

## Inhibitory effect of honey-sweetened goat and cow milk fermented with *Bifidobacterium lactis* Bb-12 on the growth of *Listeria monocytogenes*

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Received - Prispjelo: 13.03.2009.  
Accepted - Prihvaćeno: 19.05.2009.

### Summary

The aim of the study was to determine the influence of honey addition on fermentation of goat and cow milk with *Bifidobacterium lactis* Bb-12. Additionally, inhibitory potential of honey-sweetened fermented goat and cow milk against *Listeria monocytogenes* strain was examined. Two monofloral honey types, dark-colored chestnut and light-colored acacia honey were added. The basic hypothesis of this study was that addition of honey could have influence on the growth of *Bifidobacterium lactis* during the fermentation of goat and cow milk. Furthermore, higher inhibitory potential caused by honey addition against *Listeria monocytogenes* has been assumed. Compared to cow milk, higher acidity and CFU of *Bifidobacterium lactis* Bb-12 were noted in the fermented goat milk in all phases of the fermentation process. The results of this study show that both types of honey enhanced growth and acidity of the *Bifidobacterium lactis* Bb-12 in both milk types during fermentation. A disc assay has shown that development of growth inhibition zones depends on the type and concentration of honey, as well as on the milk type. The chestnut honey had generally higher inhibitory effect than acacia honey.

*Key words:* *Bifidobacterium lactis* Bb-12, fermented cow and goat milk, acacia and chestnut honey, inhibitory effect, *Listeria monocytogenes*

### Introduction

Bifidobacteria have been recognized as bacteria considered important to the health of the gastrointestinal tract (Tamime et al., 1995; Aires et al., 2009). One approach for ensuring or increasing the presence of healthful colonic bacteria is to provide them by food e. g. fermented milk.

A probiotic is a live microbial food and feed supplement, which beneficially affects the host organism by improving its intestinal microbial balance. To have an impact on the colonic flora it is important for probiotic strains to exhibit antagonism against

pathogenic bacteria via production of antimicrobial substances or competitive exclusion (Saarela et al., 2000). Several authors suggested that low molecular weight metabolites and secondary metabolites play more important role than bacteriocins, since they show wide inhibitory spectrum against many harmful organism (Saarela et al., 2000; Niku-Paavola et al., 1999; Boesten and de Vos, 2008). According to basic definition, bacteriocins are antibiotic-like substances and bactericidal proteins, which also might be produced during lactic acid fermentation (Klaenhammer, 2006).

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Growth and viability of bifidobacteria in fermented milk can be enhanced significantly by the incorporation of fructooligosaccharides (FOS) and galactooligosaccharides (GOS) in milk prior to fermentation (Benković et al., 2008; Van Den Broek and Voragen, 2008). Honey contains a variety of oligosaccharides varying in a degree of polymerization (Downey et al., 2005; Ouchemouk et al., 2007). The unique composition of honey suggests that it could enhance growth, activity and viability of bifidobacteria in milk, thus, in fermented dairy products. To evaluate this hypothesis, some studies on growth-promoting and prebiotic activity of honey on bifidobacteria were conducted (Chick et al., 2001; Shin and Ustunol, 2005; Cardarelli et al., 2007).

Due to specific composition and structure, goat milk has specific nutritional and therapeutic quality. Compared to cow milk, goat milk is better digestible (Juarez and Ramos, 1986) and has smaller and better distributed fat globules in milk (Mehaia, 1995), higher content of SCFA and MCT in the milk fat (Haenlein, 2004), higher buffering capacity (Park and Attaie, 1986), higher content of some minerals, such as potassium and chlorides (Park, 1994a), as well as better immunological and antibacterial characteristics (Park, 1994b).

In some recent studies, honey has been recognized as a promoter of lactobacilli and bifidobacteria

growth. However, there is no clear scientific information about synergistic effect of honey on growth of probiotics in milk during lactic acid fermentation. On the other hand, antimicrobial activity of honey has also been cited in many recent studies (Frankel et al., 1998; Taormina et al., 2001; Varga, 2006).

*Listeria monocytogenes* is an ubiquitous food-borne pathogen responsible for causing listeriosis, a fatal disease of public health concern. *L. monocytogenes* infections are particularly dangerous to certain risk groups, including, pregnant women, the elderly, newborns and immunocompromised patients (Doyle et al., 2001; Liu, 2006). Manifestations of listeriosis include meningoencephalitis, septicemia, abortion and a high fatality rate 30 % (Liu, 2004; Mc Lauchlin et al., 2004).

The aim of this study was to determine the influence of honey addition on growth of *Bifidobacterium lactis* Bb-12 during fermentation of cow's and goat's milk. Furthermore, the influence of honey addition as antagonist against the psychrophilic *Listeria monocytogenes* strain was examined.

