Effects of various magnesium salts for the production of milk fermented by Bifidobacterium animalis ssp. lactis Bb-12

Katarzyna Szajnar, Agata Znamirowska, and Dorota Kalicka

Department of Dairy Technology, University of Rzeszow, Rzeszow, Poland

ABSTRACT

The aim of this work was to develop of functional dairy product fortified with magnesium and contained probiotic bacteria Bifidobacterium Bb-12. Therefore, the effect of various magnesium salts on physicochemical, organoleptic and texture properties and Bifidobacterium Bb-12 survival were assessed. The best stimulator of bacterial growth was D-gluconate. Although the addition of L-lactate and acetate decreased the growth rate showed a positive effect on bacterial survival. The fortification with magnesium significantly affected hardness and decreased the brightness L* of fermented milk. The results of the organoleptic analysis indicated that magnesium D-gluconate was the most useful for milk fortification.

INTRODUCTION

In recent years, probiotics have found a permanent place in the diet of many people. Producers create new products with their addition, emphasizing their pro-health properties. Nutritional value and widespread availability make dairy products the group most frequently enriched in bacteria with a documented probiotic effect. In order to ensure good growth and survival of bacteria, external factors such as, fermentation cold storage, water activity, oxidation-reduction potential, and hydrogen ion concentration should be taken into consideration.\cite{1} Dairy products, such as fermented milks, are ideal carriers for fortification with various macronutrients that would stimulate their growth and survival. The study by Anderson et al.\cite{2} showed that some compounds, like gluconate, stimulate the number of bifidobacteria cells. Lactose present in milk has a stimulant effect on net absorption of magnesium along with calcium and manganese in human infants.\cite{3} Moreover, lactulose is an indigestible oligosaccharide that is present in low concentrations in heat-treated milk and can stimulate the growth of Bifidobacterium. Seki et al.\cite{4} demonstrated that lactulose improved the absorption of magnesium as well as calcium in adult males.

When choosing a magnesium compound, it should be taken into consideration that the ideal source of this element used for enriching dairy products should be characterized by high availability by the body. Moreover, these compounds should be cheap, safe in use, show the desired solubility and stability. It should not interact with other components or cause changes in the sensory attributes and shorten the shelf-life.\cite{5,6} The average diet does not provides the proper coverage of demand for magnesium; therefore, this element should be supplemented, for example, by eating fortified food products. Magnesium plays an essential role in a wide range of fundamental biological processes. Magnesium deficiency may induce several heart dysfunctions, such as arrhythmia, hypertension, atherosclerosis, formation of blood clots within the blood vessels and finally, myocardial infarction.\cite{7,8,9} Nutritional studies show that magnesium intake is much lower than the
recommended 300 mg per day.\textsuperscript{[10–12]} Milk and dairy products are largely seen as calcium-rich food group, whereas they are an important source of magnesium. Milk and dairy products are one of the main dietary sources of magnesium particularly for children, contributing approximately 10–30\% of the total magnesium intake.\textsuperscript{[13]} An adult human body contains 20–28 g of magnesium, which is not evenly distributed in the body. The amount of available magnesium, i.e., its potential retention in the human organism, depends, for example, on its concentration in the consumed food.\textsuperscript{[14]} The fortification of milk products is beneficial to consumers and provides opportunities for marketing for the dairy product industry. The consumers benefit from the healthy products that provide alternatives sources of micronutrients to meet their nutrient requirements, and the industry develops fortified products that are attractive for consumers. Such products can be advertised as having high mineral contents.

There are few publications regarding the effect of the addition of various magnesium salts on the properties of fermented milk and the survival of bacteria. Many works emphasize that there negligible research on the possibility of using different magnesium salts in dairy industry. According to the literature\textsuperscript{[15–17]}, there is a growing interest in using mineral compounds as a functional additive for fermented milk. In this study, the research of milk fermented by \textit{Bifidobacterium} Bb-12 fortified with various magnesium salts may form the basis for the development of functional products with different organoleptic and textural properties that could be implemented into production as innovative food products. Therefore, the aim of this work was to assess the effect of the type of the magnesium salt added on the growth and survival of \textit{Bifidobacterium} Bb-12 and on the physicochemical, organoleptic properties and texture of fermented milk.

