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ORIGINAL RESEARCH PAPER

OBTAINING FERMENTED DAIRY PRODUCTS WITH THE YOGURT CULTURE YF-L 812

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Exopolysaccharides (EPS) produced by some lactic acid bacteria cultures can efficiently replace commercial stabilizers for preventing or reducing syneresis, providing fermented milk products with suitable structure viscosity. The effect of EPS on food quality characteristics depends on the EPS properties themselves, as well as their interaction with various components of the food system. This paper was aimed at studying the influence of the environment composition on the EPS biosynthesis by starter culture YF-L 812 and at determining the properties of at yogurt obtained. High fat content of milk (3.5% and 1.5%) had a positive effect on yogurt texture. In order to reduce the syneresis phenomenon, the milk with low fat content (0.1%) was supplemented with different concentrations of milk powder and lactose. Our results indicated that the whey separated was reduced to 0.6% and 0.3% when the milk was supplemented with 2% lactose and 2% skimmed milk powder, and respectively, 3% lactose and 3% skimmed milk powder.

Keywords: exopolysaccharides, viscosity, syneresis, yogurt.

Introduction

Exopolysaccharides (EPS) produced by some lactic acid bacteria in the environment gives the possibility to obtain high quality fermented milk products with desired rheological properties and various health benefits.

The ability of lactic acid bacteria to produce EPS depends on bacterial strain, while the amount of EPS produced is influenced by the environment composition (C:N ratio) and growing conditions (pH, temperature and incubation time) (Broadbent *et al*., 2003). It was shown that an optimal balance between the carbon and nitrogen source is absolutely necessary to achieve high EPS yields (De Vuyst and Degeest, 1999).

Since EPS contributes to ensuring specific rheological characteristics of fermented dairy products, it is necessary to understand how the structural components of the EPS (e.g. nature of carbohydrate monomers, the type of linkages between

monomers, the degree of polymerization and their molecular weight) influences the final product viscosity. It should be mentioned that the influences of EPS on fermented products properties depend not only on the biopolymers properties, but also on their interaction with various components (e.g. protein) of the food system (Kleerebezem *et al.*, 1999). Current research is focused on improving the production of EPS with specific structure and size to achieve the desired functionality.

Our aim was to investigate the influence of milk composition on the EPS production by culture YF-L 812 and textural properties of the yogurt obtained.

Materials and methods

In the experiment was used the thermophilic culture YF-L 812 specific for yogurt, which is a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* in a lyophilized form. YF-L 812 is known to produce yogurt with very consistent body, moderate flavor, and very low post-acidification (AD Chr-Hansen, 2003).

Yogurt was made from UHT milk with different fat contents (3.5%, 1.5% and 0.1% fat) produced by Fulga, Albalact. The milk was heated to 45°C and inoculated with YF-L 812 yogurt culture (50 U in 250 l of milk) according to the producer's recommendation (AD Chr-Hansen, 2006). Samples were incubated to 43°C for 6 h and 30 min. The fermentation was stopped when the *p*H reached 4.6. The set yogurt samples with compact coagulum were afterwards cooled and stored at 4°C

The following physico-chemical and microbiological analyses were carried out: acidity, quantity of lactic acid, *p*H, quantity of synthesized exopolysaccharides, viscosity, syneresis and the total number of lactic acid bacteria.

Acidity was determined by titration with NaOH 0.1N and expressed in Thörner degrees.

Lactic acid was determined according to AOAC (1995) method and expressed as lactic acid (%). The *p*H measurement was made using a Hanna digital pH meter.

Exopolysaccharides were extracted according to the method of Garcia-Garibay and Marshall (1991) with slight modifications. The samples were treated with 20% trichloroacetic acid to precipitate proteins and 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 for total sugar dosage (Vata *et al.*, 2000).

Viscosity was measured with a Visco STAR viscosimeter type R.

