

## QUALITY OF YOGURT FORTIFIED WITH MAGNESIUM LACTATE

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### ABSTRACT

**Background.** Magnesium is a micronutrient which plays an important role in a wide range of fundamental cellular reactions. Deficiency of magnesium leads to serious biochemical and symptomatic changes. The present study was carried out to establish the influence of magnesium lactate fortification on the physicochemical, microbiological and rheological properties of fat-free yogurt manufactured using different starters.

**Material and methods.** In this study, yogurts were produced from fat-free milk, standardized with skimmed milk powder to 6% protein content, and then divided into two parts. One part was left without supplementation as a control and in the second part, magnesium L-lactate hydrate was added in the amount of 317.30 mg 100 g<sup>-1</sup>, which was equal to 35 mg of Mg<sup>2+</sup> 100 g<sup>-1</sup> of milk. Both mixtures were blended, pasteurized at 85°C for 30 minutes, cooled to 45°C and then divided into three parts, inoculated with: (1) YC-X11 yogurt culture, (2) YF-L811 yogurt culture and (3) VITAL yogurt culture supplemented with probiotics (*Lactobacillus acidophilus*, *Bifidobacterium lactis*) respectively. Fermentation was performed at 43°C and the final yogurts were cooled to 5°C. After 24 hours of cold storage, the pH values, titratable acidity, syneresis, color, texture profile, viscosity, sensory analysis and microbiology of the yogurts were analyzed.

**Results.** The results showed that addition of magnesium lactate significantly reduced syneresis and increased the hardness of fat-free yogurts. There was no impact on the viability of starter bacteria in the yogurts after 24 hours of refrigerated storage.

**Conclusions.** Magnesium lactate showed good potential for the fortification of dairy foods, according to physicochemical data. Further research is needed regarding the influence of storage time and to establish whether the observed effects are largely due to the magnesium cation or lactate anion.

**Keywords:** minerals, magnesium, yogurt, quality, starter culture

### INTRODUCTION

Functional dairy products with health-promoting properties are becoming more fashionable and are desired by consumers around the world (Gera et al.,

2012). With our current eating habits, the demands of the human body for minerals are not fully met, and the significant deficiencies of magnesium and calcium

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in the average diet require supplementation. Magnesium is a micronutrient which plays an important role in a wide range of fundamental cellular reactions. Relatively little research has been carried out regarding the magnesium supplementation of dairy products, and the available literature is limited. Only a few papers have reported the effects of magnesium salts on the properties of milk products (Cueva and Aryana, 2008; Szajnar et al., 2017; Znamirowska et al., 2016). Among all fermented milk products, yogurt is the most frequently consumed. In order to meet the requirements of potential buyers, manufacturers tend to increase the range of yogurts available. In particular, yogurts with functional properties (i.e. rich in minerals) and low fat content are popular amongst consumers (Khurana and Kanawjia, 2007). The magnesium content of yogurts is estimated to be 11–19 mg 100 g<sup>-1</sup> (The Dairy Council, 2016). Thickening milk with skimmed milk powder and fortification with magnesium during yogurt production could increase the amount of magnesium content to above 56 mg/100 g, which is a significant amount (i.e. 15% of the nutrient reference values of recommended daily intake; Annex XIII Regulation no 1169/2011). Moreover, the addition of magnesium cannot reduce the thermal stability of milk, especially when fortification is carried out before pasteurization. The impact of pasteurization on the mineral content of milk and mineral bioavailability should also be taken into consideration. Studies indicate that organic mineral salts such as citrates, lactates or gluconates outperform inorganic mineral sources in terms of their relative bioavailability. Organic mineral salts are also highly soluble, but their use in food is limited due to “off” tastes at higher concentrations (Gerhart and Schottenheimer, 2013). The quantity of minerals in yogurt remains the same as in milk, the only change is that, due to the reduction in pH during fermentation, these minerals are in the ionic rather than the colloidal form (Sfakianakis and Tzia, 2014). Reducing the fat content of milk products influences the taste and texture, which is why one of the most important factors influencing the qualitative properties of fat-free yogurts is an appropriate choice of starter culture. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* are the main species used in yogurt starter cultures. Unfortunately, some of the species used for yogurt production do not produce

