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EFFECT OF THE SELECTED WHEY PROTEIN PREPARATIONS ON EXOPOLYSACCHARIDES PRODUCTION BY LACTIC ACID BATERIA AND RHEOLOGICAL PROPERTIES OF YOGHURT

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Summary. Some strains of lactic acid bacteria (LAB) possess an ability to produce exopolysaccharides (EPS). Presence of these biopolymers in fermented milk products contributes to improvement of consistency and stability of the final products. This study investigated the effect of whey protein preparations on the rheological properties of yoghurt fermented by starter cultures able to EPS production. The addition of whey protein preparations into milk during yoghurt production stimulates the secretion of EPS by LAB and effects on increase viscosity of products. The highest concentrations of EPS were obtained in products containing 0.5% addition of whey powder (WP) – 127.8 mg/100 ml or 134.6 mg/100 ml of yoghurt containing different culture. At higher WP concentration negatively affected on consistency of yoghurts was observed. Inverse tendency was observed in products containing WPI. The obtained results suggest milk products fermented by cultures producing EPS combined with whey protein preparations supplementation may lead to new products considered as functional food.

Key words: whey protein preparations, exopolysaccharides (EPS), syneresis, yoghurt texture

INTRODUCTION

Yoghurt is a fermented dairy product widely consumed around the world. The main difficulties in obtaining adequate rheological parameters of yoghurt are associated with low firmness and high level of syneresis [Gustaw et al. 2006]. The content of dry matter has a significant impact on the consistency of the final product and contributes to reduc-

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tion a level of syneresis on the surface of yoghurt [Sodini et al. 2005]. Therefore, increase content of dry matter in milk is one of the basic stages of the industrial production of yoghurt.

Exopolysaccharides (EPS) are defined as long-chain polysaccharides secreted by microbial cells. Some strains belonging to lactic acid bacteria (LAB) exhibit the ability to produce EPS [Salazar et al. 2016]. Wide structural diversity and unique properties represented by EPSs allow to multiple applications of such biopolymers in the different field of the study [Fialho et al. 2008]. Over the past few decades, microorganisms exhibiting ability to EPS synthesis are used in food, pharmaceutical, biomedicals areas as well as in bioleaching and environmental bioremediation [Waśko et al. 2010]. Determination the possibilities of application in dairy manufacturing EPS-producing bacteria strains is the object of many research study. The huge scientific interest associated with these molecules concerns their water-binding ability and texture promoting properties that allow for using them instead of the commercial polysaccharide hydrocolloids [De Vuyst et al. 2011]. Stabilizers such as gelatin, starch or other plant hydrocolloids, which improve the functional properties of yoghurt are also used, however they can also be the cause of off-flavors [De Vuyst et al. 2011]. The consumption of foodstuffs containing EPS is associated with health benefits. It has been proved that EPS may improve transient colonization by probiotic bacteria, exhibit immune-modulatory effects, demonstrate antitumor properties and cholesterol-lowering activity [Hidalgo-Cantabrana et al. 2012].

Whey is a source of lactose, calcium, proteins, soluble vitamins and other valuable nutrients. Moreover, several studies indicate the positive effect of using addition of whey proteins in yoghurt production that contribute to obtaining better consistency, texture and creaminess of the final products [Skrzypczak and Gustaw 2012]. Also, the addition of whey proteins stimulates growth of LAB in dairy products [Gustaw et al. 2016]. Moreover, it is suggested that whey protein may positively influence exopolysaccharides biosynthesis carried out by yoghurt bacteria [Saija et al. 2010].

The object of this investigation was to determine the influence of selected whey preparations on a bacterial exopolysaccharides production and rheological properties of yoghurt.

MATERIALS AND METHODS

Materials. For implementation this research the following materials were used: skim milk powder – SMP (SM Gostyn, Poland) whey protein isolate – WPI and whey protein concentrates WPC65, WPC80 (Milei, Leutkirch, Germany), WPC35 (Laktopol, Warsaw, Poland), whey powder – WP (OSM Krasnystaw, Poland), demineralized whey powder – DWP (Euroserum, Port – Sur-Saone, France), whey with reduced lactose content: LLWP (Foremost Farm, Baraboo, USA). The chemical compositions of whey protein preparations are shown in Table 1.

