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Impact of enzymatic hydrolyzed lactose on fermentation and growth of probiotic bacteria in whey

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Summary

Taking in consideration the long time for whey fermentation using probiotic bacteria, the aim of this research was to determine if prior enzymatic hydrolysis of lactose influences microbial activities of *Lactobacillus acidophilus* La-5 or *Bifidobacterium animalis* subsp. *lactis* BB-12 in reconstituted sweet whey. During fermentation (at 37 °C), pH-value and viable cell counts were monitored. The fermented samples were sensory profiled. Lactose hydrolysis shortened the fermentation time of *Lactobacillus acidophilus* La-5 by 2 h, and viable cell count at the end of fermentation time was greater in hydrolyzed whey sample (~9.45 $\log_{10}$ CFU/mL) when compared with the control sample (~8.91 $\log_{10}$ CFU/mL). In contrast, lactose hydrolysis in whey did not enhance the activity of *Bifidobacterium animalis* subsp. *lactis* BB-12. Lactose hydrolysis had slightly influence on sensory score of fermented samples, probably due to sweetness that masked the acidic taste of the product.

Key words: bifidobacteria, fermentation, lactose hydrolysis, lactobacilli, whey

Introduction

Whey is a green-yellowish fluid that derives from cheese-making and casein manufacture. The compositional quality and properties of whey can differ greatly, which depends on production technology and milk quality used. The main constituents of whey's dry matter are lactose (46-52 g/L) and whey proteins (6-10 g/L) (Jelen, 2003; Adam et al., 2004; Jeličić et al., 2008).

Whey fermentation is initiated by lactose hydrolysis by lactic acid bacteria (LAB). During fermentation, around 23-30% of lactose is transferred to lactic acid. LAB do not metabolize lactose directly but, by using lactose-permease, it is transferred into the cell where it is hydrolyzed to glucose and galactose (Neves et al., 2005). Some humans do not possess sufficient amounts of β-D-galactosidase in their digestive system and, therefore, are not able to digest lactose. This undigested lactose causes problems like bowel cramps and diarrhea.

Interest for hydrolyzed lactose dairy products started in the 1970s, which is not surprising, since around 70 % of world population suffers from lactose intolerance, i.e. the lack of β-D-galactosidase (Vasiljevic and Jelen, 2003; Jeličić et al., 2008). In addition, hydrolyzed whey has potential use in biotechnology and food applications (Stehlik-Tomas et al., 2001; Jeličić et al. 2008). The enzyme (β-D-galactosidase), which is used to hydrolyze the lactose in milk and dairy products, is obtained from bacteria, yeasts (*Kluyveromyces* spp.) and moulds (*Aspergillus* spp.) (Mahoney, 2003; Vasiljevic and Jelen, 2003).

Whey is mainly utilized in dairy beverages preparation by using whey powder (WP) or a whey protein concentrate powder (WPCP) (Tratnik,

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2003; Božanić et al., 2004; Tudor et al., 2008). The fermentation of liquid whey is more economical because certain technological processes, such as ultrafiltration and drying, are avoided (Gallardo-Escamilla et al., 2005). Whey can be fermented using lactic acid bacteria or probiotic bacteria. The use of probiotic bacteria for fermentation enhances the nutritive value and health quality of the product (Maity et al., 2008). To achieve health benefit of a probiotic dairy product, minimal microbial dairy intake should be 10^6 CFU/mL, preferably 10^9 CFU/mL (Reid et al., 2003; Tamime et al., 2003; Walstra et al., 2006). The most commonly used probiotic bacteria for the preparation of dairy beverage belong to the genus *Lactobacillus* and *Bifidobacterium*. In general, the fermentation time of whey using probiotic micro-organisms is rather long (15 h or more) (Matijević et al., 2008) and, therefore, the aim of this research was to determine the influence of lactose hydrolyzed whey on the fermentation time, growth of *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 and also to profile the sensory properties of fermented whey beverages.