## Materials and methods

### Preparation of *Listeria monocytogenes* suspension

*Listeria monocytogenes*, obtained from Institute of Public Health (Osijek, Croatia), was used. *Liste-*

Table 1: Chemical composition ( $\text{g}\cdot 100\text{g}^{-1}$ ) and acidity of commercial cow and goat UHT milk  
Tablica 1: Kemijski sastav ( $\text{g}\cdot 100\text{g}^{-1}$ ) i kiselost komercijalnog UHT kozjeg i kravljeg mlijeka

Composition and acidity Sastav i kiselost	Goat milk Kozje mlijeko			Cow milk Kravlje mlijeko		
	$\bar{x}$	Range/Raspon	SD	$\bar{x}$	Range/Raspon	SD
Total solids/Suha tvar	11.45	11.24-11.92	0.122	11.41	11.37-11.45	0.024
Ash/Pepeo	0.79	0.77-0.89	0.036	0.68	0.67-0.73	0.015
Milk fat/Mliječna mast	3.20	3.20	-	3.20	3.20	-
Lactose/Laktoza	4.32	4.26-4.39	0.045	4.91	4.87-4.96	0.026
Proteins/Proteini	3.08	2.88-3.19	0.071	3.08	3.04-3.17	0.036
Acidity/Kiselost						
pH	6.55	6.49-6.67	0.088	6.64	6.60-6.69	0.061
°SH	8.05	7.85-8.26	0.131	7.23	7.16-7.32	0.047

SD - standard deviation of 20 determinations/SD - standardna devijacija 20 mjerenja

*ria monocytogenes* was cultured on Tryptic Glucose Yeast agar (MERCK KgaA, Germany) at 37 °C for 24 hours. For determination of inhibition, the inoculums were adjusted to match with 0.5 McFarland and this suspension was further diluted to obtain final concentration of  $1 \times 10^8$  CFU/mL. Final concentration was obtained as  $10^4$  dilution of initial concentration.

#### Sample preparation

Commercial UHT cow and goat milk with 3.2 % of milk fat were used for fermentation of all samples (producer "Vindija" Dairy Industry, Varaždin, Croatia). The composition of UHT cow and goat milk was determined on MILCOSCAN FT 120 (FOSS ELECTRIC, Denmark). 20 samples of both milk types were analyzed and the average chemical composition is presented in Table 1.

Samples of cow and goat milk for fermentation were prepared by adding acacia and chestnut honey at levels 3.0 %, 5.0 % and 10.0 % (w/v). Before addition, honey was pasteurized at 63 °C for 30 min.

Analyses of acacia and chestnut honey was conducted as follows: water content (moisture) was determined using a refractometric method (ATAGO RX-5000ALPHA BEV Abbe Refractometer) reading at 20 °C (AOAC Official Method 969.38); total

ash content was determined by incinerating honey samples in a muffle furnace at a temperature of 550 °C overnight (AOAC Official Method 920.181); pH was measured with pH-meter (MA 235, pH/Ion Analyzer, METTLER TOLEDO) in a solution containing 10 g honey in 75 mL of CO<sub>2</sub> free distilled water (AOAC Official Method 962.19); total acidity was determined by a titrimetric method (AOAC Official Method 962.19) and expressed as °SH; hydroxymethylfurfural was determined by the use of a spectrophotometric method according to Wunderlin et al. (1998); diastase activity was determined photometrically (AOAC Official Method 958.09) by using a buffered solution of starch and honey, which was incubated in thermostatic bath until the endpoint was reached; free amino acids were determined by the reaction between  $\alpha$ -amino acids and formaldehyde (Method N<sup>o</sup> 30, FIPJF, 1984) and sugars profile was determined by HPLC method according to Wunderlin et al. (1998). 10 samples of both honey types were analyzed. The chemical composition of honeys is presented in Table 2.

#### Fermentation of samples; analyses during fermentation

DVS monoculture of *Bifidobacterium lactis* Bb-12 (Chr. Hansen, Denmark) was used to inoculate samples which were fermented at 37 °C for 25 hours.

Table 2: Chemical composition and basic properties of acacia and chestnut honey collected from Bilogorian (west Croatian province) and East Slavonian region

Tablica 2: Kemijski sastav i osnovna svojstva bagremovog i kestenovog meda s bilogorskog i istočnoslavonskog područja

Component (Sastojak)/Property (Svojstvo)	Type of Honey/Vrsta meda	
	Acacia/Bagrem	Chestnut/Kesten
Water/Voda	16.40 %	17.40 %
Ash/Pepeo	0.06 %	0.94 %
Acidity/Kiselost	9.73 mmol/1000 g	16.2 mmol/1000 g
Water insoluble components Tvari netopljive u vodi	0.01 %	0.03 %
Reducing sugars/Reducirajući šećeri	68.30 %	75.82 %
Sucrose/Saharoza	0.40 %	1.95 %
Active Dyastase/Aktivne dijastaze	10.2 U*	24.6 U*
Hydroxymethylfurfural/Hidroksimetilfurfural (HMF)	3.4 mg/kg	4.8 mg/kg

\*U - unit/U - jedinica

The pH of samples during fermentation was measured using an MA 235 pH/Ion Analyzer.

