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FAT CONTENT IN YOGHURTS VERSUS NON-FAT FORTIFYING–A RHEOLOGICAL AND SENSORIAL APPROACH

INA VASILEAN[a], IULIANA APRODU[a], LIVIA PATRASCU[a*]

ABSTRACT. This study investigated the effect of fat content (3.5; 1.5 and 0.1 % w/w), together with total solids content on selected rheological and sensorial characteristics of yoghurt samples fermented with exopolysaccharides producing lactic acid bacteria (*Streptococcus termophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus*). Rheological flow and oscillatory tests showed that all tested yoghurt samples behave like thixotropic fluids. Pronounced hysteresis areas were obtained for skimmed yoghurts with added solids (skim milk powder and lactose, 2 and 3% w/v). Higher levels of fat favored the flow properties of the yogurt samples, enabling the formation of more stable viscoelastic gel networks. Sensorial characteristics were highly appreciated for samples with higher fat content.

Keywords: *yoghurt, exopolysaccharides, fat content, fortification, flow, viscoelastic gel*

INTRODUCTION

Fermented milk products are very popular all over the world[1], mainly because of their sensorial characteristics but also due to their potential in maintaining and even improving consumers` health [2]. The most widely used lactic acid bacteria for yogurt fermentation are *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus*, which are responsible for the development of specific taste and consistency.

The structure, microstructure and rheological characteristics of the yogurts highly influence the perception of the product during consumption [3]. It is well known that many technological parameters and starter culture can modulate textural properties of the yoghurts [4]. The knowledge of the yoghurt rheological properties can offer essential information about products texture

[a] *Universitatea Dunarea de Jos, Facultatea de Stiinta si Ingineria Alimentelor, Str. Domneasca, Nr. 111, RO-800201 Galati, Romania.*
[*] *Corresponding author: livia.mantoc@ugal.ro*

and gel stability. Skriver et al. [5] investigated both rheological and sensorial characteristics of stirred yogurts showing that gel firmness can be correlated with viscoelastic properties (G′, G″) while palatability was closely related to the shear stress. It is well known that textural and rheological characteristics of fermented lactic products with low fat and high protein content differ from those with no added solids, milk supplementation strongly influencing the texture of fermented products [6]. In order to improve rheological properties of skimmed dairy products different methods for increasing total solids content are approached. These compositional modifications are required in order to overcome problems associated with weak texture gels and whey separation [7].

Yoghurt bacteria can form a polysaccharide covering lair named glycocalyx. These macromolecular compounds can be partially released during fermentation process being generically named exopolysaccharides (EPS). The EPS producing starter bacteria ensure a stable yoghurt structure, a more consistent texture and higher viscosity by preventing gel rupture and whey releasing [8, 9, 10, 11,12]. The presence of EPS in lactic fermented milk products was found to improve rheological properties due to their thickening capacity, texture and products taste [13]. The EPS also have excellent water binding properties [14]. It was shown that yoghurt viscosity depends on EPS concentration and their specific volume [11]. However, Amatayakul et al. [15] concluded that using EPS-producing lactic acid bacteria could not compensate the reduction of total solids of yoghurt and these starter cultures are not efficient in improving the firmness of the low solid content products.

The aim of the present study was first to obtain yoghurt samples with different fat contents through fermentation with a pure culture of EPS producing lactic acid bacteria, and to characterize the obtained products in terms of rheological and sensory properties. Moreover, the influence of skim milk powder and lactose addition on the properties of skimmed yogurt was tested.

RESULTS AND DISCUSSION

Quality of the set yogurt obtained by fermenting milk with different fat contents (3.5, 1.5 and 0.1%) with EPS producing lactic acid bacteria was estimated by testing rheological behavior and sensory properties of the samples.

Rheological analyses. Two different types of tests were performed for rheological characterization of the samples: controlled shear-rate measurements by progressively increasing and decreasing the shear rate to check the viscosity dependence by the shear rate and small deformation oscillatory rheological test to gain insight into the structural particularities of the samples.

The flow parameters and hysteresis loop area obtained when performing shear thinning tests on the yoghurts with different fat contents are presented in Table 1.

