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THE SUITABILITY OF DIFFERENT PROBIOTIC STRAINS FOR THE PRODUCTION OF FRUIT-WHEY BEVERAGES*

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ABSTRACT

Background. When designing new probiotic products, one of the most important aspects is the selection of bacterial strains with high survival rates in the matrix of the product concerned. The aim of the present research was to evaluate the potential of selected strains of probiotic bacteria for the production of fruit-whey beverages.

Materials and methods. Orange, apple and blackcurrant whey beverages were produced, and each was inoculated with one of the following probiotic strains: *Bifidobacterium lactis* HN019™; *Lactobacillus acidophilus* NCFM®; *Lactobacillus paracasei* Lpc-37™; *Lactobacillus rhamnosus* HN001™. The count of probiotic bacteria as well as pH and total acidity were evaluated at the 1st, 7th, 14th, 21st and 28th day of storage.

Results. Beverages containing *L. paracasei* Lpc-37™ or *L. rhamnosus* HN001™ were characterized by a significantly higher average number of viable cells (7.02 or 7.05 log cfu/g, respectively) than products with *B. lactis* HN019™ or *L. acidophilus* NCFM® (6.43 or 6.37 log cfu/g, respectively). The use of *L. paracasei* Lpc-37 and *L. rhamnosus* HN001 strains in orange and apple drinks allows the recommended count for probiotic products, 10^6 cfu/g for 28 days of storage, to be exceeded. Survival of the *B. lactis* HN019 strain fulfills the above requirements only in the orange drink. The *L. acidophilus* NCFM® strain was found to be the least suitable for the production of beverages, as it did not reach 6 log cfu/g in any products after 28 days of storage. The highest average number of bacteria was found in the orange beverages (7.14 log cfu/g). In terms of bacteria viability, blackcurrant juice was the least suitable for the production of whey probiotic drinks, due to its high acidity.

Conclusion. The results of the present study indicate that careful selection of the fruit juice component, especially in terms of its acidity, is key to designing successful probiotic fruit-whey beverages. Other factors which should be taken into account to ensure a sufficient number of live probiotic cells, i.e. their therapeutic level in fruit-whey drinks, are the choice of probiotic strain and determination of the maximal shelf life.

Keywords: whey, fruit drink, probiotics, viability, acidity

INTRODUCTION

Consumer awareness of the impact of diet on health has been increasing in recent years (Mandal and Hati, 2016). Due to the presence of physiologically active components, functional foods provide health benefits beyond basic nutrition (Wildman and Kelley, 2007). Fruits are rich in functional components, such

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as vitamins, antioxidants and fiber and have good sensory properties. Whey, a waste product obtained from the production of cheese, contains high levels of bioactive proteins and B vitamins, but shows negative sensory characteristics. The combination of whey with fruit juices allows a product with favorable sensory properties, as well as with higher nutritional value than pure fruit drinks, to be obtained. The industrial production of whey-based beverages was launched in the 1970s, with the launch of the Rivella drink on the Swiss market. Many types of non-alcoholic whey drinks have since been developed and sold, produced from native rennet or acid whey, fermented whey, deproteinized or demineralized whey or whey powders. There are also alcoholic whey beverages, such as whey beer or wine and beverages with low alcohol content (less than 1.5%; Jeličić et al., 2008). However, the majority of whey drinks on the market are refreshing and thirst-quenching products, which do not contain live or active microflora such as lactic acid bacteria or probiotic bacteria.