Materials and methods

Reconstituted whey preparation

Sweet WP (Zdenka, Dairy Industry Ltd., Veliki Zdenci, Croatia) with following chemical composition was used for this research: lactose (73-75 g/100 g), proteins (11-14 g/100 g), ash (7-10 g/100 g), water (up to 6 g/100 g), milk fat in dry matter (up to 1 g/100 g). The powder was reconstituted by adding 60 g to 1 L of water, and pasteurized at 73 °C/15 s followed by cooling to 37 °C.

Lactose hydrolysis

Lactose hydrolysis was achieved by using β-D-galactosidase (*Kluyveromyces marxianus* var. *lactis*-E.C. 3.2.1.23), and commercially marketed as MAXILACT L2000 (DSM Food Specialtes, Netherlands). 2.5 mL/L of β-D-galactosidase was added to the processed reconstituted whey. Enzymatic activity was carried out at 37 °C during 120 min with continuous stirring (600 revolutions per minute - rpm) (Matijević et al., 2009).

Probiotic bacteria inoculum preparation

Lyophilized (DVS) bacterial monocultures (Chr. Hansen, Denmark): *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 were used. Inoculum was prepared by rehydrating 1 g of dried bacterial culture in 100 mL whey and then reactivated at 37 °C for 30 min. After reactivation, 2.5 % (v/v) of inoculum was added in whey samples.

Fermented whey samples

To evaluate the effect of lactose hydrolysis on the activity of probiotic bacteria, two whey samples were prepared. The first sample consisted of reconstituted whey (control sample), the other one contained hydrolyzed lactose whey (experimental sample) followed by inoculation of each sample with reactivated culture. Fermentation of all samples was stopped when pH 4.6 was reached.

Chemical, microbiological and sensory analyses

Acidity was measured using pH-meter "Knick" type 647 (Sabadoš, 1996). The viable cell counts of lactobacilli and bifidobacteria (CFU/mL) was determined using standard method on MRS agar (Biolife, Milano, Italy) after 3 days of incubation at 37 °C. The strain *Lactobacillus acidophilus* La-5 was incubated in microaerophilic conditions, which were obtained by one layer of MRS agar over the inoculated one (ISO 2006). Bifidobacteria was incubated in anaerobic conditions (IDF 2007) using anaerobic jars with anaerogen (Oxoid Limited, England). Samples were analyzed during fermentation after 0, 5, 8 and 10 h, and when pH value reached 4.6.

Sensory evaluation of fermented whey was profiled when fresh, i.e. after one day in the cold store. The sensory properties of fermented whey (control and experimental) was performed by 5 trained panelists using the International Dairy Federation method (IDF, 1984). The sensory attributes consisted of taste, odour, general appearance and colour, and the coefficients of significance (Fv) were: 2.0 for taste; 0.8 for odour; 0.8 for appearance and 0.4 for colour. Maximum score was 20, and the sensory scores were awarded for each attribute using a rating scale ranging between 1 and 5.

Data analysis

The experiment was replicated three times, and the data was analyzed statistically using Microsoft Office Excel 2003 software. The results are shown as mean value with standard deviation.

Results and discussion

The fermentation times of *Lactobacillus acidophilus* La-5 in whey (control and experimental samples) to reach pH 4.6 were 13 h 50 min and 11 h 40 min, respectively (Figure 1). Lactose hydrolysis was slight enhancement of the activity of the bacterium *Lactobacillus acidophilus* La-5, i.e. fermentation lasted 2 hours less. The activities of *Bifidobacterium animalis* subsp. *lactis* BB-12 in both whey samples were similar and the fermentation time was 15 h 35 min. However, the activity of the lactobacilli in the hydrolyzed whey was greater than bifidobacteria.

Influence of different carbon source, amongst others also influence of glucose and galactose, on activity of *Lactobacillus acidophilus* DSM 20079 in MRS media was investigated before (Goderska et al., 2008). Media that contained glucose contained 15 % more lactic acid after 48 h of fermentation than media that contained lactose. However, even though whey is a poor media in comparison with MRS media, hydrolyzed lactose increased activity of *Lactobacillus acidophilus* La-5. Literature data says that *Bifidobacterium bifidum* DSM 20239 in MRS media that contains glucose has a better activity compared to media that contains lactose (Goderska et al., 2008), but this research did not show better activity of *Bifidobacterium animalis* subsp. *lactis* BB-12 in whey with hydrolyzed lactose.

