The Resistance of Some Prebiotics and Probiotic Bacteria in the Stomach Model Environment
Dažu prebiotiku un probiotisko baktēriju rezistence kuņģa modelī

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Abstract. This study was done to evaluate the resistance of 4% water solution of prebiotics (inulin, maltodextrin, and lactulose) and probiotic bacteria *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* in different stomach model environments. The simplest stomach model environment was an HCl water solution and an oat gruel filtrate with 0.46% of solids at pH 2. Fermentations of oat gruel water environments with or without prebiotics and/or probiotic bacteria were carried out at 37 ºC for 30 or 60 min. In water as well as in oat gruel environments, lactulose and maltodextrin proved to be more resistant than inulin. After 60 min of incubation with raftiline, glucose, and albumin, probiotic bacteria *L. acidophilus* showed slightly higher viable cell count (VCC) than *B. lactis* Bb-12. Both cultures had similar good resistance in oat gruel-based stomach model system; however, *B. lactis* Bb-12 was found to be slightly more resistant than *L. acidophilus*. The biomass samples of *B. lactis* Bb-12 and *L. acidophilus* were discriminated by Fourier transform infrared (FTIR) spectroscopy according to the composition of growth environment in order to evaluate qualitative differences in the carbohydrate composition and quantitative differences in the content of total carbohydrates and proteins in bacterial cells. In the environment with raftiline and albumin, the amount of *B. lactis* biomass increased nearly twice and the intensity of the absorption band at 1040 cm^{-1} increased significantly, which differed from the environment with glucose.
Key words: prebiotics, probiotic bacteria, stomach model environment, resistance.

Introduction

Prebiotics are nondigestible food components that selectively stimulate the growth and/or activity of one or a limited number of bacteria in the colon that can improve the host's health. Natural prebiotics are components of different foods and can be isolated from plants or synthesized enzymatically, for example from sucrose. The prebiotic must be stable in the stomach environment, not absorbed in the gastrointestinal tract but fermented by the gastrointestinal microflora, and must selectively stimulate the growth and/or activity of intestinal bacteria associated with health and wellbeing (Gibson, 1999; 2004; Wang, 2009). Well known prebiotics are lactulose, lactitol, oligofructose, fructooligosaccharides, inulin and galacto-oligosaccharides, isomaltooligosaccharides, arabinogalactan, polydextrose and maltodextrin. Probiotics are microorganisms – certain species and strains of *Lactobacillus*, *Bifidobacterium,* and *Streptococcus* used as supplements of foods and beverages. However, not all bacteria present in fermented milk products or yogurt possess the probiotic effect. Foodstuffs with probiotic bacteria and prebiotic additives are called synbiotic or eubiotic functional foods that possess increased survivability of the administered probiotic and facilitate its inoculation in the large intestine (Scantlebury-Manning, Gibson 2004). Recent studies have shown that synbiotics modulate the gut microbiota, promoting its healthier composition; it appears that synbiotics can be more efficient than either pro- or prebiotics alone in inducing this effect (Saulnier, 2007).

There are many studies dealing with the effect of prebiotics on the organism *in vivo* experiments while few investigations are devoted to *in vitro* experiments on the impact of fructooligosaccharides on the growth of selected probiotic bacteria. The growth dynamics of *Lactobacillus acidophilus* DSM 20079, *Lactobacillus acidophilus* DSM 20242, *Bifidobacterium bifidum* DSM 20082, *Bifidobacterium bifidum* DSM 20215, *Bifidobacterium bifidum* DSM 20239, and *Bifidobacterium bifidum* DSM 20456 in media supplemented with various saccharides, including prebiotic preparations, were evaluated by Goderska, Nowak and Czarnecki (2008). An ideal probiotic would be one that can survive passage through the gastrointestinal tract, establish itself permanently in the small intestine and colon, and provide a specific health benefit for the host by eliciting an immune response; secretion, production, and synthesis of compounds such as short-chain fatty acids, lactic acid, and bacteriocins or another appropriate mechanism. As a source of energy this probiotic would selectively utilize a prebiotic, would be safe, and would have few, if any, side effects (Bezkorovainy, 2001).

Lactic acid-fermented foods have been shown to increase the absorption of iron (Fe) in human organism, possibly by lowering pH, activation of phytases, and formation of soluble complexes of Fe and organic acids. Bering, Suchdev and Sioltov (2006) tested the effect of an oat gruel fermented with *Lactobacillus plantarum* 299v on non-haem Fe absorption from a low-Fe bioavailability meal compared with pasteurized, fermented oat gruel and non-fermented oat gruels. It was shown that fermented gruel with live *L. plantarum* 299v significantly increased the absorption of Fe compared with the pasteurized and non-fermented gruels. Oat gruel is one of typical foods for infants and elderly people, and there are several oat-based functional food products on a market, thus the resistance of prebiotics in oat gruel-based stomach model environment should be estimated.