Bacteria cultures and inoculum preparation. As a starter were used MYE 95 and MYE 96 (Danisco, Poland) containing strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* that exhibit ability to EPS synthesis. Bacteria cultures in the form of lyophilized powders (4 mg) were incorporated (separately) to 50 ml of 12%

reconstituted skim milk (RSM) samples (previously pasteurized at 90°C for 30 min and cooled down to 40°C). Incubation was carried out in beakers (42°C/4 h). The obtained inoculum was used in production of yoghurts.

Table 1. Composition (%) of whey protein preparations (according to the declarations of manufacturers)

Tabela 1. Skład (%) preparatów białek serwatkowych (według deklaracji producentów)

Whey preparations Preparaty serwatkowe	Proteins Białko	Lactose Laktoza	Fat Tłuszcz	Minerals Związki mineralne	Moisture Wilgotność
WPI	91.1	0.9	1.0	2.0	5.0
WPC 35	33.9	48.5	4.4	7.7	5.5
WPC 65	65.4	19.6	4.6	5.9	4.5
WPC 80	79.6	5.0	4.7	5.5	5.2
WP	11.0	68.5	8.5	8.1	3.9
DWP	12.5	79.9	1.3	2.7	3.6
LLWP	22.3	50.4	9.6	14.6	3.1

Yoghurt production. Yoghurts were obtained from 12% reconstituted skim milk (RSM) (OSM Krasnystaw, Poland) by thermostat method. Seven variants of yoghurts differing in the type of added protein preparation were prepared. Each yoghurt variant was produced with 0.5%, 1.0% and 2.0% addition of the selected protein preparation. The control variant did not contain any additive of protein preparation. Pasteurized (at 80°C for 30 min in a water bath), samples of milk variants were inoculated using 2% (v/v) of previously prepared inoculum, subsequently were incubated at 42°C until the products achieved pH at level about 4.7.

EPS isolation. The exopolysaccharides were isolated according to the method Polak – Berecka et al. (2015) with minor modifications from yoghurts obtained with 0.5% addition of whey preparations.

Rheological properties. Rheological analysis of yoghurts included determination of the hysteresis loop in the shear rate from 0.1 to 200 in 60 s (at maximum shear rate for 30 s and held back to 0.1 in 1/s in 60 s at 20°C) were performed using dynamic rheometer RS 300 (ThermHaake, Germany) with the coaxial cylinder system Z41. The measurements were repeated three times. The values of K and n were calculated from the following Hershel – Bulkey model.

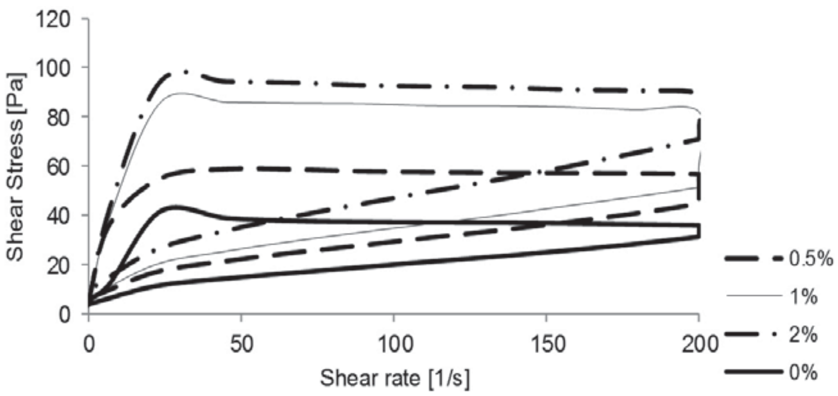
RESULTS AND DISCUSSION

Effect of the selected whey preparations on a rheological properties of yogurt

Figure 1 presents the effect of whey powder (WP) concentration on the hysteresis loop of yoghurt obtained by using a bacteria cultures able to synthesize an exopolysaccharides. All tested yoghurts have a thixotropic properties that is indicated by the course of the hysteresis loops. During the increasing shear rate from 0 to 200 [1/s] values of shear stress were higher than those received during the decreasing shear rate. The lowest values of shear stress and the smallest area of the hysteresis loop were noted for a control variant of yoghurt. Fermented products containing 0.5% addition of WP were characterized by the highest values of shear stress among all yogurts obtained with this addition (Fig. 1A). Increasing concentration of the protein preparation influenced formation a weaker yoghurt gel that was indicated by lower values of shear stress (Fig. 1A).

The adverse effect of the higher content of WPC 35 and WPC 65 in obtained fermented products might cause increase in the content of non-protein components such as lactose and fat that are present in large amounts in these type of protein preparations.