\section*{Materials and methods}

\subsection*{Materials}

Pasteurized milk, fat 2\% (Piątnica Dairy, Poland), magnesium acetate (CH\textsubscript{3}COO)\textsubscript{2}Mg·4H\textsubscript{2}O (Chempur, Poland), magnesium L-pidolate C\textsubscript{10}H\textsubscript{12}N\textsubscript{2}MgO\textsubscript{6} (Sigma, France), magnesium chloride MgCl\textsubscript{2}·6H\textsubscript{2}O (Chempur, Poland), magnesium L-lactate C\textsubscript{6}H\textsubscript{10}MgO\textsubscript{6}·H\textsubscript{2}O (Sigma-Aldrich, Spain), magnesium bisglycinate MgC\textsubscript{4}H\textsubscript{4}O\textsubscript{5}·3H\textsubscript{2}O (Sigma-Aldrich, USA), magnesium D-gluconate C\textsubscript{12}H\textsubscript{22}MgO·H\textsubscript{2}O\textsubscript{14} (Sigma-Aldrich, USA). All the reagents used in the present work were of analytical grade.

\subsection*{Fermented milk manufacture}

Milk was divided into six parts and, a different magnesium salts were added to each group in an amount of 30 mg Mg 100 g\textsuperscript{-1} of milk (the magnesium dose was calculated on the molecular weight of the magnesium salt used). Fermented milk without magnesium addition was used as a control sample. The non-fat dry matter content has not been standardized. Then, it was homogenized and re-pasteurized. Re-pasteurization was carried out at 72°C, 15 s according to the method Ramasubramanian et al.\textsuperscript{[18]} with modifications. According to (EC) No 1662/2006\textsuperscript{[19]}, the temperature used during research was 72°C. Homogenization was carried out using homogenizer (Nuoni GJJ-0.06/40, Zhejiang, China), 20MPa, 60°C. Afterward, re-pasteurized milk samples were cooled to 37°C. Each batch of milk was inoculated with a previously activated single starter culture \textit{Bifidobacterium animalis} ssp. \textit{lactis} Bb-12, DMS 15954 (Chr. Hansen, Dania) according to the method Mituniewicz-Małek et al.\textsuperscript{[20]} with some modification (in the form of a bulk activated at 40°C for 5 h, which was added to the milk in the amount of 3\%). Inoculated milk was stirred and poured into 100 ml plastic cups and fermented at 37°C for 10 h. The final products were cooled to 5° C (Cooled Incubator ILW 115, POL-EKO Aparatura, Poland). The experiment was repeated three times. Fermented milk was evaluated after 1st and 21st day of cold storage (5°C). There were evaluated five samples of fermented milk from each group.
**Physicochemical properties**

The pH determination was performed with a pH-meter (FiveEasy Mettler Toledo, Switzerland). Total acidity (g of lactic acid \( \times L^{-1} \)) of milk was estimated according to the method Jemaa et al.\(^{[21]} \)

syneresis was estimated according to the method Santillan-Urquiza et al.\(^{[22]} \) with modifications (10 g of fermented milk sample was transferred into 50 mL plastic tube and centrifuged at 1790 g for 10 min at 5°C in LC-04 CENTRIFUGE, Zenithlab, China). The syneresis was estimated as the released whey over the original weight and was an average of five determinations.

Colour was analyzed with the colorimeter Chroma Meter CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan) using the CIELAB system. The determination of \( L^* \), \( a^* \), and \( b^* \) parameters is represented by \( L^* \) the brightness (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. The temperature of the studied fermented milk samples was equal to the ambient temperature, i.e., 23–24°C.

**Parameters of texture**

Texture was determined with the CT3 Texture Analyzer with the Texture Pro CT software (Brookfield AMETEK, USA). The TPA (two cycles count) was selected with a solid-state-fermented milk sample which was placed without mixing in a 100 ml container with the following settings: sample – cylinder 66.00 mm x 33.86 mm, trigger load 0.1 N, test speed 1 mm s\(^{-1} \), table TA-BTKIT, probe TA3/100 (acrylic cylinder – 35 mm diameter).

**Microbiological analysis**

The number of characteristic microorganisms was estimated according to the method by Lima et al.\(^{[23]} \)

Viable counts of *Bifidobacterium animalis* ssp. *lactis* Bb-12 were evaluated using MRS agar (Biocorp, Poland) and incubated anaerobically at 37°C for 72 h. The results were expressed as log cfu g\(^{-1} \).