The aim of this study was to evaluate the resistance of some prebiotics (inulin, lactulose, and maltodextrin) and the growth of probiotic bacteria *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* in different simple stomach model environments.

Materials and Methods

Prebiotics under the study were: lactulose-based solution containing ≥67.0% of lactulose (Duphalac, Netherlands), ≥10.0% of galactose, and ≥6.0% of lactose; inulin (Raftiline HP, Orafti, Belgium), with inulin content ≥99.5%, polymerization degree ≥5, dry matter (DM) content – 97%; and maltodextrin (Aldrich). The probiotic bacteria – freeze-dried *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* – were obtained from Chr. Hansen (Denmark).

The stomach model environment was an HCl water solution with pH 2 and an oat gruel filtrate with 0.46% of solids at pH 2. An oat gruel filtrate was prepared from 10 g of oat flacks per liter of water, boiled for 10 min. Water solutions of prebiotics – 4% of inulin, 4% of maltodextrin, or 4% of lactulose – were stored at 37 ºC for 15, 30 or 60 min, at pH 2. Fermentations of 0.46% oat gruel water solution with or without prebiotics and/or probiotic bacteria were carried out at 37 ºC for 30 and 60 min.

Prior to fermentation, *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* were twice cultivated in MRS (de Man, Rogosa and Sharpe**)** medium at 37º C for 24 h, then centrifuged and the obtained biomass dissolved in 2 mL of peptone water, pH was regulated to 2, and 400 µL of culture were grown in water-prebiotic environment with 4% of raftiline, 4% of albumin, and 4% of glucose in several combinations at 37 ºC for 60 min. The viable cell count (VCC mL^{-1}) was tested before and after the fermentation.

The concentration of total carbohydrates was measured after hydrolysis (10% HCl) by Lane-Eynon method, and was calculated as reducing sugars (RS). Free reducing sugars were determined without hydrolysis according to Velikaya, Suxodul and Tomasevich (Великая, Суходул, Томашевич, 1964). The DM of samples was determined gravimetrically after dehydration at 105 ºC. The content of inulin was determined according to the AOAC 999.03 and AACC 32.32 methods. Lactulose was determined chromatographically by Agilent 1100, using Aminex HPX-87H column (Biorad), the refraction index detector, at 45 ºC, mobile phase – 0.005 M H_2SO_4, the speed of mobile phase – 0.6 mL min^{-1}, and the sample volume – 20 µL. The content of maltodextrin was determined as the concentration of glucose (Schmidt, 1961).

Fourier transform infrared (FTIR) absorption spectra were registered on an HTS-XT micro-plate reader (BRUKER, Germany). The samples of oat gruel media, prebiotic components (10-20 µL), and biomass of *Bifidobacterium lactis* Bb-12 (5-10 µL) were dried on a 384 place silicon plate at 50 °C, and the spectra were collected over the wavelength range of 4000-600 cm^{-1}, 32 scans, resolution –

Table 1

The concentration of prebiotics after fermentation in water solution

Prebiotics in water solution	Reaction time, min	Total carbohydrates, %	Glucose, %	Fructose, %	Inulin, %	Lactulose, %
Inulin, 4.00%	0	3.33	0.009	0.024	2.96	–
	15	3.25	0.010	0.040	2.76	–
	30	3.26	0.010	0.050	3.03	–
Lactulose, 4.00%	0	1.69	0.006	0.002	–	–
	15	1.55	0.005	0.003	–	1.89
	30	1.48	0.006	0.002	–	1.92
Maltodextrin, 4.00%	0	1.02	0.065	–	–	–
	15	0.98	0.060	–	–	–
	30	1.15	0.060	–	–	–

Table 2

The concentration of prebiotics after fermentation in stomach model environment with gruel filtrate

Prebiotics in stomach model environment	Reaction time, min	Total carbohydrates, %	Glucose, %	Fructose, %	Inulin, %	Lactulose, %
Inulin, 4.00%	0	3.60	0.011	0.029	3.17	–
	30	3.27	0.033	0.307	2.67	–
	60	3.32	0.055	0.578	2.29	–
Lactulose, 4.00%	0	1.64	0.006	0.003	–	1.808
	30	1.67	0.007	0.003	–	1.521
	60	1.57	0.007	0.004	–	1.617
Maltodextrin, 4.00%	0	1.32	0.072	–	–	–
	30	1.26	0.065	–	–	–
	60	1.24	0.068	–	–	–