exopolysaccharide (EPS), or produce only low yields of EPS, which may affect the final product quality (Han et al., 2016). Nowadays, culture laboratories produce a wide range of customized yogurt cultures. The type of starter and its composition may alter the physical characteristics of the final product, affecting its texture and appearance. Some cultures will give the final yogurt different mouth thickness and gel firmness, others influence fermentation time and post-fermentation acidification (Domagała and Wszolek, 2008). One should bear in mind the fact that supplementation with Mg<sup>2+</sup> in the body should be accompanied simultaneously by the delivery of calcium. Fermented dairy products fortified with magnesium compounds such as magnesium lactate may constitute the source of both elements.

The aim of the study was to determine the effect of magnesium fortification in the form of magnesium L-lactate hydrate on the acidity, texture profile and rheological and microbiological properties of fat-free yogurts fermented by three different starter cultures.

## MATERIAL AND METHODS

### Yogurt manufacture

UHT fat-free milk (Mlekpól Dairy, Poland) was preheated to 65 °C and standardized with skimmed milk powder (Gostyń Dairy, Poland) to 6% protein content, then divided into two parts. One part was left without supplementation as a control, and magnesium L-lactate hydrate – C<sub>6</sub>H<sub>10</sub>MgO<sub>6</sub> · H<sub>2</sub>O (Sigma-Aldrich, Spain) was added to the second part in the amount of 317.30 mg 100 g<sup>-1</sup>, which is equal to 35 mg of Mg<sup>2+</sup>·100 g<sup>-1</sup> of milk. Both mixtures were blended using a hand blender, pasteurized at 85°C for 30 minutes, cooled to 45°C and then divided into three parts inoculated with: (1) YC-X11 yogurt culture (Chr. Hansen, Denmark), (2) YF-L811 yogurt culture (Chr. Hansen, Denmark) and (3) VITAL yogurt culture supplemented with probiotics (*Lactobacillus acidophilus*, *Bifidobacterium lactis*) (Danisco-DuPont, Poland), in accordance with the planned experiment: control inoculated with starter culture YC-X11, fortified with magnesium and inoculated with starter culture YC-X11, control inoculated with starter culture YF-L811, fortified with magnesium and inoculated with starter culture YF-L811, control inoculated with

starter culture VITAL, fortified with magnesium and inoculated with starter culture VITAL. The prepared mixtures were poured into sterile 100 mL containers and coded. The fermentation was performed at 43°C, until the pH in control samples reached 4.6. The final yogurts were cooled to 5°C (Cooled Incubator ILW 115, POL-EKO Aparatura, Poland).

All of the reagents used in the present work were of analytical grade. The experiment was repeated three times. The yogurts were evaluated after 24 hours of refrigerated storage. Five samples of yogurt from each group were evaluated.

### Physicochemical analysis of yogurt

The pH values of the samples were measured using a FiveEasy pH-meter (Mettler Toledo, Switzerland). The titratable acidity (TA) of the milk was determined according to the Soxhlet-Henkel method (Budślawski, 1973) and was calculated as a percentage of lactic acid (% LA). Syneresis (whey separation) was determined according to the method described by Delikanli and Ozcan (2014), with modifications. Color measurements were performed using the CIELAB space with a portable colorimeter Chroma Meter CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan). The data was expressed in terms of  $L^*$ ,  $a^*$  and  $b^*$  parameters, where  $L^*$  represents lightness (from 0 – black to 100 – white);  $a^*$  and  $-a^*$  redness and greenness respectively; and  $b^*$  and  $-b^*$  yellowness and blueness respectively. Before testing, the instrument was calibrated on a White Calibration Plate CR-A43.

### Texture profile analysis

Texture was determined with the CT3 Texture Analyzer with Texture Pro CT software (Brookfield AMETEK, USA). A TPA (Cycle Count 2) test was performed on a 100 mL sample of solid state yogurt, without mixing in the container, with the following settings: sample – cylinder 66.00 mm × 33.86 mm, trigger Load 0.1 N, test Speed 1 mm s<sup>-1</sup>, table TA-BTKIT, probe TA3/100 (acrylic cylinder – 35 mm diameter).