**Organoleptic parameters**

The analysis was made for six fermented milk samples fortified with magnesium salts and the control sample. The organoleptic analysis was performed by a trained panel (10 women and 10 men at the age of 25–30). The panelists were served seven samples at a time (in three-digit random number of coded plastic cubs) and asked to rinse their mouths with water between samples. The panelists evaluated the – consistency and the presence of creamy-milky taste, sour taste, off-taste, fermented-odour, off-odour on a 9-point rating scale with edge markings (from 1 = not perceptible to 9 = extremely strong).\(^{[24]} \)

**Statistical analysis**

The obtained results were given as the mean and standard deviation and were calculated statistically using the Statistica v. 13.1 software (StatSoft, USA). ANOVA was used to investigate the overall effect of magnesium salts and storage time (days) on the properties of fermented milk. Significance of differences between the averages was estimated with Tukey’s test (\( P < 0.05 \)).

**Results and discussion**

**Microbiological analysis**

In the first day of storage the number of viable cells of *Bifidobacterium animalis* ssp. *lactis* Bb-12 (*Bifidobacterium Bb-12*) in the D-gluconate sample was significantly higher than the other samples, both those with salts and the control one (Figure1). In the study by Anderson et al.\(^{[2]} \), the conducted
tests showed a significant increase in the count of *Bifidobacterium* Bb-12 (by 0.43–1.48 log) during gluconate supplementation. In another study, it was found that gluconate is poorly absorbed in the upper gastrointestinal tract. It reaches the lower part of the gastrointestinal tract, and is used by the colonic microflora in which *Bifidobacterium* Bb-12 is involved. Detailed in vitro studies have shown that gluconate can be fermented by certain *Bifidobacterium* strains. Some studies emphasize the promising prebiotic potential of gluconic acid. In this study, it was confirmed that magnesium D-gluconate can stimulate growth of *Bifidobacterium* Bb-12. The number of *Bifidobacterium* Bb-12 cells on the first day was determined in the acetate sample, which means that the increase of *Bifidobacterium* Bb-12 was the slowest in this sample. After 21 days of storage, there was a significant (*P* < 0.05) reduction in the number of viable bacteria in samples with L-pidolate (by 0.47 log cfu·g⁻¹), D-gluconate (by 0.56 log cfu·g⁻¹) as well as in the control (by 0.34 log cfu·g⁻¹). A decrease in viability of *Bifidobacterium* Bb-12 during 21 days of storage was also found by Kumari et al. Gueimonde et al. after 30 days of storing fermented milk from supermarkets found a reduction in the content of *Bifidobacterium* by 0.17 ÷ 1.10 log cfu ml⁻¹. In the samples with L-lactate and bisglycinate, acetate and chloride good survival of *Bifidobacterium* Bb-12 during storage were found. The number of *Bifidobacterium* Bb-12 cells in the sample with bisglycinate increased during storage by 0.24 log cfu·g⁻¹. Bisglycinate is an amino acid chelate of magnesium. According to Gajewska and Błaszczyk, lactic acid bacteria for their growth require exogenous amino acids and peptides that are released in the proteolysis of proteins present in the raw material. Some of the bacteria require from 6 to 18 amino acids to grow in the culture medium.

Therefore, the addition of the amino acid from bisglycinate probably contributed increased survival of *Bifidobacterium* Bb-12 during storage. The addition of acetate and chloride did not significantly affect the survival of *Bifidobacterium* Bb-12. The number of bacterial cells on the 1st and 21st day of storage was similar. The study by Tabasco et al. indicated that organic
acids produced by *Bifidobacterium* Bb-12 during milk fermentation were lactic, acetic and formic acids, in quantities of: 12.2 mmol/L, 35.3 mmol/L, 6.1 mmol/L, respectively. Bunesova et al.\[^{32}\] also determined the fermentation profile and metabolites produced by *Bifidobacterium* Bb-12, which included acetate (222.87 mg l\(^{-1}\)) and lactate (167.88 mg l\(^{-1}\)). Therefore, lactic acid and acetic acid and their derivatives make the natural environment for the development of *Bifidobacterium* Bb-12. The carried out studies indicate that they did not negatively effect on the survival of bacterial cells. An increase in the viable count of *Bifidobacterium* Bb-12 in yogurts was also reported by Illupapalayam et al.\[^{33}\] during 28 days of storage at 4°C. In this study, the count of viable bifidobacteria cells increased in the control sample after 14 days of storage. In the study Marafon et al.\[^{34}\] reported that in the nonfortified product, the counts ranged between 6.63 log10 cfu/mL (1 day) and 6.78 log10 cfu/mL (28 days) and no significant differences were observed in the counts of bifidobacteria during the cold storage of the nonfortified probiotic yogurt.