Yoghurts obtained with using MYE 95 and WP addition were characterized by slightly lower values of shear stress comparing to yogurt obtained with culture MYE 96 (Fig. 1B). Increasing concentration of whey powder had an influence on decreasing values of shear stress in yoghurts produced using MYE 95.



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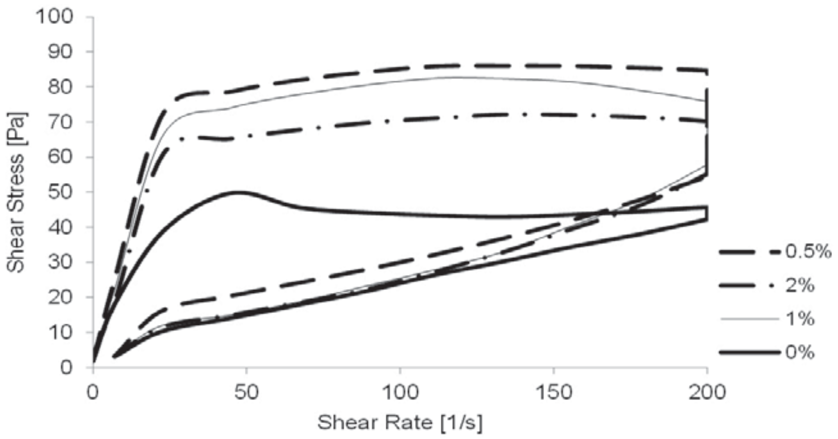

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Fig. 1. Comparison hysteresis loops of yoghurts obtained by using MYE 95 (A) or MYE 96 (B) starter and addition of whey powder in various concentration

Rys. 1. Porównanie pętli histerez jogurtów otrzymanych przy użyciu startera MYE 95 (A) lub MYE 96 (B) i zawierających dodatek serwatki w proszku o różnym stężeniu

It has been suggested that repulsive force between EPS and proteins (during yoghurt fermentation) may result in depletion induced attraction of casein micelles by EPS. This may lead to formation of acid milk gel containing EPS causing the difference in the protein aggregation mechanisms and the protein structures [de Kruif and Tuinier, 2001; Amatayakul et al. 2006]. Moreover, it is considered that two biopolymers can exist either in a single phase systems or in a phase separated systems depending on the nature of biopolymers, their concentration and solution properties. Stability of hydrocolloids (protein-polysaccharide complexes) is mainly governed by repulsive force between these two biopolymers. Higher degree of repulsion between the two neighboring biopolymers results in maximum stability due to least chances coalescence [de Kruif and Tuinier 2001]. It was also suggested that EPS have an ability to interact with the protein network and exhibit various functional and sensory properties in milk products [Folkenberg et al. 2005].

In yoghurts containing the addition of WPI obtained using starter MYE 95 or MYE 96 higher values of shear stress and bigger area of hysteresis loop between the up and down shear rate *versus* shear stress curves were observed. Yoghurts produced by using MYE 95 exhibited thixotropic properties (Fig. 2A). Increasing WPI concentration contributed to increase values of shear stress of yoghurt curd. The highest values of shear stress was observed in products containing 2% addition of WPI. Similar tendency was noted in products obtained by using the second of tested starter (Fig. 2B). These yoghurt samples also exhibited thixotropic properties. However, products obtained using starter MYE 96 characterized by higher values of shear stress compared with yoghurts containing MYE 95.

Addition of whey protein preparations exhibited a different influence on the consistency index (K) value (Table 2). The highest values of consistency index (K) were noticed in all yoghurts obtained with MYE 95 and WPI addition, while the lowest K values were noted for yoghurts supplemented with WPC80 (Table 2). The highest value of flow index (n) of products obtained with using MYE 95 were reported for yoghurts fortified with demineralized whey preparation (data not shown).

The addition of protein preparations caused increase viscosity of yoghurts that was connected with the increase in the consistency index. The obtained findings are in accordance with results presented by Ayala-Hernández et al. [2009], who observed that bacterial cells appeared attached to the protein network through filamentous strands with EPS strands emerging from the protein aggregate. These filaments were present throughout the protein network.

Obtained results correspond to Sady et al. [2009], who also reported that addition of solids to milk caused significant increase of apparent viscosity. In our study the increasing concentration of additives such as WP, WPC 65 and WPC 80 caused a decrease viscosity of yoghurt. While, WPC 35 and WPI addition increased the viscosity (enhancing concise of consistency) of the yoghurt curd respectively to increasing concentration of those preparations. Various effects of addition WPC on yoghurt viscosity were also noted by Sodini et al. [2005], who claimed that some yoghurts enrich in WPC exhibited the same viscosity as the control samples, while others exhibited lower values of viscosity parameter.