### Rheological measurements

Viscosity was determined using the material testing machine Zwick/Roell ProLine Z010 (Zwick Roell AG, Ulm, Germany) with a back extrusion rig. The measuring system consisted of a back extrusion cell

(50 mm diameter and 65 mm height) and a piston (40 mm diameter). About 90 mL of yogurt sample was carefully placed into the measurement cell and the test was carried out at a constant temperature of 6°C, with the use of a temperature chamber, and was repeated five times for each experimental group, at five shear rates (1.14, 2.28, 4.56, 9.11 and 18.22 s<sup>-1</sup>). The mean viscosity  $\eta$  (Pa·s) was calculated using the testXpert II program, which was developed specifically for viscosity testing (Kędzierska-Matysek et al., 2016). The difference in initial apparent viscosity ( $\eta_i$ ) and final apparent viscosity ( $\eta_f$ ) was calculated as % lost structure ( $L_s$ ) using the following formula:

$$L_s = \frac{n_i - n_f}{n_i} 100, \%$$

The % lost structure is a measure of the rate of thixotropic breakdown, whereas the ratio of the initial to final apparent viscosity, ( $\eta_i / \eta_f$ ), may be considered as a relative measure of the extent of thixotropy. Courses of the flow curves were described by the Ostwald-de Waele model (power law equation; Singh and Muthukumarappan, 2008).

### Microbiological analysis

Determination of the number of characteristic microorganisms was carried out by the plate method. Viable counts of *S. thermophilus* were determined on an M17 medium (Biocorp, Poland), after aerobic incubation at 37°C for 72 h. Viable counts of *L. bulgaricus* were determined on MRS agar (Biocorp, Poland) acidified with acetic acid to pH 5.4 and incubated anaerobically at 37°C for 72 h (PN-ISO7889.2007). In yogurts inoculated with the VITAL starter the total numbers of *L. bulgaricus*, as well as *L. acidophilus* and *B. lactis*, were enumerated on MRS agar (Biocorp, Poland) and incubated at 37°C, anaerobically, for 72 h (Lima et al., 2009).

### Sensory analysis

The yogurt samples were evaluated by a trained panel, consisting of 15 people who had been familiarized with sensory analysis of yogurt. The panelists were served six samples at a time (in three-digit random number coded plastic cubs) and asked to rinse their mouths between samples with water. The panelists

evaluated the presence of sour and salty tastes, bitterness and off-flavors on a nine-point rating scale with edge markings (from 1 = not perceptible to 9 = extremely strong; PN-ISO11035.1999).

### Statistical analysis

The obtained results were given as the mean and standard deviation, calculated statistically using the Statistica v. 12.0 software (StatSoft, USA). One and two way analyses of variance were performed to evaluate the influence of magnesium supplementation and type of starter culture on the properties of fermented milks. The significance of differences between the averages was estimated with Tukey's test ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

### Physicochemical properties of yogurt

Table 1 shows the pH, acidity, syneresis and values of color parameters of non-fat yogurts fermented by different starter cultures, with or without the addition of magnesium. The lowest pH and significantly higher acidity characterized yogurt C (YF-L811) which was not enriched with magnesium. Yogurt samples without magnesium fermented with YC-X11 and VITAL cultures (samples A and E) were characterized by similar pH values and titratable acidities (TA). Fortification with magnesium resulted in a decrease in TA and a significant increase in the pH of yogurt, regardless

of the type of culture used in fermentation. Both factors, i.e. starter culture and magnesium fortification, affected the overall level of acidity and pH of yogurt samples, as indicated by ANOVA ( $P < 0.05$ ). In all yogurts *L. bulgaricus* was the main bacterium responsible for acid production. Most probiotic bacteria have no significant effect on the fermentation process or the sensory properties of the yogurt (Sfakianakis and Tzia, 2014). Han et al. (2012) reported that all fermented milk samples with added  $\text{Cu}^{2+}$  showed pH levels higher than the control sample. Also, changes in fermentation time and pH during fermentation were influenced by the concentration of  $\text{Cu}^{2+}$ . They observed that, in milk fermented by *S. thermophilus*, when the concentration of  $\text{Cu}^{2+}$  increased, the pH decrease was slowed down, which indicated that a higher concentration of  $\text{Cu}^{2+}$  resulted in a longer fermentation time. Znamirowska et al. (2015) also reported that goat's yogurts enriched with 5, 10, 15, 20 mg  $\text{Mg}^{2+}$  100  $\text{g}^{-1}$  were characterized throughout 21 days of storage by higher pH values, compared to a control without added magnesium D-gluconate.