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The two-way ANOVA (Table 1) indicates that the storage time and the magnesium salts, and the interactions of these factors significantly influenced the growth and survival of *Bifidobacterium* Bb-12 cells. However, according to Russel et al.\[^{35}\], *Bifidobacterium* Bb-12 had greater ability to withstand environmental stress conditions, such as an environment with increased oxygen content, high acidity, and low temperature, than other *Bifidobacterium* strains. Populations of *Bifidobacterium* in fermented milk should be more than 6 log cfu·g\(^{-1}\) at the time of the product consumption to be considered probiotic.\[^{36}\]

In this study, all milk samples met the minimum therapeutic criterion throughout the research period.

### Physicochemical properties

Table 2 presents the effect of fortifications with various magnesium salts on the pH value of milk before fermentation. Fortification with pidolate, L-lactate, D-gluconate, acetate and chloride reduced the pH value of milk before fermentation. The fortification with bisglycinate significantly increased the pH value. A dose of 30mg Mg 100 g\(^{-1}\) is the highest dose that did not coagulate milk proteins during thermal processing of milk. Commission Directive 2008/100/EC\[^{37}\] specifies that products should be enriched with minerals in such quantity that their content in 100 g or in a package containing only one portion of the product would correspond to at least 15% of the recommended daily allowance. Fermented milk is usually sold in portions of 200 g, that gives 30 mg Mg 100 g\(^{-1}\) which is 15% RDA.

A smaller number of viable *Bifidobacterium* Bb-12 cells in the samples with acetate resulted in a higher pH compared to the other samples on the first day of storage (Table 2) and these differences were not significant. The two-way analysis ANOVA indicated that only the storage time significantly affected the

### Table 1. Analysis of variance (ANOVA) P-values on the effects of storage time (days) and magnesium salts on pH, total acidity, syneresis, consistency, milky-creamy taste, sour taste, off-taste, fermentation odor, off-odor, *Bif*. Bb-12, hardness, adhesiveness, cohesiveness of fermented milk.

| Properties                  | Storage time (days); P-values | Magnesium salts; P-values | Storage time (days)*Magnesium salts; P-values |
|-----------------------------|-------------------------------|----------------------------|-----------------------------------------------|
| pH                          | ↑ 0.0001                      | n.s.0.1422                 | n.s.0.7518                                   |
| Total acidity [g of lactic acid/L\(^{-1}\)] | ↑ 0.0017                    | n.s.0.7901                 | n.s.0.7940                                   |
| Syneresis [%]               | ↑ 0.0039                      | ↑ <0.0001                  | ↑ 0.0001                                    |
| Consistency                 | ↑ <0.0001                    | ↑ <0.0001                  | ↑ <0.0001                                   |
| Milky-creamy taste          | n.s.0.2240                   | ↑ <0.0001                  | ↑ 1.0001                                    |
| Sour taste                  | n.s.0.4333                   | ↑ <0.0001                  | ↑ 1.0001                                    |
| Off-   taste                | ↑ <0.0001                    | ↑ <0.0001                  | ↑ <0.0001                                   |
| Fermentation odour          | n.s.0.3267                   | ↑ <0.0001                  | ↑ <0.0001                                   |
| Off-   odour                 | ↑ <0.0001                    | ↑ <0.0001                  | ↑ <0.0001                                   |
| *Bif*. Bb-12                | 10.0009                      | 10.0009                    | 10.0009                                      |
| Hardness [N]                | n.s.0.1749                   | ↑ <0.0001                  | n.s.0.2903                                   |
| Adhesiveness [mJ]           | n.s.0.0059                   | ↑ <0.0001                  | 1.0016                                       |
| Cohesiveness                | n.s.0.1602                   | ↑ <0.0001                  | n.s.0.0622                                   |

Where: Storage time (days)*Magnesium salts = interaction, ↑ indicates significant effect P<0.05, n.s. no significant effect.
pH values (Table 1). However, salt fortification and interactions of these factors did not affect the pH value. This effect was also demonstrated by the two-way ANOVA analysis for total acidity. The total acidity in the samples with the addition of L-lactate, D-gluconate and chloride significantly ($P < 0.05$) increased during storage. The reason of the increase in the total acidity during storage was acid metabolites produced during *Bifidobacterium Bb-12* fermentation and the dissociation components of the magnesium salts added. The fortification of milk with magnesium salts significantly reduced syneresis compared to the control sample on the first day of storage. D-gluconate and L-pidolate limited syneresis most effectively. In these samples, syneresis was lower by 18–21% compared to the controls. This could be due to the ability of *Bifidobacterium* to produce exopolysaccharides (EPS), that could bind free water and modify the yogurt gel during coagulation. Usually, syneresis occurs due to the loss in the ability of the yogurt gel to entrap all of the serum phase due to the weakening of the gel network. Therefore, strengthening the gel can reduce syneresis. Presumably, the addition of glutamic acid derivatives (D-gluconate, L-pidolate) contributed to the increase in cross-linking of casein bonds and the creation of a better matrix to retain whey.