The viable count of *Bifidobacterium lactis* Bb-12 was determined on modified Bifidobacterium agar (according to Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) in anaerobic jars at 37 °C for 48 hours. MRS agar was modified by adding 13.5 g/100 mL Bacteriological agar (Agar Bios Special LL, Biolife, Italy) and 3 g/100 mL LiCl. pH and viable count of *Bifidobacterium lactis* Bb-12 were determined every five hours during fermentation. All measurements were performed for 4 times.

#### Agar diffusion test

Inhibitory effect of samples on *Listeria monocytogenes* was qualitatively determined by the agar well diffusion method. 10 mL of molten Mueller-Hinton agar (Biolife, Italy) were cooled at 47 °C

and seeded with 1 mL prepared suspension of *L. monocytogenes* containing 10<sup>8</sup> cells/mL. Seeded agar was poured into sterile Petri plate and overlaid with a second layer of 10 mL of sterile Mueller-Hinton agar. After solidification at room temperature, wells (9 mm) were cut in the agar using a sterile metal cork borer and filled with 150 µL of sample. After incubation at 37 °C for 24 hours, zones of inhibition (clear areas) surrounding wells were measured. According to the mentioned method (Servin, 2004), inhibition zones including the sums of wells' diameters and inhibition zones diameters were measured.

#### Statistics

All the results were statistically analyzed using Basic statistic pack in STATISTICA 7.0. Standard deviations were calculated. Influence of honey on fermentation rate, as well as on *B. lactis* cells in fer-

Table 3: Differences in count of *Bifidobacterium lactis* Bb-12 at the end of fermentation between different samples (influence of milk type, type of honey and honey content addition) (Fisher's LSD test)

Tablica 3: Razlike u broju bakterija *Bifidobacterium lactis* Bb-12 na kraju fermentacije među različitim uzorcima (utjecaj vrste mlijeka, utjecaj vrste meda, utjecaj udjela dodanog meda) (Fisher-ov LSD test)

Sample/Uzorak	log CFU of <i>Bifidobacterium lactis</i> Bb-12* log broja bakterija <i>Bifidobacterium lactis</i> Bb-12*
GM-Control	1.863abcde
GM-3%AH	1.680a
GM-5%AH	2.040ab
GM-10%AH	1.621e
GM-3%CH	2.003abc
GM-5%CH	1.950abcd
GM-10%CH	1.651e
CM-0	1.560e
CM-3%AH	1.680de
CM-5%AH	1.737cde
CM-10%AH	2.020abc
CM-3%CH	1.600e
CM-5%CH	1.737cde
CM-10%CH	1.650e

Mean values followed by the same letter in the same column and in the same row are not significantly different ( $P < 0.05$ ) - for all samples separately

\*Mean of 3 determinations

Legend for Table 3/Legenda za Tablicu 3: GM - goat milk/kozje mlijeko; CM - cow milk/kravlje mlijeko; AH - acacia honey/bagremov med; CH - chestnut honey/kestenov med; 3 %, 5 %, 10 % addition of honey (w/v)/3 %, 5 %, 10 % dodatak meda (w/v); a, b, c, d, e - samples marked with the same letters are statistically not significantly different on level of significance  $p \leq 0.05$ , a, b, c, d, e - uzorci označeni istim slovom statistički nisu značajno različiti na nivou značajnosti  $p \leq 0,05$

mented goat and cow milk was analyzed using a Fisher's LSD test in STATISTICA 7.0. The coefficient of variation (CV) was used to analyze the microbiological results (Shelley et al., 1987).

## Results and Discussion

The average chemical composition of the goat and cow milk is reported in Table 1.

Very small differences in the overall composition between UHT goat and cow milk were observed. Goat milk had inconsiderably lower average content of lactose and slightly higher level of acidity compared to cow milk. Total protein and minerals were approximately equal in goat and cow milk. Furthermore, SD values in Table 1 suggest very low

variations in composition of 20 goat and cow milk samples. This suggests good standardized quality of UHT cow and goat milk on Croatian market.

The chemical composition of the acacia and chestnut honey collected from Croatian market are shown in Table 2. According to the data, chestnut honey had, compared to acacia honey, higher contents of water, ash, reducing sugars, sucrose, higher activity of dyastases, HMF, slightly higher content of water insoluble components, as well as considerably higher acidity. It is also possible that some of these properties, such as content of ash, sucrose and reducing sugars, could have influence to fermentation rates in cow or goat milk, in order words to *Bifidobacterium lactis* Bb-12 activity in goat or cow milk.