6 cm^{-1}. Data were processed by software OPUS 6.5, and baseline corrected by the rubber band method, CO_2 bands excluded. For data processing, spectra with the absorption of 25–80% were used to ensure direct proportionality of the band intensity and concentration, and thus apply the semi-quantitative analysis. Data pre-processing for cluster analysis were vector normalization and frequency ranges 1501–727 cm^{-1}. The semi-quantitative analysis was based on the band integration (an integration area) – the vertical to the oblique line between the closest minimums of peaks: 1040 cm^{-1} for carbohydrates, and 1543 cm^{-1} for proteins (Amid II).

Results and Discussion

The resistance of prebiotics was studied in water solution and oat gruel as the simplest stomach model environment. The concentrations of prebiotics after fermentation for 15, 30 or 60 min are shown in Tables 1 and 2. The concentration of inulin, lactulose and maltodextrin in a water solution practically did not change during 30 min; however, lactulose and maltodextrin were slightly more resistant than inulin (Table 1). The stomach model environment with a gruel filtrate contained oat origin substances, including carbohydrates and one of the prebiotics under the study. During fermentation for 60 min, lactulose and maltodextrin proved to be more stable than inulin as its concentration decreased from 3.17 to 2.29% (Table 2).

The resistance of prebiotics in oat gruel was evaluated also by means of FTIR spectroscopy (Fig. 1). Infrared (IR) absorption spectra of oat gruel and with three prebiotic components varied in