Cuomo et al. concluded that Ca$^{2+}$ and Mg$^{2+}$ ions have different binding sites on the casein, phosphoserine for calcium and glutamic and aspartic acids for magnesium. The authors also suggested that the presence of calcium aids the binding of magnesium by making more sites available. This may explain the synergistic effect of calcium and magnesium ions.

With the prolongation of the storage time to 21 days, significantly lower syneresis was found in the control samples and in those fortified with L-lactate. In the other samples, no significant differences were found in syneresis between the 1st and 21st day of storage. The storage time and the magnesium salts, as well as the interactions of these factors, significantly affected syneresis (Table 1).

Differences in the color of fermented milk may be noticeable by the consumer; therefore, they may be one of the criteria for sensory assessment of the quality and choice of fermented milk beverages. According to the literature reports, magnesium salts may cause color changes in the fermented beverage. It is known that minerals change the structure of milk proteins. Augustin reported that mineral salts change the distribution of caseins between colloidal milk and serum phases and the ionic environment of proteins. Minerals could change the matrix structure of casein micelles in yogurts in such a way as to contribute to the greying of the color of the protein curd. Achanta et al. studied non-fat yogurts fortified with various minerals and reported that the average values of L* of yogurts fortified with chromium, magnesium, manganese, and molybdenum were higher than the control. Clowley et al., in their study on fortification of reconstituted skimmed milk powder with various calcium salts, showed that the calcium compounds used did not significantly affect the brightness of colors. Only fortification with calcium hydroxide significantly contributed to the lower intensity of the yellow color in the tested yogurt. In our study, it was decided to check whether the magnesium salts used would cause significant changes in color. The most important aim was to check whether the magnesium salts, rather than the storage time, significantly affect these changes. Moreover, the color was not tested on day 21 of storage because, according to literature reports, in yogurts fortified with chromium, magnesium, manganese, and molybdenum there was no significant interaction ($P > .05$) between minerals and storage time. Storage time ($P > .05$) had no effect on color parameters.

Table 3 presents the effect of the magnesium salts used on the color parameters L*, a*, b*. The brightest color (the highest L*) was determined in the control sample. Milk fortification with L-pidolate, L-lactate, D-gluconate, and bisglycinate did not significantly affect the brightness of the color compared

### Table 2. The pH values in milk after dose of magnesium salts before fermentation.

| Properties | Control | Magnesium L-pidolate | Magnesium L-lactate | Magnesium D-gluconate | Magnesium acetate | Magnesium bisglycinate | Magnesium chloride |
|------------|---------|----------------------|---------------------|-----------------------|------------------|-----------------------|-------------------|
| pH         | 6.78± 0.00 | 6.64± 0.01 | 6.68± 0.01 | 6.67± 0.00 | 6.66± 0.01 | 7.70± 0.00 | 6.59± 0.00 |

Values are means ± S.D

a, b, c – mean values in lines denoted by different letters differ significantly ($P<0.05$)
to the control sample. The darkest color was determined in the sample with acetate and chloride. The intensity of green (a *) and yellow (b *) also increased in the sample with acetate. The chloride added reduced the intensity of green and yellow, similar to L-lactate, D-gluconate, and bisglycinate.

Znamirowska et al.\[44\] concluded that with increasing dose of fortification with calcium and magnesium salts, the darkening of color of the beverages fermented by *Bifidobacterium animalis* ssp. *lactis* Bb-12 was found. Values a * and b * determined for these beverages fortified with calcium and magnesium salts indicated the change of the colour scale in space towards the green and yellow colour compared to the controlsamples . A similar tendency was observed by Bagci and Gunasekaran\[45\] in their study where the brightness of color of the yogurts fortified with FeSO\(_4\) was significantly influenced (\(P<.05\)) by the amount of mineral addition.

