The study of added prebiotics on b group vitamins concentration during milk fermentation

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ILZE BEITANE, INGA CIPROVICA
Latvia University of Agriculture, Faculty of Food Technology, 2 Liela Street, LV-3001, Jelgava, Latvia, Tel. +37163003547, Fax +37163022829, E-mail: Ilze.Bitane@llu.lv

Abstract
The effect of different concentrations of lactulose and inulin was studied during milk fermentation on B group vitamins: thiamin, riboflavin, pyridoxine and cobalamin concentration.

The added prebiotics concentrations significantly influence the content of vitamins B₁ and B₂ (p<0.05) in fermented milk samples. The highest concentration of vitamins B₁ and B₂ was observed in fermented milk samples with 3% of lactulose and 4% of inulin. There are not established significant differences between the analyzed concentrations of prebiotics. The concentration of vitamin B₆ in fermented milk is possible to increase (p>0.05) by adding lactulose in concentrations to 3% and inulin to 4%. The type and the concentration of added prebiotics have not significant influence (p>0.05) on the concentration of vitamin B₆ in fermented milk samples. The concentration of prebiotics has the significant influence on vitamin B₁₂ content in fermented milk (p<0.05). There are established significant differences between control and fermented milk samples with prebiotics in concentrations of 4% and 5%, it means to increase concentrations of added prebiotics suppress the vitamin B₁₂ synthesis in fermented milk.

Keywords: B group vitamins, prebiotics, fermented milk

Introduction
Growing consumer interest about nutrition for health and well-being provides the development of functional food market, where dairy-based functional foods account for nearly 43% of the market, which is almost entirely made up to fermented dairy products (ÖZER and KIRMACI [1]). Fermented functional products containing probiotics and prebiotics have important role in the development of functional foods. The adding probiotics and prebiotics increase the nutritional value of fermented milk product, due to synthesize of water soluble vitamins. Bifidobacterium strains are able to produce vitamins during milk fermentation (HUGENHOLTZ and SMID [2], CRITTENDEN et al. [3]). The ability to synthesize vitamins depends on the species of the microorganism (PAPASTOYIANNIDIS et al. [4]), which can be stimulated by adding prebiotics. Therefore the task of the research was to investigate the influence of lactulose and inulin on the ability of Bifidobacterium lactis to synthesize B group vitamins during milk fermentation.

Materials and Methods
The research was performed at the microbiological laboratory of the Department of Food Technology of Latvia University of Agriculture and at the laboratory of Biochemistry and Physiology of Animals of the Institute of Biology of the University of Latvia. Pasteurized milk with fat content 2.5% and the strain of Bifidobacterium lactis (Bb-12, Chr.Hansen, Denmark) were used for experiments. During the experiments, the culture was maintained at -18 °C. As prebiotics were used inulin RAFTILINE®HP (ORAFI, Belgium) with polymerization degree ≥5 and degree of purity 99.5% and syrup of lactulose (Duphalac®).
the Netherlands) with following composition (%): lactulose – no less than 67, lactose – less than 6, galactose – less than 10. Different lactulose and inulin concentrations (1; 2; 3; 4 and 5%) were added individually to 100 g of milk. Bifidobacterium lactis was inoculated with 2 ml of milk suspension (10^6 cfu ml⁻¹) and cultured at 37 °C for 16 hours. The control sample was prepared without the prebiotics for comparing results.

The content of B₁ and B₂ vitamin was determined by AOAC Official Method 986.27 and 970.65, B₆ vitamin – using J.Odincovas method (VULFA [5]) and B₁₂ vitamin – using Escherichia coli 113-3 (VALDMANIS [6]).

The differences in the concentrations of vitamins B₁, B₂, B₆ and B₁₂ were analyzed using the analysis of variance (ANOVA). t-test was applied to compare the mean values, and p-value at 0.05 was used to determine the significant differences. Experiments were carried out in triplicate.

**Results and Discussions**

During the fermentation process bifidobacteria are able to synthesize water soluble vitamins, however, the ability of species of Bifidobacterium genus is different. Consequently, Bifidobacterium lactis ability to synthesize vitamins B₁, B₂, B₆ and B₁₂, as well as the content of synthesized vitamins influenced by the type and concentration of the added prebiotics were studied.

The content of vitamin B₁ in fermented milk samples depending on the added lactose and inulin concentration is presented in Figure 1.

![Figure 1](image_url)  
**Figure 1.** The influence of lactulose and inulin concentrations on the content of vitamin B₁ in fermented milk samples

The results of the research show that the content of vitamin B₁ significantly increases in fermented milk samples by adding lactulose (p-value =0.0001<0.05) and inulin (p-value=0.0032<0.05). There is not found the significant influence of the prebiotic type on the content of vitamin B₁ in fermented milk (p-value=0.48>0.05). Comparing the obtained results (Figure 1) with the content of vitamin B₁ in milk mentioned in literature (0.4 mg·kg⁻¹) (ГОРБАТОБА [7]; CHANDAN [8]; RAYNAL-LJUTOVAC et al. [9]), it is seen that there is a radical decrease of the content of vitamin B₁ (0.21 mg·kg⁻¹). It could be explained that water soluble vitamins, including B₁, are necessary for the growth of bifidobacteria (DEGUCHI et al. [10]). The radical decrease of the content of vitamins is possible to diminish by adding prebiotics. Besides, the differences between milk and control may be
explained by the influence of thermal treatment where the concentration of thiamin decreases by 10% (OTTAWAY [11]). The obtained results show evidence that prebiotics facilitate production of vitamin B₁ and significantly increases its concentration in the final product.