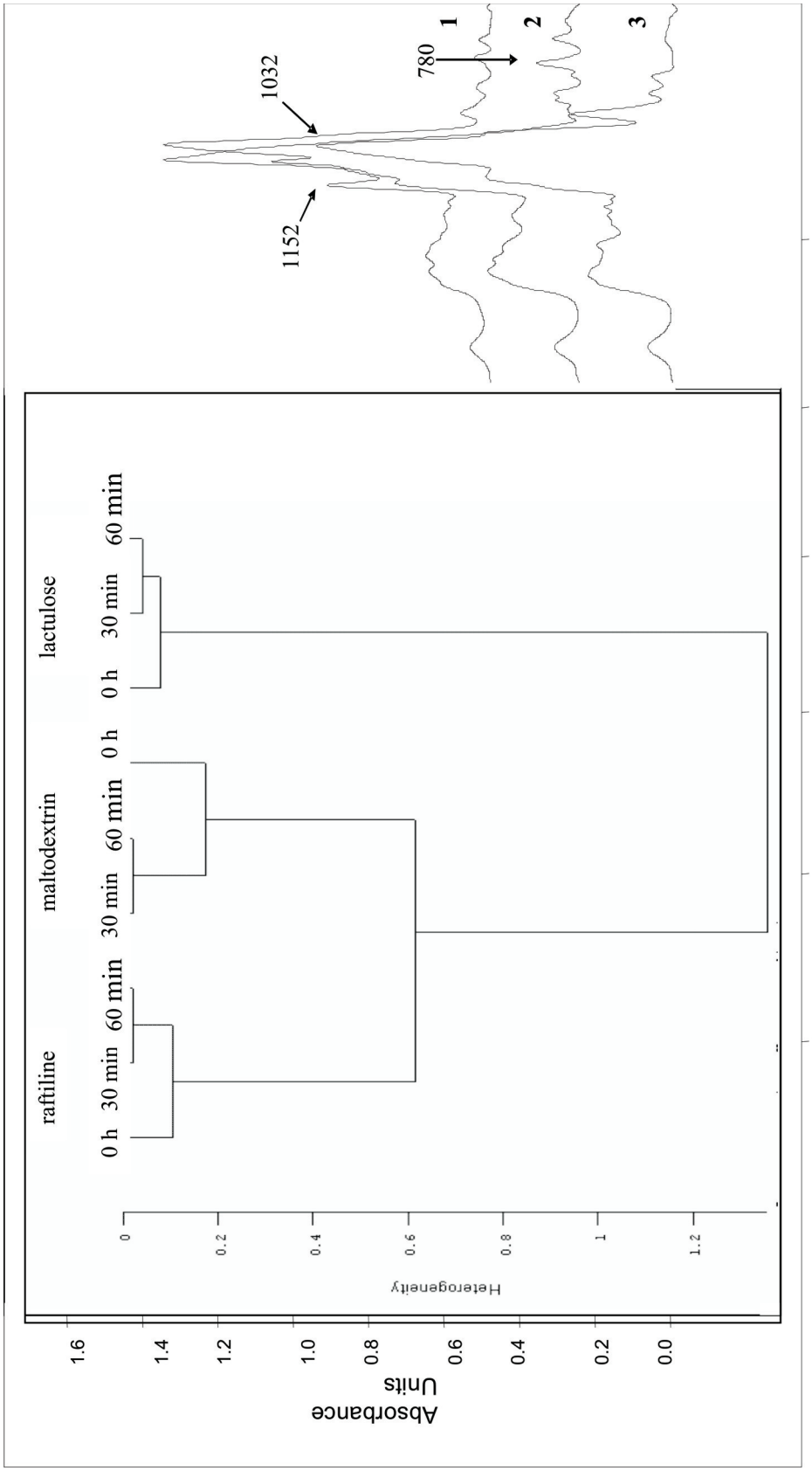

Fig. 1. FT-IR spectra of maltodextrin (1), lactulose (2) and raftiline (3), and the cluster of spectra of oat gruel-based environment with maltodextrin, or raftiline, or lactulose additives at different fermentation stages.

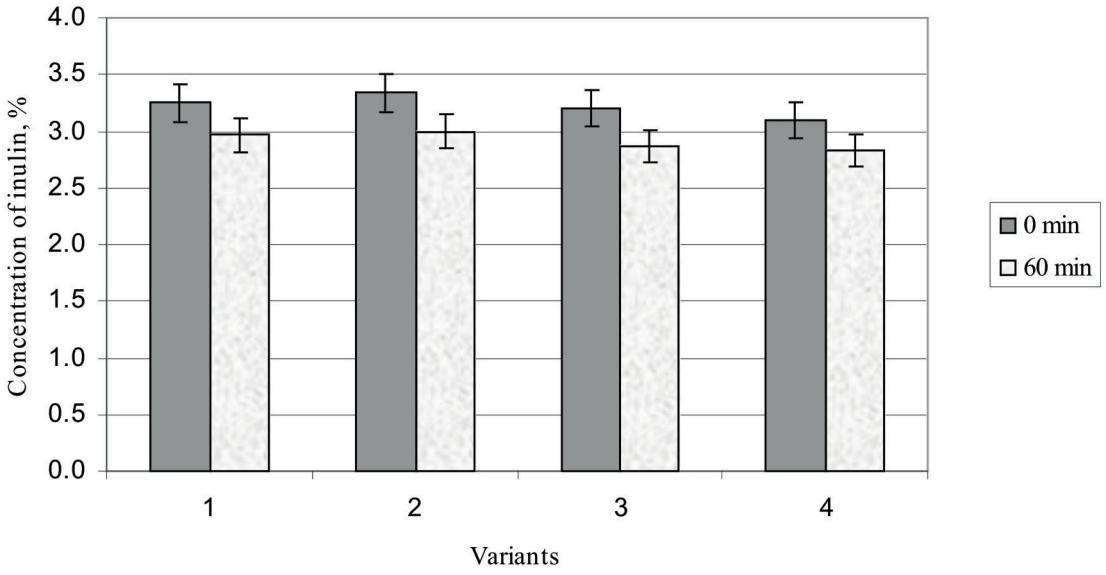

Fig. 2. The concentration of inulin in the stomach environment model during fermentation with: 1 – 4% raftiline powder in water solution; 2 – 4% raftiline + 4% glucose; 3 – 4% raftiline + 4% albumin; 4 – 4% raftiline + 4% albumin + 4% glucose.

Table 3

Viable cell count (VCC) of *Bifidobacterium lactis* Bb-12 in different stomach model environments

The stomach model environment and the reaction time	VCC, mL^{-1}	Δ (30 min-60 min)
Control		
H_2O	$(2.99\pm0.42)\cdot10^7$	
30 min	$(23.45\pm2.19)\cdot10^6$	$4.90\cdot10^6$
60 min	$(18.55\pm0.64)\cdot10^6$	
Raftiline, 4.00%		
30 min	$(26.0\pm5.66)\cdot10^6$	$8.65\cdot10^6$
60 min	$(17.35\pm0.49)\cdot10^6$	
Lactulose, 4.00%		
30 min	$(24.85\pm7.28)\cdot10^6$	$6.05\cdot10^6$
60 min	$(18.80\pm0.28)\cdot10^6$	
Maltodextrin, 4.00%		
30 min	$(23.70\pm7.5)\cdot10^6$	$4.45\cdot10^6$
60 min	$(19.25\pm0.35)\cdot10^6$	
Oat gruel, 0.46%		
30 min	$(24.75\pm3.18)\cdot10^6$	$7.35\cdot10^6$
60 min	$(17.40\pm0.57)\cdot10^6$	