**Parameters of texture**

Gel formation is one of the main texture properties of yogurt. This structure is the result of casein aggregation by pH decreasing and disulfide bonding between \(\kappa\)-casein and denatured whey proteins.\[46\] However, other parameters such as milk base composition, heat treatment applied, fermentation process, storage conditions, and starter culture, also perform a determinative role in gel structure formation.\[47\] Hardness, adhesiveness, and cohesiveness are commonly evaluated in determining yogurt texture.\[48–50\] In the study of Santillán-Urquiza et al.\[22\], the hardness of yogurt increased in samples with higher concentration of mineral and principally in yogurts fortified with nanoparticles reflecting a stronger gel structure. Philippe et al.\[51\] and Lombardi et al.\[52\] showed specific affinity of each cation for some groups, in milk proteins could be playing a more important role than plain electrostatic interaction, i.e., the ionic strength effect. Lombardi et al.\[53\] reported that stronger gels were developed in the presence of cations with higher percentage of casein-association. It was reported that the order of association of determined cations with caseins is: Zn\(^{2+}\) > Ca\(^{2+}\) > Mg\(^{2+}\). Therefore, in our study, it was decided to check how a magnesium salts addition would affect the individual textural components of cow’s milk fermented by *Bifidobacterium*- Bb12. Table 4 presents the results of hardness, adhesiveness, and cohesiveness of the control samples and magnesium fortified samples. The L-lactate and D-gluconate-fortified samples were characterized by the hardness value like the control sample. The two-way analysis ANOVA indicated that only the addition of magnesium salt (Table 1) significantly affected the hardness of the samples. The storage time and interactions of these factors were found to be not significant (Table 1). The introduction of various magnesium salts could affected the ability of the strain to produce EPS, which can explain differences in the hardness of the samples tested. However, there were differences in the pH of the samples with various magnesium salts which could also affect the hardness of the samples. Ocak and Köse\[54\] found that the addition of various minerals, e.g., Fe, to milk resulted in an increase in yogurt hardness and cohesiveness compared to the control yogurt. Philippe et al.\[51\] studied the effects of different cations on the physicochemical characteristics of casein micelles and hydrophobicity of casein micelles decreased after the addition of ferric iron and copper.

### Table 3. Effect of magnesium fortification on color of fermented milk after 1 day of cold storage.

| Properties | Control | Magnesium L-pidolate | Magnesium L-lactate | Magnesium D-gluconate | Magnesium acetate | Magnesium bisglycinate | Magnesium chloride |
|------------|---------|----------------------|---------------------|-----------------------|------------------|-----------------------|--------------------|
| L*         | 96.87\(^c\) | 96.01\(^bc\)          | 95.80\(^bc\)        | 96.39\(^bc\)          | 94.32\(^c\)      | 95.88\(^bc\)          | 95.52\(^b\)       |
| ±0.63      | ±0.36   | ±0.44                | ±0.74               | ±0.32                 | ±0.51            | 0.76                  |
| a*         | −4.38\(^c\) | −4.38\(^c\)          | −4.01\(^a\)         | −4.20\(^d\)           | −4.56\(^d\)      | −3.97\(^a\)          | −4.10\(^ab\)      |
| ±0.05      | ±0.07   | ±0.04                | ±0.05               | ±0.09                 | ±0.04            | ±0.06                 |
| b*         | 12.91\(^c\) | 12.49\(^b\)          | 12.16\(^ab\)        | 12.03\(^a\)           | 14.75\(^d\)      | 12.41\(^b\)          | 12.46\(^b\)       |
| ±0.10      | ±0.03   | ±0.40                | ±0.04               | ±0.22                 | ±0.05            | ±0.07                 |

Values are means ± S.D. for n = 15.
a, b, c – mean values between magnesium salts denoted by different letters differ significantly (\(P<0.05\)).
The two-way ANOVA analysis showed that the storage time and the magnesium salts, as well as the interactions of these two factors, significantly affected the adhesiveness of the samples (Table 1). Fortification of the samples with L-pidolate, L-lactate, bisglycinate and chloride reduced the adhesiveness compared to the control sample. However, the differences turned out to be not statistically significant. Prolonging the storage time to 21 days decreased the adhesiveness of samples (except for the sample with D-gluconate). The cohesiveness of magnesium-fortified samples and the control sample were significantly influenced by addition of magnesium salts. Therefore, only the addition of a magnesium salt that had an effect on the texture and stabilized it during storage, the time of storage of tested samples did not significantly affect the hardness, adhesiveness, and cohesiveness of milk samples fermented by *Bifidobacterium* Bb-12. There are different opinions in the literature regarding the effect of storage time on the texture of fermented milk. Alakin et al. showed that the hardness did not change during storage (P > .05) in fermented milk with SMP (skimmed milk powder). In the study by Santillán-Urquiza et al., in all samples a significant decrease in hardness and cohesiveness parameters was observed, this reduction was also associated with changes in the consistency coefficient during storage.