An important and rapidly growing category of functional foods are those containing probiotics – live microorganisms which confer health benefits to the host, when administered in sufficient amounts (Mandal and Hati, 2016). Although the major probiotic functional foods are dairy products, in particular fermented milks, a number of other products, including fruit and vegetables, cereal, soya and meat are employed as delivery vectors for probiotic microorganisms (Granato et al., 2010; Rivera-Espinoza and Gallardo-Navarro, 2010). Due to their low pH, fruits are not a good environment for probiotic bacteria, although there are still attempts to incorporate these bacteria into fruit beverages, as they are widely consumed and still increasing in popularity. The health benefits of probiotic product consumption depends on the viability of specific probiotic microorganisms during production, storage and consumption, as well as passage through the gastrointestinal tract. The most important factors affecting the stability of probiotics in products are: specific strain properties, chemical composition of the food matrix, acidity, other accompanying bacteria, storage time and conditions (Mahmoudi et al., 2013). Most of these characteristics determine the sensory properties of food in general. Sensory attractiveness is the most important factor, on the basis of which the consumer makes the decision to buy food. Therefore, when designing probiotic foods, there is limited scope for adjusting the product's parameters to improve the survival of probiotic bacteria. Thus, particular attention should be paid to the selection of a suitable probiotic strain with good technological properties for a specific novel food application (Saarela, 2009). The aim of the present study was to evaluate the potential of selected strains of probiotic bacteria for the production of fruit-whey beverages.

MATERIAL AND METHODS

Manufacturing of the beverages

Three flavors of whey beverages were produced: orange, apple and blackcurrant, each inoculated with one of the following probiotic cultures: *Lactobacillus paracasei*, *Lactobacillus acidophilus*, *Bifidobacterium lactis* or *Lactobacillus rhamnosus*. The following raw materials were used for beverage production: orange, apple and blackcurrant concentrated juice, comprising 64.5; 62.5 and 60.0% of the extract content respectively (obtained from Tymbark S.A., Poland); acid whey obtained during production of tvarog cheese in the local dairy plant (OSM Miechów, Poland); low-mineral spring water (Wosana S.A., Andrychów, Poland); and sugar. The following probiotic freeze-dried starter cultures (obtained from Danisco Poland Sp. z o.o.) were used for inoculation: *Lactobacillus paracasei* Lpc-37™; *Lactobacillus rhamnosus* HN001™; B*ifidobacterium lactis* HN019™; *Lactobacillus acidophilus* NCFM®.

It was assumed that the beverages would contain 12 ±0.1% of the extract. The extract would contain 50% fruit concentrate in the orange and apple drink, and in the blackcurrant drink 25% would be fruit concentrate. The lower proportion of concentrate in the blackcurrant beverages was due to its high acidity, which is typical for this kind of product. In all beverages, whey was added at the rate of 50% of the amount of added process water. Immediately after tvarog cheese production, whey was centrifuged to remove residual fat and pasteurized at 72°C for 15 s to ensure microbiological stability. All ingredients were measured according to the formula, pasteurized at 80°C for 15 min and cooled to 20°C. The obtained beverages were inoculated with 1% (v/v) suspension

of a suitable probiotic strain, prepared by dissolving 1 g of lyophilized culture in 100 mL of sterile water. The bacterial suspension was prepared 30 min before use to rehydrate the cells and avoid rehydration in the low pH environment of the beverages, which could have a negative impact on bacterial survival. 300 mL of inoculated beverages were poured into sterile glass bottles. After sealing the bottles, the beverages were stored at 4°C until analysis.

pH analysis

The pH of drinks was measured according to the PN-EN 1132:1999 standard, using the digital pH-meter CP 411 (Elmetron, Zabrze, Poland).

Total acidity analysis

Determination of total acidity was performed by titration and expressed as a percentage of citric acid (PN-EN 12147:2000).

Microbiological analysis

The analysis was conducted in accordance with the general requirements for microbiological testing (PN-EN ISO 7218:2008). 10-fold dilutions of samples were made according to the PN-EN ISO 6887-1:2000 standard. The count of characteristic probiotic microflora in beverages was performed in duplicate by the pour plate method. Standard MRS (de Man, Rogosa and Sharpe) agar (Biocorp, Warszawa, Poland) was used for enumeration of *L. acidophilus*, *L. paracasei* and *L. rhamnosus* (Champagne et al., 2011). All media plates were incubated aerobically with a 20% CO_2 atmosphere (incubator Shel Lab®, Sheldon Manufacturing, Inc., Cornelius, US) at 37°C for 72 h. *B. lactis* was enumerated according to the method of Tharmaraj and Shah (2003) using MRS (Biocorp, Warszawa, Poland) agar with NNLP supplement (nalidixic acid, neomycin sulphate, lithium chloride and paramomycin sulphate). The Petri dishes were incubated at 37°C for 72 h in a CO_2 atmosphere in anaerobic jars (Anaerocult® A, Merck, Darmstad, Germany). After incubation, the colonies were counted using a colony counter (LKB 2002, Pol-Eko Aparatura™ Wodzisław Śląski, Poland). The average number of colonies in the duplicates was calculated, and the results were expressed as log cfu/g.

