Original scientific paper - Izvorni znanstveni rad UDK: 637.344/579.86

# The influence of lactulose on growth and survival of probiotic bacteria *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 in reconstituted sweet whey

*Bojan Matijević*[1]*, *Rajka Božanić*[2], *Ljubica Tratnik*[2]

[1]Department of Food Technology, Polytechnic of Karlovac, Trg J.J. Strossmayera 9, Karlovac
[2]Laboratory for Technology of Milk and Dairy Products, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, Zagreb

Prispjelo - Received: 13.01.2009.
Prihvaćeno - Accepted: 05.02.2009.

**Summary**

This research was examined the influence of lactulose, a well-defined prebiotic, on the growth and activity of probiotic bacteria *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 in reconstituted sweet whey as well as their survival in fermented whey during 28 days of cool storage. Reconstituted sweet whey was supplemented with 5 and 10 g/kg lactulose. Fermentation of whey with *Bifidobacterium animalis* subsp. *lactis* BB-12 was about 1.8 h shorter (approximately 11 h) in comparison to fermentation with *Lactobacillus acidophilus* La-5 (approximately 13 h). Lactulose addition in reconstituted sweet whey prolonged the time of fermentation in both bacteria species, but it did not influence on the viable cells count at the end of fermentation. *Lactobacillus acidophilus* La-5 better grew (Δlog CFU/mL = 1.25) during fermentation in comparison with *Bifidobacterium animalis* subsp. *lactis* BB-12 (Δlog CFU/mL = 0.27), regardless to the added amount of lactulose. During storage of fermented whey viable cells count of species *Bifidobacterium animalis* subsp. *lactis* BB-12 was more stable than count of *Lactobacillus acidophilus* La-5. The obtained results show that lactulose, as a well-defined prebiotic did not have a significant effect on fermentation and survival of *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 in whey, regardless to the added amount.

*Key words*: *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus acidophilus*, lactulose, fermentation, whey

## Introduction

Whey is a by-product during the production of cheese or casein. Although whey is as old as cheese, this valuable liquid was firstly thrown away, and later was used only as an animal feed (Pomeranz, 1992). Its usage as kind of human food started after the finding out that whey is valuable source of nutrients. Usually about 50% of dry matter from milk transfers into whey (Tratnik, 1998).

Despite many problems, for a long time attempts were made for using whey in human diet, because of its valuable ingredients and the constantly increasing available quantities. Traditional way of using whey in human diet has been the production of whey cheese or beverages producted from whey. Today, whey powder has been used as an additive for different products like yoghurt, milk based spreads and bread (Jelen, 2003). Because of the high water content (about 94%) processing whey in different beverages is supposed to be the most acceptable alternative (Jeličić et al., 2008). Whey is also a good medium for the growth of many bacteria and it can be raw material for production of fermented beverages (Maity et al., 2008) which is nutritiously more valuable than juices without whey addition.

Definition of probiotic has been changed several times. The last definition of probiotic was given by the European Expert Committee - "Live microorganisms which if consumed in certain amount (at least $10^9$ CFU per day) cause the improvement of health above the boundaries of normal diet" (Guarner and Schaafsma, 1998). To improve growth and efficiency of probiotic bacteria, selective sources of carbon and energy are trying to be introduced (Benković et. al., 2008). These selective, nutritious substances are called

*Corresponding author/Dopisni autor: Phone/Tel.: +385 47 843 530; E-mail: mikrobiolog@yahoo.com

prebiotic, and they are defined as: "Indigestible ingredients in food which are useful for the host by selective stimulation of growth and/or activity of one bacteria kind or limited number of bacteria kinds in the large intestine, and by doing that they improve the health of people" (Gibson and Roberfroid, 1995). The most known prebiotics are inulin and lactulose (Gibson, 2004; Scantlebury-Manning and Gibson, 2004).

Lactulose is disaccharide made of galactose and fructose (4-O-β-D-galactopyranosyl-D-fructose), and has molecular formula ($C_{12}H_{22}O_{11}$) and molar mass (342.3 g/mol) identical to lactose. It is formed from lactose during heat processing of milk (Tratnik, 1998) or by alkali isomerisation (Mussatto and Mancilha, 2007). Unlike all the other prebiotics, lactulose found its first usage in medicine in treating hepatic encephalopathy and constipation (Schuman, 2002), and only later its prebiotic characteristics were recognized. Literature states lactulose as bifidus factor because it stimulates the growth of bifidobacteria (Shah and Lankaputhra, 2003), and some species of *Lactobacillus*, *Clostridium* and *Peptostreptococcus* in the large intestine in people (Hoffman et al., 1964; Sako et al., 1999; Kneifel et al., 2000). There are large numbers of clinical researches on prebiotic features of lactulose; however the research on its influence on fermentation, survival and characteristics in fermented dairy products is limited. In previous investigations it was shown that inulin addition had almost negligible effect on bacterial count during fermentation and cool storage (Drgalić et al., 2005).

Thus, the purpose of this work was to describe the growth and survival of *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 in fermented whey, as well as the influence of lactulose addition for the possible production of nutritiously valuable beverage based on fermented whey.