Table 1. Rheological flow parameters for yoghurt samples with different fat and total solids content

Sample	Yield stress, Pa	Hysteresis area, Pa/s	Rate index		Viscosity, Pa*s	
			up curve	down curve	up curve	down curve
FY	1.907±0.01	18.1±0.35	0.350±0.01	0.409±0.01	1.017±0.03	0.778±0.02
NY	0.348±0.01	4.5±0.01	0.644±0.01	0.679±0.00	0.107±0.01	0.091±0.01
SY	1.632±0.01	30.4±0.21	0.324±0.02	0.445±0.01	0.907±0.01	0.516±0.01
SY_1	3.210±0.3	70.0±0.74	0.263±0.01	0.384±0.1	2.007±0.2	1.107±0.1
SY_2	3.344±0.3	71.2±0.77	0.260±0.00	0.374±0.01	2.098±0.12	1.198±0.03

Values represent means of three replicates ± SD. FY - Yoghurt with high fat content (3.5% fat w/w); NY – Partially skimmed yoghurt (1.5% fat w/w); SY – Skimmed yoghurt (0.1% fat w/w); SY_1 – Skimmed yoghurt enriched with 2% skim milk powder and 2% lactose (w/v); SY_2 - Skimmed yoghurt enriched with 3% skim milk powder and 3% lactose (w/v).

The flow behavior of the yogurt samples is represented in Figures 1 and 2. In order to improve appearance and quality of low fat yoghurts we proceeded to the fortification with skim milk powder (2 and 3% w/v) and lactose (2 and 3% w/v). Rheological characteristics of skimmed yoghurt were compared with those of the fortified products (Figure 2). Flow test revealed the thixotropic behavior for tested yoghurt samples as indicated by rate index values (Table 1). The shear thinning characteristic can be also seen in viscosity curves (decreasing viscosity when increasing shear rate) (Figures 1 and 2).

Regarding hysteresis phenomenon one can see that yoghurt with normalized fat content to 1.5% (w/w) recorded the lowest value, indicating that when speaking of yoghurts, high fat content does not necessary ensure flow stability. Moreover, samples with high fat contents (3.5% w/w) requested a rather high yield stress value (calculated with Bingham equation) in order to flow, compared to the samples with 1.5% and 0.1% fat contents and no added foreign solids. Adding solids to the skimmed yoghurt samples resulted in higher values for the yield stress, with no significant differences ($p>0.05$) between SY_1 and SY_2 sample (Table 1). The yoghurt samples supplemented with 2% of lactose and skim milk powder (SY_1) presented higher values of the hysteresis area compared to the un-fortified skimmed yogurt. Further increase of the solid content (SY_2 sample) did not led to significant differences ($p>0.05$) in terms of hysteresis area.

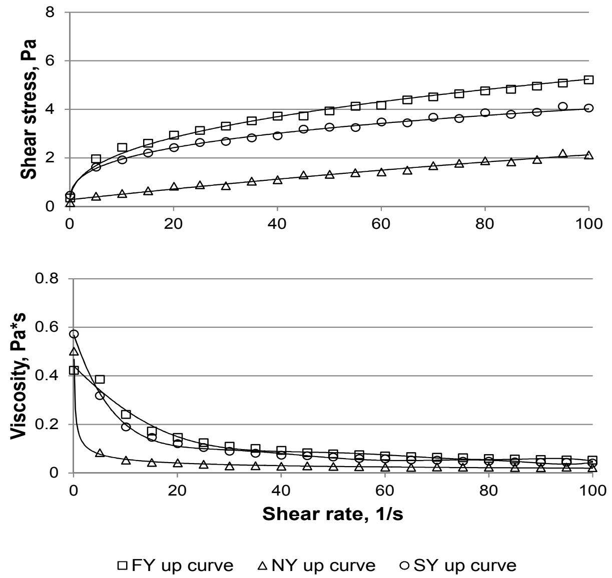

Figure 1. Flow behaviour of yoghurt samples with different fat contents. FY - Yoghurt with high fat content (3.5% fat w/w); NY – Partially skimmed yoghurt (1.5% fat w/w); SY – Skimmed yoghurt sample (0.1% fat w/w);