Statistical analysis

All experiments were carried out in triplicate, and each sample was analyzed in duplicate. Statistical analysis was performed using the Statistica 9 software (StatSoft, USA). All results were submitted to the 3-factor multi-way analysis of variance (MANOVA). The significance of the differences between means was determined by the Tukey's test at $p \leq 0.05$.

RESULTS

The number of microflora in beverages depended on the flavor, the strain used for production and the storage time (Table 1). Beverages containing *L. paracasei* or *L. rhamnosus* were characterized by a significantly higher number of viable cells (on average by 0.6 log cfu/g) than products with *L. acidophilus* or *B. lactis*. The highest average number of bacteria was found in the orange beverages (7.14 ±0.07 log cfu/g); it was 0.4 logarithmic cycles higher than in the apple beverages and nearly one order of magnitude higher than the blackcurrant drinks. During storage, the average number of viable bacteria in the beverages decreased significantly every 7 days. This resulted in a 100-fold decrease in the average number of bacteria after 28 days of storage. The initial number of bacteria was similar (7 log cfu/g) for all samples as the same types and levels of starter cultures were used in all of the beverages. Significant interactions ($p \leq 0.05$) between the factors strain and storage as well as flavor and storage indicate the variations in the dynamics of changes in bacteria viability in beverages depending on its flavor and the strain used.

Probiotic bacteria showed the greatest viability in orange beverages (Fig. 1). The number of *L. paracasei* was stable (>7 log cfu/g) throughout the storage period. A slightly lower survival rate was observed for *L. rhamnosus* and *B. lactis*; their numbers decreased significantly ($p \leq 0.05$) after 28 days of storage by 0.7 and 1 logarithmic unit respectively, in relation to their initial counts in the orange beverages. *L. acidophilus* was characterized by the lowest survivability; its number significantly ($p \leq 0.05$) decreased after 14 and then after 28 days of storage, after which it dropped to 5 log cfu/g.

The apple beverage environment was more detrimental to probiotic bacteria than orange drinks (Fig. 2).

Table 1. Effect of probiotic strain, flavor and storage time on the viable bacteria count, pH and total acidity in fruit-whey beverages (the least squared means and means standard errors)

Factor	Group	Bacteria count log cfu/g	pH	Total acidity %
Strain	*L. paracasei*	$7.02^{A} \pm 0.11$	$3.90^{A} \pm 0.03$	$0.60^{A} \pm 0.03$
	L. rhamnosus	$7.05^{A} \pm 0.10$	$3.90^{A} \pm 0.03$	$0.59^{A} \pm 0.03$
	B. lactis	$6.43^{B} \pm 0.15$	$3.91^{A} \pm 0.02$	$0.60^{A} \pm 0.03$
	L. acidophilus	$6.37^{B} \pm 0.13$	$3.90^{A} \pm 0.02$	$0.59^{A} \pm 0.03$
Flavor	orange	$7.14^{A} \pm 0.07$	$4.17^{A} \pm 0.00$	$0.49^{A} \pm 0.00$
	apple	$6.75^{B} \pm 0.10$	$3.96^{B} \pm 0.00$	$0.42^{B} \pm 0.00$
	blackcurrant	$6.25^{C} \pm 0.12$	$3.58^{C} \pm 0.00$	$0.89^{C} \pm 0.00$
Storage, day	0	$7.67^{A} \pm 0.03$	$3.90^{A} \pm 0.04$	$0.59^{A} \pm 0.03$
	7	$7.27^{B} \pm 0.05$	$3.91^{A} \pm 0.04$	$0.60^{A} \pm 0.03$
	14	$6.71^{C} \pm 0.09$	$3.89^{A} \pm 0.03$	$0.60^{A} \pm 0.03$
	21	$6.28^{D} \pm 0.12$	$3.90^{A} \pm 0.04$	$0.60^{A} \pm 0.03$
	28	$5.65^{E} \pm 0.15$	$3.90^{A} \pm 0.04$	$0.61^{A} \pm 0.02$

A–E – different letters with mean values indicate statistically significant differences at $p \leq 0.05$.