## Materials and methods

*Preparation of reconstituted sweet whey*

Powdered sweet whey was obtained from dairy Zdenka d.d. from Veliki Zdenci, and its chemical composition (g/kg) was: lactose (730-750), proteins (110-140), ash (70-100), water (up to 60) and fat in dry matter (up to 10). Whey powder (60g/kg) was dissolved in 1 kg of water. To examine the influence on growth and survival of probiotic bacteria *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12, three samples were prepared as follows: (a) the first sample was without added lactulose (i.e. control), and (b) lactulose was added to the other two samples at a rate of 5 and 10 g/kg, respectively. Whey was pasteurized at 73 °C during 15 s, cooled to 37 °C and inoculated with probiotic culture.

*Used probiotic culture*

Used cultures were DVS *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 (Chr. Hansen, Denmark). Inoculum was prepared from 100 mL pasteurized whey, cooled to 37 °C, in which 1 g of culture *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 was added. After 30 min of activation, 2.5% (v/v) of inoculum was added in reconstituted whey. Inoculated samples were incubated at 37 °C and fermentation was stopped at pH ≈ 4.6.

*Chemical and microbiological analyses*

Acidity of whey samples was analysed as pH and titratable acidity. pH was measured by pH metre "Knick" type 647-1, and titratable acidity (°SH) by Soxhlet-Henkel method.

The viable cells count of bacteria (i.e. expressed as colony forming units (CFU)/mL) was determined by the standard method on MRS agar plates (Biolife, Milano, Italy) at 37 °C for 3 days. *Lactobacillus acidophilus* La-5 was incubated in microaerophilic conditions, which were obtained by a layer of MRS agar over the MRS agar inoculated with culture (IDF, 1995). *Bifidobacterium animalis* subsp. *lactis* BB-12 was incubated in anaerobic conditions which were obtained in an anaerobic jar with Anaerogen (Oxoid Limited, Hampshire, England) (Østlie et al., 2003).

The samples were analysed during fermentation (at the start of fermentation, after 5, 8, 10 hours and at the end of fermentation at pH ≈ 4.6). Survival of probiotic cells in fermented whey during the storage was observed after 1, 7, 14, 21 and 28 days of cool storage at 5 °C.

The experiments were replicated five times. The results were statistically analysed and shown as means with standard deviations.

## Results and discussion

Due to its complex chemical composition whey is good media for growth of different species of probiotic bacteria (Drgalić et al., 2005). Prebiotics stimulate the growth and colonisation of probiotic bacteria having beneficial effects when ingested. The most common used prebiotics are inulin, fructo-oligosaccharides and lactulose (Özer et al., 2005).

In order to improve the growth of probiotic bacteria in whey and functional characteristics of fermented products, in this research 5 and 10 g/kg of lactulose were added in reconstituted sweet whey. Fermentation was performed at 37 °C with two species of probiotic bacteria *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 until pH 4.6 was reached. According to the literature data when *Lactobacillus* species was used in culture media

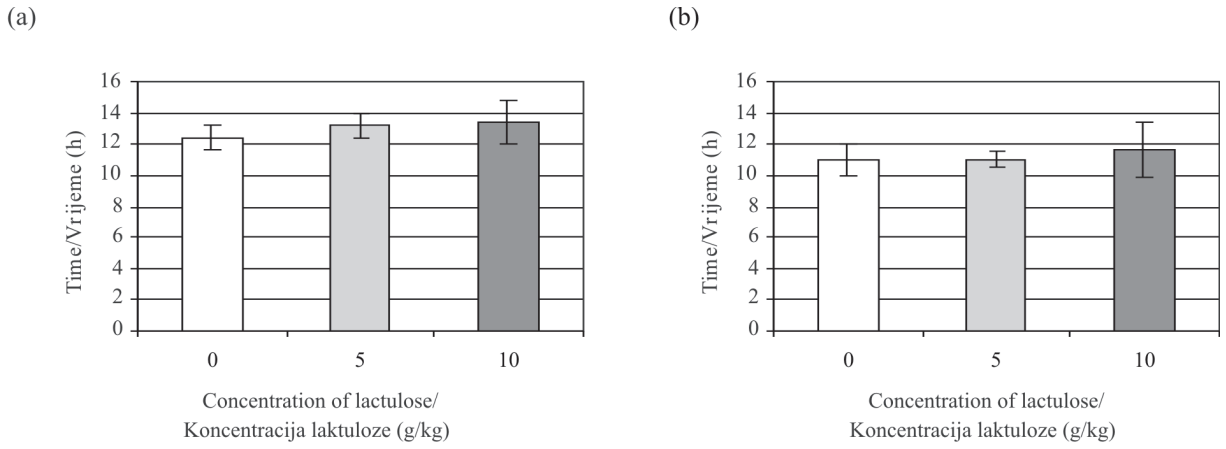


Fig. 1: Fermentation time of whey without (□), with 5 (■) and with 10 (■) g/kg addition of lactulose fermented by *Lactobacillus acidophilus* La-5 (a) and *Bifidobacterium lactis* BB-12 (b)

