THE STUDY OF CARBOHYDRATES FERMENTATION ABILITY OF *B.LACTIS* IN MILK

Ilze Beitane*, Inga Ciprovica

Latvia University of Agriculture, Faculty of Food Technology, Jelgava, LV-3007, Latvia
*Tel. +37163005647, Fax +37163022829, *e-mail: Ilze.Beitane@llu.lv*

Abstract

The ability of *Bifidobacterium lactis* (Bb-12) to hydrolyse lactose, lactulose and inulin was studied during milk fermentation. For this purpose, the content of lactose, lactulose and inulin was determined before and after fermentation of milk samples. Pasteurized milk, freeze-dried culture Bb-12 (Chr. Hansen, Denmark), inulin - RAFTILINE®HP (ORAFI, Belgium) with polymerization degree ≥5 and degree of purity 99.5%, syrup of lactulose (Duphalac®, the Netherlands) were used for experiments. The different concentrations (1; 2; 3; 4 and 5%) of lactulose and inulin were used for studying of *B.lactis* ability to assimilate of milk sugar and prebiotics during fermentation. The fermentation process of milk samples enriched with lactulose or inulin was produced at 37 ^{o}C for 16 hours. The content of lactose and lactulose was determined by IDF standard 147B:1998 procedure, the content of inulin by AOAC Official Method 999.03 and by AACC Official Method 32.32.

Results showed that bifidobacteria poorly assimilate lactose at the presence of prebiotics in milk. The lactose assimilation decreases together with increase of added prebiotics concentration in milk. However bifidobacteria are able to hydrolyse up to 50% of lactulose in the product, except sample with 5% of lactulose. There was a decrease of lactulose by 2/3. The changes of lactulose content are significant ($p<0.05$). Consequently *B.lactis* possesses ability to assimilate lactulose. Inulin assimilation degree in fermented milk samples was low (10–20%), because it depends on the inulin polymerization degree and the degree of purity. The inulin assimilation decreases together with the increase of inulin polymerization degree and the degree of purity. The obtained results confirm that most suitable substrate for growing of bifidobacteria in milk is lactulose at any analyzed concentration.

Key words: bifidobacteria, lactose, inulin, lactulose, fermented milk

Introduction

Bifidobacteria are the most popular probiotics, they have been associated with health promoting effects. Many studies suggest that prebiotics such fructo-oligosaccharide, inulin, galacto-oligosaccharide, lactulose, isomalto-oligosaccharide are able to stimulate the growth of probiotic bacteria as well bifidobacteria (Özer et al., 2005; Martinez-Villaluenga et al., 2006) but not all prebiotics are suitable substrate for growing of bifidobacteria in milk. Bifidobacteria ferment various types of carboxydrates, the fermentation ability depends on the species. One of most popular species of *Bifidobacterium spp*., which is used for production of dairy products, is *B.lactis*. *B.lactis* is isolated from animal faeces and adapted in milk (Klein et al., 1998). It possesses a relative oxygen and acid tolerance which is not observed in some *Bifidobacterium* species. Therefore, *B.lactis* is able to grow in milk. Many studies indicate different results about the ability of bifidobacteria to hydrolyze prebiotics. Semjonovs et al. (2004) observed that *B.lactis* is not capable of utilizing inulin, whereas levan can be metabolized in relatively small amounts. Kontula et al. (1999) reported that bifidobacteria are able to utilize lactulose and lactitol, Reyed (2007) indicated that bifidobacteria can ferment lactose and lactulose, too. Therefore, the objective of this study was to investigate the ability of *Bifidobacterium lactis* to hydrolyse lactose, lactulose and inulin.

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 the Department of Microbiology and Biotechnology of the Faculty 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) was used for experiments. During the experiments, the culture was maintained at -18 ^{o}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 with the obtained results.
The content of lactose and lactulose was determined by IDF standard 147B:1998 procedure, the content of inulin by AOAC Official Method 999.03 and by AACC Official Method 32.32.