Syneresis is one of the most important physical attributes of yogurt and a crucial factor in its sensorial quality. Syneresis produced by funnel drainage does not represent the usual breakage of the yogurt matrix, but reflects the capability of the whole gel structure to retain water (Bagci and Gunasekaran, 2016). In the performed experiment there were no significant

**Table 1.** The effect of starter cultures and magnesium lactate on the pH, titratable acidity (TA), syneresis and color parameters of yogurts after 24 hours of cold storage

Parameter	YC-X11		YF-L811		VITAL	
	A	B	C	D	E	F
pH	4.420 <sup>b</sup> ± 0.150	4.500 <sup>c</sup> ± 0.060	4.240 <sup>a</sup> ± 0.020	4.830 <sup>d</sup> ± 0.070	4.420 <sup>b</sup> ± 0.010	4.750 <sup>d</sup> ± 0.130
TA, LA %	1.090 <sup>b</sup> ± 0.060	0.910 <sup>a</sup> ± 0.04	1.340 <sup>c</sup> ± 0.060	1.070 <sup>b</sup> ± 0.040	1.110 <sup>b</sup> ± 0.050	1.060 <sup>b</sup> ± 0.060
Syneresis, %	20.26 <sup>b</sup> ± 3.710	16.78 <sup>a</sup> ± 2.430	23.42 <sup>b</sup> ± 1.820	19.08 <sup>ab</sup> ± 3.260	22.53 <sup>b</sup> ± 2.270	14.00 <sup>a</sup> ± 1.730
<i>L</i> *	96.70 <sup>bc</sup> ± 0.510	96.26 <sup>ab</sup> ± 0.020	97.01 <sup>c</sup> ± 0.060	98.53 <sup>d</sup> ± 0.410	96.07 <sup>a</sup> ± 0.030	96.31 <sup>ab</sup> ± 0.050
<i>a</i> *	-5.04 <sup>c</sup> ± 0.030	-5.14 <sup>d</sup> ± 0.080	-4.61 <sup>a</sup> ± 0.020	-4.69 <sup>a</sup> ± 0.060	-4.88 <sup>b</sup> ± 0.010	-5.08 <sup>cd</sup> ± 0.050
<i>b</i> *	12.23 <sup>b</sup> ± 0.100	11.87 <sup>a</sup> ± 0.080	12.99 <sup>c</sup> ± 0.050	13.24 <sup>c</sup> ± 0.200	12.53 <sup>d</sup> ± 0.010	12.15 <sup>b</sup> ± 0.130

*L*\* – lightness, 0 – black, 100 – white, - *a*\* greenness, *b*\* yellowness.

A, C, E – control samples, B, D, F – Mg fortified samples. Values are means ± S.D. for  $n = 15$ .

Mean values denoted with different letters within a row differ significantly ( $P < 0.05$ ).