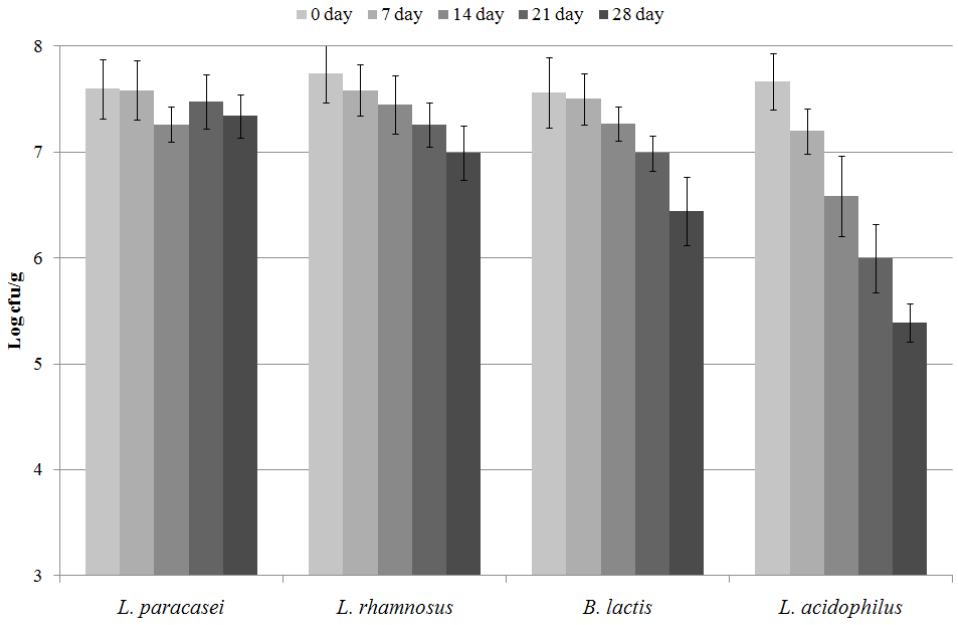

Fig. 1. Changes of viable probiotic bacteria in orange beverages during storage (means and standard deviations)

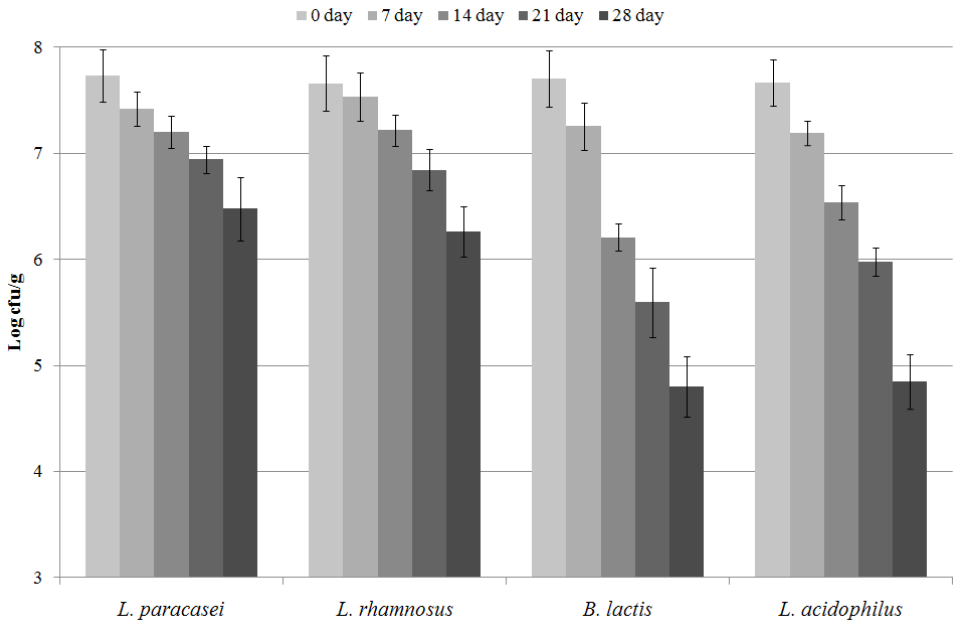

Fig. 2. Changes of viable probiotic bacteria in apple beverages during storage (means and standard deviations)