### Organoleptic parameters

Organoleptic 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. Previously conducted studies have indicated that the result of the preference test showed that the most panelists selected beverage...
with magnesium addition, especially in amount 30 mg, taking into account the intense milky-cream flavour, moderately sour and undetectable off-taste.^[44]^ Table 5 presents the results of the organoleptic analysis of magnesium-fortified samples and the control sample on the 1st and 21st day of storage. In the panelists’ opinion, the most tight, smooth and homogeneous consistency was indicated in the sample with D-glucanate and in the control sample on the first day of storage. At that time, significantly lower consistency notes were given to samples with L-pidolate and bisglycinate. In assessment of the texture components (Table 2), the hardness of the samples with L-pidolate and bisglycinate was also lower than in the control sample. After 21 days of storage, lower notes for consistency were given to all samples (except for the sample with L-lactate) than on the first day of storage. The two-way ANOVA indicates that storage time (days), magnesium salts, and the interaction of these factors (storage times, days*magnesium salts) significantly influenced consistency (Table 1). According to Znamirowska et al.^[55]^ fortification of goat’s yogurt with magnesium (20 mg of magnesium for 100 g of milk) had a positive effect on consistency.

The taste evaluation was based on three different features: milky-creamy, sour and off-taste. The most intense off-taste and flavor was found in the samples with acetate. The high notes were the result of the acetic taste, which intensity increased with the prolongation of the storage time. On the first day, more intense sour taste was found in the samples with chloride, L-pidolate, L-lactate, bisglycinate than in the control sample. However, after 21 days of storage, in the samples with magnesium salts, the sour taste was less intense than in the control. It turns out that the addition of some magnesium salts to milk beverages intensified the milky-creamy taste and these salts include D-glucanate and acetate. However, in the L-lactate samples, the panelists found a reduction in the intensity of the milky-creamy taste. After 21 days of storage, the milky-creamy taste was also barely perceptible in the L-pidolate samples. The most intense off-odor was found in the sample with acetate, subsequently followed by the samples with chloride and L-pidolate. Zbikowska and Zbikowski^[12]^ also came to similar conclusions. In their study, there where found that the analyzed samples of bio-yogurt produced with the thermostat method

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**Table 5. Effect of magnesium fortification on organoleptic parameters of fermented milk after 1 and 21 days of cold storage.**

| Properties         | Days of cold storage | Control | Magnesium L-pidolate | Magnesium L-lactate | Magnesium D-glucanate | Magnesium acetate | Magnesium bisglycinate | Magnesium chloride |
|--------------------|----------------------|---------|----------------------|---------------------|-----------------------|-------------------|------------------------|---------------------|
| Consistency        | 1                    | 7.60^a^ | 6.30^a^             | 7.10^a^             | 7.60^a^               | 7.20^a^           | 6.90^a^                | 7.00^a^             |
|                    | ±0.69                | ±0.94   | ±1.10                | ±0.84               | ±1.22                 | ±1.85             | ±0.88                  | ±0.48               |
|                    | 21                   | 7.22^a^ | 6.27^aba             | 7.44^a^             | 7.44^a^               | 5.77^a^           | 5.33^a^               | 5.77^a^             |
|                    | ±0.66                | ±0.66   | ±0.72                | ±1.01               | ±0.44                 | ±0.50             | ±0.83                  | ±0.83               |
| Milky – creamy taste | 1                    | 2.90^a^ | 2.90^a^             | 2.70^a^             | 4.00^a^               | 4.70^a^           | 3.10^a^                | 3.50^a^             |
|                    | ±0.87                | ±0.99   | ±0.82                | ±0.66               | ±1.05                 | ±0.99             | ±0.52                  | ±0.52               |
|                    | 21                   | 3.22^a^ | 2.00^a^             | 2.77^ca             | 4.77^ca               | 4.66^a^           | 4.22^ca                | 4.33^ca             |
| Sour taste         | 1                    | 2.50^a^ | 4.06^ba             | 3.30^a^             | 2.00^a^               | 3.80^a^           | 3.10^a^                | 5.00^ca             |
|                    |                      | ±0.52   | ±0.94                | ±0.82               | ±0.66                 | ±1.47             | ±0.87                  | ±0.52               |
|                    | 21                   | 5.11^b^ | 4.88^gb             | 3.66^a^             | 1.66^a^               | 3.66^a^           | 3.00^a^                | 3.00^a^             |
| Off- taste         | 1                    | 1.30^a^ | 1.80^a^             | 2.00^a^             | 1.30^a^               | 3.20^a^           | 2.20^a^                | 2.30^a^             |
|                    |                      | ±1.05   | ±0.78                | ±0.86               | ±0.50                 | ±0.50             | ±0.70                  | ±0.86               |
|                    | 21                   | 1.55^a^ | 3.33^b^             | 2.22^ba             | 1.55^a^               | 5.66^b^           | 2.22^bba                | 3.55^b^             |
| Fermentation odour | 1                    | 3.96^a^ | 1.60^a^             | 1.80^a^             | 1.70^a^               | 4.90^a^           | 3.40^a^                | 3.10^a^             |
|                    |                      | ±1.19   | ±0.69                | ±0.91               | ±0.82                 | ±1.52             | ±1.07                  | ±1.28               |
|                    | 21                   | 3.11^ab^ | 2.66^ab             | 3.22^ab             | 2.22^a^               | 4.00^a^           | 2.66^a^                | 3.33^ab^             |
| Off-odor           | 1                    | 1.00^a^ | 1.50^a^             | 1.80^a^             | 1.30^a^               | 2.90^a^           | 1.70^a^                | 2.30^a^             |
|                    |                      | ±0.33   | ±0.70                | ±0.97               | ±0.66                 | ±1.00             | ±0.70                  | ±1.11               |
|                    | 21                   | 1.00^a^ | 2.3^b^              | 1.88^ba             | 2.00^b^               | 7.33^b^           | 1.77^ba                | 2.88^b^             |
|                    |                      | ±0.00   | ±0.50                | ±0.33               | ±0.86                 | ±1.00             | ±0.66                  | ±0.33               |

