FOOD SCIENCES

THE CHANGES OF THE CONCENTRATIONS OF THIAMIN AND RIBOFLAVIN IN MILK ENRICHED WITH PREBIOTICS AND PROBIOTICS

Ilze Beitāne, Inga Ciproviča
Latvia University of Agriculture
e-mail: ptfuzt@llu.lv

Abstact

Fermentation of milk with probiotics and prebiotics has been shown to increase the concentration of B group vitamins in fermented milk products.

The task of the study was to investigate the influence of prebiotics on the increasing of thiamin and riboflavin concentrations in milk during fermentation by *Bifidobacterium lactis*.

Lactulose (Duphilac, Netherlands) and inulin (Raftiline, Belgium) were added (1, 2, 3, 4, and 5%) into 100 g of milk. The milk samples were inoculated with *Bifidobacterium lactis* (BB-12, Chr. Hansen, Denmark) and incubated at 38 °C for 16 hours. The amount of thiamine and riboflavin was determined by fluorometric method.

Laboratory studies have shown that the increasing of thiamin and riboflavin concentrations in milk depends on the concentration of lactulose and inulin.

Data on the final thiamin and riboflavin concentrations in fermented milk indicates that increasing the lactulose concentration from 1% to 3% enhanced the production of thiamin and riboflavin. The similar data was obtained with inulin. Increasing of inulin concentration from 1% to 4% enhanced the production of vitamins thiamin and riboflavin.

The present results furthermore indicate that finding optimal combination of prebiotics and probiotic pairs where the prebiotic would benefit the specific probiotic strain, e.g. during production and formulation into foods, is one of the research priority for functional foods.

Key words: thiamin, riboflavin, inulin, lactulose, bifidobacteria, milk.

Introduction

Vitamins are organic substances that are present in human natural foods. They cannot be made by the body itself and are essential for growth, maintenance and normal metabolism.

Whole cows' milk contains about 37 mg of thiamine 100^{-1}g, mostly in the free form. The main physiological function of thiamin is to serve as a cofactor in intermediary metabolism, i.e. in the oxidative decarboxylation of a-ketoacids and in transketolase reactions. When looking at changes in thiamin concentration during milk processing, the most dramatic reductions occur when high temperatures are applied over a long period of time or when the product is stored over an extended period. The best dietary sources of thiamin are pork, beef, whole grain cereals, and legumes. Milk can contribute up to 20% to the daily thiamine requirements of adults in Latvia where milk and dairy products are commonly consumed.

Milk and milk products are important sources of riboflavin in human nutrition, accounting for up to 70% of total daily intake in industrial countries. Riboflavin is heat stable in the absence of light, but extremely photosensitive. As it is known, dairy products compile the important part of daily intake, therefore dairy products are considered as one of the most import sources of B group vitamins, which provide significant part of B group vitamins in the total daily intake.

The amount of thiamin and riboflavin in milk can increase during fermentation using lactic acid bacteria. As it is known about the possibilities to increase the concentration of B group vitamins, one of the most important questions is to determine the suitable concentrations of prebiotics for stimulation of the growth of *Bifidobacterium lactis* in milk and to achieve the higher concentrations of thiamin and riboflavin in fermented milk. Probably, there is connection between the growth of bifidobacteria and the synthesis of thiamin and riboflavin during fermentation, depending on the concentration of the prebiotic. Summarizing the above mentioned information, the task of the study was to investigate the influence of prebiotics on the increasing of thiamin and riboflavin concentrations in milk during fermentation by *Bifidobacterium lactis*.

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 University of Latvia.

The strain of *Bifidobacterium lactis* (BB-12, Chr.Hansen, Denmark) was used. During the experiments, the culture was maintained at - 18 °C.

The lactulose syrup (Duphalac®, Netherland) was used for growing of bifidobacteria in milk. The composition of the

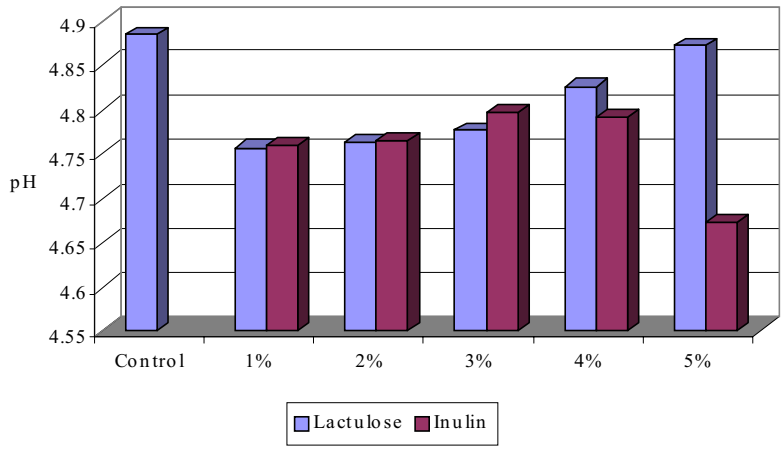

Figure 1. pH of fermented milk samples with different concentrations of prebiotics.

syrup of lactulose was as follows (%): lactulose 67, lactose less than 6, galactose less than 10.