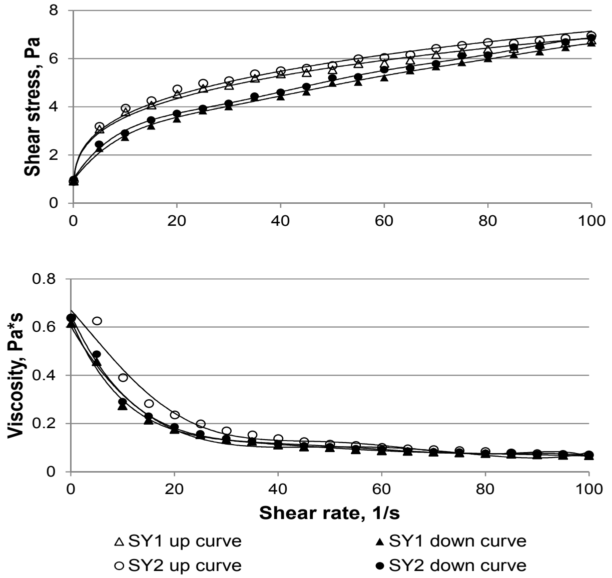

Figure 2. Flow behaviour of the skimmed and fortified yoghurt samples. SY_1 – Skimmed yoghurt sample enriched with 2% skim milk powder and 2% lactose (w/v); SY_2 - Skimmed yoghurt sample enriched with 3% skim milk powder and 3% lactose (w/v)

Protein addition to the skimmed milk samples (SY_1 and SY_2) resulted in higher viscosity and shear stress values compared to the skimmed yoghurt with no added solids (SY) (Figure 1 and 2). Therefore, protein addition might be considered appropriate for improving yogurt structure by favoring a thicker gel network formation. These findings are in agreement with Bhullar et al. [16], who stated that supplementation of the yogurt samples with 2% (w/v) of whey protein concentrate led to increased viscosity and reduced syneresis. The lack of fat in yoghurt structure could affect EPS interaction with product components, resulting in higher hysteresis areas during flow (Figure 2). This phenomenon can be explained by the presence of higher number of protein-protein links, which result in a denser gel network and fewer protein-water links [17, 18, 15].

EPS presence into the samples highly influences the viscosity and other rheological properties of the yogurts. Patel *et al*. [19] stated that EPS exhibit remarkable thickening and shear-thinning properties, and display high intrinsic viscosities. Broadbent et al. [20] found that the amount of EPS produced in lactic fermented milk products is influenced by the environment composition (C:N ratio) and growing conditions. In our case the amount of synthesized EPS, reported by Vasilean et al. [21] when using the same yogurt preparation procedure, was relatively close in case of the samples obtained from whole fat and partially skimmed milk (71.46 and 71.07 mg/l respectively), and lower in case of the yogurt obtained from skimmed milk (63.65 mg/l). The addition of protein and lactose to the skimmed milk before fermentation did not favor the EPS amount from yoghurt samples (63.51 mg/l for SY_1 sample and 68.89 mg/l for SY_2). Thus the viscosity increment in fortified samples could not be necessarily attributed to EPS presence.

Analyzing oscillatory rheology results presented in Figure 3, the soft solid structure can be observed for all yoghurt samples. For the entire range of tested frequency, G’ values overcame the G``, indicating that yoghurt samples behaved like elastoviscous fluids. The yoghurt sample with high fat content (FY) presented the highest value of elastic modulus. In agreement with the flow test observations, the SY sample presented a more elastic (solid like behaviour) structure with respect to NY sample. We appreciate that this phenomenon could be explained by different molecule distribution within the gel network and by the different bond type participating to network formation and stabilization. As indicated by Van Vliet et al. [22] the rheological properties of yoghurts are influenced by the strength of protein-protein interactions, the number of bonds per cross-section of the strand, relaxation times for the network bonds, and the orientation of the strands within the matrix. Thus presence of higher amount of protein-fat-protein links alowed higher fluidity in comparison to the samples where the protein-protein interactions are predominant.