differences in the amount of whey released in yogurt samples produced with different starter cultures ( $P < 0.05$ ). However, fortification with  $Mg^{2+}$  decreased syneresis. In yogurt D ( $Mg^{2+}$ , YF-L811), syneresis was lower by 4.3%, in the yogurt fermented by YC-X11 (B) by approx. 3.5%, and in yogurt F supplemented with potential probiotic cultures (VITAL) the amount of separated whey was reduced by as much as 8.5% compared to the control. That the occurrence of syneresis in yogurts is reduced by fortification with magnesium is important information for manufacturers of dairy products. In the previously published results (Szajnar et al., 2017), fortification of milk with magnesium compounds in the amount of  $30 \text{ mg } Mg^{2+} 100 \text{ g}^{-1}$  of milk caused a decrease in syneresis in yogurts with magnesium chloride, magnesium pidolate and magnesium gluconate compared to control yogurts, but the addition of magnesium bisglycinate and magnesium lactate hydrate did not impair syneresis. When the color parameters of yogurt were analyzed, there was significantly lower  $L^*$  value (whiteness) determined in the probiotic yogurt (E) compared to the yogurts A and C with classic yogurt cultures, depending on the type of starter cultures used for fermentation. Fortification with magnesium lactate significantly increased the  $L^*$  value only in yogurt D ( $Mg^{2+}$ , YF-L811;  $P < 0.05$ ). Different cultures used in yogurt manufacture significantly affected the  $a^*$  and  $b^*$  color characteristics of yogurt ( $P < 0.05$ ). All yogurt samples exhibited a negative  $a^*$  value, indicating greenness, and positive  $b^*$  values, indicating a yellowish color. Among the non-supplemented yogurts, sample A (YC-X11) showed the deepest greenness, followed by samples

E (VITAL) and C (YF-L811). Fortification with magnesium increased the greenness of yogurts. The effect of fortification with magnesium was found to be significant regarding the  $a^*$  value for the yogurts B and F when compared to the controls (A and E respectively;  $P < 0.05$ ). The highest  $b^*$  value was shown in sample C (YF-L811). An increase in the intensity of the yellow color was only found in fortified yogurt D (YF-L811) in comparison to the control C, while the opposite effect of fortification with magnesium was observed in yogurts B and F, for which a reduction of the intensity of the yellow color compared to the corresponding control samples A and E was found. Bagci and Gunasekaran (2016) showed significantly lower  $L^*$  values for yogurts fortified with  $FeSO_4$  compared to a control.

#### Texture of yogurts

Problems with texture are one of the main reasons for lower consumer acceptance of yogurts. The excessively loose texture caused by the thermostatic method and the too runny consistency and low viscosity of yogurt produced with the tank method are the most common defects in texture (Domagała and Wszolek, 2008). Table 2 presents the results of the texture analysis of fat-free yogurts. The hardness of the fat-free yogurts was significantly influenced by the type of starter culture used for fermentation. The lowest value of hardness was found in yogurt A. Fortification with magnesium led to a significant increase in hardness of the fat-free yogurt samples (B, D and F) in comparison to non-enriched yogurts ( $P < 0.05$ ). Furthermore, enriching yogurts with magnesium significantly reduced

**Table 2.** The effect of starter cultures and magnesium lactate on the textural properties of yogurts

Parameter	YC-X11		YF-L811		VITAL	
	A	B	C	D	E	F
Hardness, N	2.590 <sup>a</sup> ± 0.120	3.230 <sup>c</sup> ± 0.080	3.070 <sup>b</sup> ± 0.150	4.020 <sup>d</sup> ± 0.210	3.130 <sup>b</sup> ± 0.170	3.520 <sup>c</sup> ± 0.110
Adhesiveness, mJ	8.610 <sup>ab</sup> ± 0.910	7.840 <sup>a</sup> ± 0.370	11.03 <sup>b</sup> ± 1.300	11.00 <sup>b</sup> ± 2.920	10.45 <sup>ab</sup> ± 1.110	10.70 <sup>ab</sup> ± 1.150
Cohesiveness	0.410 <sup>b</sup> ± 0.020	0.370 <sup>a</sup> ± 0.000	0.410 <sup>b</sup> ± 0.020	0.380 <sup>a</sup> ± 0.010	0.410 <sup>b</sup> ± 0.010	0.400 <sup>b</sup> ± 0.020
Gumminess, N	1.040 <sup>a</sup> ± 0.030	1.200 <sup>b</sup> ± 0.030	1.280 <sup>bc</sup> ± 0.070	1.530 <sup>d</sup> ± 0.130	1.290 <sup>bc</sup> ± 0.070	1.410 <sup>d</sup> ± 0.030

A, C, E – control samples, B, D, F – Mg fortified samples. Values are means ± S.D. for  $n = 15$ . Mean values denoted with different letters within a row differ significantly ( $P < 0.05$ ).

cohesiveness and simultaneously increased gumminess only in samples B and D, compared to their control. Domagała and Wszolek (2008) found that the type of culture applied significantly affected the quality of fermented goat's milk products. In studies by these authors, the yogurts obtained with the use of the classic yogurt starter YC-180 were characterized by a more desirable texture and overall sensory quality, and slightly higher susceptibility to syneresis, than bio-yogurts.