Analyzing the content of vitamin B₂ in the fermented milk samples with prebiotics (Figure 2), similar tendencies can be observed.

![Figure 2](image_url)

**Figure 2.** The influence of lactulose and inulin concentrations on the content of vitamin B₂ in fermented milk samples

The presence of lactulose (p-value=0.0005<0.05) and inulin (p-value=0.0069<0.05) significantly increased the content of vitamin B₂ in fermented milk samples. The type of prebiotics has not the significant effect (p-value=0.422>0.05) on the content of vitamin B₂ in fermented milk samples.

Comparing the obtained results with the content of vitamin B₂ in milk which is indicated in literature (1.5-1.7 mg·kg⁻¹) (ГОРБАТОВА [7]; CHANDAN [8]; RYNAL-LJUTOVAC et al. [9]), it is apparent that with certain concentrations of prebiotics it is possible to increase the content of vitamin B₂ in fermented milk samples. It relates to the studies reported in literature on the ability of bifidobacteria to synthesize water soluble vitamins, including B group vitamins (DEGUCHI et al. [10]). This should be evaluated as a positive aspect if you take into consideration that during the thermal treatment the content of vitamin B₂ in milk decreases. It follows that in the presence of certain prebiotics, it is possible significantly increase the content of vitamin B₂ in the sample.

The results of vitamin B₆ in fermented milk samples with prebiotics (Figure 3) show that the higher indices are reached in milk samples with prebiotics in certain concentrations – for lactulose from 1% to 3% (0.51 to 0.60 mg·l⁻¹) and for inulin from 1% to 4% (0.49 to 0.58 mg·l⁻¹).

In the higher lactulose and inulin concentration (5%), the content of vitamin B₆ is 0.30 mg·l⁻¹ and 0.28 mg·l⁻¹, respectively. It is remarkably lower than that of control (0.42 mg·l⁻¹). As it is known, the content of dry matter ranges from 11% to 14% (OZOLA and CIPROVICA [12]) in milk, but in fermented milk samples from 14% to 18% if prebiotics are added in concentrations up to 5%. If the content of dry matter is increased, the water activity in the product is changed and multiplication of microorganisms is prolonged, particularly Gram-positive ones. *B.lactis* abilities to produce vitamin B₆ decrease if 5% of lactulose or inulin is added. The content of vitamin B₆ decreases in these samples up to 0.28 and 0.30 mg·l⁻¹, respectively.
Figure 3. The influence of lactulose and inulin concentrations on the content of vitamin B\(_6\) in fermented milk samples

By applying t-test, it was established that neither presence of lactulose (\(p\)-value=0.35>0.05) or inulin (\(p\)-value=0.21>0.05) influences significantly the content of vitamin B\(_6\) in fermented milk samples. The vitamin B\(_6\) content in milk varies from 0.4 to 0.6 mg·1\(^{-1}\) (TOPBATOA [7]; CHANDAN [8]; RAYNAL-LJUTOVAC et al. [9]), it is possible to increase the content of vitamin B\(_6\) in fermented milk by adding lactulose and inulin. Despite the fact mentioned in literature that vitamin B\(_6\) needs for successfully growth of bifidobacteria (DEGUCHI et al. [10]; BALLONGUE [13]), the results showed that it is possible to increase the vitamin B\(_6\) content in fermented milk by adding prebiotics.

The content of vitamin B\(_{12}\) in fermented milk samples with prebiotics is presented in Figure 4.

Figure 4. The influence of lactulose and inulin concentrations on the content of vitamin B\(_{12}\) in fermented milk samples

The obtained results have shown that the content of vitamin B\(_{12}\) in fermented milk samples with different concentrations of lactulose and inulin decreases, with the exception of the sample with 3% of lactulose. In higher concentrations (4% to 5% lactulose and 3% to 5% inulin) the content of vitamin B\(_{12}\) in fermented milk samples decreases up to 0.01 mg·1\(^{-1}\). The decrease of vitamin B\(_{12}\) may be explained with the needs of bifidobacteria for growing and multiplication (BALLONGUE [13]). It follows that during fermentation vitamin B\(_{12}\) synthesis is essentially less in comparison with its usage for providing the life processes of bifidobacteria cells.
The results of dispersion analysis have shown that the concentration has significant effect on the vitamin B₁₂ content in fermented milk (p<0.05). There are significant differences between control and fermented milk samples with prebiotics in concentrations of 4% to 5%.

Whereas between the concentrations of lactulose in fermented milk samples were not significant differences of vitamins B₁, B₂ and B₆ content, except vitamin B₁₂. The research results showed that 3% concentration of lactulose could be taken for the optimal concentration. There were the highest content of vitamins B₁, B₂ and B₁₂ in fermented milk samples.

Among the fermented milk samples with inulin the highest content of B group vitamins were determined by different concentrations of inulin (3% and 4%). Therefore it is difficult to mention one optimal concentration for inulin.

Conclusions

The added prebiotics concentrations significantly influenced the content of vitamins B₁, B₂ and B₁₂ (p<0.05), whereas they did not have a significant effect of vitamins B₆ concentration (p>0.05) in synbiotic fermented milk sample.

The optimal concentration of lactulose is 3%, which is confirmed by the highest content of vitamins B₁, B₂ and B₁₂ in fermented milk samples, whereas it is not possible to mention one optimal concentration of inulin.

References

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