Values are means ± S.D. for n = 15.

a, b, c – mean values in lines denoted by different letters differ significantly (P<0.05)

A,B – Mean values in columns denoted by different letters differ statistically significantly (P <0.05) depending on the storage time.
fortified with 20 mg Mg L\(^{-1}\) and 60 mg Mg L\(^{-1}\) were characterized by a slightly acidic dense curd, a pure taste with a slightly noticeable aftertaste of the salt used and homogeneous compact consistency. Moreover, the authors stipulated that the addition of minerals in high concentrations (600 mg Ca + 60 mg Mg L\(^{-1}\)) to milk produced a metallic aftertaste in the obtained bio-yogurt.

The most intense fermentation odor was determined in the sample with acetate. In the other samples with magnesium, the intensity of fermentation odor was reduced compared to the controls. It should also be added that the addition of magnesium salt gives the samples an off-odor, and its intensity depends on the type of salt and storage time (Table 1).

In this study, the lowest concentrations of off-odor on the first day of storage were found in the samples with L-pidolate, L-lactate, D-gluconate. After 21 days, a barely noticeable off-odor was found in the samples with L-lactate and bisglycinate. Singh et al.\textsuperscript{[56]} found that some calcium compounds give an unpleasant odor and color as well as sandy consistency. In a study by Znamirowska et al.\textsuperscript{[44]} magnesium lactate gave a barely perceptible off-odor. \textit{Bifidobacteria} strains which do not produce much acetic acid during fermentation and storage are more appropriate for fermented milk products due to the low acetic odor in fermented dairy products.\textsuperscript{[57]}

**Conclusion**

Fortification of milk fermented by \textit{Bifidobacterium} Bb-12 with various magnesium salts and their production on an industrial scale will be of great importance in eliminating magnesium deficiencies and improving the health of the society. This study also has a considerable practical aspect and can be used by entrepreneurs to use the conclusions to produce milk and functional beverages. This study showed that the addition of magnesium at a dose of 30 mg Mg 100 g\(^{-1}\) may favorably affect the number of viable \textit{Bifidobacterium} Bb-12 cells. The best stimulator of bacterial growth was D-gluconate. Although the addition of L-lactate and acetate decreased the growth rate of \textit{Bifidobacterium} Bb-12, and it had a positive effect on their survival. All fortified fermented milks samples met the minimum therapeutic criterion, i.e., containing more than 6 log cfu g\(^{-1}\). The addition of D-gluconate-limited syneresis most effectively. Fortification with magnesium significantly affected hardness and decreased the brightness L\(^{*}\) of fermented milk. The milk with the addition of D-gluconate and L-pidolate showed the color parameters L\(^{*}\) a\(^{*}\) b\(^{*}\) most like the control sample. The results of the organoleptic analysis indicated that magnesium D-gluconate was the most useful for milk fortification. With a better understanding of magnesium in the dairy system, there is potential for milk and dairy products to be developed to deliver increased levels of bioavailable magnesium.

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**ORCID**

Katarzyna Szajnar \(\text{http://orcid.org/0000-0001-6399-1380}\)
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