Syneresis was monitored during yogurt storage at 4°C (after 12 and 84 hours) by measuring the quantity of whey spontaneously separated on the surface of 100 g yogurt with firm curd and assessed using the relationship (Folkenberg *et al*., 2006):

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

The cultural method was employed to determine the total number of lactic acid bacteria, on MRS medium agarose gel in double layer: the first layer - solid - 1.5% and the second layer - semisolid - 0.75% agar. From each sample of yogurt, decimal dilutions were made in sterile (0.9 % NaCl) saline solution, and the dilutions 6, 7 and 8 were used for inoculations in Petri dishes over the first layer of solid MRS. After the sample was uniformly distributed on the surface of the plate, the second layer of MRS was added. Samples were incubated at 37°C for 24, 48 or 72 hours.

Statistical analysis was performed by one-way ANOVA method. The decision was made by comparing the calculated Fisher statistics (F) with the statistical tables (Fcrit) for a significance level (α) of 0.05 and degrees of freedom associated.

Results and discussion

The data in Table 1 shows that all yogurt samples reached values of *p*H and acidity optimal for fermented dairy products.

Table 1. Physico-chemical and microbiological characteristics of yogurt samples

Yogurt sample*	Acidity, °T	*p*H	Acid lactic, %	Viscosity, mPa·s	Syneresis, %	EPS, mg/l	Lactic bacteria, CFU/ml
IG	87	4.56	0.79	2660	0	71.46	$1.32 \cdot 10^7$
IM	84	4.57	0.75	2010	0	71.07	$1.30 \cdot 10^7$
IS	86	4.57	0.77	1600	1.25	63.65	$3.2 \cdot 10^6$

* IG - yogurt sample made from UHT milk with high fat content (3.5%); IM - yogurt sample made from UHT milk with a medium fat content (1.5%); IS - yogurt sample made from UHT milk with low fat content (0.1%)

The obtained results indicate that the viscosity of yogurt samples decreased by reducing the fat content. The high level of fat (3.5%) increased the yogurt viscosity by 40% in comparison with the yogurt sample obtained from milk with 0.1% fat. At the same time, the EPS biosynthesis by lactic acid bacteria decisively influenced the yogurt viscosity. The amount of EPS synthesized was comparable in case of the IG and IM samples (71.46 mg EPS/l and 71.07 mg EPS/l, respectively), and higher than the sample (63.65 mg EPS/l).

According to Hassan *et al.* (2002), in order to obtain high quality fermented milk products, it is not necessarily needed a large amount of EPS, because it is not directly proportional to environment viscosity. Thickening effect produced by the EPS depends on other characteristics, such as size and structure of the molecules and their interaction with other components of the fermentation environment (Hassan *et al.*, 2002).

It was noted that yogurt samples IG and IM, with a high and respectively medium content of fat, showed no syneresis phenomenon. This can be explained by the fact that fat has participated along with the protein to gel network formation, thus

resulting in an elastic gel network with alveolar-capillary cavities, large enough, which managed to retain all whey quantity in the network.

The number of viable lactic acid bacteria was different, depending on the nature of samples, being $1.3 \cdot 10^7$ CFU/ml in IG and IM yogurt samples and $3 \cdot 10^6$ CFU/ml in the IS sample.

Since the ability of lactic acid bacteria to produce EPS and to influence the consistency and texture of the yogurt depends on the bacterial strain and on the composition of the fermentation media (C:N ratio), to stimulate the synthesis of carbohydrate biopolymers, the UHT milk with low fat (0.1%) content was supplemented with various additives as carbon and nitrogen sources.

To increase nitrogen intake, the milk was fortified with skimmed milk powder in proportion of 1%, 2%, 3% and 4%. Table 2 presents the physico-chemical and microbiological characteristics of yogurt samples obtained from milk with different protein contents.

