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EFFECT OF PREBIOTIC ADDITIVES TO CARROT JUICE ON THE SURVIVABILITY OF *LACTOBACILLUS* AND *BIFIDOBACTERIUM* BACTERIA

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Key words: probiotics, prebiotics, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, functional beverage, survivability

The study is an attempt to apply *Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20215 bacteria, of properties earlier characterised by the authors, to carrot juice with the addition of prebiotic preparations, oat gruel and homogenised banana fruit. The best survivability of the *Lactobacillus acidophilus* DSM 20079 bacteria up to the 28th day of refrigerated storage was obtained in the carrot juice with the addition of oat gruel. However, the product obtained in this way, even though it fulfilled the requirements expected from probiotic products, obtained the lowest score of sensory assessment. Sensoric evaluation of the *Lactobacillus acidophilus* containing juice has shown that addition of homogenised banana to the juice beneficially contributed to its flavor and aroma. The number of the live bacterial cells of the *Bifidobacterium bifidum* DSM 20215 strain in the carrot juice supplemented with the prebiotic preparations exceeded the level of 10^6 cfu/mL of juice during the entire storage period.

INTRODUCTION

Many strains of *Lactobacillus* and *Bifidobacterium* bacteria are characterised by probiotic properties and their natural habitat is the human and animal gastrointestinal and urinary-reproductive systems. Both *L. acidophilus* and *B. bifidum* bacteria are found in intestines and, therefore, various strains of these bacteria are recommended as components of probiotic preparations and products [Gomes & Malcata, 1999; Libudzisz, 2002].

The observed development of functional foodstuffs is associated, to a large extent, with products containing probiotics and prebiotics which contribute to the supplementation of the bacterial flora in the intestines [Matilla-Sandholm *et al*., 2002; Mountzouris *et al*., 2002]. The consumption of food products containing probiotics facilitates greatly the introduction of this microflora to the gastrointestinal tract. This is particularly true about refrigerated products as they provide good environment allowing to maintain live and active microorganisms for extended periods of time. In the situation where they can be proliferated in a food product until reaching required quantities, costs of their production would be significantly lower than in the situation in which they would have to be multiplied in a separate medium and later introduced to the given food product. In the case of functional food, it is generally assumed that the minimum number of live probiotic microflora should range from 10^5 to 10^6 cfu/mL or gram of the product. It was demonstrated that, in order to achieve evident health results, it is necessary to consume at least approximately 10^8 to 10^9 of live microbial cells daily in fermented milk [Libudzisz, 2002; Usajewicz, 1999]. The substrates required for the growth of bacteria in the intestines include dietary sources and the content of food components which are not absorbed in the upper parts of the gastrointestinal tract [Tannock, 2002]. These include: starch resistant to digestion, dietary fibre, sugars, oligosaccharides, proteins, peptides and amino acids. Endogenous sources, such as mucine, exert a smaller influence [Fooks *et al*., 1999].

Bifidobacterium genus can, with the assistance of extracellular enzymes, break down polysaccharides which undergo conversion into glucose and fructose phosphates and can then be metabolised in a manner characteristic for bifidobacteria [Gomes & Malcata, 1999].

Probiotic cultures employed for the production of fermented food products should, additionally, posses a number of important technological characteristics allowing to manufacture products of desirable sensory qualities and acquired health properties.

Apart from widely available probiotic products of animal origin, plant-derived probiotic product deserve attention, among others, fruit and vegetable juices. Recently, products manufactured from oatmeal have been gaining in popularity since they contain soluble and insoluble fibres and the fermentation process of these products can contribute to increased interest in oatmeal as a raw material for, what is often called, 'new functional food'. It was demonstrated that the above-mentioned products have a positive impact on the level of cholesterol in blood [Blandino *et al*., 2003]. The application of probiotic cultures in non-milk products and environments poses a challenge for scientists. The vitality of probiotics in the matrix of a food product depends on such factors as: pH, storage temperature, oxygen levels as well as the presence of competitive microorganisms and inhibitors.

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The aim of this work was to evaluate the survival of *Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20215 in fermented carrot juice supplemented with prebiotic substances and kept in cold-storage facilities. Moreover, consumer acceptability of the presence of bacteria in the juice was examined.