The inulin RaftilineāHP (ORAFI Active Food Ingredients, Belgium) was used for growing of bifidobacteria in milk. The composition of inulin was as follows (%): inulin - more than 99.5; glucose, fructose and sucrose - less than 0.5.

Control sample without inulin and lactulose was prepared for comparison of obtained results.

Bifidobacterium lactis was incubated in milk. Different lactulose and inulin contents (1, 2, 3, 4, and 5%) were added individually in 100 g of milk. *Bifidobacterium lactis* was inoculated with 2 ml of milk suspension (about $1*10^6$ bifidobacteria) and cultured at 36 °C for 16 hours.

pH of fermented milk was determined by pH-meter WTW series inoLAB pH 720. The amount of thiamin and riboflavin was determined by fluorometric methods. Thiamin was determined according to AOAC Official Standard Method 986.27 and riboflavin – AOAC Official Standard method 970.65.

Fermentations were performed in triplicate, and the analyses were carried out in duplicate. The data given here are the mean values of the measurements.

Results and Discussion

In contrast with lactobacilli, bifidobacteria exhibit a weak growth in milk or do not grow at all in milk (Mizota, 1996). The addition of prebiotics such as inulin and lactulose in milk stimulates growth of bifidobacteria (Rastall et al., 2002, Strohmaier, 1998).

The changes of pH value (Figure 1) is an important factor in milk fermentation by bifidobacteria. The chemical composition of the fermentation medium for growth (for instance, the carbohydrate source, total solid content, availability of nutrients and growing parameters, dissolved oxygen content) and the cultivation conditions (for instance, the level of inoculation, the incubation temperature, the fermentation time) can influence changes of pH value. The final active acidity may affect the viability of probiotic organisms in fermented products. As obtained results showed, medium active acidity can be increased by addition of 5% of inulin and 2-3% of lactulose.

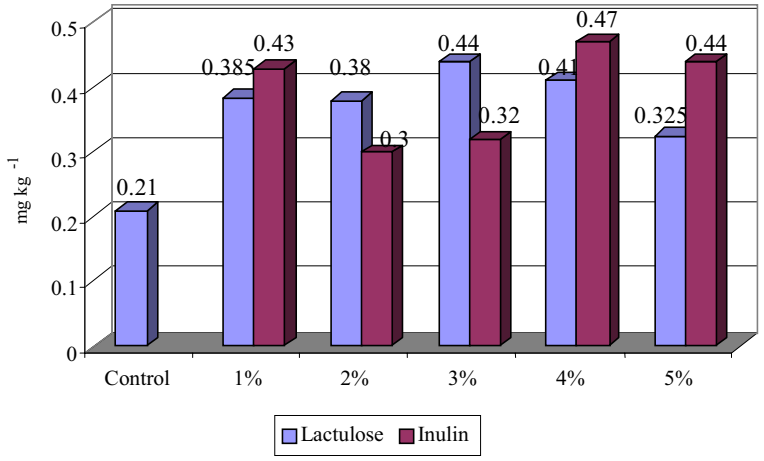

Figure 2. Thiamin concentration in fermented milk samples.

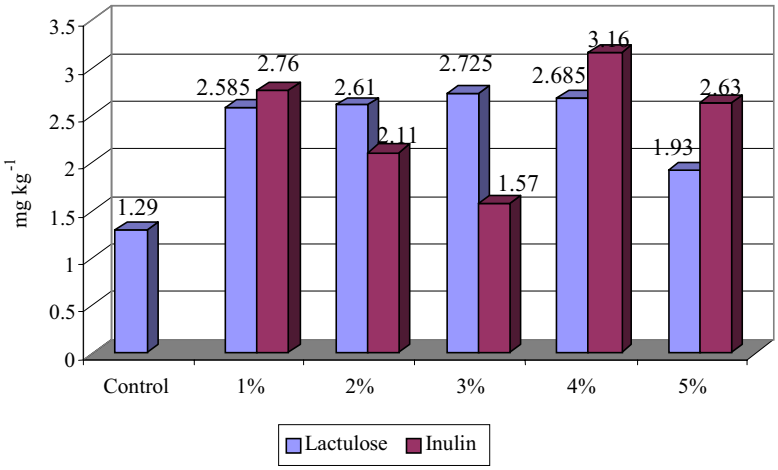

Figure 3. Riboflavin concentration in fermented milk samples.