Among all analyzed cultures *L. paracasei* and *L. rhamnosus* showed the best viability in apple beverages, as a result of which drinks with these species contained a significantly higher ($p \leq 0.05$) number of bacteria compared to the *L. acidophilus* or *B. lactis* drinks. During storage, a significant reduction in the number of all species of bacteria was noted. In *L. paracasei* or *L. rhamnosus* beverages, the number of bacteria decreased significantly ($p \leq 0.05$) by one order of magnitude after 21 days, and remained at the level of 6 log cfu/g until the end of the storage period. *L. acidophilus* and *B. lactis* were characterized by lower viability, which reduced by one order of magnitude by the 14th day of storage, and, after 28 days, the drink contained live cell counts (4 log cfu/g) nearly three orders of magnitude lower than the beverages directly after production.

The lowest survival rate of all probiotic bacteria used was found in the blackcurrant beverages (Fig. 3). *L. paracasei* and *L. rhamnosus* were characterized by the highest viability in the blackcurrant drinks, as in those made from apple and orange juice, but their numbers were stable only for the first 7 days of storage. The beverages contained lower numbers of viable cells by more than 1 and 2 orders of magnitude after 14 and 28 days respectively, compared to the beverages directly after production. In the drinks with *L. acidophilus* or *B. lactis*, a significant 10-fold reduction in bacteria count was observed after 7 days, and longer storage resulted in a further reduction in their number. As a result, after 4 weeks the drinks had a live cell count of about 4 log cfu/g, which was more than 3 orders of magnitude lower than the drinks directly after production.

The acidity of beverages was determined by their flavor, i.e. the species of fruit from which the concentrate used as a drink component was produced (Table 1). The highest total acid and the lowest pH were found for the blackcurrant beverages, with average values of 0.89% and 3.58, respectively. In comparison to these values, apple and orange drinks showed a higher average pH by 0.38 and 0.59 respectively, and the total acid was lower by an average of 0.47 and 0.40% respectively. The type of strain applied had no significant effect on the total acid and pH of the analyzed beverages. The value of both parameters was also stable during storage.

