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Potential of Exopolysaccharides in Yoghurt Production

Jana Feldmane, Pavels Semjonovs, and Inga Ciprovica

Abstract**—**Consumer demand for products with low fat or sugar content and low levels of food additives, as well as cost factors, make exopolysaccharides (EPS) a viable alternative. EPS remain an interesting tool to modulate the sensory properties of yoghurt. This study was designed to evaluate EPS production potential of commercial yoghurt starter cultures (Yo-Flex starters: Harmony 1.0, TWIST 1.0 and YF-L902, Chr.Hansen, Denmark) and their influence on an apparent viscosity of yoghurt samples. The production of intracellularly synthesized EPS by different commercial yoghurt starters varies roughly from 144.08 to 440.81mg/l. Analysing starters' producing EPS, they showed large variations in concentration and supposedly composition. TWIST 1.0 had produced greater amounts of EPS in MRS medium and in yoghurt samples but there wasn't determined significant contribution to development of texture as well as an apparent viscosity of the final product. YF-L902 and Harmony 1.0 starters differed considerably in EPS yields, but not in apparent viscosities ($p>0.05$) of the final yoghurts. Correlation between EPS concentration and viscosity of yoghurt samples was not established in the study.

Keywords**—**Exopolysaccharides, yoghurt starters, apparent viscosity.

I. Introduction

EXOPOLYSACCHARIDES (EPS) from lactic acid bacteria (LAB) have found their most valuable application in the improvement of the texture properties of fermented milk products as well yoghurt and have been studied extensively over the past decades [1]-[3]. Their production significantly contributes to texture, mouth feel, taste perception and stability of the final products.

The increasing demand by consumers for healthy and consumers' friendly dairy products motivates food industry to better understand the effects of EPS on existing products and to search for new EPS-producing strains with different properties, because in the world the number of strains producing EPS with well-studied characteristic is limited. From other hand, there is a high consumer demand for products with low fat or sugar content and low levels of food additives especially stabilizers and thickeners, as well as cost factors, make EPS a viable alternative [4],[5].

Yogurt is a fermented dairy product resulting from the symbiotic growth of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* during milk fermentation. Classical yoghurt starter produces a smooth viscous gel with a desirable fermented product aroma and flavor. Consistency is very important characteristic evaluating the quality of yoghurt. In order to stabilize the gel structure of product and stop the syneresis, hydrocolloids of plant origin and microbial polysaccharides were used in the past decades and nowadays, too. Since many of these hydrocolloids are chemically modified, and their application is restricted in some countries, a viable alternative is application of exopolysaccharides synthesized LAB starter cultures [6]. The production of EPS by different yoghurt starters varies roughly from 45 to 350 mg/l [2]. A lot of studies are showed that EPS amounts and properties are strain dependent.

Polysaccharides derived from *Streptococcus thermophilus* and *Lactobacillus bulgaricus* show large variation in composition, charge, spatial arrangement, rigidity and ability to interact with proteins, no defining correlation between EPS concentration and viscosity has yet been established in realized studies [7]-[9]. This correlation is particularly important because it will provide a foundation for a strategy aimed at producing functionally valuable polysaccharides, which will behave in a relatively predictable fashion when incorporated into food products [9].

This study was designed to evaluate EPS production potential of commercial yoghurt starter cultures and their influence on the apparent viscosity of yoghurt samples.

II. Materials and Methods

Commercial starters used in this study are listed in Table I.

TABLE I
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*	Chr. Hansen, Denmark
Twist 1.0	*Streptococcus thermophilus, Lactobacillus johnsonii, Lactobacillus delbrueckii* subsp. *bulgaricus*	
YF-L902	*Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus*	

According to starters' manufacturer Chr. Hansen (Denmark) recommendations Yo-Flex® starters Harmony 1.0 and Twist 1.0 are characterized with EPS production potential that allow diminishing the application of stabilizers in fermented dairy products production; Yo-Flex® starter YF-L902 is a starter for yoghurt production which contributes to very mild yoghurt

J. Feldmane and I. Ciprovica are with the Latvia University of Agriculture, Jelgava, LV 3001 Latvia (phone: +371 6 30 05720; fax: +371 6 30 05720; e-mail: jana@baltais.lv, inga.ciprovica@llu.lv).

flavor intensity and extra high viscosity development during milk fermentation [10].

