SUMMARY

This doctoral dissertation consists of a series of 5 papers published in the following journals: two publications in the International Journal of Food Properties, two papers in the Journal of Elementology, and one paper in the Acta Scientiarum Polonorum Technologia Alimentaria.

The scientific purpose of the study was to assess the effect of six calcium compounds and six magnesium compounds used for milk fortification on selected properties of fermented milk beverages. The utilitarian purpose, in turn, was to assess the applicability of these compounds in the production of fermented milk beverages.

Nine research hypotheses were formulated.

The subject of the study were yogurts (YC-X16) and milks fermented by two dairy monocultures *Bifidobacterium* Bb-12 and *Lactobacillus rhamnosus* enriched with six different calcium compounds or six different magnesium compounds. Yogurts fermented by YC-X11, YF-L811, VITAL inoculums fortified with magnesium lactate were also tested.

Acidity, syneresis, texture profile (TPA) and instrumentally color were determined in fermented beverages. Organoleptic assessment and microbiological analysis were performed. The obtained data were subjected to statistical analysis in the Statistica software.

Six magnesium compounds (30 mg Mg $100g^{-1}$ of milk and lactate 35 mg Mg $100g^{-1}$ of milk) and six calcium compounds (80 mg Ca $100g^{-1}$ of milk) used for milk enrichment did not cause denaturation of milk proteins during pasteurization. It has been shown that the addition of calcium bisglycinate, calcium carbonate, calcium citrate and also magnesium bisglycinate causes alkalization of milk before fermentation. Moreover, calcium chloride, calcium D-gluconate and calcium lactate as well as magnesium L-pidolate, magnesium L-lactate, magnesium D-gluconate, magnesium acetate and magnesium chloride were indicated as milk acidifying compounds. After 24 hours of storage, all calcium or magnesium fortified beverages had the typical acidity characteristic for fermented milk beverages. Fortification of milk with calcium bisglycinate resulted in obtaining the highest optical density OD_{640} during fermentation using *Lactobacillus rhamnosus* and *Bifidobacterium* Bb-12. Milk fermented by *Lactobacillus rhamnosus* fortified and not fortified with calcium compounds was characterized by more intense growth of bacterial cells compared to milk fermented by *Bifidobacterium* Bb-12. Compounds that best stimulated the growth of *Bifidobacterium* Bb-12 cells during fermentation and on the first day of storage were: calcium chloride, calcium citrate and calcium carbonate. The highest level of cell survival of the *Bifidobacterium* Bb-12 strain was found in milk fortified with calcium carbonate, citrate and bisglycinate. Along with extending the storage time from one to twenty-one days, there was a reduction in the population of live cells of the *Bifidobacterium* Bb-12 strain and the *Lactobacillus rhamnosus* strain in fermented milk, both not fortified and fortified with calcium. The growth and survival of live *Lactobacillus rhamnosus* cells was stimulated by milk fortification with calcium carbonate, citrate, bisglycinate and D-gluconate. Of the six tested magnesium compounds, the addition of magnesium D-gluconate created the best conditions for the proliferation of *Bifidobacterium* Bb-12 cells during fermentation. However, milk fortification with magnesium bisglycinate had a particularly positive effect on the survival of *Bifidobacterium* Bb-12 cells during refrigerated storage. Milk fortification with magnesium

lactate created less favorable conditions for the proliferation of *Streptococcus thermophilus* in yogurts fermented by YC-X11, YF-L811 and probiotic VITAL. More *Lactobacillus bulgaricus*, *Lactobacillus acidophilu*s and *Bifidobacterium lactis* colonies were determined in probiotic yogurt fortified with magnesium lactate than in the control. In terms of the number of bacterial cells, yogurts and fermented milks fortified with calcium or magnesium met the minimum therapeutic criteria throughout the study period. Yogurts enriched with magnesium chloride and calcium carbonate were characterized by the most similar L*a*b* color parameters to the control yogurts. Yellow and green colors were found in all yogurts fortified with magnesium and calcium compounds. In most cases, enrichment with calcium compounds of milk fermented by *Bifidobacterium* Bb-12 and *Lactobacillus rhamnosus* probiotic monocultures caused a change in color to darker and reduced or not yellow color intensity. The added calcium compounds increased the proportion of green color, the intensity of which increased with increasing storage time.

