Fermentation of Honey-Sweetened Soymilk with *Bifidobacterium lactis* Bb-12 and *Bifidobacterium longum* Bb-46: Fermentation Activity of Bifidobacteria and *in vitro* Antagonistic Effect against *Listeria monocytogenes* FSL N1-017

Vedran SLAČANAC, Mirela LUČAN, Jovica HARDI, Vinko KRSTANOVIĆ and Daliborka KOCEVA KOMLENIĆ

Faculty of Food Technology, University Josip Juraj Strossmayer in Osijek, Osijek, Croatia

Abstract

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The influence of the honey addition on the fermentative activity of *Bifidobacterium lactis* Bb-12 and *Bifidobacterium longum* Bb-46 in soymilk was determined. Additionally, the inhibitory potential of honey-sweetened fermented soymilk against *Listeria monocytogenes* strain was examined. Two monofloral honey types were added to soymilk before the fermentation: dark-coloured chestnut honey and light-coloured acacia honey. On the basis of our previous studies on cow and goat milks, the basic hypothesis of this study was that the addition of honey could influence the growth of *Bifidobacterium lactis* and *Bifidobacterium longum* during the fermentation of soymilk. The addition of honey also influenced the decrease of raffinose and stachyose contents during fermentation. Furthermore, a higher inhibitory potential was assumed against *Listeria monocytogenes* caused by the honey addition. The obtained results show that both types of honey influenced the fermentative activity and numbers of *Bifidobacterium lactis* Bb-12 and *Bifidobacterium longum* Bb-46 viable cells in soymilk. Chestnut honey strongly influenced the acidity increase during the soymilk fermentation. A disc assay showed that the development of the inhibition zones of the growth depended on the type and concentration of honey, as well as on the type of milk. The chestnut honey had generally a higher inhibitory effect than acacia honey.

Keywords: bifidobacteria; fermenttive acivity; fermented soymilk; acacia honey; chestnut honey; inhibitory effect

The production of non-dairy fermented food has been pointed out as a novel trend in functional food production (Kano *et al.* 2002). Soymilk is suitable for the growth of lactic acid bacteria, especially bifidobacteria (Chou & Hou 2000a,b; Božanić *et al.* 2008a,b). Being free of cholesterol, gluten, and lactose, soymilk is suitable for lactose-intolerant consumers, vegetarians, and milk-allergy patients (Liu & Lin 2000). Soy-based foods may provide a range of health benefits to consumers due to their hypolipidemic, anticholesterolemic, and antiatherogenic properties, as well as reduced allergenicity (Favaro Trindade *et al.* 2001; Donkor *et al.* 2007). Bifidobacteria have been recognised as important for the health of the gastrointestinal tract (GI) (Tamime *et al.* 1995; Aires *et al.* 2009). One approach for ensuring or increasing the presence of healthful colonic bacteria is to provide suitable food, e.g. fermented milk.

The term "probiotic" should be used only for such products that meet the scientific criteria

for this term-namely, the products that contain an adequate dose of live microbes that have been documented in target-host studies to confer a health benefit. To have an impact on the colonic flora, it is important for the probiotic strains to exhibit antagonism against pathogenic bacteria via the production of antimicrobial substances (such as acetic and lactic acids, hydrogen peroxide etc.) or competitive exclusion (Leeber *et al*. 2010). Several authors suggested that low molecular weight metabolites and secondary metabolites play a more important role than bacteriocins, since they show a wide inhibitory spectrum against many harmful organisms (Niku-Paavola *et al*. 1999; Saarela *et al*. 2000; Slačanac *et al*. 2005; Boesten & de Vos 2008). According to the basic definition, bacteriocins are antibiotic-like substances and bactericidal proteins, which could be also produced during lactic acid fermentation (Klaenhammer 2006).