the carbohydrate region of 900-1200 cm^{-1} (CO, CC, stretching vibrations and COH, COC deformation vibrations of carbohydrates) with maximums at 1080, 1075, or 1032 cm^{-1} in the maltodextrin, lactulose, or inulin additive samples correspondingly. The spectra of oat gruel and oat gruel with maltodextrin were similar, and no band can be assigned as a characteristic for maltodextrin in this mixture. Absorption bands of inulin and lactulose overlapped with oat carbohydrate bands thus changing the shape of total carbohydrate band. In the spectrum of oat gruel with lactulose, a separate band at 780 cm^{-1} was more pronounced than in oat gruel spectrum and therefore can be used for quantitative evaluations. In the spectrum of oat gruel with inulin, a separate band at 936 cm^{-1} was much higher than in oat gruel spectrum. Our previous studies showed that it can be assigned as a characteristic for inulin, and therefore was used to control the changes in inulin concentration in Jerusalem artichoke or chicory (Bekers, Grube, Upite, 2007). Evaluation of the spectra by cluster analysis did not show any significant changes during the reaction time. Nevertheless, the cluster analysis demonstrated pronounced differences between the spectra depending on the applied prebiotic additive.

A more complicated stomach environment model was water solution of 0 or 4% of inulin/raftiline, albumin and glucose. The initial concentration of inulin as well as after 60 min of fermentation was determined, and the results are shown in Fig. 2. The study showed that albumin stimulates the destruction of inulin.

The benefits of probiotics depend on their viability and growth under specific environmental conditions. As probiotic bacteria are the most important components of the symbiotic complex acting in the intestine and effectively diminishing the risk of many illnesses, the effect of inulin, lactulose and maltodextrin in gruel filtrate and of albumin and glucose on the resistance of *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* was studied (Tables 3 and 4). Both cultures showed similar good resistance in an oat gruel-based stomach model

Table 4

Viable cell count (VCC) of *Lactobacillus acidophilus* in different stomach model environments

The stomach model environment and the reaction time	VCC, mL^{-1}	Δ (30 min-60 min)
Control		
H_2O	$(3.00\pm0.01)\cdot10^6$	
30 min	$(10.70\pm0.61)\cdot10^5$	$6.60\cdot10^5$
60 min	$(4.1\pm0.85)\cdot10^5$	
Raftiline, 4.00%		
30 min	$(15.0\pm2.83)\cdot10^5$	$8.05\cdot10^5$
60 min	$(6.95\pm1.34)\cdot10^5$	
Lactulose, 4.00%		
30 min	$(9.95\pm1.34)\cdot10^5$	$2.20\cdot10^5$
60 min	$(7.75\pm0.35)\cdot10^5$	
Maltodextrin, 4.00%		
30 min	$(8.97\pm1.79)\cdot10^5$	$4.04\cdot10^5$
60 min	$(4.93\pm0.9)\cdot10^5$	
Oat gruel, 0.46%		
30 min	$(10.67\pm1.15)\cdot10^5$	$5.67\cdot10^5$
60 min	$(5.0\pm1.0)\cdot10^5$	

Table 5

Viable cell count (VCC) of *L. acidophilus* and *B. lactis* Bb-12 in environment with different prebiotics

The strain and the stomach model environment	VCC, mL^{-1}
Control *L .acidophilus*	$(3.5\pm0.71)\cdot10^7$
L .acidophilus + 4.00% raftiline	$(2.8\pm1.13)\cdot10^7$
L. acidophilus + 4.00% raftiline + 4.00% glucose	$(3.1\pm0.14)\cdot10^7$
L. acidophilus + 4.00% raftiline + 4.00% albumin	$(2.25\pm1.77)\cdot10^7$
L. acidophilus + 4.00% raftiline + 4.00% albumin + 4.00% glucose	$(4.35\pm0.92)\cdot10^7$
Control *B. lactis* Bb-12	$(8.9\pm0.14)\cdot10^7$
B. lactis Bb-12 + 4.00% raftiline	$(2.15\pm0.76)\cdot10^7$
B. lactis Bb-12 + 4.00% raftiline + 4.00% albumin	$(1.97\pm0.85)\cdot10^7$
B. lactis Bb-12 + 4.00% raftiline + 4.00% albumin + 4.00% glucose	$(2.24\pm1.14)\cdot10^7$

system; however, *Bifidobacterium lactis* Bb-12 was slightly more resistant than *Lactobacillus acidophilus* (VCC – 10^6 and 10^5 per mL correspondingly).