### Rheological characteristics

Table 3 shows the effect of the type of starter culture and the addition of magnesium on all determined rheological parameters of the tested fat-free yogurts. The yogurts fermented by three different starters which were not fortified with magnesium were characterized by similar rheological parameters, as evidenced by the lack of significant differences between them in terms of the mean, initial and final viscosity, and the consistency coefficient,  $K$ . Only yogurt A (YC-X11) differed from the other two types, with a significantly lower ratio of the initial to final apparent viscosity and %  $L_s$  (a reduction of 82.88%), and a higher value of flow behavior index,  $n$  ( $P < 0.05$ ). The addition of magnesium resulted in a significant decrease in the mean, initial and final viscosity of yogurts B (YC-X11) and F (VITAL). Yogurt D ( $Mg^{2+}$ , YF-L811) showed the highest mean viscosity (35.88 Pa·s), initial apparent viscosity

(82.20 Pa·s) and %  $L_s$  (a reduction of 87.61%), and the differences were significant compared to other fortified yogurts (B, F). Power law index,  $n$  expresses the flow behavior as Newtonian when it is close to 1 or non-Newtonian when it is far from 1. Yogurts are typical examples of shear-thinning, non-Newtonian fluids. The flow behavior index of the analyzed yogurts showed significant differences depending on the starter cultures ( $P < 0.05$ ), and decreased in yogurts fortified with magnesium compared to the controls. The addition of magnesium caused a significant decrease in the viscosity ( $\eta$ ) and consistency coefficient ( $K$ ) of yogurts B and F compared to the controls. It can be assumed that these differences in the rheological parameters of yogurts are related to the different texture and consistency perceived by the consumer. In comparison, Singh and Muthukumarappan (2008) also showed a decrease in consistency and increase in flow behavior index in yogurt fortified with calcium citrate. The authors obtained lower values of apparent viscosity and %  $L_s$ , which could be the result of different solid content and manufacturing technology. They also found that the addition of calcium caused an increase in colloidal calcium phosphate (CCP) cross-linking between casein micelles, which resulted in comparatively higher water holding capacity (WHC) and final apparent viscosity, and comparatively less shear thinning when compared with a control fruit yogurt.

**Table 3.** Rheological parameters of yogurts

Parameter	YC-X11		YF-L811		VITAL	
	A	B	C	D	E	F
Mean viscosity $\eta$ , Pa·s	30.58 <sup>b</sup> ± 4.270	18.58 <sup>a</sup> ± 2.450	32.63 <sup>b</sup> ± 4.620	35.88 <sup>b</sup> ± 4.830	33.01 <sup>b</sup> ± 4.260	19.38 <sup>a</sup> ± 2.490
Initial viscosity $\eta_i$ , Pa·s	65.92 <sup>b</sup> ± 9.900	41.50 <sup>a</sup> ± 2.630	72.14 <sup>b</sup> ± 9.720	82.20 <sup>b</sup> ± 0.960	72.87 <sup>b</sup> ± 4.870	43.20 <sup>a</sup> ± 0.960
Final viscosity $\eta_f$ , Pa·s	10.89 <sup>b</sup> ± 1.040	5.96 <sup>a</sup> ± 0.400	10.96 <sup>b</sup> ± 1.100	10.18 <sup>b</sup> ± 0.080	11.22 <sup>b</sup> ± 0.650	6.26 <sup>a</sup> ± 0.190
$\eta_i/\eta_f$	5.910 <sup>a</sup> ± 0.300	6.970 <sup>b</sup> ± 0.040	6.470 <sup>b</sup> ± 0.250	8.080 <sup>c</sup> ± 0.070	6.480 <sup>b</sup> ± 0.120	6.910 <sup>b</sup> ± 0.080
Lost structure $L_s$ , %	82.88 <sup>a</sup> ± 0.800	85.64 <sup>b</sup> ± 0.080	84.45 <sup>b</sup> ± 0.550	87.61 <sup>c</sup> ± 0.110	84.54 <sup>b</sup> ± 0.300	85.52 <sup>b</sup> ± 0.170
Consistency coefficient $K$ Pa·s <sup>n</sup>	66.64 <sup>b</sup> ± 10.29	42.33 <sup>a</sup> ± 2.620	72.96 <sup>bc</sup> ± 10.12	86.10 <sup>c</sup> ± 1.060	73.38 <sup>bc</sup> ± 3.960	44.16 <sup>a</sup> ± 0.920
Flow behavior index $n$	0.360 <sup>c</sup> ± 0.010	0.300 <sup>b</sup> ± 0.000	0.330 <sup>d</sup> ± 0.010	0.240 <sup>a</sup> ± 0.000	0.330 <sup>cd</sup> ± 0.000	0.300 <sup>bc</sup> ± 0.000