Table 2. Physico-chemical and microbiological characteristics of yogurt samples obtained from low-fat milk supplemented with skimmed milk powder

Yogurt sample*	Acidity °T	pH	Lactic acid %	Viscosity mPa·s	Syneresis %		EPS mg/L	Lactic bacteria CFU/ml
					12 h	84 h		
IS	86	4.57	0.77	1600	1.25	1.01	63.65	$3.2 \cdot 10^6$
IS1P	93	4.65	0.82	1670	1.82	1.30	60.51	$1.01 \cdot 10^6$
IS2P	101	4.68	0.9	2540	1.66	1.23	65.09	$1.06 \cdot 10^6$
IS3P	104	4.68	0.92	2870	1.83	1.68	70.08	$2.3 \cdot 10^6$
IS4P	110	4.68	0.98	2950	1.43	1.13	105.20	$6 \cdot 10^6$

* IS1P - addition of 0.35% protein (1% skimmed milk powder); IS2P - addition of 0.72% protein (2% skimmed milk powder); IS3P - addition of 1.80% protein (3% skimmed milk powder); IS4P - addition of 1.44% protein (4% skimmed milk powder)

The results in Table 2 demonstrate once again that high protein content in milk increases the amount of lactic acid in yogurt. Although the acidity increases, the pH of the sample remains relatively high, due to the buffering properties of proteins.

It should also be noted that all the samples presented the syneresis phenomenon. The amount of whey separated from all samples supplemented with skimmed milk powder was higher than IS control sample (sample of yogurt made from UHT milk with low fat content (0.1%)). IS3P sample recorded syneresis values with 46.4% higher than the control sample, followed by the IS1P sample with 45.6%, and by the IS2P sample with 32.8%. This was due to a higher number of protein-protein links, which resulted in a denser gel network and fewer protein-water links. Similar results were reported by other researchers (Hassan *et al*., 1996; Marshall and Rawson, 1999; Amatayakul *et al*., 2006). The incompatibility between EPS and proteins may be the explanation. When the concentration of the biopolymers increases, the system may become unstable, and the proteins and polysaccharides tend to separate. This is a depletion phenomenon and any excess of polymer

concentration leads to separation of phases rich in protein and polysaccharide (Kleerebezem *et al*., 1999).

Regarding the effects of EPS biosynthesis, it can be observed a 43% increase of the viscosity of the sample supplemented with 4% skimmed milk powder compared to the sample supplemented only with 1%. It was also recorded an EPS content increase, depending on the amount of the supplement; the largest EPS content (105.2 mg/l) occurred in the sample supplemented with 4% skimmed milk powder. However, larger quantities of EPS in the environment did not favor the obtaining of a good texture of yogurt.

The results also indicate that a higher protein level did not significantly affect the lactic acid bacteria growing, (approximately 10^6 CFU/ml sample of yogurt) which was comparable to the control sample.

The UHT milk with low fat content (0.1%) was afterwards supplemented with different types of carbohydrates – carbon sources – (lactose, glucose, fructose, galactose). The obtained results indicate that all yogurt samples, regardless of the nature of carbohydrates, have reached optimal levels of pH and acidity for fermented dairy products. Table 3 presents the physico-chemical and microbiological properties of yogurt samples obtained from milk enriched with different carbohydrates.

Regarding the syneresis phenomenon it was noted that, after 12 hours of storage at 4°C, all samples had separated over 1% whey (g whey /100 g yogurt).

The addition of lactose to the milk favored the development of a higher number of lactic bacteria; in all samples with lactose addition, lactic acid bacteria number was of the order 10^8 CFU/ml, higher compared to samples with glucose addition (10^6 CFU/ml), fructose (10^7 CFU/ml) and galactose (10^6 CFU/ml).