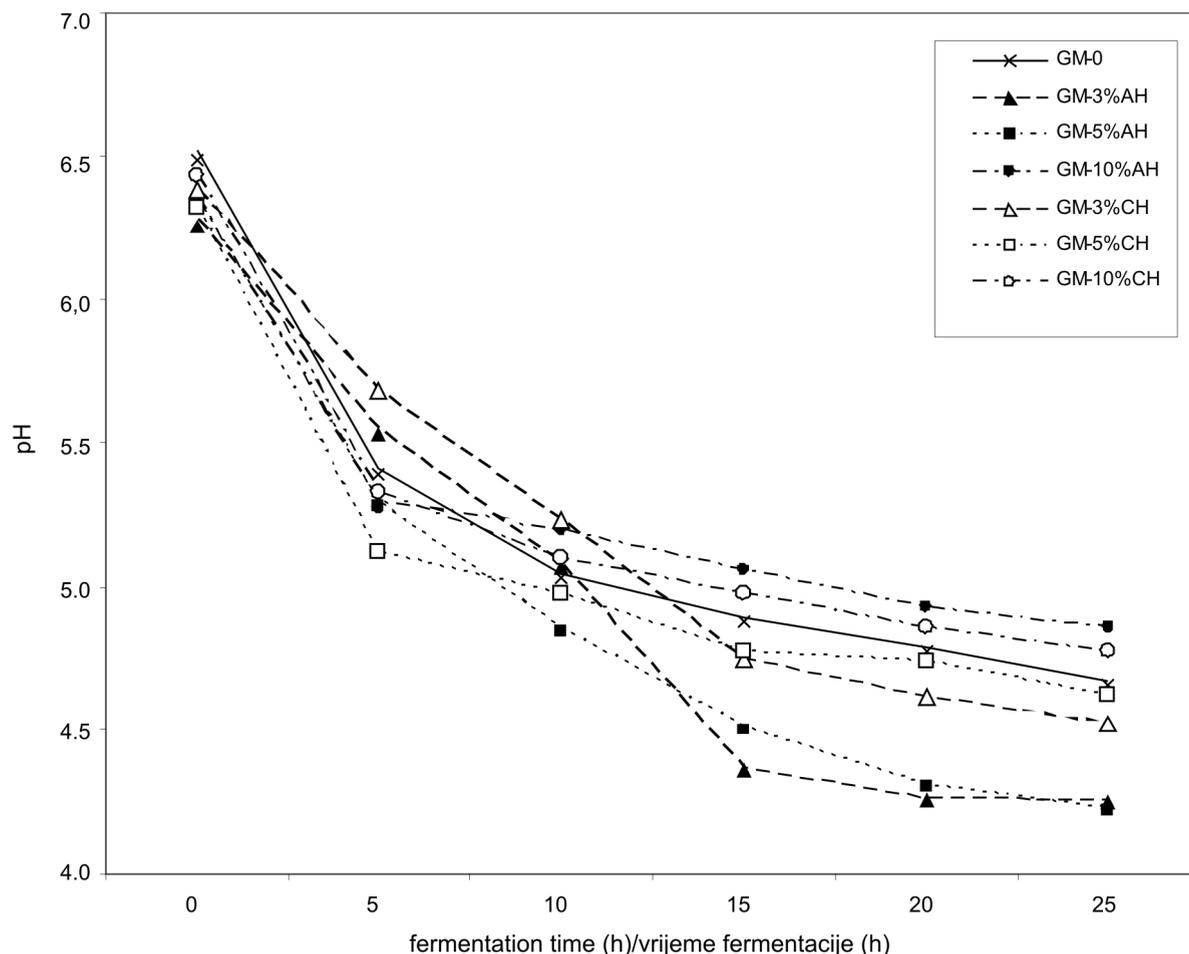


Fig. 1: Change of pH during fermentation of honey-sweetened (AH-acacia; CH-chestnut) goat milk with *Bifidobacterium lactis* Bb-12

Grafikon 1: Promjena pH tijekom fermentacije kozjeg mlijeka s *Bifidobacterium lactis* Bb-12 uz dodatak meda (AH-bagremov; CH-kestenov)