Results and Discussion
Martinez-Villaluenga et al. (2006) have indicated that *B.lactis* is characterized by a pronounced ability to ferment lactulose in concentrations from 0.5% to 2%, Özer et al. (2005), in turn, have stressed that *Bifidobacterium bifidum* BB-02 and *Lactobacillus acidophilus* LA-5 more effectively assimilated lactulose, if compared with inulin. Taking into consideration these authors' conclusions and many contradictory data in literature, the content of lactose, lactulose and inulin in milk was determined before and after fermentation. The content of lactose in milk before and after fermentation is shown in Figures 1 and 2.

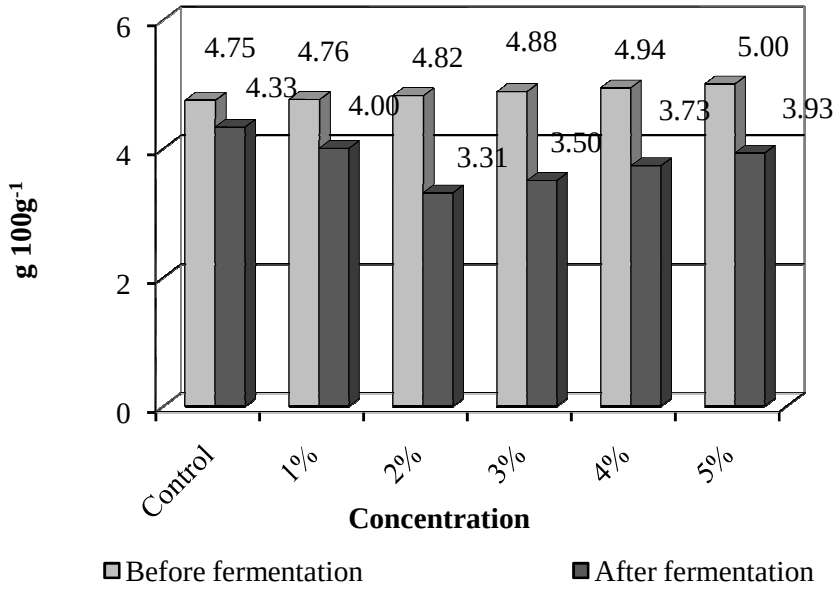

Figure 1. The content of lactose in milk before* and after fermentation depending on the concentration of lactulose

*The increasing of the content of lactose in milk samples before fermentation is connected with the presence of lactose in lactulose syrup.

The obtained results confirm the conclusions mentioned in literature that bifidobacteria poorly assimilate lactose (Modler, 1994). As it is seen in Figure 1, during the fermentation bifidobacteria have been able to utilize 0.42 g 100 g^{-1} of lactose in control sample. In the milk samples with lactulose, in turn, the changes of lactose content depend on the added lactulose concentration. It is reported in literature that lactose assimilation depends on the added bifidobacteria species, and with the higher assimilation ability are *B.bifidum*, *B.breve* and *B.infantis* (Lamoureux et al., 2002). It is possible to facilitate the process by adding prebiotics. In order to evaluate the effect of lactulose concentration on lactose assimilation in the samples, a dispersion analysis was applied. The obtained results show that different lactulose concentrations do not have the significant effect on the ability of bifidobacteria to

assimilate lactose (p>0.05) during fermentation. Evaluating the decrease of lactose content in the analyzed control and samples, and comparing with the initial lactose content in milk, it should be considered as significant (p<0.05). That could be explained by *B.lactis* properties. *B.lactis* is adapted in milk (Klein et al., 1998), consequently *B.lactis* is able to grow in milk and use lactose as a nutrient for the cell energy metabolism.
Similar tendencies are could observe to analyze the data in Figure 2.