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

In some recent studies, honey has been recognised as a promoter of lactobacilli and bifidobacteria growth. However, there is no clear scientific information on the synergistic effect of honey on the growth of probiotics in milk during lactic acid fermentation. On the other hand, antimicrobial activity of honey has been also reported in many recent studies (Frankel *et al*. 1998; Taormina *et al*. 2001; Varga 2006). Molan (1992b) listed the results of *in vitro* studies which confirmed a strong antibacterial and a slightly lower antifungal activities of different types of honey. Most of the antimicrobial activity of honey is derived from its low water activity (osmolytic effect, high acidity (its pH being between 3.2 and 4.5), and its catalase or glucose oxidase activity resulting in hydrogen-peroxide formation (Molan 1992a).

Listeria monocytogenes is a ubiquitous foodborne 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). The manifestations of listeriosis include meningoencephalitis, septicemia, abortion, and a high fatality rate of 30% (Liu 2004; McLauchlin *et al*. 2004).

The aim of this study was to determine the influence of honey addition on the growth of *Bifidobacterium lactis* Bb-12 and *Bifidobacterium longum* Bb-46 during the soymilk fermentation. Furthermore, the influence of the addition of honey to fermented milk on the antagonistic effect against the psychrotrophic *Listeria monocytogenes* FSL N1-017 strain needs to be examined.

MATERIALS AND METHODS

Preparation of** Listeria monocytogenes **suspension, *Listeria monocytogenes* FSL N1-017, obtained from the Institute of Public Health (Osijek, Croatia), was used. *L. monocytogenes* FSL N1-017 was cultured on Tryptic Glucose Yeast agar (MERCK KgaA, Darmstadt, Germany) at 37°C for 24 hours. For the determination of inhibition, the inocula were adjusted to match with 0.5 McFarland and each suspension was further diluted to obtain the final concentration of 1×10^8 CFU/ml. The final concentration was obtained as 10^{-4} dilution of the initial concentration.

Preparation of soymilk. Soymilk was prepared according to the method of Mulimani and Ramalingam (1995). Soybean seeds were ground to flour. The soybean flour was defatted with hexane (1:1 w/v) (Prashant & Mulimani 2005), suspended in 10-fold volume of distilled water and heated to boiling. The undissolved residue was separated from soymilk by centrifugation at 5000 rpm (Multifuge 3L-R; Heraeus, Buckinghamshire, UK) for 5 minutes. The pasteurised supernatant (65°C/20 min) containing soymilk was used for fermentation with bifidobacteria.

Determination of oligosaccharides in soymilk, fermented soymilk, and honey-sweetened soymilk. Soymilk, fermented soymilk, and honey-sweetened soymilk (15 ml) were poured into 35 ml of absolute ethyl alcohol and centrifuged at 6000 rpm and 37°C for 15 minutes. The supernatants were dissolved in

15 ml of distilled water. The amounts of raffinose and stachyose were determined by the simplified method of Tanaka *et al*. (1975) using a Thin Layer Chromatography method. TLC was performed on aluminium sheets (20 × 20 cm) coated with silica gel G260; Merck KgaA, Darmstadt, Germany). 10 µl of soymilk was applied onto TLC plate. The solvent used was n-propanol-ethyl acetate-water (7:2:1). After the run, the plates were dried and the sugars were spotted by spraying with 1% α-naphthol in absolute alcohol containing 10% orthophosphoric acid. The spots were identified based on the retention factor. The identified spots in TLC were cut out and each spot was separately soaked in 2 ml of distilled water for total dissolution of the sugars. 0.1 ml of 5% phenol was added to each dissolved sugar followed by a rapid addition of 0.5 ml concentrated sulphuric acid. The tubes were then placed in a water bath to cool for 20 minutes. The absorbance was read at 490 nm and the corresponding concentrations were determined from the standard curves prepared from each reference sugar. The measurements were done 5 times.