Grafikon 1: Vrijeme fermentacije sirutke bez (□), te sa 5 (■) i 10 (■)g/kg dodatka laktuloze primjenom sojeva *Lactobacillus acidophilus* La-5 (a) i *Bifidobacterium lactis* BB-12 (b)

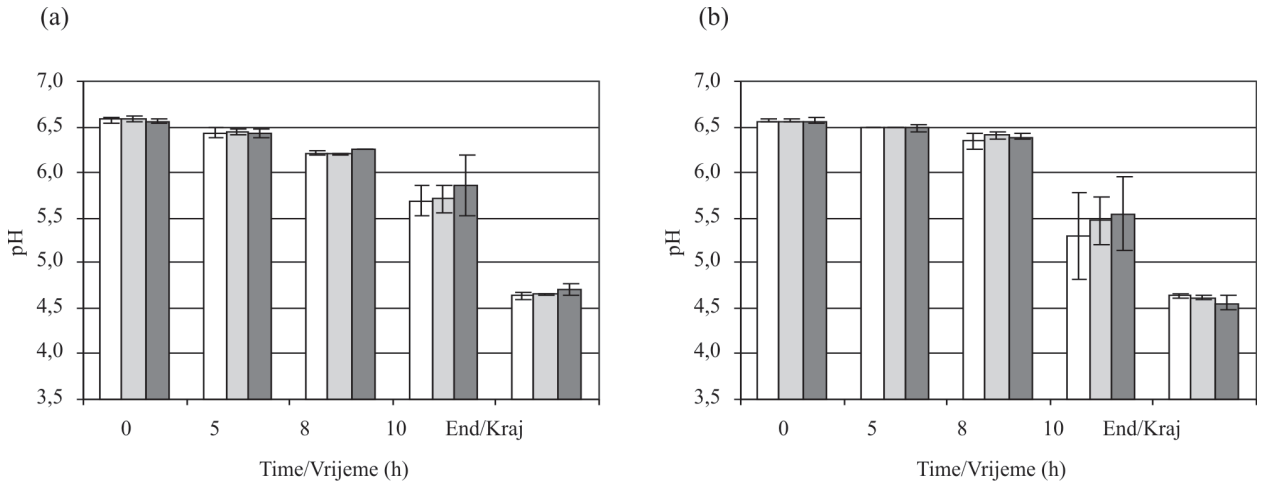


Fig. 2: Changes in the pH-value of whey without (□), with 5 (■) and 10 (■) g/kg addition of lactulose during fermentation with *Lactobacillus acidophilus* La-5 (a) and *Bifidobacterium lactis* BB-12 (b)

Grafikon 2: Promjena pH-vrijednosti sirutke bez (□), te sa 5 (■) i 10 (■) g/kg dodatka laktuloze tijekom fermentacije s *Lactobacillus acidophilus* La-5 (a) i *Bifidobacterium lactis* BB-12 (b)

in aerobic and anaerobic conditions the best growth was observed in culture media supplemented with 5 and 10 g/kg lactulose (Saarela et al., 2003).

Results for the fermentation rates of all samples are presented Fig. 1. Added lactulose did not shorten the fermentation time of reconstituted whey as it was expected in our hypothesis (Fig. 1). In reconstituted whey with addition of 10 g/kg lactulose, the fermentation occurred longer than the fermentation of whey without lactulose, for the both of bacteria species. Furthermore, fermentations with *Bifidobacterium animalis* subsp. *lactis* BB-12 were generally shorter for 1.8 h than fermentations with *Lactobacillus acidophilus* La-5.

At the beginning of fermentation, the pH of reconstituted whey was 6.5 and it was decreased after first 5 hours in all samples, regardless of the added lactulose amount (Fig. 2). After 8 h, the pH of reconstituited whey was lower in samples fermented with *Bifidobacterium animalis* subsp. *lactis* BB-12 than these fermented with *Lactobacillus acidophilus* La-5 ($\Delta pH \approx 0.36$). Similar results for reconstituted whey fermentation were obtained in our previous research (Drgalić et al., 2005; Matijević et al., 2008a; Matijević et al., 2008b), with the same probiotic microorganisms. These results show that for both probiotic species pH value started