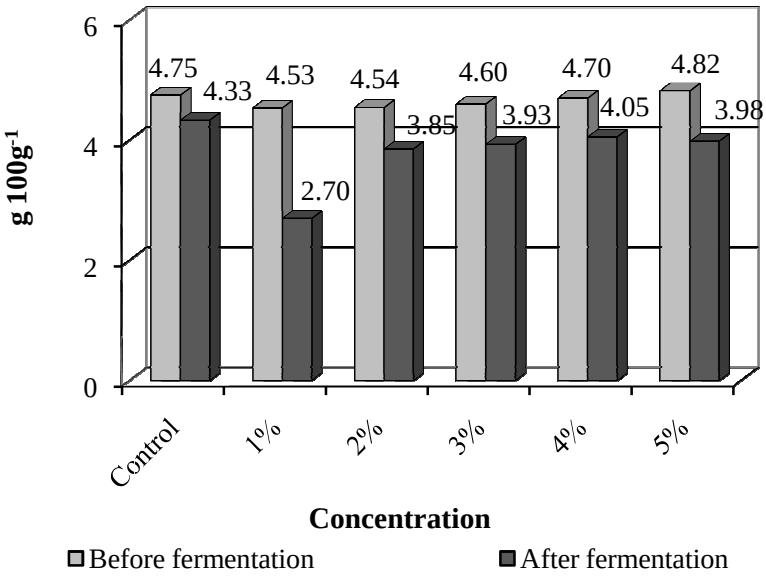

Figure 2. The content of lactose in milk before* and after fermentation depending on the concentration of inulin

*The changes of the content of lactose in milk samples before fermentation are connected with the composition of inulin. The content of glycose in inulin is calculated into the total content of lactose according to the standard method for determination of lactose.

In the sample with 5% of inulin, lactose assimilation increases, however, there are not established significant differences between the fermented milk samples with 2%, 3% and 4% of inulin and with control samples (p>0.05).
The lactose content in milk samples with inulin and control before and after fermentation (Figure 2) was established as significant (p<0.05). However, it should be remarked that lactose assimilation depends on the type and concentration of the added prebiotics. In literature can find indications about the ability of bifidobacteria to assimilate lactulose (Özer et al., 2005) and derivatives of raffinose (Martinez-Villaluenga et al., 2006). When evaluating results, it should be taken into consideration that *B.lactis* is adapted in milk and consequently the speed of multiplication in milk is higher than the other bifidobacteria species. It does explain the decrease of lactose content in fermented milk samples.
The lactulose and inulin content is analysed in the research before and after milk fermentation in order to be able to find out regularities among lactose, lactulose or inulin assimilation in milk under the influence of *B.lactis*. The content of lactulose in milk before and after fermentation is reflected in Figure 3.
The obtained results show that bifidobacteria are able to ferment up to 50% of lactulose in the product, except milk sample with 5% of lactulose where there was a decrease of lactulose by 2/3. When the lactulose concentration increases, the lactulose assimilation also increases in milk and resulting in the increase of *B.lactis* in samples that indicates to a mutual interaction. The obtained results confirm conclusions mentioned in literature about the bifidogenic effect

of lactulose (Palframan et al., 2002; Bouhnik et al., 2004) and bifidobacteria ability to assimilate lactulose (Saarela et al., 2003).