MATERIALS AND METHODS

The following two bacterial strains were used in the test: *Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20215 which were obtained from a museum collection of strains from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Bacterial strains chosen for the study fulfilled the basic criterion for probiotic bacteria, *i.e.* survival at low pH and ability to grow in the presence of bile salts (data not shown). In earlier studies the inhibitory effect of *Lactobacillus acidophilus* DSM 20079 on the growth of *Helicobacter pylori*, *Salmonella enteritidis and Escherichia coli* was determined *in vitro. Bifidobacterium bifidum* DSM 20215 was found to exhibit only minor sensitivity towards oxytetracycline and thus its application in post-antibiotic therapy is regarded as helpful (data not shown).

Carrot juice of the 10.5°Bx extract content was obtained from an ordinary commercial carrot concentrate manufactured by "Ogród Podlaski" Ltd. company in Siemiatycze, Poland.

Application of bacteria to the carrot juice. The applied carrot juice was supplemented with the addition of 2% (w/v) of the following selected prebiotics: inulin (Fluka), Raftilose®P95 and Raftiline®HP or the addition of 10% (w/v) banana fruit as well as 6% saccharose (w/v) (POCh, Gliwice). The product was pasteurized for 15 min at 80°C. The inoculum of the examined bacteria at the amount of 10% (v/v) was added to the juice. *Bifidobacterium bifidum* bacterial cells were grown on Medium 58 (DSMZ) and *Lactobacillus acidophilus* on MRS medium. Bacterial cultures were centrifuged, washed and suspended in saline solution to obtain a concentration of bacteria at 10^9 cfu/mL. Each bacterial strain was added to the juice independently and carrot juice was fermented at 25°C for 12 h. In addition, *Lactobacillus acidophilus* DSM 20079 mixed with oat gruel was also introduced into the examined juice [Molin, 2001]. The oat gruel was mixed with the carrot juice in the amount of 5% (w/v). Oat gruel was mixed with carrot juice at a 5% (w/v) ratio to eliminate the flour taste and to obtain an appropriate clarity of the final product. The experiment was carried out at five repetitions and the presented results are means obtained from these replications.

The experimental juice after fermentation was stored for 28 days at 4°C and, throughout this period, the number of live (cfu/mL) bacteria in the juice was determined using the Koch's plate method [Burbianka *et al.*, 1983]. *Bifidobacterium bifidum* was counted on the Medium 58 and *Lactobacillus acidophilus* on MRS (Merck) at 37°C. The cultures of *Lactobacillus acidophilus* were run in the atmosphere modified by 10% proportion of CO_2, while the *Bifidobacterium bifidum* cultures were run in special anaerobic generators also modified by 10% CO_2 [Schlegel, 2000].

Determination of the quantities of lactic acid [Ball, 1990]. Lactic acid was determined after fermentation on a Waters liquid chromatographer equipped in a refractometric detector (type Waters 410). The applied column was that of the Merck Company type POLYSPHER^R OAKC. A solution of sulphuric acid of 0.005 mol/L concentration was used as eluent at a flow rate of 0.5 mL/min. Assays were carried out at 32 RIU/F.S sensitivity of the detector. After passing through 0.22 μm microbiological filters, 20 μL samples were injected into the column. The samples were diluted depending on the predicted concentration of lactic acid. Measurements, file integration as well as the calculation of results were carried out with the assistance of the Millenium 32 software. Calculations and measurements were performed in relation to the previously prepared and investigated model solutions of the examined substance.

Assays of the L(+)and D(-) isomers of lactic acid. Assays of the L(+)and D(-) isomers of lactic acid were carried out with the chromatographic method using a Waters liquid chromatographer with a UV detector (type Waters 2487). The description of the method used in this experiment can be found in the Daicel Chemical Industries, Ltd. guidebook. A Baker B.V. type Chiralpak MA (+) column was used to carry out assays. A $CuSO_4$ solution of 2 mmol/L concentration and 0.6 mL/min flow was used as an eluant. Measurements, file integration as well as the calculation of results were carried out with the assistance of the Millenium 32 software.

Sensory assessment of the applied carrot juice. The sensory evaluation was carried out only on the carrot juice supplement with the *Lactobacillus acidophilus* DSM 20079 bacteria and the tested additives. The five-point sensory assessment was carried out according to the Tilgner's method [Gawęcka & Jędryka, 2001] which was performed on the last day when the determined bacterial count of live cells was still at the level of 10^6 cfu/mL of the juice. The following parameters were evaluated: clarity, colour, smell and taste. The sensory panel consisted of 20 persons.