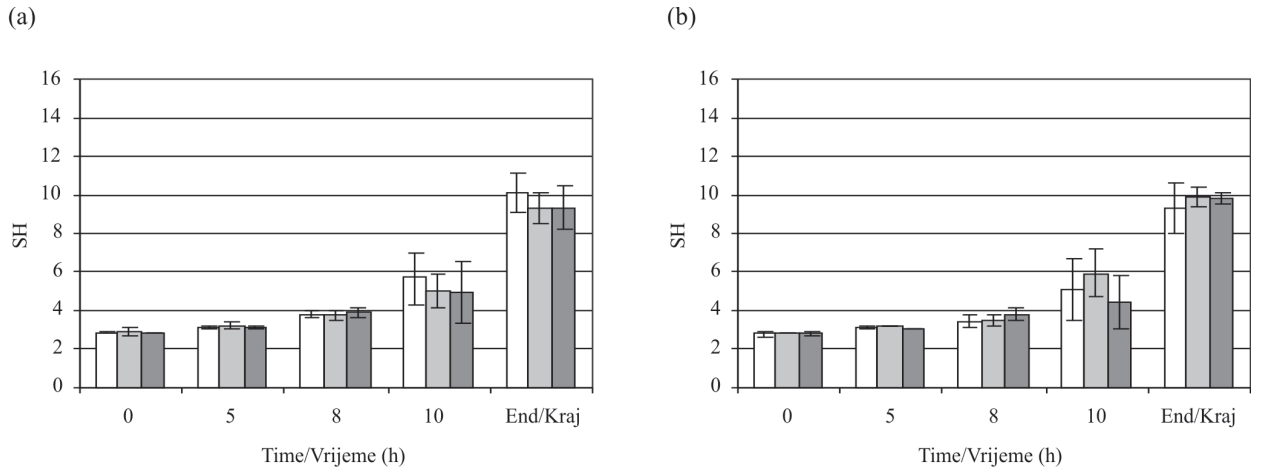


Fig. 3: Changes in the titratable acidity (°SH) of whey without (□), with 5 (■) and 10 (■) g/kg addition of lactulose during fermentation with *Lactobacillus acidophilus* La-5 (a) and *Bifidobacterium lactis* BB-12 (b)

Grafikon 3: Promjena titracijske kiselosti (°SH) sirutke bez (□), te sa 5 (■) i 10 (■) g/kg dodatka laktuloze tijekom fermentacije s *Lactobacillus acidophilus* La-5 (a) i *Bifidobacterium lactis* BB-12 (b)

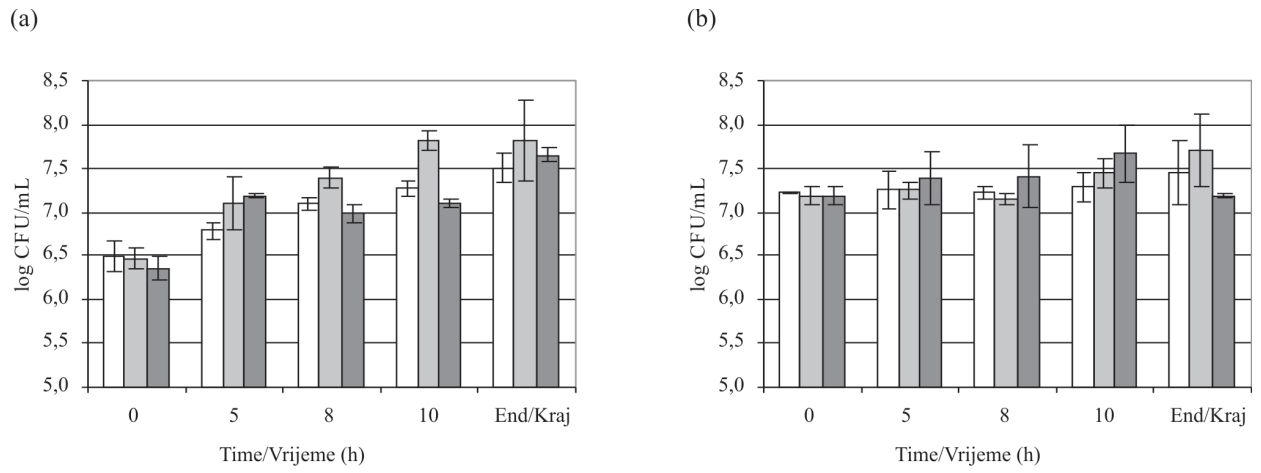


Fig. 4: Changes in viable cells count of whey without (□), with 5 (■) and 10 (■) g/kg addition of lactulose during fermentation with *Lactobacillus acidophilus* La-5 (a) and *Bifidobacterium lactis* BB-12 (b)

Grafikon 4: Promjena broja živih bakterija u sirutki bez (□), te sa 5 (■) i 10 (■) g/kg dodatka laktuloze tijekom fermentacije s *Lactobacillus acidophilus* La-5 (a) i *Bifidobacterium lactis* BB-12 (b)

to decrease after 5 h and faster decrease was noticed with *Lactobacillus acidophilus* La-5. Titratable acidity of samples at the beginning of the fermentation was around 2.8 °SH (Fig. 3). The change in titratable acidity during fermentation of reconstituted whey was not noticeable as the change in pH value. Significant increase of titratable acidity was recorded after 10 h of fermentation. Higher titratable acidity occurred in whey without added lactulose (i.e. control) fermented with *Lactobacillus acidophilus* La-5. Whey with 5 g/kg of lactulose fermented with *Bifidobacterium animalis* subsp. *lactis* BB-12 had higher titratable acidity. At the end of fermentation titratable acidity was 9.3-10.1 °SH in all samples. Similar values of titratable acidity were obtained in our previous research (Drgalić et al., 2005; Matijević et al., 2008a; Matijević et al., 2008b).