However, yogurt made from milk supplemented with lactose showed the highest amounts of EPS biosynthesized and had viscosities higher than of samples from milk supplemented with glucose, fructose and galactose, on which viscosity values were relatively similar. Thus, samples with the addition of lactose had 36.1% higher EPS amounts compared to the samples with fructose addition, 20.7% higher than samples with galactose addition and 16.3% higher than those with glucose. It can be noted that lactose was more preferred by lactic acid bacteria. Van Den Bogaard *et al.* (2000) showed that *Streptococcus thermophilus*, a homofermentative thermophilic lactic acid bacterium, is highly adapted to growth on lactose as the primary carbon and energy source. They demonstrated that *S. thermophilus*, unlike many other gram-positive bacteria, prefers lactose over glucose as the primary carbon and energy source.

The study has continued using combined supplements; the milk was supplemented with different contents of skimmed milk powder and lactose. In Table 4 are registered the physical and chemical characteristics of yogurt made from UHT milk (0.1% fat) supplemented with various proportions of skimmed milk powder and lactose.

Table 3. Physico-chemical and microbiological characteristics of yogurt samples obtained from low-fat milk and the addition of carbohydrates

Yogurt sample*	Acidity, °T	*p*H	Lactic acid, %	Viscosity, mPa·s	Syneresis, %		EPS, mg/l	Lactic bacteria, CFU/ml
					12 h	84 h		
IS1L	87	4.57	0.77	1510	2.06	1.06	64.08	$1.05 \cdot 10^8$
IS2L	87	4.57	0.77	1530	1.80	0.2	63.59	$1.04 \cdot 10^8$
IS3L	86	4.58	0.76	1640	2.32	0.3	63.11	$1.07 \cdot 10^8$
IS4L	85	4.58	0.76	1700	1.78	0.38	67.71	$1.13 \cdot 10^8$
IS1G	91	4.60	0.81	1150	2.64	0.94	51.11	$7 \cdot 10^6$
IS2G	90	4.60	0.80	1220	2.25	0.55	57.71	$2 \cdot 10^6$
IS3G	89	4.59	0.79	1280	2.01	0.46	54.41	$1 \cdot 10^6$
IS4G	89	4.59	0.79	1290	2.86	0.98	59.36	$1.09 \cdot 10^6$
IS1F	87	4.58	0.77	1010	2.97	1.43	50.08	$2.12 \cdot 10^7$
IS2F	87	4.58	0.77	1210	3.29	1.74	49.47	$1.11 \cdot 10^7$
IS3F	85	4.57	0.76	1290	2.84	1.36	49.47	$2.8 \cdot 10^7$
IS4F	84	4.57	0.75	1310	1.71	0.38	42.87	$2 \cdot 10^7$
IS1Ga	84	4.56	0.75	1000	2.55	1.52	50.51	$5.9 \cdot 10^6$
IS2Ga	84	4.56	0.75	1290	1.76	0.42	57.60	$1 \cdot 10^6$
IS3Ga	82	4.55	0.73	1270	1.76	0.34	54.41	$1.19 \cdot 10^6$
IS4Ga	81	4.55	0.72	1280	1.67	0.75	52.66	$2.52 \cdot 10^6$

* IS1L - addition of 1% lactose; IS2L - addition of 2% lactose; IS3L - addition of 3% lactose; IS4L - addition of 4% lactose; IS1G - addition of 1% glucose; IS2G - addition of 2% glucose; IS3G - addition of 3% glucose; IS4G - addition of 4% glucose; IS1F - addition of 1% fructose; IS2F - addition of 2% fructose; IS3F - addition of 3% fructose; IS4F - addition of 4% fructose; IS1Ga - addition of 1% galactose; IS2Ga - addition of 2% galactose; IS3Ga - addition of 3% galactose; IS4Ga - addition of 4% galactose

Table 4. Physico-chemical and microbiological characteristics of yogurt samples obtained from low-fat milk with mixed addition of skimmed milk powder and lactose