A, C, E – control samples, B, D, F – Mg fortified samples. Values are means ± S.D. for  $n = 15$ . Mean values denoted with different letters within a row differ significantly ( $P < 0.05$ ).

### Microbiological results

In the analyzed yogurts (A, B, C, D), the count of *S. thermophilus* was much higher than the count of lactobacilli (Table 4). In yogurts E and F, the count of *S. thermophilus* was significantly lower than in the others ( $P < 0.05$ ). Fortification with magnesium did not have a noticeable effect on the viability of the used starters. Only in yogurt B was there a higher count of *L. bulgaricus*. In yogurts with starter YF-L811, the lowest count of bacilli was noticed. In yogurts E and F, the counts of *L. bulgaricus* + *L. acidophilus* + *B. lactis* were similar. Most yogurts have a ratio of cocci to bacilli between 1:1 and 2:1. The bacilli must never be allowed to gain the upper hand, as the flavor will then become too acidic (Bylund, 2015). In the studies of Cueva and Aryana (2008) the addition of vitamins and minerals to yogurt had no significant effect on the microbial counts. Simova et al. (2008) stated that the Fe fortification of milk with the addition of ferrous lactate in concentrations of  $\text{Fe}^{2+}$  8, 14 and 27  $\text{mg}\cdot\text{kg}^{-1}$  of milk had no significant influence on the growth and

viability of yogurt bacteria during milk fermentation and yogurt storage. This could be explained by the characteristics of their physiology. Lactic acid bacteria are organisms with complex nutritional needs, but are reported to have no  $\text{Fe}^{2+}$  requirements. However,  $\text{Mg}^{2+}$  was reported to be essential for their growth (Hébert et al., 2004). Studies (Rauch et al., 2017) have shown that this divalent cation is necessary for Gram-positive bacteria to grow. The authors also found that there is a time-lag between the incubation of magnesium and growth that was not observed in Gram-negative bacteria, despite the fact that they are similarly dependent on magnesium for growth. This time-lag was only measurable in low concentrations of magnesium.

### Sensory evaluation

Table 5 shows the results of sensory analysis. Fortified yogurt D showed the strongest sour taste in comparison to the other samples. Significant differences were observed in the sourness of yogurts fortified with magnesium lactate ( $P < 0.05$ ). Fortified

**Table 4.** Viable counts of *S. thermophilus*, *L. bulgaricus*, *L. acidophilus*, *Bifidobacterium lactis* in yogurts,  $\log \text{CFU}\cdot\text{g}^{-1}$

	YC-X11		YF-L811		VITAL	
	A	B	C	D	E	F
<i>S. thermophilus</i>	9.930 <sup>d</sup>	9.710 <sup>bcd</sup>	9.860 <sup>c</sup>	9.270 <sup>b</sup>	5.850 <sup>a</sup>	5.670 <sup>a</sup>
<i>L. bulgaricus</i>	5.240 <sup>b</sup>	5.790 <sup>c</sup>	4.080 <sup>a</sup>	4.020 <sup>a</sup>	–	–
<i>L. bulgaricus</i> + <i>L. acidophilus</i> + <i>B. lactis</i>	–	–	–	–	7.170 <sup>d</sup>	7.240 <sup>d</sup>

A, C, E – control samples, B, D, F – Mg fortified samples.