When milk (goat's and cow's) was fermented with *Bifidobacterium animalis* subsp. *lactis* BB-12 the pH 4.6 was reached after 28 h (Božanić and Tratnik, 2001). The difference in recorded value of titratable acidity in whey and milk was probably due to lower whey buffer capacity. Lower buffering capacity possibly causes faster decrease in pH value and less increase in titratable acidity, and this is probably the

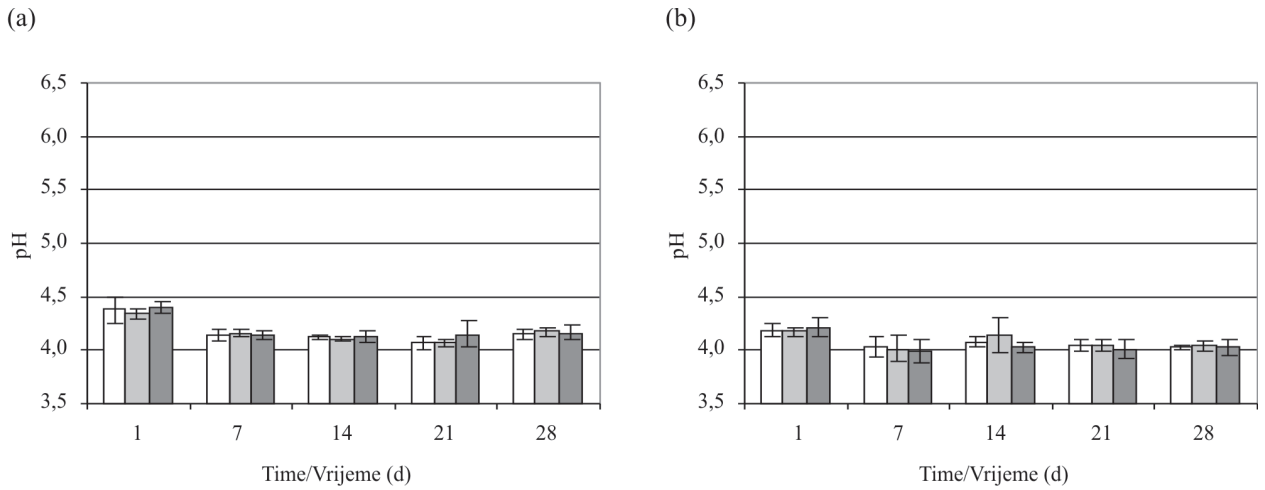


Fig. 5: Changes in the pH-value of whey without (□), with 5 (■) and 10 (■) g/kg addition of lactulose and fermented with *Lactobacillus acidophilus* La-5 (a) and *Bifidobacterium lactis* BB-12 (b) during 28 days of cool storage

Grafikon 5: Promjena pH-vrijednosti sirutke bez (□), te sa 5 (■) i 10 (■) dodatka g/kg laktuloze fermentirane s *Lactobacillus acidophilus* La-5 (a) i *Bifidobacterium lactis* BB-12 (b) tijekom 28 dana hladnog čuvanja

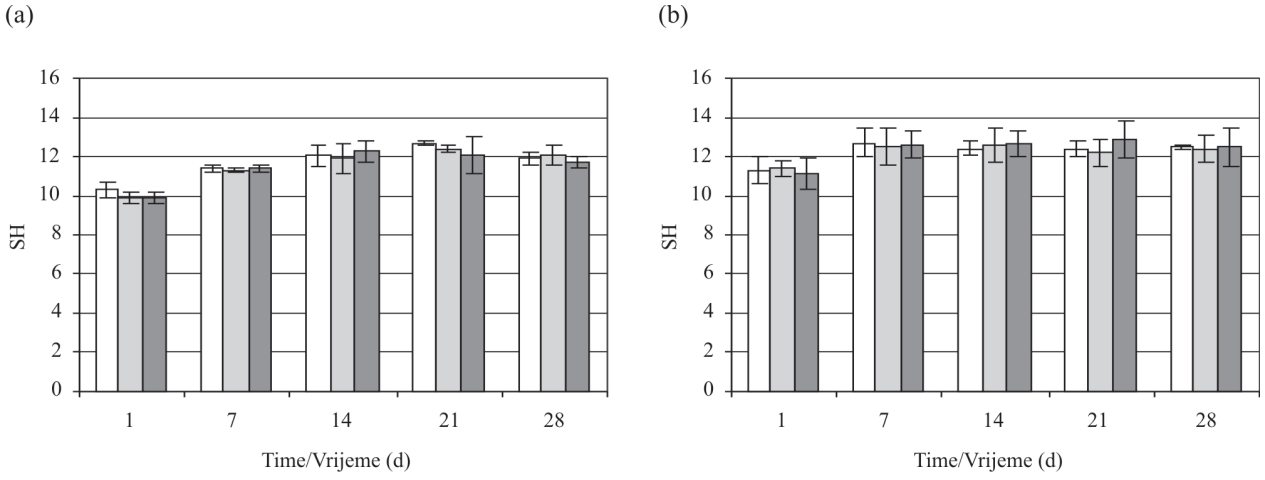


Fig. 6: Changes in the titratable acidity (°SH) of whey without (□), with 5 (■) and 10 (■) g/kg addition of lactulose and fermented with *Lactobacillus acidophilus* La-5 (a) and *Bifidobacterium lactis* BB-12 (b) during 28 days of cool storage

Grafikon 6: Promjena titracijske kiselost (°SH) sirutke bez (□), te sa 5 (■) i 10 (■) dodatka g/kg laktuloze fermentirane s *Lactobacillus acidophilus* La-5 (a) i *Bifidobacterium lactis* BB-12 (b) tijekom 28 dana hladnog čuvanja

reason for the shorter fermentation time of reconstituted whey in comparison with milk.