Statistical analysis. In the course of the performed statistical analysis of results with the assistance of the Excel 2000 software, all the experimental designs were analysed employing mean descriptive statistics and one-way analysis of variance at $p<0.05$. In order to select mathematical models for some research experiments, the CurveExpert 1.3 software was employed.

RESULTS AND DISCUSSION

Survivability of *L. acidophilus* and *B. bifidum* bacteria in the juice supplemented with selected prebiotics

When considering therapeutic properties of probiotic food products, it is essential to remember that they must contain a satisfactory number of live and active cells at the moment of consumption. In the case of the examined carrot juice supplemented with Raftilose, the number of live cells of the *Lactobacillus acidophilus* DSM 20079 strain remained at the level of 10^6 cfu/mL up to day 19 of storage and this period was longer in comparison with the juice with no addition of prebiotic substances but shorter when compared with the

juice to which *Bifidobacterium bifidum* DSM 20215 bacteria and prebiotic were added. The number of live cells of the *Bifidobacterium bifidum* bacteria in the juice supplemented with Raftilose was 5.24×10^6 cfu/mL on day 28 of the storage (Figures 1 and 2). Carrot juice supplemented with Raftiline was determined to contain *Lactobacillus acidophilus* bacteria at a concentration of 10^6 cfu/mL. The calculated number of bacterial cells decreased after 10-day storage period, while in the juice to which inulin was added the number bacterial cell remained at a similar level only one day longer (11 days) (Figures 3 and 4). Although on day 28 of storage, in the case of all the three treatments, live cells of *L. acidophilus* bacteria were still present; their numbers were significantly lower than those of the *Bifidobacterium bifidum* bacteria on the last day of storage of juices containing prebiotics. When inulin was added, the number of *Bifidobacterium bifidum* bacteria was reduced to 3.41×10^6 cfu/mL of the product. The best influence on the survivability of the *Bifidobacterium bifidum* DSM 20215 was exerted by Raftiline as the number of live cells of these bacteria remained at the level of 1.36×10^7 cfu/mL up to day 28. Changes in the number of live bacteria determined in juices supplemented with Raftilose, Raftiline and inulin can be presented using two different mathematical models. In the case of the *Lactobacillus acidophilus* DSM 20079 strain, it is the Gaussian's model: y=a*exp((-(b-x) ^ 2)/(2*c ^ 2)), whereas for the *Bifidobacterium bifidum* DSM 20215 bacteria in the carrot juice – it is the model of rational function: y=1/(a*x+b). Both these models provide the best match for the dynamics of the reduction of tested bacteria in the carrot juice.

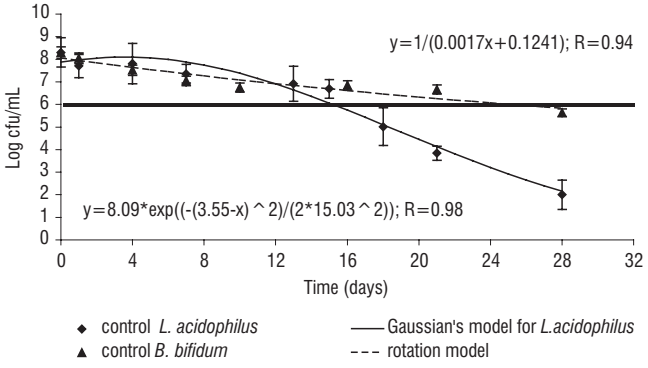

FIGURE 1. Changes in numbers of live *L. acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20215 bacteria in the carrot juice without additives = control.

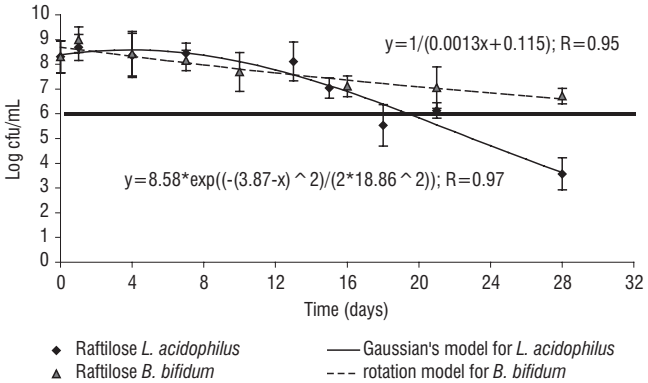

FIGURE 2. Changes in numbers of live *L. acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20215 bacteria in the carrot juice with the addition of Raftilose.