Growth of *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* in the stomach model environment with raftiline, albumin and glucose was studied. The VCC showed that both cultures, *L. acidophilus* and *B. lactis* Bb-12, were able to maintain their populations in different environments (Table 5). No significant differences were observed in the VCC after 60 min of incubation in the stomach model water environment with raftiline (4%), and with raftiline and one of the additives – either albumin (4%) or glucose (4%). It was established that *L. acidophilus* had slightly higher VCC after 60 min of incubation in the stomach model environment with 4% of raftiline, 4% of glucose, and 4% of albumin. In the same environment, the VCC of *L. acidophilus* was slightly higher than that of *B. lactis* Bb-12. This suggests that prebiotic effect of raftiline/inulin might be somewhat more efficient in an environment with glucose and albumin.

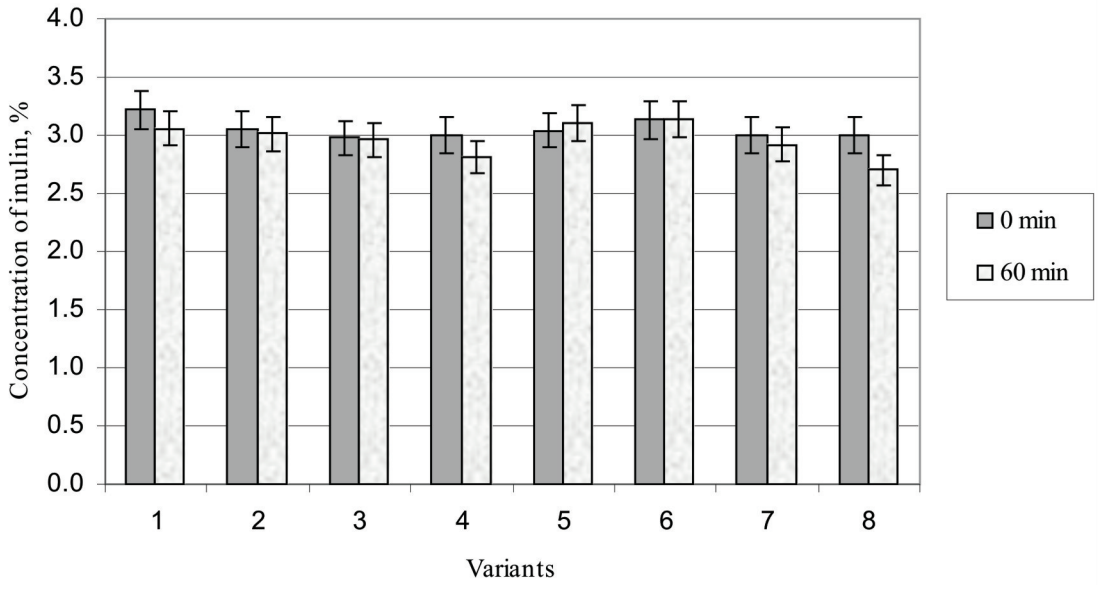

Fig. 3. The concentration of inulin in samples of *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* grown in water solution with different concentrations of raftiline, albumin and glucose:
1 – *L. acidophilus* + raftiline; 2 – *L .acidophilus* + raftiline + glucose;
3 – *L .acidophilus* + raftiline + albumin; 4 – *L. acidophilus* + raftiline + glucose + albumin;
5 – *B. lactis* Bb-12 + raftiline; 6 – *B. lactis* Bb-12 + raftiline + glucose;
7 – *B. lactis* Bb-12 + raftiline + albumin; 8 – *B. lactis* Bb-12 + raftiline + glucose + albumin.

The concentration of inulin in environment with raftiline, albumin and glucose (0 or 4%) after 60-min fermentation with *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* is demonstrated in Fig. 3. In environment with raftiline, glucose and albumin, the most significant loss of inulin was observed during fermentation with *Bifidobacterium lactis* Bb-12, whereas inulin proved to be more stable in albumin-free environment with glucose fermented either with *Lactobacillus acidophilus* or *Bifidobacterium lactis* Bb-12. Thus, in artificial stomach model environment prebiotics may negatively influence the survival and growth of probiotic bacteria.