A. Preparation of Samples

Pasteurized and cooled milk samples were inoculated with different starters (given in Table I) and incubated at 43°C for 6 h until pH of coagulum reached 4.6. Fermented milk samples were stirred and maturated at 4-6°C for 10-12 hours. Yoghurt samples were analyzed an apparent viscosity by DV III Ultra Brookfield viscosimeter with special spindle SC4-16, pH (pH-meter Jenway 3520) and amount of EPS synthesized during fermentation.

EPS were determined in yoghurt starter samples, inoculated milk samples and yoghurt samples after fermentation according to below mentioned methodology [11]. MRS medium (de Man Rogosa & Sharpe; Scharlau, Spain) was used for cultivation of starter lactic acid bacteria for analyzing of EPS production potential in medium.

B. Isolation of EPS

300-500ml of fermented sample were put into the laboratory flask and boiled in water bath at 100^{0}C for 30 minutes. After cooling samples were centrifuged at 8000rpm for 10 minutes and 17ml of 85% trichloracetic were added to 100 l of sample. Samples were cooled up to 4^{0}C and again centrifuged at 8000 rpm for 10 minutes. Precipitation of EPS from samples was provided using cold ethanol (-20^{0}C, 1:3). Samples were stood in the fridge for 48h and late centrifuged (4^{0}C, 8000rpm, for 10 minutes), dissolved precipitation in dH_2O and defined EPS.

C. Quantification of EPS

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

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

After that, 2ml 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^{0}C. Samples at 490nm in quartz cuvettes were measured and compared with the control sample. The amount of EPS (mg) was calculated using glucose calibration line.

The factors as milk composition and milk quality were not taken into consideration in the research.

Descriptive statistics was carried out to determine the differences of produced EPS concentration in different samples by Microsoft Windows for SPSS (SPSS 17.0, SPSS Inc. Chicago, Illinois, USA). Correlation analysis was used for determination of differences between EPS production amount of the starter and viscosity of yoghurt samples.

III. Results and Discussion

Exopolysaccharide production of the LAB is an important attribute for the fermented dairy products [4]-[7]. Many of thermophilic microorganisms produce exopolysaccharides and are of great technological importance. All tested starter cultures are EPS producers. The production of intracellularly synthesized EPS by different commercial yoghurt starters varies roughly from 144.08 to 440.81mg/l of yoghurt samples. The results showed that TWIST 1.0 starter had produced greater quantities of EPS (440.81mg/l), while Harmony 1.0 lower amounts of EPS (144.08mg/l) (Table II).

Analyzing Table II data there is quite different EPS production potential in analyzing mediums.

TABLE II
EPS Potential of Commercial Yoghurt Starter Cultures Used in the Study

Starter	EPS, mg/l, in MRS medium	EPS, mg/l, in inoculated milk samples	EPS, mg/l, in yoghurt samples
Harmony 1.0	140,67	25,28	144,08
YF-L902	125,89	69,05	293,02
TWIST 1.0	176,48	84,40	440,81

For better understanding the influence of different yoghurt starters on texture properties of yoghurt samples pH of samples during fermentation was studied. The results are shown in Fig. 1.

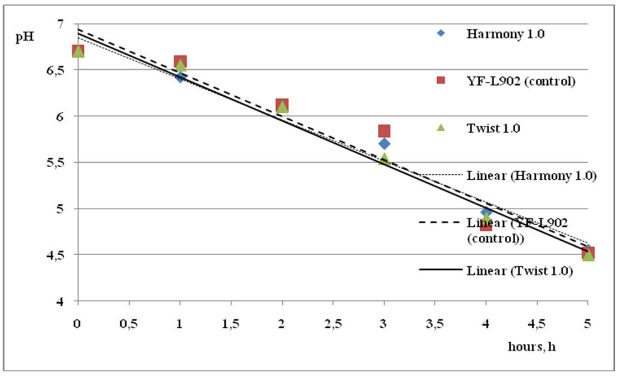

Fig. 1 Dynamics of pH during fermentation of yoghurt samples

pH dynamics are quite similar during fermentation of samples there aren't significant differences between samples.