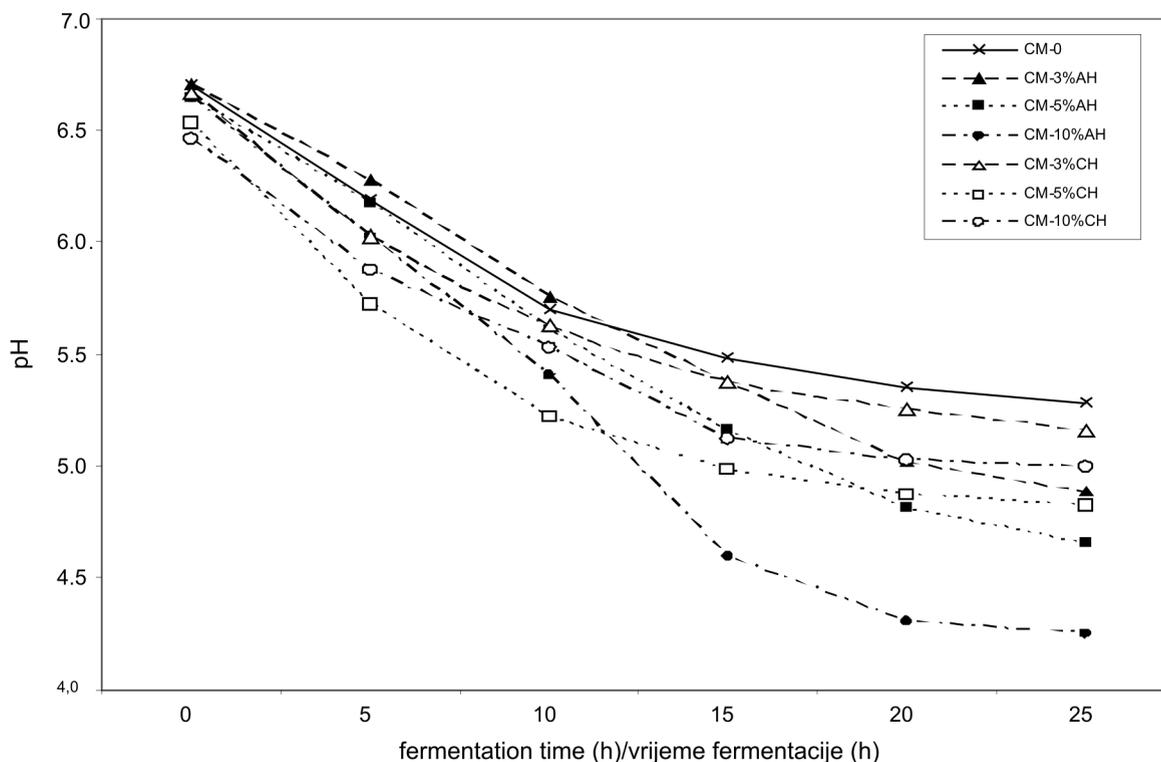


Fig. 2: Change of pH during fermentation of honey-sweetened (AH-acacia; CH-chestnut) cow milk with *Bifidobacterium lactis* Bb-12

Grafikon 2: Promjena pH tijekom fermentacije kravljeg mlijeka s *Bifidobacterium lactis* Bb-12 uz dodatak meda (AH-bagremov; CH-kestenov)

Table 4: Inhibition of *Listeria monocytogenes* growth with agar diffusion test

Tablica 4: Inhibicija rasta *Listeria monocytogenes* utvrđena restom difuzije na agaru

FT (h)/VF (h)	GM-0%H	GM-3%AH	GM-5%AH	GM-10%AH	GM-3%CH	GM-5%CH	GM-10%CH
0	±	±	±	±	+	+	++
15	±	+++	++	+++	++	++	+++
25	±	++	++	++	++	++	++
FT (h)/VF (h)	CM - Control	CM-3%AH	CM-5%AH	CM-10%AH	CM-3%CH	CM-5%CH	CM-10%CH
0	±	±	±	±	±	±	±
15	±	±	±	+	±	±	+++
25	±	±	±	++	±	++	+++

Legend for Table 3/Legenda za Tablicu 3: GM - goat milk/kozje mlijeko; CM - cow milk/kravlje mlijeko; AH - acacia honey/bagremov med; CH - chestnut honey/kestenov med; 3 %, 5 %, 10 % - addition of honey (w/v)/3 %, 5 %, 10 % - dodatak meda (w/v); FT (h) - fermentation time (h)/vrijeme fermentacije (h)

Inhibition/Inhibicija: ± - inhibition zones not clearly expressed and difficult to measure/inhibicijske zone nisu jasno izražene i teško su mjerljive; + - inadequate inhibition (<10 mm), difficult to measure/vrlo slaba inhibicija (<10mm), teško mjerljivo; ++ - clear inhibition zones (10-15 mm)/jasne zone inhibicije (10-15 mm); +++ - clear inhibition zones, >15 mm/jasne zone inhibicije, > 15 mm