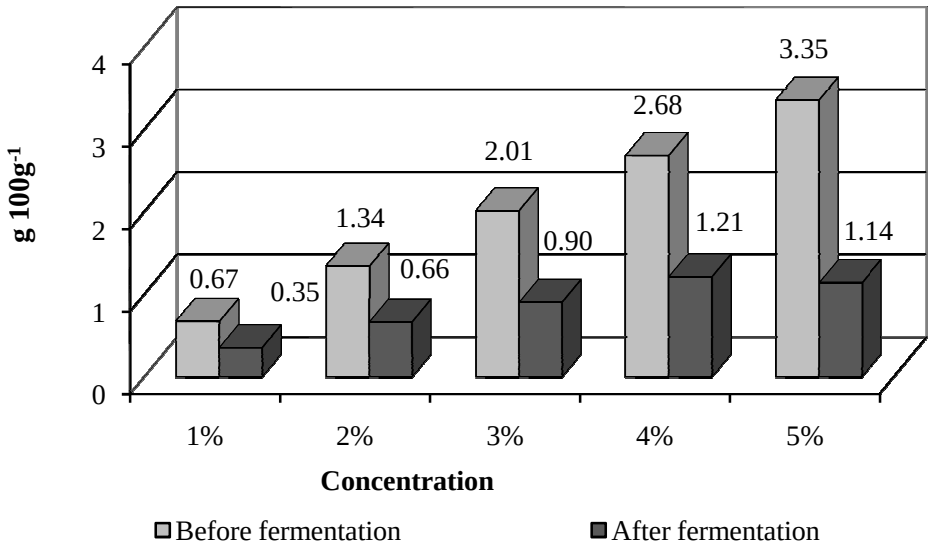

Figure 3. The content of lactulose in milk before and after fermentation

The changes of lactulose content are significant ($p<0.05$). Consequently, a conclusion can be drawn that *B.lactis* possesses ability to assimilate lactulose. It relates to conclusions found in literature that bifidobacteria better multiply in the presence of lactulose (Rycroft et al., 2001). Kontula et al. (1999), in turn, indicated that several microorganisms of the large intestine, including also bifidobacteria, are able to utilize lactulose and lactitol.

When comparing the changes of lactose and lactulose content in the fermented milk samples, it is apparent that *B.lactis* is able better to assimilate lactulose (the content of assimilated lactulose increases from 47% to 66%) in comparison with lactose (the content of assimilated lactose is from 9% to 31%). Evaluating the obtained data, it could be concluded that *B.lactis* in combination with lactulose is suitable for the development of a synbiotic dairy product.

The content of inulin in milk before and after fermentation is given in Figure 4.

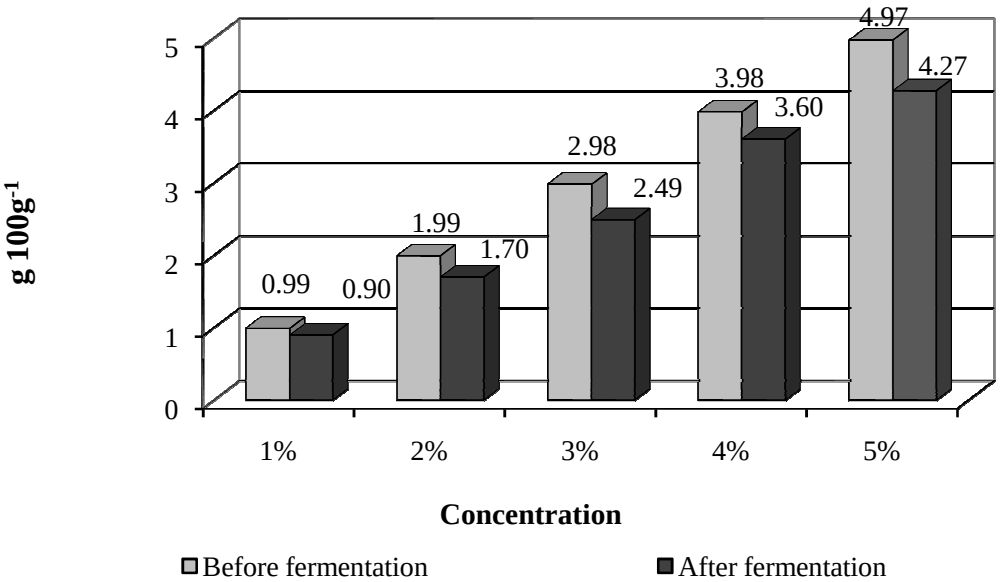

Figure 4. The content of inulin in milk before and after fermentation

The obtained results confirm that bifidobacteria poorly assimilate inulin (10–20%). It is reported in literature that *B.lactis* cannot assimilate inulin (Semjonovs et al., 2004). Biedrzycka and Bielecka (2004) have indicated that the ability of bifidobacteria to assimilate inulin is depending on the polymerization degree and the degree of purity. The inulin assimilation decreases together with the increase of inulin polymerization degree and the degree of purity. That does explain the results of inulin assimilation degree in fermented milk samples.