Starting pH-value of all samples was 6.4 (Figure 2). During fermentation of control whey sample and hydrolyzed lactose whey sample with *Lactobacillus acidophilus* La-5, significant pH decrease in both samples can be observed after the 5th h of fermentation. Results also show that hydrolyzed whey sample had somewhat lower pH-value than control sample after the 5th h of fermentation. Obtained results for control sample, support the literature data (Drgalić et al., 2005). *Lactobacillus acidophilus* has low β-D-galactosidase activity, which is probably the cause of faster pH-value change in hydrolyzed whey (Wang et al., 1997). During fermentation of control sample and hydrolyzed lactose sample with *Bifidobacterium animalis* subsp. *lactis* BB-12 significant pH decrease was noticed after the 8th h of fermentation (Figure 2). The reason might be the fact that bifidobacteria produce less lactic acid than lactobacilli, but also the fact that 37 °C fermentation temperature is more suitable for *Lactobacillus acidophilus* La-5 while bifidobacteria grow the best at 37 to 41 °C (Shah, 2006). Similar pH-value dynamics are described in

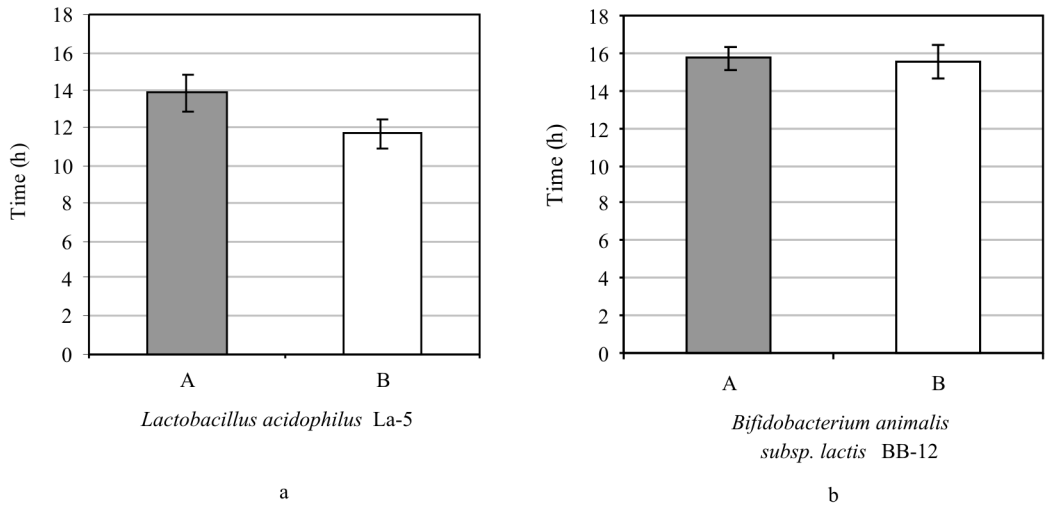

Figure 1. Average values of fermentation time for control whey samples (A) and hydrolyzed lactose samples (B) using monocultures *Lactobacillus acidophilus* La-5 (a) and *Bifidobacterium animalis* subsp. *lactis* BB-12 (b)