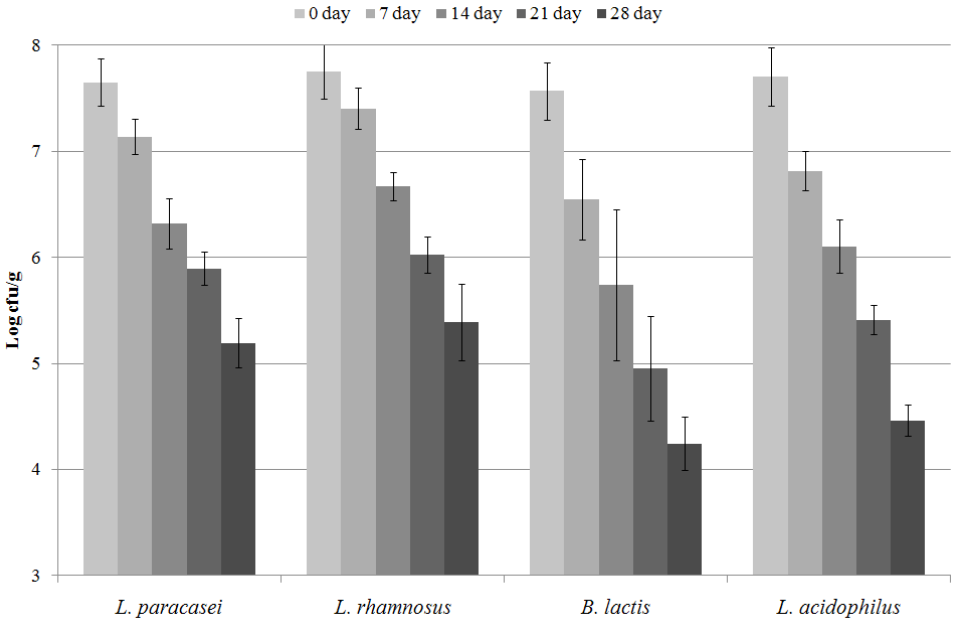

Fig. 3. Changes of viable probiotic bacteria in blackcurrant beverages during storage (means and standard deviations)

DISCUSSION

Probiotic strains used, storage temperature, food matrix type (i.e. acidity) and concentration of organic acids and other metabolites (i.e. dissolved oxygen, buffering capacity) are the main factors that affect the viability of probiotic bacteria (Shori, 2016). The optimum pH for the growth of probiotic bacteria is between 6.5 and 4.5, and at values below 4 their growth is usually inhibited (Rivera-Espinoza and Gallardo-Navarro, 2010). As the pH of the evaluated beverages directly after inoculation ranged from 3.57 to 4.18, the fermentation process ceased and the initial number of microflora in products was the result of a fixed level of starter culture additive. The differences in the survival rate of probiotic bacteria in the fruit or dairy-fruit beverages, depending on the fruit species, type of a strain and storage time, are widely reported in the literature. As mentioned above, orange drinks were a better environment for probiotic bacteria than apple and blackcurrant drinks. Results similar to those presented herein were obtained by Sheehan et al. (2007), who tested the resistance of *Lactobacillus* and *Bifidobacterium* species in fruit juices. They showed higher bacterial survival in orange and pineapple juices compared to cranberry juice, due to the acid tolerance of the tested bacteria. In the studies by these authors, *Lb. casei*, *Lb. rhamnosus* and *Lb. paracasei* showed a high viability, at levels above 8.0 and 7.0 log cfu/mL, whereas the number of *B. lactis* was above 7.0 and 6.0 log cfu/mL in orange and pineapple juices respectively, for at least 4 weeks of storage. Drastic losses in viability of all strains, more than 3–4 log cycles, was observed in cranberry juice just two days after inoculation, which was probably the result of the very low pH of this juice (2.5) and its benzoic acid content (Sheehan et al., 2007). The better survival rate of probiotic bacteria in orange juice compared to apple juice was demonstrated by Marhamatizadeh et al. (2012). The difference in the number of bacteria between beverages was on average 0.6 and 0.7 logarithmic units, for *L. acidophilus* and *B. bifidum* respectively. It was established that, although the orange juice had a significantly lower average pH (2.97) than apple juice (3.61), the effect of the pH on bacterial viability was probably limited due to the short six-day storage period (Marhamatizadeh