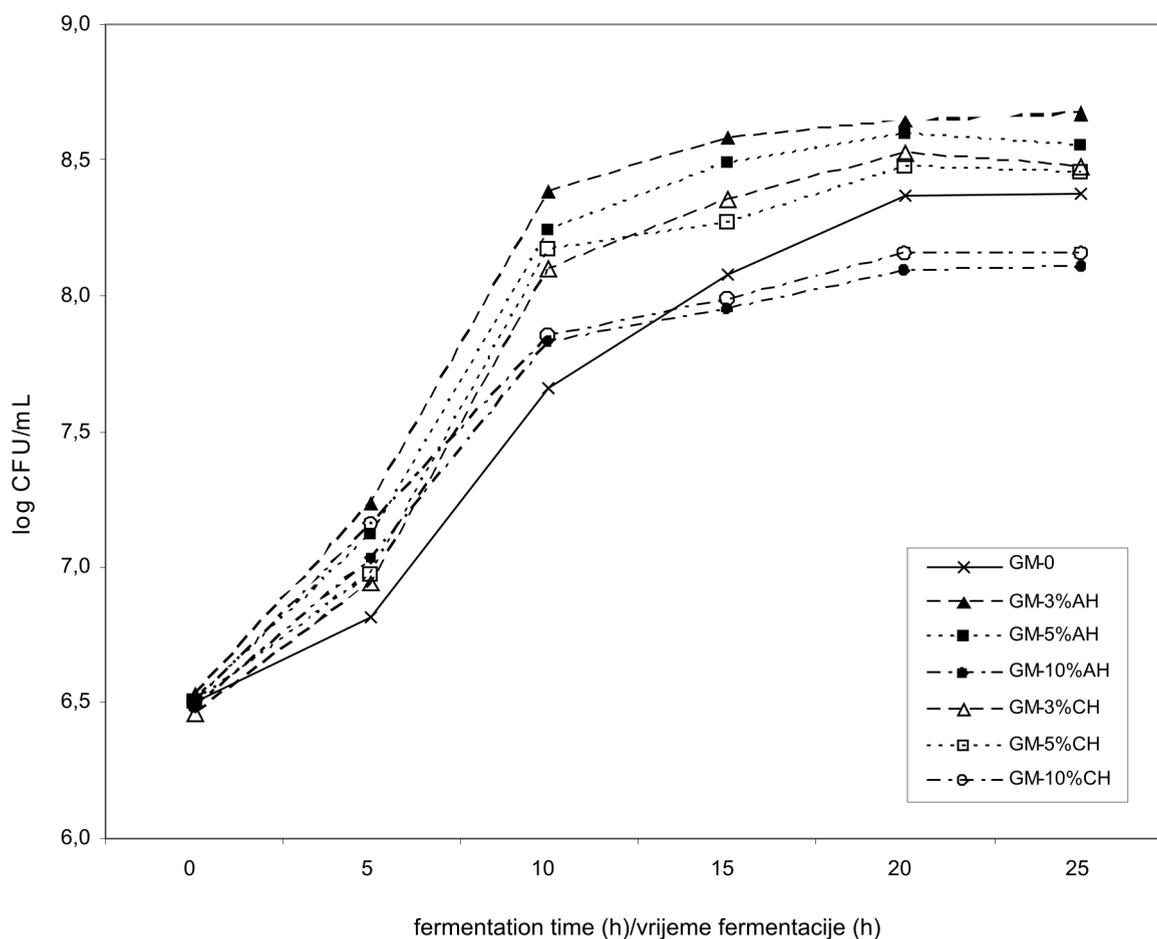


Fig. 3: Count of *Bifidobacterium lactis* Bb-12 (CFU/mL) during fermentation of honey-sweetened (AH-acacia; CH-chestnut) goat milk

Grafikon 3: Promjene broja bakterija *Bifidobacterium lactis* Bb-12 (CFU/mL) tijekom fermentacije kozjeg mlijeka uz dodatak meda (AH-bagremov; CH-kestenov)

The results on fermentation presented in Figs. 1-4 and Table 3 partially support the assertion mentioned above. In general, the addition of both types of honey had a certain influence on goat and cow milk fermentation with *Bifidobacterium lactis* Bb-12. However, significant differences between the type of added honey, as well as type of milk, are obvious. The first conclusion is that addition of acacia honey to both types of milk had higher promoting effect on the growth of *Bifidobacterium lactis* Bb-12 than chestnut honey. In goat milk, best promotion effect was achieved by the addition of 3 and 5 percent of acacia honey. Consequently, samples of fermented goat milk with addition of 3 % and 5 % of acacia honey, resulted in higher CFU of *Bifidobacterium lactis* Bb-12, and the lowest pH values was observed at the end of fermentation (Fig. 1, Fig. 3).

In contrary, 5 and 10 percent of chestnut honey to goat milk inhibited the growth of *Bifidobacterium lactis* Bb-12 thus reduced the acid production during fermentation. Unlike in goat milk, higher addition of acacia honey, particularly 10 %, showed the best promoting effect on growth of *Bifidobacterium lactis* Bb-12 in cow milk (Fig. 4). Consequently, pH values decreased more rapidly during fermentation of cow milk with 10 % of added acacia honey, than with 5 % and 3 % (Fig. 3). Compared to goat milk, addition of 5 % and 10 % of chestnut honey to cow milk did not inhibit the growth of bifidobacteria. Compared to goat milk fermentation, addition of 5 % and 10 % of chestnut honey to cow milk had weak supporting effect to the growth of *Bifidobacterium lactis* Bb-12.