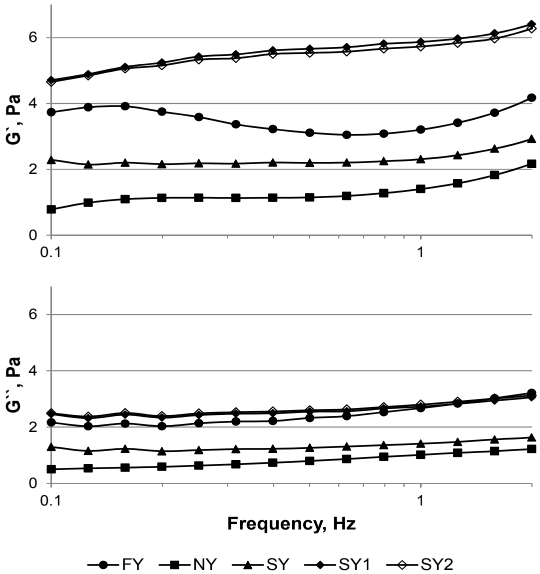

Figure 3. The influence of total fat content and milk fortification on viscoelastic moduli of set yoghurt samples. FY - Yoghurt with high fat content (3.5% fat w/w); NY – Partially skimmed yoghurt (1.5% fat w/w); SY – Skimmed yoghurt (0.1% fat w/w); SY_1 – Skimmed yoghurt enriched with 2% skim milk powder and 2% lactose (w/v); SY_2 - Skimmed yoghurt enriched with 3% skim milk powder and 3% lactose (w/v).

Addition of 2% skim milk powder together with 2% lactose to the skimmed yoghurt samples determined an increase of the gel network density. The fortification most probably led to the reduction of pores dimensions thus causing the increase of viscoelastic moduli values. There observations are in agreement with the literature [23, 24, 5, 4, 25]. Lee and Lucey [26] stated that the physical and sensory properties of yogurt gels are greatly influenced by the total solids content of the yogurt milk, especially the protein content, the G′ values of yogurt increasing with the increase of the total solids content. However, further increase of the total solids quantity in case of SY2 sample did not led to significant variation of G` and G`` values.

Sensorial analysis. The results of sensorial analysis of the yoghurt samples are represented in figure 4. As one can see fat content together with product fortification significantly influenced products sensorial characteristics ($p<0.01$).

The color of the samples with higher fat contents (3.5% and 1.5%) was better appreciated by the assessors probably because of their higher luminosity, and no significant differences between these two samples ($p>0.05$) was obtained. Concerning the samples with low fat content, regardless of the enrichment with skim milk powder and lactose, the color was medium appreciated (Figure 4). The non-fat solid addition to the skimmed milk before fermentation with EPS producing lactic acid bacteria had no significant influence on the color attribute (p>0.05).

Panelists found no odor for the yoghurt samples with 3.5 and 1.5% fat (FY and NY), with no significant differences between the two samples (p>0.05). On the other hand, some unspecific smell was identified in case of the skimmed yogurt samples, regardless of non-fat solids supplementation (p>0.05).

The same trend was observed in case of the syneresis attribute. No syneresis phenomenon was observed for FY and NY samples (p>0.05). The highest amount of separated whey was obtained in case of skimmed yoghurt sample (SY), and the addition of skim milk powder and lactose resulted in the decrease of released whey, with significant differences between SY2 and SY or SY1 samples.

Regarding firmness, the most appreciated sample was FY, while the lowest score was obtained for SY sample. The firmness values (p<0.01) varied significantly with the yogurt sample, except for NY and SY2 samples when no differences were registered (p>0.05). The addition of higher amounts (3% w/v) of skim milk powder and lactose to the skimmed yoghurts determined the perception of firmness attribute similar to yoghurts with medium fat content. The same trend was observed in case of consistency.