The influence of yoghurt starter cultures on viscosity of yoghurt samples is shown in Fig. 2.

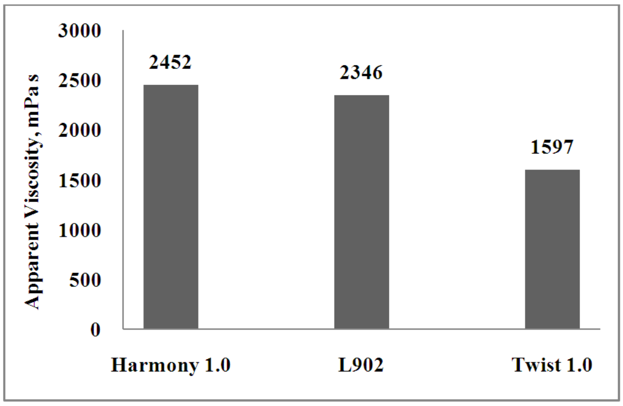

Fig. 2 The influence of different starters on yoghurt apparent viscosity

There are found interesting results analyzing viscosity of yoghurt samples. According to information given in Table II the most perspective EPS producing starter culture for yoghurt production was TWIST 1.0 but the highest viscosity was observed in yoghurt samples inoculated with starter Harmony 1.0. Although its production of high EPS amounts, TWIST 1.0 resulted in relatively thin yoghurt, so that texture values (an apparent viscosity) did not directly correlate with EPS production capacity. Viscosity has also been correlated with increasing molecular mass in some instances [12]. Polysaccharides derived from commercial starters show large variation in concentration and potential for implementation in dairy products production, but no defining correlation between EPS concentration and viscosity has been established in the present study.

Our findings we could explain with the results of Kleerebezem and co-authors that the effect of EPS on the physical and sensory properties of yoghurt depends on various factors, such as the type of EPS and the molecular and chemical characteristics of the EPS with the type of linkages, degree of branching and molecular weight being the most important factors [13]. 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 [14].

According to starter's producer's recommendations [10], YF-L902 characterizes with extra high mouth thickness and high gel firmness. Extra high texture relates to an apparent viscosity above 1900mPa s [10]. The higher viscosity of yoghurt samples inoculated with Yo-Flex starters Harmony 1.0 and YF-L902 we could explain with symbiosis of starters' technological properties and EPS producing potential. The combinations of flavor producing yoghurt strains and EPC producing strains get the desirable texture and flavor characteristics.

EPS is economically important because it can impart functional effects to foods and confer beneficial health effects - enhancing colonization by probiotic bacteria [15], as well as LAB EPS have been claimed to have antitumor effects [16], immunostimulatory activity [17], [18] and to lower blood cholesterol [19].

IV. Conclusion

Analyzing starters' producing EPS showed large variations in concentration from 144.08 to 440.81mg/l of yoghurt samples.

TWIST 1.0 produced greater amounts of EPS in MRS medium and in yoghurt samples but there wasn't determined significant contribution to development of texture of the final products comparing to other testing starter cultures.

Correlation between EPS concentration and viscosity of yoghurt samples was not established in the study.

The effect of EPS on the physical and sensory properties of yoghurt depends on various factors, such as the type of EPS and the molecular and chemical characteristics of the EPS with the type of linkages, degree of branching and molecular weight being the most important factors.

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Jana Feldmane, Ms.sc.ing. Author was born in Tukums, Latvia at 1971. Main research areas are: fermented dairy products and exopolysaccharides of dairy starters, their potential in dairy products production.

She is doctoral student in Latvia University of Agriculture.

Inga Ciprovica, Dr.sc.ing. professor. Author was born in Riga, Latvia at 1969. She has received Dr.sc.ing. in Food science at 1997 at Latvia University of Agriculture, Jelgava, Latvia. Main scientific directions are: milk and milk product quality, cheese quality, functional food, food additives.

She is professor, elected in Latvia University of Agriculture at 2010 but previous associate professor from 2000 till 2010.