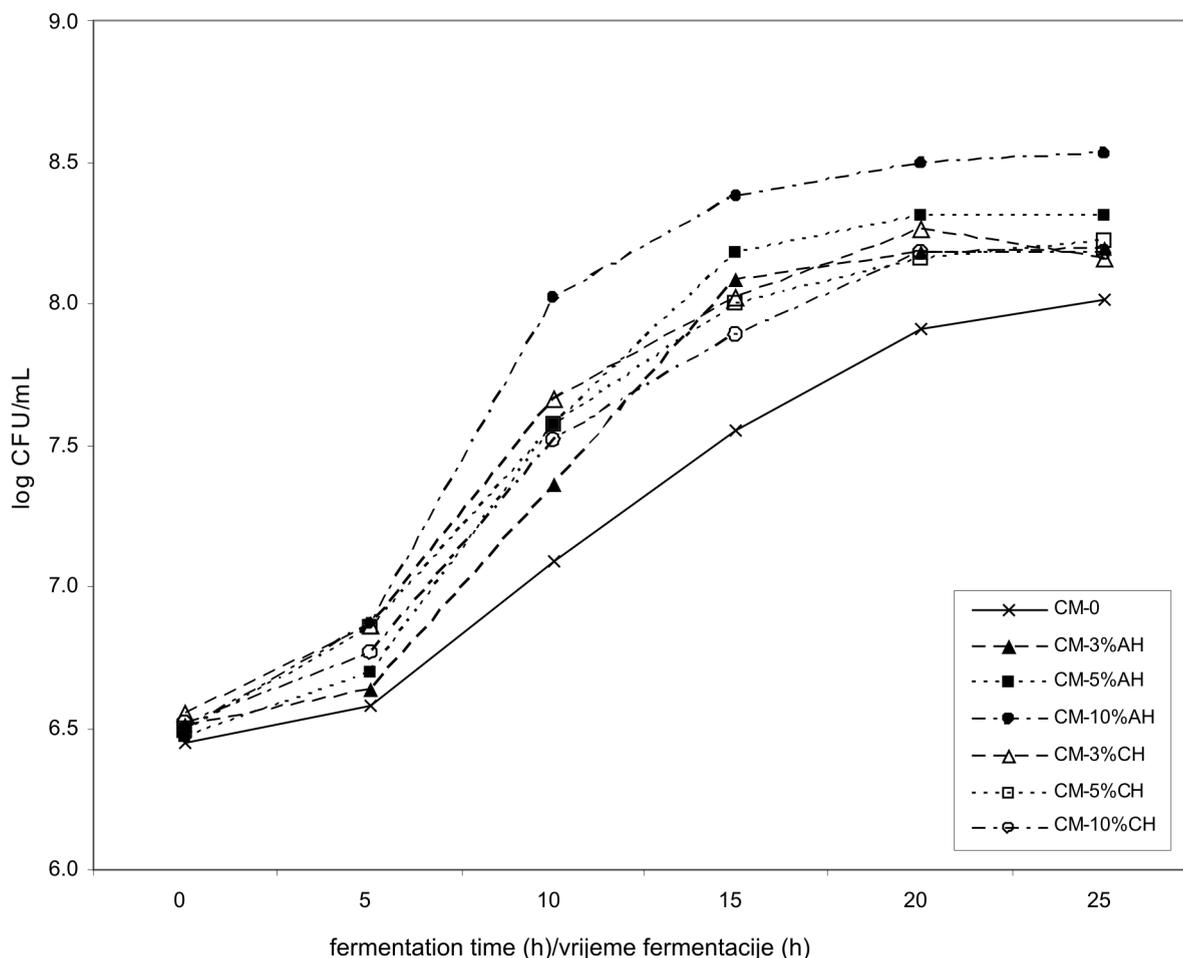


Fig. 4: Count of *Bifidobacterium lactis* Bb-12 (CFU/mL) during fermentation of honey-sweetened (AH-acacia; CH-chestnut) cow milk

Grafikon 4: Promjene broja bakterija *Bifidobacterium lactis* Bb-12 (CFU/mL) tijekom fermentacije kravljeg mlijeka uz dodatak meda (AH-bagremov; CH-kestenov)

These results suggested diversity in microbial sensitivity to components from honey during fermentation of milk. The results also suggest different fermentation courses in goat milk than in cow milk. Furthermore, obtained results obviously show that content of sugar (sucrose or reduced sugars), as a type of metabolic fuel, for examined bifidobacterial strain was not a limiting factor. In spite of significantly higher contents of sucrose and reduced sugars in chestnut honey (Table 2), acacia honey promoted the growth of *Bifidobacterium lactis* cells in both milk types significantly better (statistical analysis, Table 3). Moreover, number of bifidobacterial viable cells at the end of fermentation process in fermented goat milk without honey addition was statistically overlapped with samples of fermented goat milk with honey addition. Opposite to fermented goat

milk, number of bifidobacterial viable cells at the end of fermentation process in fermented cow milk without honey addition, as well as in samples of fermented cow milk with 3 % of chestnut addition, was significantly lower in comparison with other samples of fermented cow milk (Table 3).

Inhibitory effect of honey-sweetened fermented goat and cow milk samples against pathogenic bacteria *Listeria monocytogenes* is presented in Table 4. Opposite to the influence of honey addition to fermentation kinetics, where acacia honey strongly effected on increase of *Bifidobacterium lactis* growth in both milk types in comparison with chestnut addition, chestnut honey addition showed higher inhibitory potential against examined pathogen. Based on results, is clear that increase of chestnut honey addition proportionally influenced the higher inhibi-

tory potential of goat, and especially cow milk samples. Results presented in Table 4 show that samples of goat and cow milk without honey addition had very low antagonistic activity against *Listeria monocytogenes*, regardless to fermentation stage. Furthermore, results show that unfermented goat milk with 10 % of chestnut honey addition inhibited growth of *Listeria monocytogenes* considerably stronger than unfermented cow milk with 10 % of chestnut honey addition. These observations suggest higher inhibitory potential of goat milk-honey mixture than cow milk-honey mixture. Higher inhibitory potential of fermented goat milk against some pathogens in comparison with fermented cow milk was detected in some of our previous studies (Slačanac et al., 2004; Slačanac et al., 2007a; Slačanac et al., 2007b). However, the highest inhibitory effect detected in this study, were recorded for cow milk with 10 % of chestnut honey addition samples, fermented for 15 and 25 hours, as well as for goat milk samples with 10 % addition, fermented for 15 hours. It proves that addition of chestnut honey considerably influenced to inhibitory potential of cow milk against *Listeria monocytogenes*.