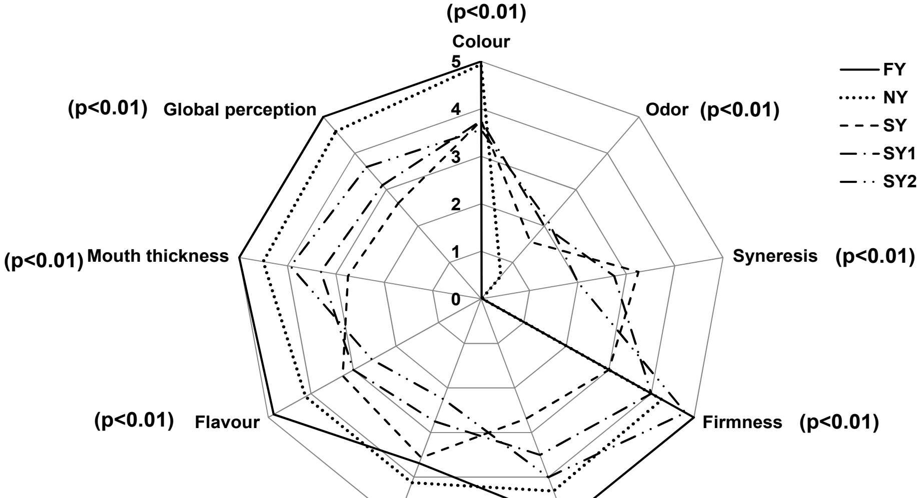

Figure 4. Sensorial characteristics of set yoghurt types fermented with EPSs releasing starter culture, as a function of fat and total solids content. FY - Yoghurt with high fat content (3.5% fat w/w); NY – Partially skimmed yoghurt (1.5% fat w/w); SY – Skimmed yoghurt (0.1% fat w/w); SY_1 – Skimmed yoghurt enriched with 2% skim milk powder and 2% lactose (w/v); SY_2 - Skimmed yoghurt enriched with 3% skim milk powder and 3% lactose (w/v).

The sourish taste of the yogurt was masked by the fat content and lactose addition, being perceived with similar intensity for FY, NY and SY samples ($p>0.05$). The SY_1 with SY_2 samples registered the lowest scores with no significant differences between values ($p>0.05$), but with significant differences with respect to FY, NY and SY samples ($p<0.01$).

The flavour of the fat containing yogurt samples (FY and NY) was better appreciated, whereas the addition of lactose and skim milk powder had no contribution to flavour attribute.

Regarding mouth thickness, the skimmed yoghurt got the lowest appreciation scores. The addition of non-fat solids to the skimmed milk before lactic fermentation led, to some extent, to the improvement of the yogurt thickness.

When considering the global perception, the yogurt samples with fat in composition (FY and NY) the most appreciated by the panelists, while with the skimmed yoghurt sample got the lowest scores (Figure 4). The same conclusion was drawn based on the real score values estimated by taking into account the importance coefficient of every attribute as seen in Figure 5.

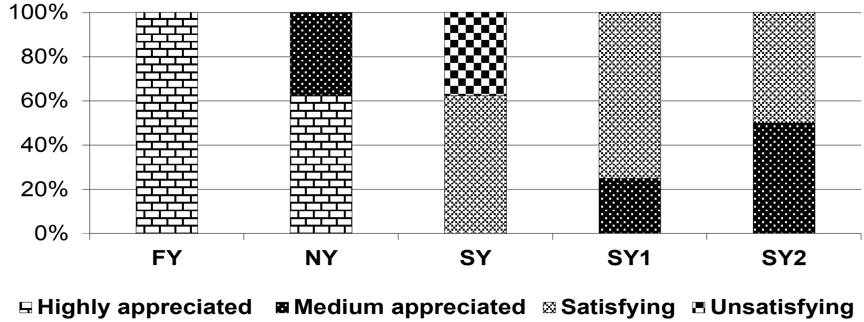

Figure 5. Global perception of yoghurt samples as indicated by importance coefficient of every assessed sensory attribute.

CONCLUSIONS

Yogurt samples with different fat contents were obtained through fermentation with EPS producing lactic acid bacteria. Rheological tests showed that all yoghurt samples behave like viscoelastic gels. We appreciate that the interactions between EPS released by lactic acid bacteria and other macromolecular components, such as proteins, allowed formation of viscoelastic network with proper flow characteristics. Better rheological properties were obtained in case of the yogurts with high fat levels. When considering the skimmed yoghurt, increasing the total solids level proved to be an appropriate method of improving the rheological characteristics of the gel. This observation is in good agreement with sensorial appreciation showing that increasing the non-fat solid content of yogurts support denser and firmer gel structure formation. However, sensorial characteristics were better appreciated in case of the samples with 3.5% and 1.5% fat with respect to the skimmed yogurts.