After inoculation of whey with *Lactobacillus acidophilus* La-5 the viable cells count in all three samples was 6.3-6.5 log CFU/mL (Fig. 4). During fermentation there has been an increase in *Lactobacillus acidophilus* La-5 viable cells count in all three samples. In whey with 5 g/kg addition of lactulose, the growth of lactobacilli was the best, compared to whey without added lactulose. At the end of fermentation viable cells count of *Lactobacillus acidophilus* La-5 in whey with addition of 5 g/kg lactulose reached 7.8 log CFU/mL, and in whey without lactulose 7.5 log CFU/mL. Previous research have shown (Drgalić et al., 2005) that during fermentation of reconstituted whey without added lactulose the viable cells count of *Lactobacillus acidophilus* La-5 after 12 h was almost identical to the results obtained in this paper (Δlog CFU/mL = 1.2).

The fermentation of reconstituted whey with *Bifidobacterium animalis* subsp. *lactis* BB-12 the viable cells count did not change significantly (Fig 4). In all three samples at the beginning and at the end of fermentation the viable cells count was about 7 to 7.5 log CFU/mL, regardless of the concentration of added lactulose. Results of some previous research showed (Drgalić et al., 2005) that the viable cells

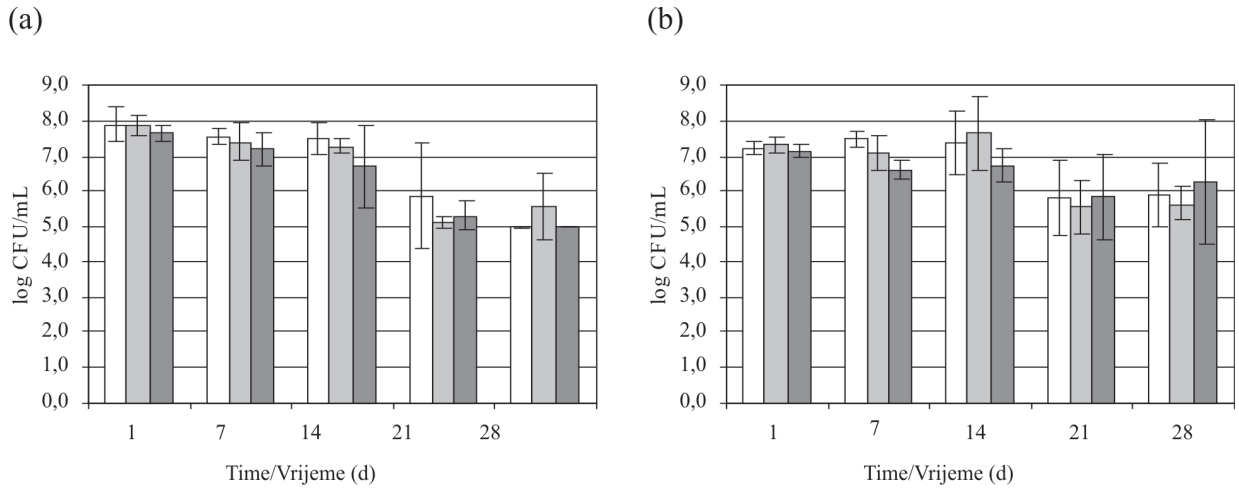


Fig. 7: Viable cells counts of *Lactobacillus acidophilus* La-5 (a) and *Bifidobacterium lactis* BB-12 (b) in fermented whey without (□), with 5 (■) and 10 (■) g/kg addition of lactulose during 28 days of cool storage

Grafikon 7: Broj živih bakterijskih stanica *Lactobacillus acidophilus* La-5 (a) i *Bifidobacterium lactis* BB-12 (b) u sirutki bez (□), te sa 5 (■) i 10 (■) g/kg dodatka laktuloze tijekom 28 dana hladnog čuvanja

count of *Bifidobacterium animalis* subsp. *lactis* BB-12 did not significantly change during the 12 h of fermentation, and the increase in the viable cells count was achieved only after 24 h of fermentation (Δlog CFU/mL ≈ 0.6). In milk fermented with *Bifidobacterium animalis* subsp. *lactis* BB-12 the highest viable cells count increased during the first 12 h of fermentation (from 1 to 1.65 logarithms) (Božanić and Tratnik, 2001).

