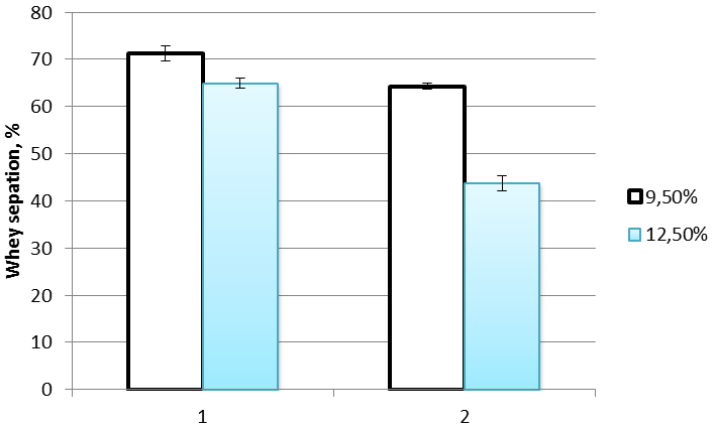

Fig. 2. The level of spontaneous whey separation determined by the centrifugation method in set yougurt made at 9,5% and 12,5% total solid using non-EPS and ropy EPS-producing starter cultures; error bars represent standard deviation

The difference in the patterns of syneresis detected from each method may suggest that these methods measured different data. The siphon method determines the level of spontaneous whey separated on the surface of gels. This method would represent the level of spontaneous syneresis in yogurts. The centrifugation method measures the level of whey separated from the collapsed gels as a result of centrifugal force. The whey collected as a result of centrifugation would be influenced by other factors such as the rigidity and rheological properties of gels. In general, the level of whey separation in yogurts as determined by the siphon method decreased due to an increase in solids content and the use of EPS starter cultures. This result is in agreement with that of others [2,9].

The level of syneresis as determined by the centrifugation method in the products made at 9,5% solids using EPS starter cultures was higher than that made using non-

EPS starter cultures. Hassan et al. [10] observed larger pore sizes in the microstructure of set yogurts made using EPS starter cultures compared to those made using non-EPS starter cultures. In general, when mixing milk proteins and polysaccharides together at a certain concentration, the solution may show phase separation into milk protein-rich phase and polysaccharide-rich phase if they are incompatible. Was observed that the EPS separated from the protein matrix after yogurt was stirred and formed a large phase.

In our study, the centrifugation method is likely to accelerate the phase separation between milk proteins and EPS in stirred yogurt made at 9,5% solids. Yogurts made at 12,5% solids with EPS starter cultures showed lower level of syneresis than those made with non-EPS starter cultures. Many factors may be responsible for those results including a decrease in the phase separation of milk protein EPS, an increase in EPS concentration causing an increase in viscosity as well as water adsorption as the total solids was elevated to 12,5%.

Conclusions

The whey separation as determined by the siphon method and centrifugation method was different. The level of syneresis could be influenced by method of determination as well as by the level of solids content and the type of starter culture. Therefore, it is important to select the right method for the determination of syneresis and to be cautious of other factors as well as the interpretation of the results. By comparing between the two methods, the siphon method would be more appropriate in the determination of the level of spontaneous whey separation on the surface of set yogurt.

EPS strains of *Streptococcus thermophilus* using in this study increases resistance of yogurt coagulum to thermal and physical shock and reduce the syneresis of yoghurt. This capacity would contribute to better stability of yogurt during storage time and increase the shelf-life of the product.

References

1. **Hassan, A. N., et. al.** Textural properties of yogurt made with encapsulated nonropy lactic cultures. Journal of Dairy Science. 1996, **79**, 2098–2103. ISSN 0022-0302.
2. **Jaros, D., et. al.** Influence of the starter cultures on the relationship between dry matter content and physical properties of set-style yogurt. Milchwissenschaft Journal. 2002, **57** 325–326. ISSN 0026-3788
3. **Broadbent, J. R., et al.** Biochemistry, genetics, and applications of exopolysaccharide production in Streptococcus thermophilus: a review. Journal of Dairy Science. 2003, **86**, 407–423. ISSN 0022-0302.
4. **De Vuyst, L. and Degeest, B.** Heteropolysaccharides from lactic acid bacteria. FEMS Microbiology Reviews. 1999, **23**, 153–177. ISSN 1574-6976.
5. **Amrane, A. and Prigent, Y.** Lactic acid production rates during the different growth phases of Lactobacillus helveticus cultivated on whey supplemented with yeast extract. Biotechnology Letters. 1998, **20,** 379–383. ISSN1573-6776
6. **Hassan, A. N.** Factors affecting capsule size and production by lactic acid bacteria used as dairy starter cultures. International Journal of Food Microbiology. 2001, **64,** 199–203. ISSN 0168-1605.

7. **Zisu, B. and Shah, N. P.** Effects of pH, temperature, supplementation with whey protein concentrate, and adjunct cultures on the production of exopolysaccharides by Streptococcus thermophilus 1275. Journal of Dairy Science. 2003, **86**, 3405–3415. ISSN 0022-0302.
8. **Amatayakul, T., et.al.** Physical characteristics of set yoghurt made with altered casein to whey protein ratios and EPS-producing starter cultures at 9 and 14% total solids. Food Hydrocolloids. 2006, **20**, 314-324. ISSN 0268-005X.
9. **Amatayakul, T., et.al.** Syneresis in set yogurt as affected by EPS starter cultures and levels of solids. International Journal of Dairy Technology. 2006, **59**(3), 216–221. ISSN 1471-0307
10. **Hassan, A. N.** Formation of yogurt microstructure and threedimensional visualization as determined by confocal scanning laser microscopy. Journal of Dairy Science. 1995, **78** 2629–2636. ISSN 0022-0302.