Analyses of acacia (A) and chestnut (C) honey. Water content (moisture) was determined using a refractometric method (RX-5000ALPHA BEV Abbe Refractometer; ATAGO, Tokyo, Japan) with reading at 20°C (AOAC Official Method 969.38). Total ash content was determined by incinerating the honey samples in a muffle furnace at a temperature of 550°C overnight (AOAC Offical Method 920.181). pH values were measured with pH-meter (MA 235, pH/Ion Analyzer; METTLER TOLEDO, Giessen, Germany) in a solution containing 10 g honey in 75 ml of CO_2- free distilled water (AOAC Official Method 962.19). Total acidity was determined by a titrimetric method using 0.1M NaOH in accordance with AOAC Official Method No. 962.19 and expressed as miliequivalents of NaOH/kg. Hydroxymethylfurfural was determined by the 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 a thermostatic bath until the endpoint was reached. Free amino acids content was determined by the reaction between α-amino acids and formaldehyde (Method No. 30; FIPJF 1984). The sugar profiles of acacia and chestnut honey were determined by HPLC method according to Wunderlin *et al*. (1998). Ten samples of each types of honey were analysed.

Fermentation conditions and analyses during fermentation. DVS monocultures of *Bifidobacterium longum* Bb-46 and *Bifidobacterium lactis* Bb-12 (Chr. Hansen, Hørsholm, Denmark) were used to inoculate the samples which had been fermented for 25 h at 37°C.

Acacia and chestnut honeys were added to soymilk before the fermentation in levels of 5 and 10%. Before the addition, honey was pasteurised for 30 min at 63°C.

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

The viable counts of *B. longum* Bb-46 and *B. lactis* Bb-12 were determined on modified Bifidobacterium agar (according to Deutche Sammlung von Microorganismen und Zelkulturen GmbH, Braunschweig, Germany) in anaerobic jars (Merck KGaA, Darmstadt, Germany) at 37°C after 48 hours. Anaerocult C (Merck KgaA, Darmstadt, Germany) was used for semi-anaerobic conditions in the jars. MRS agar (Merck KGaA, Darmstadt, Germany) was modified by adding 13.5 g/100 ml Bacteriological agar (Agar Bios Special LL; Biolife, Milan, Italy) and 3 g/1000 ml LiCl.

pH and viable counts of bifidobacteria were determined every five hours during the fermentation. All measurements were done 4 times.

Agar diffusion test. The agar well diffusion method was used to detect *in vitro* the inhibitory effects of the samples on *L. monocytogenes* growth. 10 ml of molten Mueller-Hinton agar (Biolife, Milan, Italy) were cooled at 47°C and seeded with 1 ml of the prepared suspension of *L. monocytogenes* containing 10^8 cells/ml. The samples of soymilk and fermented soymilk were centrifuged at 6200× *g* and 4°C for 10 min before the antibiotic assay. The clear supernatant was applied on the antibiogram susceptibility walls. The seeded agar was poured into a sterile Petri plate and overlaid with a second layer of 10 ml of sterile Mueller-Hinton agar. After solidification at room temperature, wells (diameter 9 mm) were cut in the agar using a sterile metal cork borer and were filled with 150 µl of sample. After incubation at 37°C for 24 h, the zones of inhibition (clear or proximate clear areas) surrounding the wells were measured according to the method mentioned (Servin 2004), including the sums of wells diameters and inhibition zones diameters. The measurements were done 3 times.

Statistics. All the results were statistically analysed using Basic statistic pack in STATISTICA 8.0.

The standard deviations of all corresponding results series were calculated. The influence of honey on the fermentation rate (related to pH values decrease), as well as on *B. longum* Bb-46 and *B. lactis* Bb-12 cells in fermented soymilk, was analysed using Fisher's *LSD* test in STATISTICA 8.0. The coefficient of variation (*CV*) was used to analyse the microbiological results (Shelley *et al*. 1987).