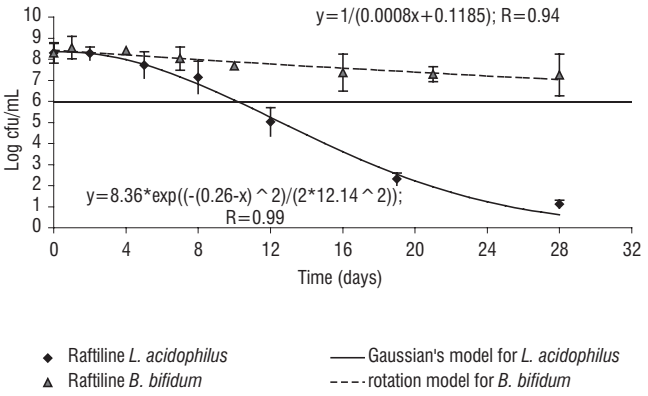

FIGURE 3. Changes in numbers of live *L. acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20215 bacteria in the carrot juice with the addition of Raftiline.

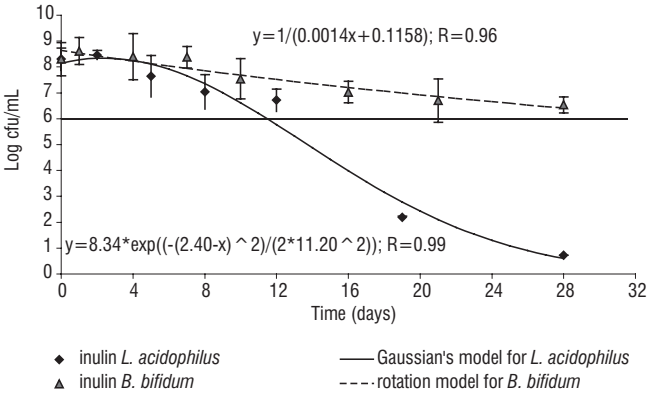

FIGURE 4. Changes in numbers of live *L. acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20215 bacteria in the carrot juice with the addition of inulin.

Function formulas as well as the fitting of the experimental data to the proposed mathematical model (R) are presented in Figures 1–4.

In experiments carried out by Trząskowska *et al*. [2003], they found that carrot juice fermented at 32°C for 15 h was characterised by the best quality after it was inoculated with *Lactobacillus acidophilus* bacteria. In our previous studies, the fermentation performed at 30°C and 37°C with the addition of both bacterial strains, *i.e. Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20215, failed to affect positively their survivability and, therefore, further experiments were abandoned. Despite the negative results during juice fermentation at high temperatures, the carrot juice was fermented at 25°C for 12 h. We believe that juice supplemented with prebiotics and probiotic bacteria could serve as an alternative for milk products containing probiotics. Chosen strains were found to exhibit beneficial probiotic values *in vitro*.

Bacterial survivability in the juice with the addition of oat gruel and banana

The addition of oat gruel influenced significantly the survivability of the *Lactobacillus acidophilus* DSM 20079 bacteria during refrigerated storage. The juice after 28-day storage period was found to contain 10^8 cfu/mL of live bacteria. The number of live *Lactobacillus acidophilus* cells supplemented with homogenised banana, which is a carrier of prebiotic

substances, decreased during storage. However, their numbers remained at the level of 10^6 cfu/mL until day 20 of the storage, so that on day 28 of storage, the live bacterial count was reduced to the level of nearly 10^4 cfu/mL (Figure 5). Observations made during this study provide evidence on the beneficial influence of natural additives in juice containing prebiotic substances during an approximately 3-week storage period. The oat gruel enhanced significantly the survivability of the *Lactobacillus acidophilus* DSM 20079 bacteria when compared with the Raftilose and Raftiline preparations or inulin. Changes in the number of the *Lactobacillus acidophilus* DSM 20079 live cells in the carrot juice supplemented with oat gruel and banana are described by the Gaussian's mathematical model. Figure 5 shows the fitting of this model to the number of live bacterial cells determined at definite time intervals together with coefficient values of these equations.