Mean values denoted with different letters within a row differ significantly ( $P < 0.05$ ).

**Table 5.** Sensory parameters of yogurts

Parameter	YC-X11		YF-L811		VITAL	
	A	B	C	D	E	F
Sour taste	2.880 <sup>a</sup> ± 1.900	4.550 <sup>b</sup> ± 1.810	3.550 <sup>ab</sup> ± 1.230	6.660 <sup>c</sup> ± 1.730	3.550 <sup>ab</sup> ± 1.870	4.440 <sup>b</sup> ± 1.740
Salty taste	1.220 <sup>a</sup> ± 0.440	3.000 <sup>b</sup> ± 0.800	1.220 <sup>a</sup> ± 0.440	2.000 <sup>a</sup> ± 1.220	1.220 <sup>a</sup> ± 0.440	2.220 <sup>a</sup> ± 1.200
Bitterness	1.110 <sup>a</sup> ± 0.330	1.770 <sup>a</sup> ± 1.560	1.110 <sup>a</sup> ± 0.330	1.660 <sup>a</sup> ± 1.410	1.220 <sup>a</sup> ± 0.440	2.110 <sup>a</sup> ± 1.560
Off-flavors	1.000 <sup>a</sup> ± 0.000	1.000 <sup>a</sup> ± 0.000	1.000 <sup>a</sup> ± 0.000	1.110 <sup>a</sup> ± 0.330	1.000 <sup>a</sup> ± 0.000	1.220 <sup>a</sup> ± 0.440

A, C, E – control samples, B, D, F – Mg fortified samples. Values are means ± S.D. for  $n = 15$ .

The differences between the mean values denoted with the same letter within a row are not statistically significant ( $P > 0.05$ ).

samples showed higher sourness than their controls, but in samples with VITAL starter the difference was insignificant. The addition of magnesium lactate also caused a slightly stronger salty taste in the yogurts, but the difference was significant only in sample B. The  $Mg^{2+}$  dose of  $35 \text{ mg} \cdot 100 \text{ g}^{-1}$  did not significantly influence the bitterness and off-flavors of yogurts. Sensory properties of fortified dairy products are influenced by the type of mineral source and the amount of the component which is added to the product. The salts of divalent cations such as calcium and magnesium are characterized primarily by bitter and salty tastes, and to a lesser extent by other basic tastes and metallic, astringent and irritative sensations (Lawless et al., 2003). Two principal off-flavors have been created with iron-fortified yogurt: oxidized flavor and metallic flavor, which emerge due to the catalytic role of iron and the presence of iron salts respectively (Gahruie et al., 2015). Münchbach and Gerstner (2010) reported that high levels of calcium, particularly insoluble forms such as carbonates and phosphates, tend to produce a chalky mouthfeel and may promote astringency or bitter taste in the finished product. The results of Znamirowska et al. (2015) showed that fortification with magnesium gluconate decreases the sour taste of the yogurts in comparison to non-fortified ones. No significant differences were observed in the flavor scores of yogurts fortified with iron chloride, casein chelated iron, and whey protein chelated iron (Achanta et al., 2007).

## CONCLUSIONS

Fortification with magnesium significantly raised the pH value of yogurts, regardless of the type of starter. The reduction of syneresis and improvement of hardness in magnesium-supplemented yogurts were also observed. The rheological properties of yogurts and the values of their color parameters depended on the type of starter cultures as well as on the addition of magnesium lactate. Fortification with magnesium did not cause a noticeable effect on the viability of starter bacteria. Significant differences were found in the sour taste of the fat-free yogurts, except for the sample with VITAL starter, for which the difference was insignificant. Magnesium lactate showed the potential to be appropriate for dairy food fortification, according to

physicochemical and sensory data. Yogurt fortified with 35 mg magnesium could be a good source of this mineral in the everyday human diet. A portion of about 150 g would constitute over 20% of the recommended daily intake of that mineral. Further research should be carried out regarding the influence of storage time and to establish whether the observed effects are largely due to the magnesium cation or lactate anion.

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