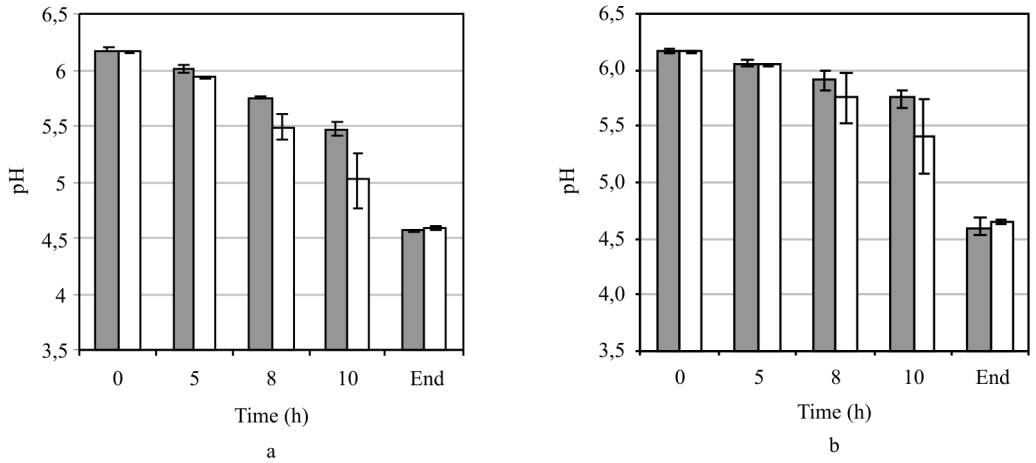

Figure 2. Change of pH-values control whey samples (■) and hydrolyzed lactose samples (□) during fermentation with *Lactobacillus acidophilus* La-5 (a) and *Bifidobacterium animalis* subsp. *lactis* BB-12 (b)

the literature regarding whey fermentation with *Bifidobacterium animalis* subsp. *lactis* BB-12 (Drgalić et al., 2005). Probable cause of similar pH-value change in control sample and hydrolyzed whey sample is possibility of bifidobacteria to use galactose and produce oligosaccharides made of 3 galactose units (Lamoureux et al., 2002). *Bifidobacterium animalis* subsp. *lactis* BB-12 contains izoenzyme β-galactosidase and can produce oligosaccharides. The more hydrolyzed lactose in milk, the more oligosaccharides *Bifidobacterium animalis* subsp. *lactis* BB-12 produces, but not lactic acid, therefore fermentation will not be more intense (Nguyen et al., 2009).

The viable cell count of *Lactobacillus acidophilus* La-5 on the beginning of the fermentation in both whey samples was around 6.62 $\log_{10}$ CFU/mL (Figure 3). During fermentation, increase of lactobacilli occurred in both samples, but at the end of fermentation there was more *Lactobacillus acidophilus* La-5 in hydrolyzed whey samples (~9.45 $\log_{10}$ CFU/mL), than in control sample (~8.91 $\log_{10}$ CFU/mL). Milk fermentation with *Lactobacillus acidophilus* La-5 resulted in 9 $\log_{10}$ CFU/mL (Božanić et al., 2001), which is similar to whey control sample meaning that whey is just as good media for *Lactobacillus acidophilus* La-5 growth than milk. Glucose is