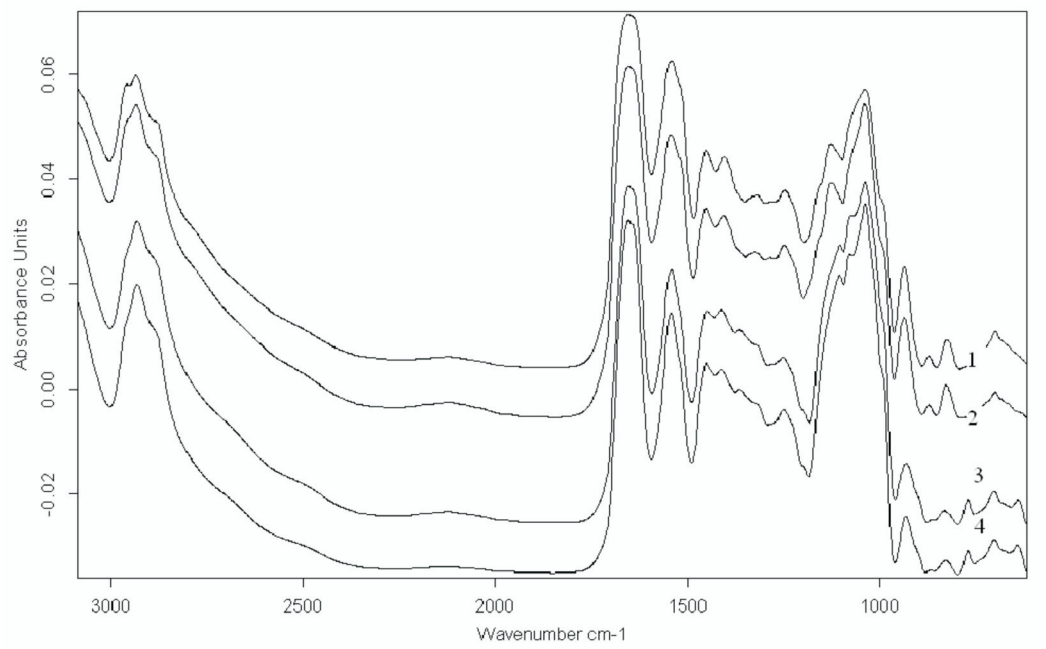

Fig. 4. FTIR spectra of the *Bifidobacterium lactis* Bb-12 biomass grown in various environments: 1 – raftiline + albumin before neutralization for 0 min; 2 – raftiline + albumin after neutralization for 60 min; 3 – raftiline + albumin + glucose after neutralization for 60 min; 4 – raftiline + albumin + glucose before neutralization for 0 min.

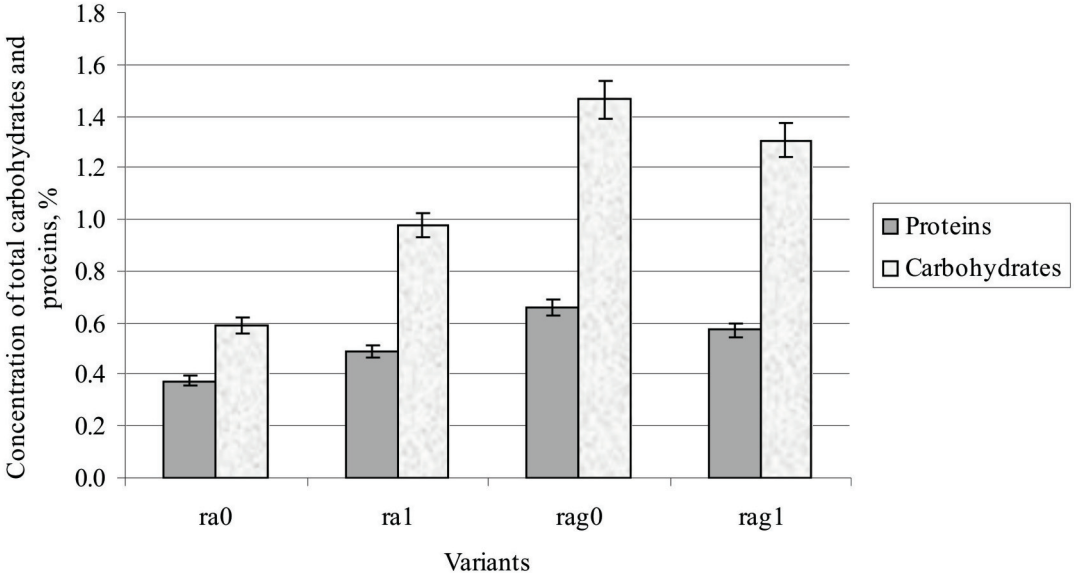

Fig. 5. The concentration of total carbohydrates and proteins in the *Bifidobacterium lactis* Bb-12 biomass grown in various environments containing: ra0 and ra1 – raftiline and albumin after 0 min and 1 h of growth; rag 0 and rag1 – raftiline, albumin and glucose after 0 and 1 h of growth.