Sample*	Lactic acid, %	Viscosity, mPa·s	Syneresis, %	
			12 h	36 h
IS1L1P	0.84	2120	2.32	2.30
IS2L1P	0.80	2150	2.03	1.96
IS3L1P	0.79	2100	1.70	1.65
IS1L2P	0.88	2620	2.49	2.40
IS2L2P	0.87	2710	0.61	0
IS3L2P	0.82	2700	1.50	1.41
IS1L3P	0.93	2930	2.01	1.98
IS2L3P	0.91	2750	1.01	0.97
IS3L3P	0.90	2900	0.3	0

* IS1L1P - addition of 1% lactose and 1% skimmed milk powder; IS2L1P - addition of 2% lactose and 1% skimmed milk powder; IS3L1P - addition of 3% lactose and 1% skimmed milk powder; IS1L2P - addition of 1% lactose and 2% skimmed milk powder; IS2L2P - addition of 2% lactose and 2% skimmed milk powder; IS3L2P - addition of 3% lactose and 2% skimmed milk powder; IS1L3P - addition of 1% lactose and 3% skimmed milk powder; IS2L3P - addition of 2% lactose and 3% skimmed milk powder; IS3L3P - addition of 3% lactose and 3% skimmed milk powder

As a consequence of combined supplementation of milk with different amounts of skimmed milk powder and lactose, the samples IS3L3P and IS2L2P recorded the smallest amounts of separate whey (0.61% and 0.3%) after 12 hours of storage at

4°C. After 36 hours of storage, the entire quantity of whey has retracted into the gel network.

Therefore, IS2L2P (with the addition of 2% lactose and 2% skimmed milk powder) and IS3L3P (with the addition of 3% lactose and 3% skimmed milk powder) samples were considered optimal for obtaining a desired quality for yogurt (no effect of syneresis). These variants were analyzed in terms of physico-chemical and microbiological characteristics (Table 5).

Table 5. Physico-chemical and microbiological characteristics of yogurt samples obtained from low-fat milk with addition of skimmed milk powder and lactose

Sample	*p*H	Lactic acid, %	Viscosity, mPa·s	Syneresis, %			EPS mg/l	Lactic bacteria CFU/ml
				12 h	36 h	84 h		
IS2L2P	4.62	0.87	2710	0.61	0	0	63.51	$1.3 \cdot 10^6$
IS3L3P	4.64	0.90	2900	0.30	0	0	68.89	$1.6 \cdot 10^6$

Based on the obtained results we can appreciate that the yogurt samples made from milk with 0.1% fat content and supplemented with 2% or 3% skimmed milk powder and lactose had adequate quality characteristics (*p*H, acidity), were stable over time, had a high level of EPS and an acceptable number of colony forming units of lactic acid bacteria.

The influence of milk supplementation with skimmed milk powder and lactose on the production of EPS was examined using one-way ANOVA method. It was intended to determine if "addition factor" influences the EPS production, by checking statistical hypothesis of homogeneity of EPS concentration, obtained in experiments. In all analyzed cases the F-statistic was higher than F-critical. Null hypothesis was rejected and it was concluded, with a probability of 95% that the "addition factor" significantly influenced the EPS production.

Conclusions

High fat content (3.5% and 1.5%) from milk positively affects yogurt texture, obtained with EPS-producing lactic acid bacteria. In either case, the quantity of synthesized EPS was about 71 mg EPS/l. Due to the relatively high fat content, the yogurt samples formed an elastic gel network, without showing syneresis phenomenon.

The addition of skimmed milk powder to the low-fat milk (0.1%) caused a deterioration of the yogurt texture, due to the protein-protein links formed during yogurt fermentation. The formed gel matrix had narrow capillaries, expelling whey at the surface.

Concerning the tests designed to study the influence of sugars addition, our results indicated that lactose was preferred by lactic acid bacteria, allowing to obtain yogurt with high amounts of EPS.

The combined addition of milk powder (2 or 3%) and lactose (2 or 3%) allowed obtaining yogurts with the lowest syneresis phenomenon.

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