The differences in minor composition (non-protein nitrogen content) and in the level of whey protein denaturation probably are associated with differences in the functional properties of WPC in yoghurts [Sodini et al. 2005]. Moreover, Bhullar et al. [2002]

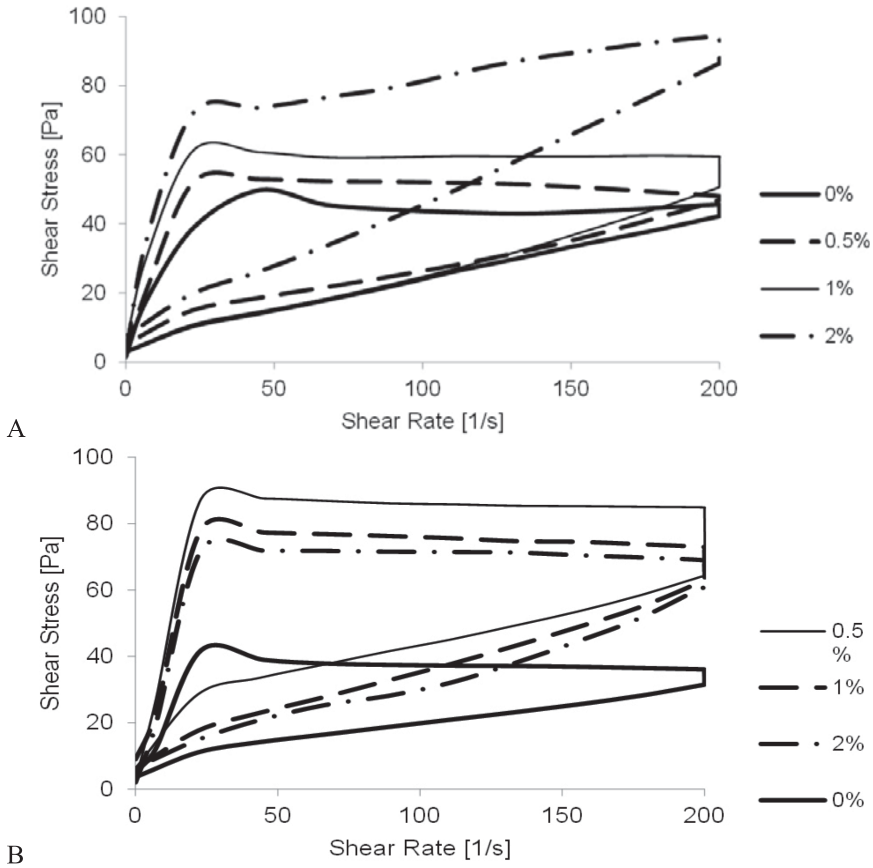

Fig. 2. Comparison hysteresis loops of yoghurts obtained by using MYE 95 (A) or MYE 96 (B) starter and addition of whey protein isolate (WPI) in various concentration

Rys. 2. Porównanie pętli histerez jogurtów otrzymanych przy użyciu startera MYE 95 (A) lub MYE (B) 96 i zawierających dodatek izolatu białka serwatkowego (WPI) w różnych stężeniach

reported that the supplementation with 2% (w/v) of WPC not only increased viscosity but also reduced syneresis. In a recent study, it was found that EPS produced by LAB modifies the functional properties of WPC, for example, increases the hardness of whey protein gels [Deep et al. 2012]. Interactions between milk proteins and EPS are known to enhance viscosity of fermented milk and to reduce syneresis and graininess [Ayala-Hernandez et al. 2009]. They also emphasized the fact that the presence of EPS – protein interactions and the changes in viscosity might be attributed to an increase in the EPS amount present in the media. In carried study they indicated that the increased viscosities obtained in fermented media (containing different protein concentrations) were caused not by the increasing protein content but by the effect of the EPS production. Also, during the process of acidification, the formation of whey protein aggregates were observed. It

Table 2. Comparison the average values of consistency coefficient (K – Pa s^n) of yoghurts obtained using MYE 95 and MYE 96 starters and addition of selected whey protein preparations addition

Tabela 2. Porównanie średnich wartości współczynnika konsystencji (K – Pa s^n) jogurtów otrzymanych przy wykorzystaniu starterów MYE 95 i MYE 96 oraz zawierających dodatek wybranych preparatów białek serwatkowych