RESULTS AND DISCUSSION

The composition and basic properties of acacia and chestnut honeys obtained from the Croatian honey producers are presented in Table 1. Like in our previous studies (Lučan *et al*. 2009; Slačanac *et al*. 2011), the data presented in Table 1 show that the ash content, reducing sugar content, active diastase units, and hydroxymethylfurfural (HMF) content were higher in chestnut honey than in acacia honey. Because a specific fermentation environment during the fermentation was created, some of these parameters could have influenced the fermentation activity of probiotic starters in milk (Slačanac *et al*. 2011). Ustunol and Gandhy (2001) specified honey as a complex additive with a wide spectrum of positive effects in fermented milk. Possible prebiotic effects are one of these effects.

The fermentation of soymilk by bifidobacteria has been investigated in many studies in the last 15 years. The growth of bifidobacteria in soymilk and their survival in the fermented soymilk have been recognised as basic functional requirements (Chou & Hou 2000). Fermentative activities of *B. longum* Bb-46 and *B. lactis* Bb-12 in soymilk determined in this study are presented in Tables 2 and 3. Related to bifidobacteria activity without honey addition, pH values and viable cells attained after 25 h of fermentation in this study (Tables 2 and 3) are in

Table 1. Chemical composition and basic properties of added acacia and chestnut honey collected from Bilogorian (West Croatian province) and East Slavonian region

Component /Property	Type of honey ($\bar{x}$ ± SD)	
	accacia	cheesnut
Water (%)	16.25 ± 1.93	17.63 ± 8.1
Ash (%)	0.058 ± 0.03	0.87 ± 2.07
Acidity (miliequivalents of NaOH/kg)	9.73 ± 3.76	16.2 ± 3.54
Water insoluble components (%)	0.011 ± 0.008	0.036 ± 0.013
Reducing sugars (%)	67.69 ± 7.05	77.82 ± 12.25
Sucrose (%)	0.51 ± 0.14	1.84 ± 0.55
Active dyastase (U)	14.2 ± 2.93	25.8 ± 5.49
Hydroxymethylfurfural (mg/kg)	3.4 ± 0.90	4.8 ± 1.70
pH value	3.95 ± 0.41	3.6 ± 0.27

$\bar{x}$ – mean value of 10 determinations; SD – standard deviation

Table 2. Changes in pH values*** during the fermentation of honey-sweetened soymilk with *Bifidobacterium longum* Bb-46 and *Bifidobacterium lactis* Bb-12

Sample	Fermentation time (h)					
	0	5	10	15	20	25
Bb46*	7.03	6.20	5.18	4.77	4.68	4.67^{abc}
Bb46 – 5%A	6.95	5.66	4.89	4.69	4.43^{IP}	4.53^{ab}
Bb46 – 10%A	6.85	5.58	4.86	4.82	4.60^{IP}	4.42^{bc}
Bb46 – 5%C	6.85	5.31	4.73	4.53^{IP}	4.38	4.39^{bc}
Bb46 – 10%C	6.55	5.23	4.76	4.80	4.63^{IP}	4.43^{bc}
Bb12**	6.97	5.69	5.07	4.83	4.71	$4.64^{IP\ a}$
Bb12 – 5%A	6.80	5.35	4.91	4.61^{IP}	4.57	4.47^{bc}
Bb12 – 10%A	6.82	5.36	4.89	4.71	4.49^{IP}	4.42^{bc}
Bb12 – 5%C	6.86	5.25	4.77	4.62^{IP}	4.48	4.37^{bc}
Bb12 – 10%C	6.59	5.25	4.76	4.57^{IP}	4.46	4.35^{c}

A – acacia honey addition; C – chestnut honey addition; ^{IP}isoelectrical point of soymilk proteins was attained; *samples fermented with *Bifidobacterium longum* Bb-46; **samples fermented with *Bifidobacterium lactis* Bb-12; ***mean value of 4 determinations; $^{a–c}$values in the same column with different letters were significantly different by Fisher's *LSD* test ($P < 0.05$)