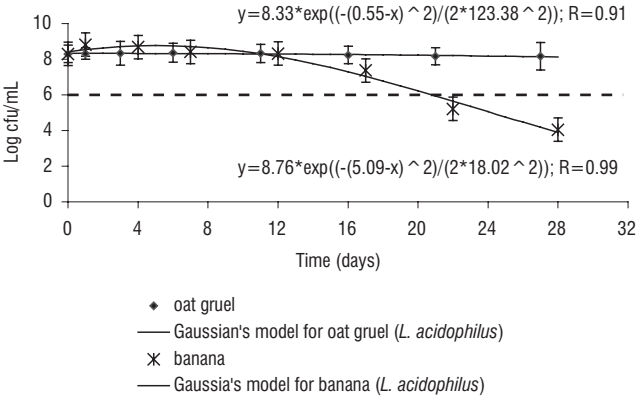

FIGURE 5. Changes in numbers of live *L. acidophilus* DSM 20079 bacteria in the carrot juice with the addition of 5% oat gruel and 10% addition of homogenised banana.

Investigations carried out by Mårtensson *et al*. [2002] provide an example of production of new fermented food products based on oatmeal. The additives used in this production were probiotic bacteria such as: *Bifidobacterium bifidum, Lactobacillus reuteri* and *Lactobacillus acidophilus* which were supposed to contribute to the beneficial effect of non-milk products on the human organism. Experiments were carried out during 30 days of storage of the product. The amount of probiotic bacteria required in food products is 10^6 cfu/mL of the product [Libudzisz, 2002; Usajewicz, 1999]. The strain of *Lactobacillus reuteri* exhibited survivability at the level of 10^8 cfu/mL in all of the examined test products after 30 days of storage. The *Lactobacillus acidophilus* DSM 20079 strain applied in our studies showed survivability at the level of 10^6 cfu/mL of the product up to day 28 of storage when oat gruel was added. Investigations conducted by Mårtensson *et al*. [2002] showed that fermented non-milk products manufactured from oatmeal constituted appropriate substrates and could support high cell survivability of probiotic bacteria of various strains during refrigerated storage (30 days).

Experiments carried out by Molin [2001] aimed at obtaining a product which would not contain milk. Fruit juice containing 5% addition of oat gruel was used as a substrate for multiplication of *Lactobacillus plantarum* 299v bacteria (approximately 1×10^{12} cfu/L). The product reaching the consumer contained about 5×10^{10} cfu/L and could be stored in a refrigerator for the period of 1 month without bacteria losing their vitality. Our experiments, which employed the *Lactobacillus acidophilus* DSM 20079 strain, allowed to obtain product stability during storage but the initial concentration of the bacterial cells in the product was lower and reached 10^8 cfu/mL.

Content of lactic acid and its isomeric forms in the carrot juice with the addition of *Lactobacillus acidophilus* DSM 20079 bacteria and prebiotic preparations

Also the amounts of the produced lactic acid as well as the percentage content of its isomeric forms were determined in the fermented carrot juice with the addition of Raftilose, Raftiline and inulin as well as the *Lactobacillus acidophilus* DSM 20079 bacteria. The quantity of the lactic acid fluctuated from 5.79 g/L for the juice supplemented with Raftilose; through 6.70 g/L - for the juice with the addition of Raftiline to 5.80 g/L - for the juice to which inulin was added. The concentration of the L(+) form of lactic acid, irrespective of the applied additive, exceeded 70% and reached: 78.17%; 77.11% and 74.04%, respectively. It can, therefore, be said that the fermented product in the form of the obtained carrot juice supplemented with prebiotic substances met the FAO/WHO recommendations concerning the content of the L(+) form of lactic acid [Grzybowski *et al.*, 1997].

A rapid increase of production of new fermented products has been observed in the course of the last decade and, at the present time, these types of products – depending on the country – constitute from several to a few dozen percent of all fermented products. However, products fermented with the assistance of intestinal bacteria are characterised by a taste different from that found in traditional products; they are mildly sour and of vague aroma. Their taste is usually corrected by the addition of herbs, various aromas, sweetening as well as by increasing the concentration of milk dry matter. It is proposed that the juice could supplement newly developed milk products with specific prebiotic saccharides, *e.g.* lactulose, fructosaccharides or galactosaccharides, which selectively stimulate the growth of bifidobacteria in the gastrointestinal tract [Libudzisz, 2002].