et al., 2012). Similar results were observed by Sadaghdar et al. (2012) who analyzed the viability of probiotics in flavored fermented milks. They showed that peach flavored products were characterized by lower pH and higher probiotic bacteria compared to strawberry flavored beverages. Similarly to our study, they also found that probiotic sensitivity to flavoring agents was strain dependent: *L. casei* L01, *L. casei* LAFTI L26, *L. paracasei* Lpc-37 and *L. rhamnosus* HN001 showed greater viability in comparison to *L. acidophilus* La-5 in both peach and strawberry drinks. The opposite results were obtained by Khezri et al. (2016) who investigated the stability of probiotics in fermented fig juice. They showed that *L. casei* DSMZ 20011 had the lowest survival rate after 2 weeks of storage, decreasing from 9.14 to 3.32 log cfu/ml, while in the same period *L. plantarum* DSMZ 20179 and *L. delbrueckii* DSMZ 15996 decreased from 8.77 to 7.11 and from 8.83 to 7.50 log cfu/ml respectively. According to Saarela et al. (2006), if a freeze-dried culture is used, the stability of bifidobacteria in probiotic juice depends on the type of cryoprotectant applied during production of the culture. After 4 weeks of storage, the number of *B. animalis* subsp. *lactis* in fruit juice (a mix of orange, grapefruit and passion fruit, pH = 3.7) was above 6 log cfu/mL when sucrose-protected cells were used and above 4 log cfu/mL for juice inoculated with skimmed milk-protected cells. Generally, in fruit drinks, the stability of probiotics is poorer than in dairy products, therefore the addition of whey to fruit juices probably improves the survival of probiotic bacteria in these products. The number of *B. bifidus* NCDC 255 bacteria in a whey beverage with aloe vera juice (whey to juice ratio was 70:30) was 7 log cfu/g and was stable during the 30-day storage period, although the pH decreased from 4.40 to 4.00 (Sasi Kumar, 2015). Shukla et al. (2013), who analyzed whey-pineapple drinks (whey to pineapple juice ratio 65:35) containing *L. acidophilus* NCDC-015 probiotic strain, found similar results in the number of probiotic bacteria and pH during 28 days of storage. High survivability of the probiotic *L. heleveticus* strain MTCC 5463 in whey beverages with 10% orange juice and 1.5% inulin was reported by Shah et al. (2016); during 28 days of storage the number of bacteria remained at a high level of 8 log cfu/g, while the pH of the beverage decreased from 3.82 to 3.67. The high viability of *L. helveticus*, despite the relatively low pH of the beverages, is probably due to the strain's acid tolerance, as well as the protective effect of fructooligosaccharides (FOS) present in inulin.

The high acidity of our whey beverages was due to the use of acid whey as well as fruit juices as flavoring agents. Acid whey contains 0.64–0.75 g of lactic acid in 100 mL and is characterized by a pH value of approximately 4.5 (Jelen, 2003). Since the whey content in all beverages was similar, the differences in acidity between them were mainly due to the different acid content of the fruit concentrates. Blackcurrant fruit is characterized by a particularly high acid content of about 3% citric acid, the value of this parameter for orange and apple fruits is 0.6% on average (Jarczyk and Płocharski, 2010). As a result, blackcurrant drinks showed levels of acidity nearly twice as high as orange and apple drinks, despite the fact that the amount of concentrate used for production was 50% lower. Similar results were obtained by Jaworska et al. (2011), who found that the acid content of blackcurrant-whey beverages was 0.91–0.93% citric acid. Sady et al. (2013) and Jaworska et al. (2014) reported that the acid content of orange and apple whey drinks was 0.60 and 0.61% citric acid respectively. The significantly higher pH values stated in these studies resulted from the adjustment of the acidity of the beverages, by adding citric acid, carried out by the authors.

CONCLUSION

One of the key factors in the quality of probiotic products is maintaining a high abundance of live probiotic bacteria until the end of the intended shelf life. Due to the relatively high acidity of whey beverages, particular attention should be paid to the selection of a suitable probiotic strain that is resistant to such environmental conditions. Among the tested strains, the best viability during storage was observed for *L. paracasei* Lpc-37 and *L. rhamnosus* HN001, and therefore these cultures seem to be the most useful for the production of fruit-whey drinks. The addition of these strains to orange and apple drinks allows the value of 10^6 cfu/g during 28 days of refrigerated storage recommended for probiotic products to be exceeded. Survival of the *B. lactis* HN019 strain allows the above requirements to be fulfilled only in the orange drink. The lowest suitability for

the production of fruit-whey beverages was found for the *L. acidophilus* NCFM strain, as the number of live cells in any of the products supplemented with this bacteria did not reach the level of 10^6 in 1 g after 28 days. The significant differences in the number of probiotic bacteria, depending on the type of fruit concentrate and the associated acidity, indicates that it is necessary to take into account these factors in the design of probiotic fruit-whey beverages. Among the products studied, the use of orange juice is the most beneficial in terms of the number of bacteria, thus allowing for a wider range of probiotic strains to be used, even those more sensitive to acidity. The blackcurrant juice, due to its high acidity, is the least suitable for the production of whey probiotic drinks. Its use would shorten the shelf life of the product to 7–14 days due to bacterial inviability in these conditions.

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