Table 3. Changes in a number of *Bifidobacterium longum* Bb-46 and *Bifidobacterium lactis* Bb-12 viable cells (log CFU/ml)*** during the fermentation of honey-sweetened soymilk with

Sample	Fermentation time (h)					
	0	5	10	15	20	25
Bb46*	4.92	5.05	5.25	5.78	6.31	$6.70^{HN\ cd}$
Bb46 – 5%A	4.96	6.37	7.46	7.83^{HN}	7.68	7.19^{b}
Bb46 – 10%A	5.51	6.35	7.45^{HN}	7.41	7.38	7.33^{ab}
Bb46 – 5%C	5.74	5.76	6.63	7.61^{HN}	7.50	7.39^{a}
Bb46 – 10%C	5.28	6.80	6.78	7.51^{HN}	6.84	6.79^{c}
Bb12**	5.17	5.51	5.79	5.81	5.85	$6.38^{HN\ f}$
Bb12 – 5%A	4.74	4.72	4.91	5.29	6.64	$6.71^{HN\ cd}$
Bb12 – 10%A	5.20	5.19	5.80	5.81	5.85	$6.39^{HN\ ef}$
Bb12 – 5%C	5.28	5.22	5.77	5.97	6.58^{HN}	6.49^{ef}
Bb12 – 10%C	5.20	5.45	6.17	6.29	6.37	$6.56^{HN\ de}$

A – acacia honey addition; C – chestnut honey addition; ^{HN}highest number of viable cells; *samples fermented with *Bifidobacterium longum* Bb-46; **samples fermented with *Bifidobacterium lactis* Bb-12; ***mean value of 4 determinations; $^{a–f}$values in the same column with different letters were significantly different by Fisher's *LSD* test ($P < 0.05$)

correlation with the results of the previous studies which also investigated the growth of bifidobacteria in soymilk (Chou & Hou 2000a,b; Wang *et al*. 2002, 2003, 2004; Božanić *et al*. 2008b). Overall, the results showed that the addition of both types of honey had stimulatory effects on the growth of *B. longum* Bb-46 and *B. lactis* Bb-12 in soymilk. However, the growth promotion effect of honey in soymilk was significantly lower than that in cow or goat milks determined in our previous studies (Lučan *et al*. 2009). From the data shown in Tables 1 and 2, it is evident that the addition of acacia and chestnut honeys resulted in lower pH values and a higher number of bifidobacteria cells. The actions of acacia and chestnut honeys in soymilk were approximately proportional, but some differences during fermentation were still noted. In the case of *B. longum* Bb-46 fermentation, the lowest pH values were reached after 20 h of fermentation when the acacia honey was added, while the highest number of viable cells was determined after 15 h of fermentation. The addition of 5% of chestnut honey influenced the faster pH value decrease as compared to other samples. At the same time, the samples with 10% acacia honey added contained the highest number of *B. longum* Bb-46 cells already after 10 h of fermentation. The final pH values and number of *B. longum* Bb-46 cells (25 h of fermentation) were approximately proportional in all samples regardless of the type of honey and amount of honey addition. In the case of *B. lactis* Bb-12 fermentation, the addition of acacia and chestnut honeys influenced faster pH values decrease, but the final number of *B. lactis* Bb-12 cells in fermented soymilk was statistically significantly lower in comparison with *B. longum* Bb-46 cells number (Tables 1 and 2). The addition of acacia and chestnut honeys also resulted in the higher number of *B. lactis* Bb-12 cells compared to the samples without the honey addition, but less than in the case when the soymilk was fermented with *B. longum* Bb-46.