EXPERIMENTAL SECTION

Yoghurt preparation. Commercial UHT treated milk with different fat contents was used for yogurt preparation: whole milk with 3.5% (w/w) fat and 11.82% (w/w) total solids; partially skim milk with 1.5% (w/w) fat and 10.67 % (w/w) total solids and skim milk with 0.1% (w/w) fat and 9.02% (w/w) total solids.

In order to increase the total solids content, the skim milk was fortified with skim milk powder (2 and 3% w/w) and lactose (2 and 3% w/w). The addition of lactose to the milk is favorable for lactic acid bacteria growth [21]. Set yoghurt was produced using mixed culture of thermophilic lactic acid bacteria containing *Streptococcus termophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus* (commercial name YF-L 812, Chr. Hansen). This lactic acid bacteria mixture was shown previously to produce EPS in yoghurt samples [21].

The milk was first tempered at 45°C and afterwards was inoculated with the starter culture according to producer specifications. The inoculated milk was immediately poured in plastic containers and was incubated at 43°C until pH 4.6 was reached. Yoghurt samples were subsequently cooled and were stored in refrigeration at 4°C before analysis. All samples were obtained in triplicate.

Rheological analyses. The rheological behaviour of the yogurt samples was determined after 24h of storage 4°C with an AR2000ex rheometer (TA Instruments, Ltd). Analyses were performed using a cone - plate geometry with 40 mm in diameter and 2^{o} cone angle, and a gap of 1000 μm. Prior to analyses yoghurt samples were stirred in order to homogenize composition with released whey if any.

In order to observe flow behaviour of tested samples, a stepped flow step was applied and shear rate was increased from 0.1 to 100 s^{-1}, and then decreased back to 0.1 s^{-1}. Temperature was set to 20 ^{o}C. Tests were performed in triplicate and mean values were represented in graphs.

The viscoelastic domain was determined by performing strain sweep test and a critical strain of 5% was estimated. The frequency sweep tests were further performed at a controlled strain of 0.5% and the elastic modulus (*G'*) and viscous modulus (*G"*) were monitored while increasing the oscillation frequency from 0.1 to 2 Hz.

The rheology data were analyzed using TA Rheology Advantage Data Analysis Software V 4.8.3 and the mathematical models of Power Low and Bingham were used to fit the experimental results.

Sensory evaluation. The sensory analysis of the yoghurt was performed after 24 hours of storage at 4^{o}C by a panel consisting on twenty trained assessors, equally distributed by gender, aged between 20 to 30 years in proportion of 70 % the other 30 % were aged between 30 to 40 years old. The samples (~100 mL of yoghurt) were purred in plastic cups and tempered at 10÷12 ^{o}C before testing.

The ten attributes assessed are listed and defined in Table 2. Products quality was appreciated by a 5 points system. The Importance Coefficients (Table 2) for each attribute was decided based on the relevance to the global quality of the yogurt. The real/final sensory score was estimated by multiplying the score of each attribute by its importance coefficient [27]. The importance coefficient is established individually, depending on the research aim so that the most significant characteristics for the study could be followed.

Table 2. Attributes used by the sensory panel for describing the sensory properties of yoghurt samples

Attribute	*Definition*	*Importance coefficient*
Odor	Identification of characteristic smell or any flavour defects as unspecific, foreign or masked odor	0.05
Flavour	General evaluation of flavour and identification of any defects	0.05
Colour	Colour evaluation (white, whitish, yellow or yellowish)	0.1
Syneresis	Visual observation of separated whey on the surface of the set yoghurt	0.15
Gel firmness	Estimation of gel fragility, hardness, gelatinous or gumminess structure	0.15
Consistency	Evaluation of gel viscosity by stirring the yoghurt sample with a spoon	0.15
Sourish taste	Evaluation of the sour taste intensity	0.05
Sweetness	Evaluation of the sweet taste intensity	0.05
Mouth thickness	Perceived as the degree of thickness when swallowing the yogurt at normal-high eating rate	0.1
Global perception	Global appreciation of the product considering the above mentioned attributes	0.15

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