Conclusions

1. The ability of *B.lactis* hydrolyse lactose depends on the type and concentration of added prebiotics.
2. *B.lactis* is characterized as being able to assimilate lactulose to 66% in comparison with lower assimilation level of lactose (to 37%) and inulin (to 20%).
3. Lactulose should be considered as the most suitable substrate for growing of *B.lactis* in milk.

References

1. Biedrzycka, E., Bielecka, M. (2004) Prebiotic effectiveness of fructans of different degrees of polymerization. *Trends in Food Science&Technology*, 15, pp. 170–175.
2. Bouhnik, Y., Attar, A., Joly, F.A., Riottot, M., Dyard, F., Flourie, B. (2004) Lactulose ingestion increases faecal bifidobacterial counts: A randomised double-blind study in healthy humans. *European Journal of Clinical Nutrition*, 58, pp. 462–466.
3. Klein, G., Pack, A., Bonaparte, C., Reuter, G. (1998) Taxonomy and physiology of probiotic lactic acid bacteria. *Int. J. Food Microbiol.*, 41, pp. 103–125.
4. Kontula, P., Suihko, M.L., Von Wright, A., Mattila-Sandholm, T. (1999) The effect of lactose derivatives on intestinal lactic acid bacteria. *Journal of Dairy Science*, 82, pp. 249–256.
5. Lamoureux, L., Roy, D., Gauhiert, S.F. (2002) Production of oligosaccharides in yogurt containing bifidobacteria and yogurt cultures. *Journal of Dairy Science*, 85, pp. 1058–1069.
6. Martinez-Villaluenga, C., Frias, J., Gomez, R., Vidal-Valverde, C. (2006) Influence of addition of raffinose family oligosaccharides on probiotic survival in fermented milk during refrigerated storage. *International Dairy Journal*, 16, No 7, pp. 768–774.
7. Modler, H.W. (1994) Bifidogenic factors – Sources, Metabolism and Applications. *International Dairy Journal*, 4, pp. 383–407.
8. Özer, D., Akin, S., Özer, B. (2005) Effect of Inulin and Lactulose on Survival of *Lactobacillus Acidophilus* LA-5 and *Bifidobacterium Bifidum* BB-02 in Acidophilus-Bifidus Yoghurt. *Food Sci. Tech. Int.*, 11, No 1, pp. 019–6.
9. Palframan, R.J., Gibson, G.R., Rastall, R.A. (2002) Effect of pH and dose on the growth of gut bacteria on prebiotic carbohydrates *in vitro*. *Anaerobe*, 8, pp. 287–292.
10. Reyed, R.M. (2007) Isolation, identification of human autochthonous bifidobacteria and comparison of its growth on different natural food products. *The Internet Journal of Microbiology*, Vol. 3, Number 2.
11. Rycroft, C.E., Jones, M.R., Gibson, G.R., Rastall, R.A. (2001) A comparative *in vitro* evaluation of the fermentation properties of prebiotic oligosaccharides. *Journal of Applied Microbiology*, 91, pp. 878–887.
12. Saarela, M., Hallamaa, K., Mattila-Sandholm, T., Mättö, J. (2003) The effect of lactose derivatives lactulose, lactitol and lactobionic acid on the functional and technological properties of potentially probiotic *Lactobacillus* strains. *International Dairy Journal*, 13, pp. 291–302.
13. Semjonovs, P., Marauska, R., Linde, R., Grube, M., Zikmanis, P., Bekers, M. (2004) Development of *Bifidobacterium lactis* Bb12 on β-(2,6)-Linked Fructan-Containing Substrate. *Eng.Life Sci.*, 4, No 5, pp. 433–437.