Stacchyose and raffinose, the principal oligosaccharides in soymilk, are believed to cause flatulence in humans after eating soybean foods (Scalabrini *et al*. 1998). Galactosidase is known as raffinose and stacchyose hydrolysing enzyme (Wang *et al*. 2003). The production of galactosidase by bifidobacteria has been reported (Hughes & Hoover 1995; Tamime *et al*. 1995). The changes in the contents of raffinose and stacchyose in soymilk fermented with *B. longum* Bb-46 and *B. lactis* Bb-12 are shown in Table 4. Regardless of which bifidobacteria were used, the content of both raffinose and stacchyose decreased proportionally with the fermentation time increase (Table 4). The reduction of raffinose content during the soymilk fermentation with *B. longum* Bb-46 and *B. lactis* Bb-12 was 17.36 and 18.89%, respectively. The reduction of stacchyose content during

Table 4. Changes in raffinose and stachyose contents (mmol/l)* in soymilk fermented with *Bifidobacterium longum* Bb-46 and *Bifidobacterium lactis* Bb-12

	Fermentation time (h)	Fermentation with			
		Bb46	Bb46 – 5%H	Bb12	Bb12 – 5%H
Raffinose	0	1.44 ± 0.05^{a}	1.44 ± 0.05^{a}	1.43 ± 0.03^{a}	1.43 ± 0.03^{a}
	10	1.39 ± 0.05^{ab}	1.36 ± 0.08^{ab}	1.41 ± 0.08^{ab}	1.35 ± 0.12^{ab}
	15	1.33 ± 0.02^{b}	1.27 ± 0.10^{bc}	1.31 ± 0.07^{b}	1.26 ± 0.10^{bc}
	25	1.19 ± 0.09^{c}	1.15 ± 0.09^{d}	1.16 ± 0.04^{c}	1.11 ± 0.09^{de}
Stachyose	0	6.06 ± 0.06^{a}	6.06 ± 0.06^{a}	6.06 ± 0.06^{a}	6.06 ± 0.06^{a}
	10	5.80 ± 0.05	5.70 ± 0.07	5.83 ± 0.04	5.66 ± 0.06
	15	4.75 ± 0.09	4.41 ± 0.18	4.81 ± 0.17	4.78 ± 0.10
	25	3.37 ± 0.11	3.22 ± 0.11	3.29 ± 0.09	3.12 ± 0.05

H – 5% addition of mixture of acacia and chestnut honey (1:1 v/w); *mean value of 4 determinations ± SD; $^{a–e}$values for raffinose and stachyose content in the same row and column with different letters were significantly different by Fisher's *LSD* test ($P < 0.05$)

the soymilk fermentation with *B. longum* Bb-46 and *B. lactis* Bb-12 was even higher, 44.38 and 45.70, respectively. The reduction of raffinose and stachyose observed was in accordance with the results presented in some previous reports (Cumchuere & Robinson 1999; Wang *et al*. 2003; Donkor *et al*. 2007). The addition of acacia and chestnut honeys resulted in further degradation of raffinose and stacchyose during the soymilk fermentation with *B. longum* Bb-46 and *B. lactis* Bb-12. The reduction of raffinose content during the fermentation of honey-sweetened soymilk with *B. longum* Bb-46 and *B. lactis* Bb-12 was 20.14 and 22.28%, respectively, whereas the reduction of stacchyose content was 44.39 and 48.51%, respectively. The same results were obtained when either acacia or chestnut honey was added, with negligible differences. These data could have an important nutritional significance, because they indicate that the honey addition improves bifidobacterial glucosidase activity during the soymilk fermentation.