After 1 day of storage at 5 °C, the pH of reconstituted whey fermented with *Lactobacillus acidophilus* La-5 was 4.3-4.4 (Fig. 5). After 7 days of storage pH dropped slightly in all samples fermented with *Lactobacillus acidophilus* La-5 (to 4.1 pH), and remained at the same level until the end of storage period. Identical change in pH happened within samples fermented with *Bifidobacterium animalis* subsp. *lactis* BB-12. At the beginning of storage they had lower pH value than samples fermented with *Lactobacillus acidophilus* La-5 (ΔpH 0.1-0.2). This difference remained the same until the end of storage period. Titratable acidity was a little higher in whey fermented with *Bifidobacterium animalis* subsp. *lactis* BB-12 (Δ°SH ≈ 1.2). Concentration of lactulose had no influence on change of pH value and titratable acidity during 28 days of storage at 5°C).

Viable cells count of probiotics bacteria decreased in fermented whey in all analyzed samples (Fig. 7). Significant decrease in the viable cells count was after the 14$^{th}$ d of storage. During the storage of whey fermented with *Bifidobacterium animalis* subsp. *lactis* BB-12 was more stable then *Lactobacillus acidophilus* La-5. After 14 days the viable cells counts in all samples dropped below 6 log CFU/mL, regardless of the amount of added lactulose in reconstituted whey.

Published data suggest that lactulose does not affect the viable cells count of bacteria belonging to *Lactobacillus* species during the storage at 4 °C (Saarela et al., 2003). Similar results were reported by Özer et al. (2005) in which the effects of lactulose were observed. Results of their research show that lactulose is good growth promoter for *Bifidobacterium bifidum* BB-02 and *Lactobacillus acidophilus* La-5 in order to keep the viable cells count at therapeutic minimum in AB yoghurt during storage.

In present study lactulose did not influence the survival of probiotic bacteria *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 in fermented reconstituted sweet whey (Fig. 7).

**Conclusion**

The results show that lactulose, a well-defined prebiotic, does not have a significant effect on fermentation and survival of *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 in whey, regardless of the added amount. Fermentation of whey with *Bifidobacterium animalis* subsp. *lactis* BB-12 was about 1.8 hours shorter (approximately 11 hours) in comparison with fermentation with *Lactobacillus acidophilus* La-5 (approximately 13 hours). Lactulose addition prolonged whey fermentation slightly with both bacteria species, but did not affect on the increase in viable cells count at the end of fermentation. Viable cells count of *Lactobacillus acidophilus* La-5 in whey during fermentation was higher (Δlog CFU/mL = 1.25) in comparison with *Bifidobacterium animalis* subsp. *lactis* BB-12 (Δlog CFU/mL = 0.27), regardless of the amount of lactulose addition. During storage *Bifidobacterium animalis*

subsp. *lactis* BB-12 was more stable than La-5. After 14th d of storage the viable cells count in all samples has dropped below 6 log CFU/mL regardless of the concentration of lactulose in reconstituted whey. All result had statistically analyzed by fisher's LSD test (least significant differences) at significant level 95%. Statistic analysis of results had shown that lactulose had any influence on whey fermentation for both probiotic cultures.

## *Utjecaj laktuloze na rast i preživljavanje probiotičkih bakterija Lactobacillus acidophilus La-5 i Bifidobacterium animalis* subsp. *lactis BB-12 u rekonstituiranoj slatkoj sirutki*

**Sažetak**

Ovaj rad istražuje utjecaj laktuloze, dobro poznatog prebiotika, na rast i aktivnost probiotičkih bakterija *Lactobacillus acidophilus* La-5 i *Bifidobacterium animalis* subsp. *lactis* BB-12 u slatkoj rekonstituiranoj sirutki i njihovo preživljavanje u fermentiranoj sirutki tijekom 28 dana hladnog skladištenja. Koncentracija laktuloze u slatkoj rekonstituiranoj sirutki bila je 5 i 10 g/kg. Fermentacija sirutke s *Bifidobacterium animalis* subsp. *lactis* BB-12 bila je 1,8 sati kraća (≈11 sati) u odnosu na fermentaciju s *Lactobacillus acidophilus* La-5 (≈13 sati). Dodatak laktuloze u rekonstituiranu slatku sirutku produžio je vrijeme fermentacije oba probiotika, ali nije utjecao na broj živih bakterijskih stanica na kraju fermentacije. *Lactobacillus acidophilus* La-5 je bolje rastao (Δlog CFU/mL = 1,25) tijekom fermentacije u usporedbi s *Bifidobacterium animalis* subsp. *lactis* BB-12 (Δlog CFU/mL = 0,27), bez obzira na količinu dodane laktuloze. Tijekom čuvanja fermentirane sirutke broj živih bakterijskih stanica *Bifidobacterium animalis* subsp. *lactis* BB-12 bio je stabilniji u odnosu na *Lactobacillus acidophilus* La-5. Rezultati pokazuju da laktuloza, dobro poznati prebiotik nije imala značajan utjecaj na fermentaciju i preživljavanje *Lactobacillus acidophilus* La-5 i *Bifidobacterium animalis* subsp. *lactis* BB-12 u sirutki bez obzira na dodanu količinu.