Yoghurt variant – Wariant jogurtu		Variant of starter – Wariant startera (kultury bakterii)					
Containing addition of protein preparation Zawierający preparat białkowy	Control sample Próba kontrolna	MYE 95			MYE 96		
		Addition of protein preparation (%) – Dodatek preparatu białkowego (%)					
		0.5	1.0	2.0	0.5	1.0	2.0
WPC 35	29.75^{E} ±1.83	44.7^{Dd} ±2.3	119^{baC} ±2.8	213.5^{bA} ±13.3	44.4^{dcD} ±3.9	133^{bC} ±4	164.6^{bB} ±7.6
WPC 65	29.75^{E} ±1.83	42.5^{dD} ±3	85.3^{bcB} ±4.1	105.9^{cA} ±5.9	61^{cC} ±2.9	33.5^{dED} ±3.3	23.3^{dE} ±4
WPC 80	29.75^{A} ±1.83	33.9^{dA} ±3.4	34.1^{dA} ±3.6	35.3^{dA} ±3.1	36.5^{dA} ±2.6	34.9^{dA} ±2.4	34.5^{dA} ±3.9
WPI	29.75^{E} ±1.83	68.6^{cbED} ±7.1	143.7^{aCB} ±30.6	327.2^{aA} ±35.9	93.1^{bCD} ±7	194.8^{aB} ±20.6	371^{aA} ±21.9
DWP	29.75^{C} ±1.83	58.5^{cBA} ±4	64.1^{dcB} ±5	55.6^{dBA} ±3.9	59.7^{cBA} ±7.6	64.1^{cBA} ±8.7	73.9^{cA} ±5
WP	29.75^{C} ±1.83	107.6^{aBA} ±4.6	88.3^{bcBC} ±11	71.7^{dcC} ±5.7	117.4^{aA} ±8.9	82.7^{cC} ±7.3	72.2^{cC} ±6.6
LLWP	29.75^{D} ±1.83	71.8^{bA} ±1.9	54.9^{dcC} ±4.4	50.5^{dC} ±2.2	55.1^{cBC} ±6.2	67.7^{cBA} ±7.7	62.5^{cBAC} ±4.2

Explanatory notes: Mean values in the same column ± standard deviation ($\bar{x}$±s/SD; n = 3) designated by different small letters are significantly different; means ± standard deviation ($\bar{x}$±s/SD; n = 3) in the same row with different capital letter differ statistically significantly ($P \leq 0.05$).

Objaśnienia: Wartości średnie w tej samej kolumnie ± odchylenie standardowe ($\bar{x}$±s/SD; n =3) oznaczone różnymi małymi literami różną się statystycznie istotnie ($P \leq 0,05$); wartości średnie w tym samym wierszu ± odchylenie standardowe ($\bar{x}$±s/SD; n = 3) oznaczone różnymi dużymi literami różną się statystycznie istotnie ($P \leq 0,05$).

can be assumed that these aggregates form a complex with the EPS produced by LAB. This is the result electrostatic interactions between anionic EPS and positive charged milk proteins [Gentes et al. 2013]. These complexes significantly contribute to improving structure features in milk fermented with EPS-producing bacteria cultures.

The effect of the selected whey protein preparations on EPS concentration in set yoghurt

The obtained results indicate that on EPS production had an impact the type of applied protein preparation as well as variant of starter culture (Table 3). The highest concentrations of EPS were obtained in products supplemented with WP and WPI. It has previously been shown that increasing the amount and availability of nitrogen, e.g. by supplementation with whey protein preparations, increases the amount of EPS produced in milk by LAB [Amatayakul et al. 2006]. The lowest EPS concentration in fermented by MYE 95 (42.0 mg/100 ml) products was noted for yoghurt enriched with WPC 80, whereas for yoghurt containing MYE 96 the lowest content of EPS (24.5 mg/100 ml) was noted for products belonging to control variant.