In a number of studies the antagonistic activity of dairy products fermented with probiotics against many harmful microorganisms was studied (Salminen & Ouwehand 2003). In our previous studies, the inhibitory effects of fermented cow's and goat's milks on the growth of some intestinal and urogenital pathogens were investigated (Slačanac *et al*. 2011). However, related to lactose malabsorption, the tendency for non-dairy food fermented with probiotics has existed for the last 15 years (Donkor *et al*. 2007). In this study, the agonistic effect of honey-sweetened fermented soymilk against dairy critical pathogen *L. monocytogenes* was investigated. The data in Table 5 suggest certain inhibitory effects of honey-sweetened soymilk fermented with *B. longum* Bb-46 and *B. lactis* Bb-12 on the *L. monocytogenes* growth. The results obtained show that the soymilk fermented with *B. longum* Bb-46 had a significantly higher inhibitory effect on the *L. monocytogenes* growth than the soymilk fermented with *B. lactis* Bb-12. Furthermore, the data in Table 5 show that honey definitely heightened the inhibitory potential of fermented soymilk. This is logical because honey per se have antimicrobial effects. For that reason, significantly larger zones of inhibitions were measured around the discs of soymilk with honey added. In combination with fermented food which has incorporated probiotic cells, the positive functional effect could be multiplied. Furthermore, the results obtained show a higher inhibitory potential of fermented soymilk with the addition of acacia honey compared to that of chestnut honey. According to the inhibitions zones measured, the samples of nonfermented and fermented soymilk with 10% of acacia honey added had the highest inhibitory impact on *in vitro L. monocytogenes* growth. It is very hard to define all complex biochemical and microbiological interactions between bifidobacterial starters, pathogen, and compounds from the additive (honey), but the differences in the composition between acacia and chestnut honeys might be a reason for different levels of the inhibitory action against *L. monocytogenes*.

Table 5. Inhibition of *Listeria monocytogenes* growth by soymilk fermented with *Bifidobacterium longum* Bb-46 and *Bifidobacterium lactis* Bb-12: agar diffusion test (mean values of 3 determinations)

Sample	Uninoculated samples	Unfermented samples	Fermentation time (h)	
			15	F
Bb46	11.2[a]	12.5*[b]	13.0[b]	12.6[b]
Bb46 – 5%A	11.8[ab]	18.5[g]	14.0[c]	18.2[g]
Bb46 – 10%A	12.2[b]	21.3[h]	15.8[de]	17.5[f]
Bb46 – 5%C	12.0[b]	14.7[cd]	13.0[b]	15.5[d]
Bb46 – 10%C	11.3[a]	13.9[c]	14.3[c]	15.8[de]
Bb12	±	±	11.80	±
Bb12 – 5%A	±	11.6[a]	12.1[ab]	14.14[c]
Bb12 – 10%A	11.9[ab]	16.8[ef]	16.2[e]	15.9[de]
Bb12 – 5%C	±	±	±	11.7[a]
Bb12 – 10%C	±	11.5[a]	±	11.5[a]

A – acacia honey addition; C – chestnut honey addition; 15 – samples fermented for 15 h; F – fully fermented samples (pH value of samples 4.6); *measured inhibition zones including the sums of wells' diameters and inhibition zones diameters; [a–f]values in the same row and column with different letters were significantly different by Fisher's *LSD* test ($P < 0.05$); ± – inhibition zones were unclear and hard to measure

CONCLUSION

There is no real scientific information on the addition of honey to soymilk before the start of the fermentation process. The results obtained in this study show a certain stimulatory effect of the addition of acacia and chestnut honeys on the *Bifidobacterium longum* Bb-46 and *Bifidobacterium lactis* Bb-12 growth in soymilk. The addition of honey had a positive efect resulting in the largest number of bifidobacterial cells in fermented soymilk, a faster pH value decrease, and the degradation of principal oligosaccharides (raffinose and stachyose) during fermentation. The results obtained also show that the addition of honey improved the inhibitory potential of soymilk fermented with the selected probiotic strains against one of the dairy most important psychrotrophic pathogen *Listeria monocytogenes*. All results obtained could have a nutritional impact and give directions for further investigations.

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Corresponding author:

Ph.D. Vinko Krstanović, Josip Juraj Strossmayer University, Faculty of Food Technology, Franje Kuhača 20, 31 000 Osijek, Croatia
tel. + 38 531 224 356, e-mail: vinko.krstanovic@ptfos.hr