There are poor scientific information on the addition of honey to the milk before the start of fermentation process, but Chick et al. (2001) showed that addition of honey to skim milk supported growth of 4 lactobacilli and bifidobacteria strains. McNaught and MacFie (2001) have also cited a possible symbiotic effect of honey and fermented milk in clinical nutrition, but like many other authors emphasized indispensability of further investigations. Al-Wabel et al. (2007) prepared symbiotic fermented milk by mixing fermented milk with honey and other components and used it against lead acetate contamination in rats. Opposite to the above mentioned authors, Varga (2006) shows that addition of acacia honey at concentration 1-5 % (W/v) did not significantly influence viability of characteristic yoghurt starter microorganisms during refrigerated storage.

## Conclusion

The results presented in this study show that addition of acacia and chestnut honey had certain influence on goat and cow milk fermentation with *Bifidobacterium lactis* Bb-12. Addition of acacia

honey promoted growth of *Bifidobacterium lactis* Bb-12 in both milk types stronger than addition of chestnut honey. In goat milk, best promoting effect was achieved by the addition of 3 % and 5 % of acacia honey. In cow milk, best promoting effect was achieved by the addition of 10 % of acacia honey. In contrast to cow milk, addition of 10 % of acacia honey to goat milk inhibited the growth of *Bifidobacterium lactis* Bb-12. Opposite to acacia honey, addition of 5 % and 10 % of chestnut honey inhibited the growth of *Bifidobacterium lactis* Bb-12 in goat milk, but stimulated the growth of *Bifidobacterium lactis* Bb-12 in cow milk. In all cases (independently from the milk type, type of added honey or content of added honey), changes of pH values during fermentation were proportional to the rates of *Bifidobacterium lactis* growth. Opposite to the influence of honey addition to fermentation kinetics, where acacia honey strongly positively effected the growth of *Bifidobacterium lactis* in both milk types in comparison with chestnut addition, chestnut honey addition had higher inhibitory potential against *Listeria monocytogenes* growth. The highest antagonistic potential against *Listeria monocytogenes* had cow milk samples with 10 % of chestnut addition, fermented for 15 and 25 hours, as well as samples of goat milk with added 10 % of chestnut honey fermented for 15 hours.

## *Inhibicijski učinak kozjega i kravljega mlijeka s dodatkom meda, fermentiranog s Bifidobacterium lactis Bb-12 na rast bakterije Listeria monocytogenes*

### Sažetak

U radu je ispitivan utjecaj dodatka meda na tijek fermentacije kozjega i kravljega mlijeka probiotičkom bakterijom *Bifidobacterium lactis* Bb-12. Također, ispitivan je utjecaj medom zaslađenoga fermentiranoga kozjega i kravljeg mlijeka na inhibicijsko djelovanje rasta bakterije *Listeria monocytogenes*. Mlijeku su dodavane dvije vrste meda - tamna vrsta kestenova meda i svijetla vrsta bagremova meda. Osnovna pretpostavka u radu bila je da dodatak meda može utjecati na tijek fermentacije kozjega i kravljeg mlijeka, te na brzinu rasta *Bifidobacterium lactis* Bb-12 u mlijeku. Pretpostavljen je i jači inhibicijski učin fermentiranog mlijeka na rast *Listeria*

*monocytogenes* uzrokovan dodatkom meda mlijeku prije fermentacije. Rezultati ispitivanja pokazali su da obje vrste meda poboljšavaju rast i aktivnost *Bifidobacterium lactis* Bb-12 u obje vrste mlijeka tijekom fermentacije. Istovremeno, u kozjem je mlijeku tijekom cijelog razdoblja fermentacije zabilježena viša kiselost i veći broj stanica *Bifidobacterium lactis* Bb-12 nego u kravljem mlijeku. Testovi inhibicije rasta bakterije *Listeria monocytogenes* pokazali su da su veličine zona inhibicije ovisile o svim ispitivanim čimbenicima - vrsti mlijeka, vrsti dodanog meda, kao i o udjelu dodanog meda mlijeku prije fermentacije. Uzorci fermentiranog mlijeka s dodatkom kestenova meda jače su inhibirali rast bakterije *Listeria monocytogenes* nego uzorci s dodatkom bagremova meda.

**Ključne riječi:** *Bifidobacterium lactis* Bb-12, fermentirano kozje i kravlje mlijeko, bagremov i kestenov med, inhibicijski učinak, *Listeria monocytogenes*

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Presented results were obtained from scientific project (Functional properties of different types of milk and whey fermented by probiotics) supported by Ministry of Science, Education and Sports, Republic of Croatia; as well as from bilateral scientific project with Hungary (HR-HU-bifido-05-07-001-Direction and evaluation of bioactive components produced by bifidobacteria).