Table 3. The concentrations of exopolysaccharides produced by MYE 95 and MYE 96 starters in yoghurts supplemented with 0.5% selected whey protein preparations

Tabela 3. Zawartość egzopolisacharydów wyprodukowanych przez startery MYE 95 i MYE 96 w jogurtach wzbogaconych 0,5% dodatkiem wybranych preparatów białek serwatkowych

Yoghurt variant	MYE 95 – EPS (mg/100ml)	MYE 96 – EPS (mg/100ml)
control	60^{b} ±1.8	24.5^{c} ±1.5
with WPC 35	60.07^{b} ±2.7	72.2^{b} ±5.3
with WPC 65	64.4^{b} ±2.5	67.27^{b} ±4.4
with WPC 80	42^{b} ±3.4	51.5^{cb} ±1.9
with WPI	104.2^{a} ±8.4	76.1^{b} ±5.4
with DWP	67.13^{b}± 5	59.1^{cb} ±8.9
with WP	127.8^{a} ±23.4	134.6^{a} ±31.7
with LLWP	62.3^{b} ±7.1	61.9^{b} ±11.6

Explanatory notes: Mean values in the same column ± standard deviation ($\bar{x}$ ±s/SD; n = 3) designated by different small letters differ statistically significantly (P ≤ 0.05).

Objaśnienia: wartości średnie w tej samej kolumnie ± odchylenie standardowe ($\bar{x}$ ±s/SD; n = 3) oznaczone różnymi literami różną się statystycznie istotnie (P ≤ 0,05).

Zisu and Shah [2003] observed that 0.5% addition of WPC 80 to RSM (10%) influenced enhancement of the EPS production by *S. thermophilus* during 24 h fermentation, while Amatayakul et al. [2006] observed decrease concentration of EPS when the ratios of casein to whey proteins was reduced in set yoghurts, also shorter time of fermentation may limit EPS production due to limitation of available nutrients (peptides and sugar) essential for bacterial growth. Prasanna et al. [2012) investigating the effect of the addition of various milk protein preparations found an increase in exopolysaccharides production by bifidobacteria, especially in the case of casein hydrolysate, WPC and WPI. Similar results were obtained during fermentation of milk supplemented with WPC by yoghurt cultures that deliver large amounts of exopolysaccharides (Buldo et al. 2016).

CONCLUSIONS

Obtained results demonstrate that using 0.5% addition of whey protein preparations in technology of yoghurt production influences increase viscosity of final products. While, increasing concentration of WP, WPC 65 and WPC 80 caused decrease viscosity of the fermented milk beverages. Whereas, addition of WPC 35 and WPI enhanced the rheological properties of yogurt respectively to increasing concentration of protein preparations. Yoghurts containing WP addition (0.5%) exhibited the highest concentrations of EPS, which interacted with the formation of protein network through bridging links (e.g. via electrostatic interactions). Further increase in the concentration of WP results in an increase in the concentration of non-protein components that negatively affect the consistency of yoghurts. Inverse tendency was noted in products with WPI addition, the increase in protein concentration stabilizes the yoghurt gel matrix. This highlights the importance of selecting the right combination of added protein preparation and yoghurt culture in order to obtain the desired rheological properties. The use of cultures producing exopolysaccharides as well as the addition of whey proteins in the yoghurt production will also improve potential of health promoting properties.

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WPŁYW WYBRANYCH PREPARATÓW BIAŁEK SERWATKOWYCH NA WYTWARZANIE EGZOPOLISACHARYDÓW PRZEZ BAKTERIE MLEKOWE ORAZ WŁAŚCIWOŚCI REOLOGICZNE JOGURTU

Streszczenie. Wybrane szczepy bakterii fermentacji mlekowej (LAB) mają zdolność wytwarzania egzopolisacharydów (EPS). EPSy obecne w fermentowanych produktach mlecznych poprawiają ich konsystencję i przeciwdziałają synerezie. Celem badań było określenie wpływu dodatku wybranych preparatów białek serwatkowych na właściwości reologiczne jogurtów otrzymanych przy użyciu kultur startowych zdolnych do wytwarzania EPS. Dodatek preparatów białek serwatkowych do mleka podczas produkcji jogurtu wpłynął na zwiększenie wydzielania EPS przez LAB oraz wzrost lepkości produktu. Najwyższe stężenie EPS oznaczono w jogurtach otrzymanych z 0,5% dodatkiem serwatki w proszku (WP) – 127,8 mg/100 ml lub 134,6 mg/100 ml w zależności od zastosowanego startera. Wyższe stężenie WP miało negatywny wpływ na konsystencję otrzymanych jogurtów. W przypadku izolatu białek serwatkowych (WPI) zaobserwowano odwrotną zależność. Na podstawie otrzymanych wyników możemy stwierdzić, że zastosowanie kultur bakteryjnych posiadających zdolność wydzielania EPS można wykorzystać w produkcji żywności funkcjonalnej.

Słowa kluczowe: preparaty białek serwatkowych, egzopolisacharydy (EPS), synereza, tekstura jogurtu