In the milk fermented by *Bifidobacterium* Bb-12, magnesium acetate gave the most intense green and yellow color. The most effective reduction of yogurt syneresis resulted from the addition of magnesium chloride in a dose of 30 mg Mg $100g^{-1}$ of milk and addition of magnesium lactate in a dose of 35 mg Mg $100g^{-1}$. In milk fermented by *Bifidobacterium* Bb-12, the addition of magnesium D-gluconate or L-pidolate most effectively reduced whey leakage. Among calcium compounds that have proved to be the most effective in reducing syneresis of milk fermented by probiotic monocultures (*Bifidobacterium* Bb-12 or *Lactobacillus rhamnosus*), calcium citrate was indicated. The texture profile most similar to the control yogurts was obtained in yogurts with the addition of calcium carbonate. However, in milk fermented by *Lactobacillus rhamnosus*, the addition of calcium citrate gave texture features similar to the control milk. Milk with magnesium lactate fermented by *Bifidobacterium* Bb-12 also did not differ from the control in terms of the values obtained characterizing the texture components. The organoleptic assessment made it possible to determine which of the added magnesium or calcium compounds seem to be most useful for fortification of milk. In the opinion of the panelists, the VITAL yogurt enriched with magnesium lactate was characterized by the organoleptic properties most similar to the control yogurt. Magnesium D-gluconate gave a good consistency of milk fermented by *Bifidobacterium* Bb-12, especially smooth and homogeneous. Moreover, the addition of magnesium D-gluconate in this milk most effectively reduced the acidic taste and intensified the milky-cream taste. In the opinion of the panelists, among the calcium compounds used for the fortification of probiotic milk (*Bifidobacterium* Bb-12 and *Lactobacillus rhamnosus*), the addition of calcium citrate changed the organoleptic characteristics in the slightest degree.

The results obtained are very important when designing new functional dairy products that combine probiotic benefits with an increased content of magnesium or calcium. Beverages fermented and fortified with various magnesium compounds in the amount of 30 mg/35 mg Mg $100g^{-1}$ of milk or calcium compounds in the amount of 80 mg Ca $100g^{-1}$ can be a good source of supplementing mineral deficiencies in the daily human diet. According to the current Regulation of the European Parliament and of the Council (EU) No. 1169/2011, the daily reference value of calcium intake for adults is -800 mg, and for magnesium - 375 mg. Therefore, a proposed portion of 150 g of fermented beverage fortified with calcium compounds would constitute about 47% and fortified with magnesium compounds about 19-

21% of the daily reference intake value. Moreover, nutrition standards for the Polish population indicate that the daily requirement for calcium for adults should be about 1200 mg, and for magnesium about 420 mg (men) or about 320 mg (women). According to these recommendations, a proposed portion of 150g of fermented beverage fortified with calcium would account for about 31% of the daily reference intake, and for magnesium 17-19% (men) and 22-24% (women). According to the Regulation of the European Parliament and of the Council (EU) No. 1169/2011, the introduction of minerals in a significant amount must provide a minimum of 15% of the reference intake value, per portion, if the packaging contains only one portion. It can therefore be concluded, taking into account the content of these macronutrients in milk, that the use of calcium additive in the amount of 80 mg Ca $100g^{-1}$ of milk and magnesium in the amount of 30 mg/35 mg Mg $100g^{-1}$ of milk constitute a significant amount of minerals introduced into 1 portion of the proposed fermented beverage ensuring this minimum. The obtained study results allow for determination of new directions of research on the possibility of using macronutrients for the fortification of beverages fermented by other probiotic bacteria and using milk other than cow's.