*Ključne riječi*: *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus acidophilus*, laktuloza, fermentacija, sirutka

**References**

1. Benković, M., Kos, B., Tonković, K., Leboš, A., Šušković, J., Gregurek, LJ. (2008): Utjecaj probiotičkog soja *Bifidobacterium animalis* subsp. *lactis* LAFTI® B94, inulina i transglutaminaze na svojstva čvrstog jogurta, *Mljekarstvo* 58 (2), 95-115.
2. Božanić, R., Tratnik, LJ. (2001): Quality of cow´s and goat´s fermented bifido milk during storage, *Food Technology and Biotechnology* 39 (2), 109-114.
3. Drgalić, I., Tratnik, LJ., Božanić, R. (2005): Growth and survival of probiotic bacteria in reconstituted whey, *Le Lait 85*, 171-179.
4. Gibson, G.R. (2004): Fibre and effects on probiotic (the prebiotic concept), *Clinical Nutrition Supplements* 1, 25-31.
5. Gibson, G.R., Roberfroid, M.B. (1995): Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics, *Journal of Nutrition* 125, 1401-1412.
6. Guarner, F., Schaafsma G.J. (1998): Probiotics, *International Journal* of *Food Microbiology* 39, 237-238.
7. Hoffman, K., Mossel, D.A.A., Korus, W., Van de Kamer, J.H. (1964): Untersuchungen über die Wirkumgsweise der Lactulose (β-Galactosido-fructose) im Darm, *Klinische Wochenschrift* 42, 126-130.
8. International Dairy Federation (1995): Detection and enumeration of *Lactobacillus acidophilus* culture media, *IDF Buletin* 306, 23-33.
9. Jelen, P. (2003): Whey processing, *Encyclopedia of dairy sciences*. vol. 4, Elsevier Science Ltd., London/San Diego.
10. Jeličić, I., Božanić, R., Tratnik LJ. (2008): Napitci na bazi sirutke - nova generacija mliječnih proizvoda, *Mljekarstvo* 58 (3), 257-274.
11. Kneifel, W., Rajal, A., Kulbe, K.D. (2000): In vitro growth behaviour of probiotic bacteria in culture media with carbohydrates of prebiotic importance, *Microbial Ecology in Health and Disease* 12, 27-34.
12. Maity, T.K., Kumar, R., Misra, A.K. (2008): Development of healthy whey drink with *Lactobacillus rhamnosus*, *Bifidobacterium bifidum* and *Propionibacterium freudenreichii* subsp. *shermanii*, *Mljekarstvo* 58 (4), 315-325.
13. Matijević, B., Božanić, R., Tratnik, LJ., Jeličić, I. (2008a): Utjecaj koncentrata proteina sirutke na rast i preživljavanje probiotičkih bakterija u sirutki, *Mljekarstvo* 58 (3), 243-255.
14. Matijević, B., Lisak, K., Božanić, R., Tratnik, LJ. (2008b): Utjecaj različitih početnih koncentracija probiotičkih bakterija na fermentaciju slatke sirutke, *Mljekarstvo* 58 (4), 387-401.
15. Mussatto, S.I., Mancilha, I.M. (2007): Non-digestible oligosaccharides: A review. *Carbohydrate Polymers* 68, 587-597.
16. Østlie, H.M., Helland M.H., Narvhus, J.A. (2003): Growth and metabolism of selected strains of probiotic bacteria in milk, *International Journal of Food Microbiology* 87, 17-27.
17. 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 Science and Technology International* 11 (1), 19-24.
18. Pomeranz, Y. (1992): Whey: Composition, properties, processing and uses, *Encyclopedia of food science and technology*. vol. 4, John Wiley and Sons, Inc., New York.
19. Saarela, M., Hallamaa, K., Mattila-Sandholm, T., Mättö, J. (2003): The effect of lactose derivatives lactulose, lactitol and lactobionic acid on the funcional and technological properties of potentially probiotic *Lactobacillus* strains, *International Dairy Journal* 13, 291-302.

20. Sako, T., Matsumoto, K., Tanaka, R. (1999): Recent progress on research and applications of non-digestible galacto-oligosaccharides, *International Dairy Journal* 9, 69-80.
21. Scantlebury-Manning, T., Gibson, G.R. (2004): Prebiotics, *Best Practice and Research Clinical Gastroenterology* 18 (2), 287-298.
22. Schuman, C. (2002): Medical, nutrition and technological properties of lactulose, An update, *European Journal of Clinical Nutrition* 41 (1), 17-25.
23. Shah, N.P., Lankaputhra, W.E.V. (2003): *Bifidobacterium* spp., *Encyclopedia of dairy sciences*. vol. 1, Elsevier Science Ltd., London/San Diego.
24. Tratnik, LJ. (1998): *Mlijeko-tehnologija, biokemija i mikrobiologija*, Hrvatska mljekarska udruga, Zagreb.

Presented results were obtained from scientific project (Sweet and fermented products based on whey and soya milk) supported by Ministry of Science, Education and Sports, Republic of Croatia.