FT-IR spectra of the *Bifidobacterium lactis* Bb-12 biomass after growth in environment with raftiline/inulin and albumin, and raftiline, albumin and glucose were recorded, and the obtained spectra are shown in Fig. 4. The spectra are qualitatively similar while the intensities of absorption bands are variable thus identifying changes in the content of carbohydrates, proteins and inulin during the growth of bacteria. Biomass samples were discriminated by cluster analysis according to the composition of the growth media and approved qualitative differences in the carbohydrate composition (profiles in the 870–1200 cm^{-1} region) and the content of total carbohydrates and proteins (variable intensities of bands at 1040 and 1543 cm^{-1}) in biomass.

In an environment with raftiline and albumin, the content of total carbohydrates in *Bifidobacterium lactis* biomass increased nearly twice and also the intensity of the absorption band at 1040 cm^{-1} increased significantly. In the spectra of the *Bifidobacterium lactis* biomass grown in environment with raftiline, albumin and glucose, the content of initial carbohydrates was higher due to the added glucose. After 60-min growth of *Bifidobacterium lactis*, the content of total carbohydrates as well as proteins slightly decreased. In order to compare all four samples, the spectra were normalized and integrals of carbohydrate and protein bands were measured (Fig. 5). The results showed that metabolism of *Bifidobacterium lactis* Bb-12 cells was significantly influenced by the growth environment, particularly by glucose.

Conclusion

This study showed that in a simple stomach model environment – water and prebiotics –, the concentration of inulin, lactulose and maltodextrin was stable during fermentation for 30 min. In oat gruel-based model environment, the loss of inulin reached 28% but lactulose and maltodextrin remained stable during 60-min fermentation. *Bifidobacterium lactis* Bb-12 proved to be more resistant than *Lactobacillus* in the stomach model environment with prebiotics, oat gruel, glucose and/or albumin. Probably some prebiotics and/or proteins can negatively influence the survival and growth of probiotic bacteria in a particular stomach environment.

Thus, in order to create and establish the benefits of synbiotic foodstuffs and offer them as valuable functional food products to the market it is necessary to study and control the resistance and efficiency of prebiotics and probiotic bacteria and their synbiotic activity/value by complex studies of biochemical processes in variable *in vitro* and *in vivo* environments.

The stomach model systems *in vitro* certainly are very simplified as *in vivo* prebiotics and probiotic bacteria interact with enzymes, microflora and other systems of the stomach and more complex gut and intestine environment. However, this preliminary study showed the resistance of some prebiotics and probiotic bacteria and indicated the necessity and importance of subsequent studies evaluating the inhibitory effects of different food components on probiotic bacteria and symbiotic complexes.

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Anotācija

Pētījuma mērķis bija noskaidrot prebiotiķu – inulīna, malto dekstrīna un laktulozes – 4% ūdens šķīduma un probiotisko baktēriju *Bifidobacterium lactis* Bb-12 un *Lactobacillus acidophilus* rezistenci kuņģa modeļa vidēs. Kā vienkāršākais kuņģa modelis tika izmantots sālsskābes ūdens šķīdums ar 0.46% auzu tumes filtrātu pie pH 2. Auzu tumes fermentācija ilga 15, 30 un 60 minūtes ar vai bez probiotiskajām baktērijām 37 ºC temperatūrā. Laktuloze un malto dekstrīns ūdens vai auzu tumes vidē bija rezistentāki nekā inulīns. Salīdzinot ar *B. lactis* Bb-12, *L. acidophilus* koloniju veidojošo vienību skaits pēc 60 minūšu inkubācijas ar raftilīnu, glikozi un albumīnu bija nedaudz lielāks. Kuņģa vides modelī ar auzu tumi *B. lactis* Bb-12 bija rezistentāks par *L. acidophilus*. *B. lactis* Bb-12 un *L. acidophilus* biomasu paraugi tika diskriminēti, izmantojot Furjē transformācijas infrasarkano spektru klastera analīzi, lai novērtētu kvalitatīvas atšķirības ogļhidrātu sastāvā un kvantitatīvas atšķirības kopējo ogļhidrātu un proteīnu daudzumā atkarībā no barotnes sastāva. Audzējot *B. lactis* kuņģa modeļa vidē ar raftilīnu/inulīnu un albumīnu, tika konstatēts gandrīz divkāršs biomasas palielinājums, bet vidē ar glikozi varēja novērot pretēju efektu. Mēģinājumi parādīja, ka kuņģa modeļa vidēs pārbaudītie probiotiķi ir rezistentāki par prebiotiķiem.