Sensory assessment of the carrot juice with additives

The performed sensory evaluations included only the carrot juice with the *Lactobacillus acidophilus* DSM 20079

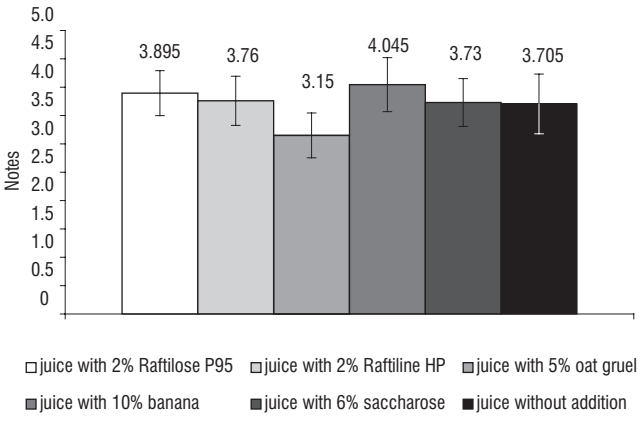

FIGURE 6. Sensory assessment of carrot juice with different additives.

bacteria and the addition of Raftilose, Raftiline, saccharose, oat gruel, homogenised banana fruit as well as the carrot juice without any additives. Juice with the addition of homogenised banana was characterised by the highest taste-smell quality (Figure 6), while the lowest scores, due to a flour off-taste and poor clarity, were awarded to the juice supplemented with oat gruel. The results of sensory assessment show that the addition of the remaining prebiotic preparations did not deteriorate the quality of the experimental carrot juice stored under refrigerated conditions.

Data reported by Trząskowska *et al*. [2003] indicate that the type of the applied bacterial strain exerts a significant influence on the sensory evaluation of the examined product. The above-mentioned researchers examined carrot juice with an addition of apple juice. They tested eight probiotic bacterial strains and found that juice fermented with the assistance of the strain of *Lactobacillus acidophilus* CH-2 bacteria was characterised by the most desirable taste and the highest quality. The authors reported the following optimal duration and temperatures of the fermentation process: 20 h and 32°C and 37°C. Sensory investigations on fermented milk products obtained using different bacterial cultures also indicate that the type of the employed microflora was the most important factor affecting the content of individual taste-aroma compounds.

It can, therefore, be assumed that the sensory assessment carried out for the carrot juice containing *Lactobacillus acidophilus* DSM 20079 bacteria was influenced not only by the type of the applied additives but also by the properties of the bacterial strain itself.

CONCLUSIONS

1. The number of live *Bifidobacterium bifidum* DSM 20215 cells was maintained at the level of 10^6 cfu/mL in juice supplemented with Raftiline, Raftilose and inulin during the first 28 days of cold-storage when 10^8 cfu/mL of bacterial cells were initially added to the juice.

2. The level of live *Lactobacillus acidophilus* DSM 20079 cells in the fermented juice during cold-storage remained stable when the bacterial cells were introduced on oat gruel as a carrier. Raftilose was determined to be the most beneficial prebiotic substance for introducing *L. acidophilus* bacteria into the juice.

3. The content of the L(+) form of lactic acid in fermented carrot juice supplemented with *Lactobacillus acidophilus* DSM 20079 and prebiotic substances was above 70%.

4. The highest sensoric rating was obtained for the carrot juice with *Lactobacillus acidophilus* DSM 20079 and 10% of homogenised banana.

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WPŁYW DODATKÓW PREBIOTYCZNYCH DO SOKU MARCHWIOWEGO NA PRZEŻYWALNOŚĆ BAKTERII *LACTOBACILLUS* I *BIFIDOBACTERIUM*

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W pracy podjęto próbę aplikacji bakterii *Lactobacillus acidophilus* DSM 20079 i *Bifidobacterium bifidum* DSM 20215, o właściwościach wcześniej scharakteryzowanych przez autorów, do soku marchwiowego z dodatkiem preparatów prebiotycznych, kleiku owsianego i homogenizowanego owocu banana. Najlepszą przeżywalność bakterii *Lactobacillus acidophilus* DSM 20079 do 28 dnia przechowywania w warunkach chłodniczych uzyskano w soku marchwiowym z dodatkiem kleiku owsianego (rys. 5). Tak otrzymany produkt, choć spełnia wymagania stawiane produktom probiotycznym, uzyskał jednak najniższe noty oceny sensorycznej. Analiza sensoryczna soku marchwiowego z *Lactobacillus acidophilus* i homogenizowanym bananem potwierdziła jego korzystny wpływ na smak i zapach soku (rys. 6). W czasie przechowywania soku marchwiowego z dodatkiem prebiotyków liczba żywych komórek szczepu *Bifidobacterium bifidum* DSM 20215 utrzymywała się na poziomie wyższym niż 10^6 jtk/mL soku.