Shin, Lee, Petska, and Ustunol (2000) were able to show that adding large quantities (5%) of fructo-oligosaccharides to skimmed milk enhanced the survival of the two *Bifidobacterium* strains and promoted the development of acidity of skimmed milk samples. One of the explanations of the above mentioned conclusion is the chemical composition of fructo-oligosaccharides and lacto-oligosaccharides and the ability of bifidobacteria to ferment that substrate.

The changes of the concentrations of thiamin and riboflavin in fermented milk samples depending on the concentration of the prebiotic are showed in Figures 2 and 3.

One of the most important properties of bifidobacteria is ability to produce different concentrations of B group vitamins (Roberfroid, 2002). As it is known, bifidobacteria exhibit a weak growth in milk and the addition of growth promoting factors would stimulate not only the development of bifidobacteria but would also promote the increasing of the concentrations of B group vitamins in milk (Modler, 1994). Our hypothesis is that there is connection between the growth of bifidobacteria and the synthesis of thiamin and riboflavin during milk fermentation depending on the concentration of prebiotic. With the exception of some B group vitamins, for example, thiamin, riboflavin, cobalamin and pyridoxine, changes in the vitamin and mineral content of fermented milk products are negligible. Moreover, the pasteurisation of milk prior to fermentation may destroy some vitamins such as thiamin, riboflavin, pyridoxine and cobalamin while the level of thermostable vitamins (niacin and pantothenic acid) remains unchanged.

Laboratory studies have shown that the level of thiamin and riboflavin in fermented milk depends on the concentration of prebiotics and probiotic properties and may be lower or higher than in control sample and non-cultured milk.

Data on the final thiamin and riboflavin concentrations in fermented milk indicate that increasing the lactulose concentration from 1% to 3% enhanced the production of thiamin and riboflavin, but increasing the lactulose concentration from 4% to 5% decreased the production of thiamin and riboflavin. Similar data was obtained with inulin. Increasing of inulin concentration from 1% to 4% enhanced the production of thiamin and riboflavin, but increasing the inulin concentration till 5% decreased the production of thiamin and riboflavin. From the investigation of R.Palframan, G.Gibson and R.Rastall was observed that FOS and inulin demonstrated the greatest bifidogenic effect at pH 6.8 and 1% (w/v) carbohydrate, whereas GOS, IMO and lactulose demonstrated their greatest bifidogenic effect at pH 6 and 2% (w/v) carbohydrate. From this we can try to explain also our research results.

For detection of most suitable concentrations of inulin and lactulose there are planned to determine the concentrations of pyridoxine and cobalamin in fermented milk.

Conclusions

1. The addition of large quantities (till 5%) of inulin to milk enhanced the survival of *Bifidobacterium lactis* and increased pH of fermented milk.

2. The increasing of concentrations of thiamin and riboflavin in milk is dependent on the ability of bifidobacteria to grow in milk enriched with different concentrations of lactulose and inulin.

3. The most suitable concentrations of inulin and lactulose are 4% and 3% based on the results of optimal concentrations of thiamin and riboflavin.

References

1. Mizota T. (1996) Lactulose as a growth promoting factor for Bifidobacterium and its physiological aspects. International Dairy Federation, Bulletin 313, pp. 43-48

2. Modler H.W. (1994) Bifidogenic factors – Sources, Metabolism and Applications. International Dairy Journal, 4, pp. 383-407.
3. Rastall R.A., Maitin V. (2002) Prebiotics and synbiotics: towards the next generation. Current Opinion in Biotechnology 13: pp. 490-496
4. Roberfroid M. (2002) Functional food concept and its application to prebiotics. Digest Liver Dis 34 (Suppl.2): S 105-110.
5. Shin H.S., Lee J.H., Petska J.J. and Ustunol Z. (2000) Growth and viability of commercial Bifidobacterium spp. in skim milk containing oligosaccharides and inulin. Journal of Food Science 65: pp. 884-887.
6. Strohmaier W. (1998) Lactulose: Status of health-related applications. International Dairy Federation, Bulletin 9804, pp. 262-271.
7. 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