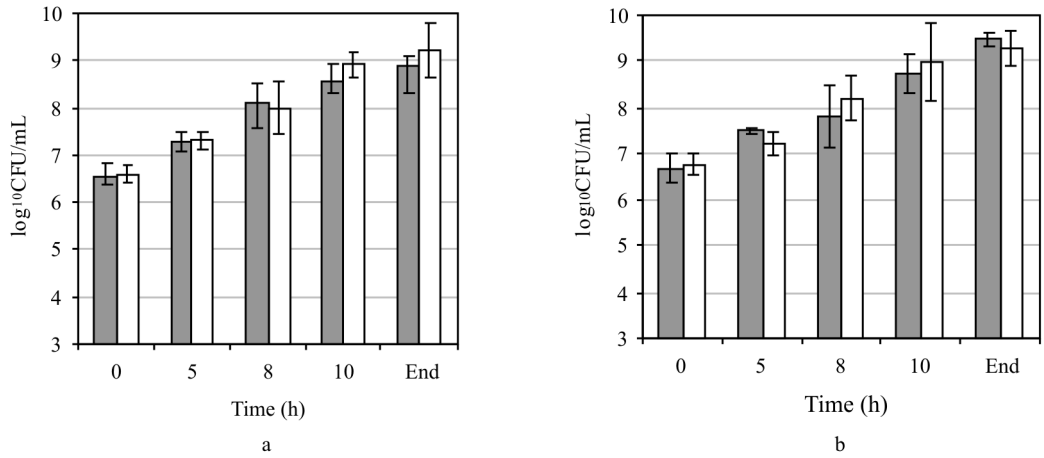

Figure 3. Change of viable cells count ($\log_{10}$ CFU/mL) in control whey samples (■) and hydrolyzed whey samples (□) during fermentation with *Lactobacillus acidophilus* La-5 (a) and *Bifidobacterium animalis* subsp. *lactis* BB-12 (b)

regarded as a better carbon source for *Lactobacillus acidophilus* than lactose, resulting in better growth. Increase of *Lactobacillus acidophilus* DSM 20079 viable cell count in MRS media was greater when glucose was source of carbon (~2.45×10^8 CFU/mL) than lactose (1.46×10^6 CFU/mL) (Goderska et al., 2008) and results of this research can confirm this.

The viable cell count of *Bifidobacterium animalis* subsp. *lactis* BB-12 on the beginning of the fermentation was around 6.78 $\log_{10}$ CFU/mL (Figure 3) in both whey samples. Their number, at the end of the fermentation was somewhat greater in control sample (~9.51 $\log_{10}$ CFU/mL), than in hydrolyzed whey sample (~9.37 $\log_{10}$ CFU/mL). Activity of *Bifidobacterium animalis* subsp. *lactis* BB-12 would probably be greater if besides β-D-galactosidase addition, hydrolyzed proteins and some extracts were added (Lourens-Hattingh and Viljoen, 2001; Gaudreaua et al., 2005). Better growth of bifidobacteria in MRS media was achieved when glucose was the source of carbon instead of lactose (Goderska et al., 2008) but this was not achieved in experiments with whey (Figure 3).

Lactose hydrolysis produces monosaccharide (glucose and galactose) that give sweeter taste than lactose (Mahoney, 2003). Therefore, sensory properties of fermented whey samples and hydrolyzed whey samples were determined. Weighted points for all samples appearance were 2.64-2.72 (from maximum 4) (Table 1), because all samples were cloudy and with some sediment. Whey was cloudy immediately after pasteurization (73 °C/15 s) which is probably consequence of whey proteins denaturation. After one day of cool storage, sediment increase was noticeable. Cryogenic IgM that sediments at temperatures below 15 °C is probably responsible, and it usually combines bacteria in the sediment because they bond as antigens with IgM (Walstra et al., 2006).

Colour of all samples was white-greenish and therefore the score for this property ranged from 1.56 to 1.64 out of possible 2.00 points. Odour of all samples was pleasant and characteristic for fermented whey (3.68 to 4.00 which is maximum). Sensory results for taste show some significant differences. Fermented hydrolyzed lactose whey samples were defined as sweeter. Both whey samples (control and sample with hydrolyzed lactose) fermented with *Lactobacillus acidophilus* La-5 scored equally for taste (9.20 and 9.40 of maximum 10 points) (Table 1). They tasted mildly acidic with noticed freshness. The highest score for taste was given to the control whey samples fermented with *Bifidobacterium animalis* subsp. lactis BB-12 (9.80), and the lowest score (7.20) was given to the hydrolyzed lactose whey samples fermented with *Bifidobacterium animalis* subsp. *lactis* BB-12 because of noticed off-flavour.

In general, samples fermented with bifidobacteria had more expressed acidity compared to samples fermented with lactobacilli (Figures 2 and 3). Besides that, during fermentation with bifidobacteria, except L (+) lactic acid, higher quantities of acetic acid were produced (Sarkar, 2008) and flavor profile was changed. Maybe sweet taste in hydrolyzed lactose sample is suitable for milky-acidic taste, but not suitable for acetic-acidic taste which resulted in worse score.

Table 1. Weighed point values for appearance, odour, colour and taste of control whey samples (A) and hydrolyzed whey samples (B) fermented with *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12

Samples	Appearance (4)	Odour (4)	Colour (2)	Taste (10)
La-5 (A)	2,72	3,76	1,60	9,40
La-5 (B)	2,72	3,84	1,64	9,20
BB-12 (A)	2,64	4,00	1,56	9,80
BB-12 (B)	2,64	3,68	1,56	7,20

In research Drgalić et al. (2005) sensory properties of whey fermented with lactobacilli and bifidobacteria after 28 days of cool storage at 4 °C were determined. Whey fermented with lactobacilli had acceptable sensory grade, while whey fermented with bifidobacteria had unacceptable grade due to the taste of bitterness and uncharacteristic acidity and odour. In this research, control sample fermented with bifidobacteria scored the best grades (18.00 points out of 20.00) while hydrolyzed whey fermented with bifidobacteria scored the worst grades (Figure 4) of all samples (14.90 out of 20.00).

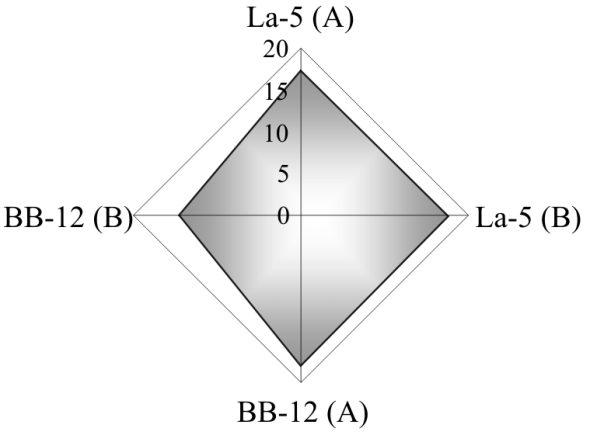

Figure 4. Total weighed point values of sensory properties for control whey samples (A) and hydrolyzed whey samples (B) fermented with *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12

Conclusions

Lactose hydrolysis shortened fermentation with *Lactobacillus acidophilus* La-5 for around 2 h, but in fermentation with bifidobacteria, it did not influence fermentation time. The viable cell count of *Lactobacillus acidophilus* La-5 was at the end of the fermentation higher in hydrolyzed whey samples (~9.45 log10 CFU/mL), compared to control samples (~8.91 log10 CFU/mL). The highest sensory grades were given to control whey fermented with bifidobacteria, while samples with hydrolyzed lactose scored somewhat worse. Lactose hydrolysation did not significantly influence the sensory score of fermented whey samples, because taste of sweetness covers the taste of probably produced acid.

Utjecaj enzimski hidrolizirane laktoze na fermentaciju i rast probiotičkih bakterija u sirutki

Sažetak

Uzimajući u obzir dužinu fermentacije sirutke probiotičkim bakterijama, cilj ovog rada bio je ispitati utječe li prethodna enzimska hidroliza laktoze na aktivnost bakterije *Lactobacillus acidophilus* La-5 ili *Bifidobacterium animalis* subsp. *lactis* BB-12 u rekonstituiranoj slatkoj sirutki. Tijekom fermentacije (pri 37 °C) praćena je promjena pH-vrijednosti i broj živih bakterijskih stanica. Fermentirani uzorci su senzorski analizirani. Hidroliza laktoze skratila je fermentaciju sirutke s *Lactobacillus acidophilus* La-5 za oko 2 sata, a broj živih bakterija na kraju fermentacije bio je nešto veći u hidroliziranoj sirutki (~9,45 $\log_{10}$ CFU/mL), u odnosu na kontrolni uzorak (~8,91 $\log_{10}$ CFU/mL). Međutim, hidroliza laktoze nije utjecala na aktivnost bakterije *Bifidobacterium animalis* subsp. *lactis* BB-12. Hidroliza laktoze neznatno je utjecala na senzorska svojstva fermentirane sirutke, jer vjerojatno stvorenu kiselinu prikriva osjet slatkoće.

Ključne riječi: bifidobakterije, fermentacija, hidroliza laktoze, laktobacili, sirutka

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