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# FERMENTED PROBIOTIC BEVERAGES BASED ON ACID WHEY

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**ABSTRACT**

**Background.** Production of fermented probiotic beverages can be a good method for acid whey usage. The obtained products combine a high nutritional value of whey with health benefits claimed for probiotic bacteria. The aim of the study was to define quality properties of beverages based on fresh acid whey and milk with addition of buttermilk powder or sweet whey powder.

**Material and methods.** Samples were inoculated with two strains of commercial probiotic cultures: *Lactobacillus acidophilus* La-5 or *Bifidobacterium animalis* Bb-12. After fermentation, samples were stored at refrigerated conditions. After 1, 4, 7, 14 and 21 days sensory characteristics, hardness, acetaldehyde content, titratable acidity, pH acidity and count of bacteria cells were evaluated.

**Results.** Throughout all storage period, the number of bacteria was higher than 8 log cfu/ml in the all samples. Beverages with La-5 strain had higher hardness and acidity, whilst samples with Bb-12 contained more acetaldehyde. Samples with buttermilk powder had better sensory properties than with sweet whey powder.

**Conclusions.** Obtained products made of acid whey combined with milk and fortified with buttermilk powder or sweet whey powder, are good medium for growth and survival of examined probiotic bacteria strains. The level of bacteria was sufficient to provide health benefits to consumers.

**Key words:** acid whey, buttermilk powder, sweet whey powder, fermented probiotic beverages, psychico-chemical parameters, *Lactobacillus acidophilus*, *Bifidobacterium animalis*

## INTRODUCTION

Acid whey is by-product obtained from milk during tvarog cheeses and acid casein production. Manufacture of tvarog is based on acid coagulation of casein, thus acid whey has low pH under value of 5.0. Content of dry matter in whey is approximately 6–7% and the main component is milk sugar-lactose. Another important ingredient of whey are whey proteins which occur in amount of about 0.7%. Those proteins are a rich source of essential amino acids and have a wide range of health benefits such as antioxidant and anticancer properties. They also have very high nutritional value. Moreover, whey contains valuable minerals like easily absorbed calcium and phosphorus and it is a source of vitamins B and vitamin A (Bednarski, 2008; Smithers, 2008).

Development of cheese industry is the reason for the constant increase in whey production. Over the past years, the amount of whey obtained in The European Union steadily increased. In 2012 production of whey in Poland was 1.2 million tonnes and it was higher than in previous year by 4.7% (Seremak-Bulge, 2012). Such a big amount of whey should be properly used. Nowadays whey is not seen as pollutant and it has more and more applications. One of ways of using whey is production of sweet whey powder which has wide range of application in food industry.

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However, there are still problems with management of acid whey. It is because of high acidity and high mineral content in this kind of whey in comparison with sweet whey obtained during rennet cheese production.

An interesting method of whey utilization may be fermented beverages production. Fermented whey has equal nutritional value as unprocessed whey, and thanks to fermentation process, it has a lower level of lactose (Jeličić et al., 2008). Mixing whey with milk aimed to get beverages similar to popular fermented milk drinks such as yoghurt. Improvement of beverages quality can be achieved by fortification with sweet whey powder and buttermilk powder. Buttermilk is another dairy by-product obtained during butter production. It is a rich source of beneficial for health lecithin, proteins and minerals. In order to obtain beverages with functional properties, they were fermented by probiotic bacteria strains with desirable properties and documented clinical effects. Two of the most popular in food manufacture probiotic genera were used: *Lactobacillus acidophilus*, and *Bifidobacterium* (Kailasapathy and Chin, 2000; Shah, 2007).

The goal of this study was to evaluate selected quality characteristic of probiotic fermented beverages based on acid whey and milk fortified with sweet whey powder or buttermilk powder.

## MATERIAL AND METHODS

Tested beverages were prepared from acid whey fermented with commonly used probiotic strains: *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* Bb-12 (both of Chr. Hansen Company). Beverages were manufactured in laboratory conditions using the thermostat method. There were obtained four batches of fermented beverages: two variants with La-5 strain contained 5% addition of buttermilk powder of Spomlek Company (variant La-B) or 5% addition of sweet whey powder of Mogador Company (La-W) and two variants of beverages with Bb-12 strain with the same composition (variants Bb-B and Bb-W). According to the manufacturer, buttermilk powder contained no less than 28% proteins and no more than 9% fat and sweet whey powder contained approximately 12.7% proteins, 0.5% fat and 75.8% carbohydrates. Acid whey obtained from local dairies came from the production of tvarog cheese. Acid whey was pasteurised in temperature of 72°C by 15 minutes, subsequently it was mixed with UHT cow milk contained 3.2% of fat (Paturages Company) in ratio 1:1. After that the mixture was divided into four equal parts. To two parts 5% m/v buttermilk powder and to the other two the same amount of sweet whey powder were added. Obtained mixtures were heated to temperature of 42°C, inoculated with suitable probiotic strain and thoroughly mixed. Bacterial strains was added in amount of 5% v/v as DVS cultures dissolved in sterilized skim milk (0.1 g/1000 ml). Count of bacteria in these inoculum was 8.74 log cfu/ml for *Lactobacillus acidophilus* La-5 and 9.50 log cfu/ml for *Bifidobacterium animalis* Bb-12. In consecutive step beverages were poured into PE-LD cups in amount of approximately 50 ml and covered with aluminium lids. Fermentation process was carried on at temperature of 42°C for about 6 hours until gel receiving. Although this temperature is not optimal for probiotic bacteria (optimum temperature for *Bifidobacterium* is 37–41°C and for *L. acidophilus* – 35–40°C), their growth may occur at temperature up to 45°C (Gomes and Malcata, 1999). Temperature of 42°C enables the acceleration of the fermentation process and it leads to the reduction of titratable acidity of the product (Bulatović et al., 2014). After fermentation, the beverages were cooled to temperature of 5 ±1°C and stored under refrigerated conditions by period of 21 days. After 1, 4, 7, 14 and 21 days of storage, sensory evaluation, estimation of physicochemical properties and microbiological analysis were performed.

Sensory evaluation included assessment of appearance, taste, smell and consistency of beverages. It was performed with the participation of 6 evaluators. There was used scoring method and each quality factor got marks from 1 to 5, where mark 1 means very poor and mark 5 very good quality of the indicator (Baryłko-Pikielna and Matuszewska, 2009; PN-ISO 6658:1998). Evaluation of physico-chemical properties included measure of titratable acidity in Soxhlet-Henkel degrees °SH (PN-75/A-86130), active acidity using pH-meter model Q150 according to the instruction manual and acetaldehyde content using diffusive method with hydrazone hydrochloride (Lees and Jago, 1969). There was also made texture profile analysis (TPA) using texture analyzer TA.XT plus Stable Micro System. The samples were penetrated with an aluminum cylinder with diameter 20 mm at a speed of

5 m/s and a trigger force of 1 G to a depth of 25 mm. Study was limited to hardness analysis which is the major texture parameter of fermented milk beveragas (Salvador and Fiszman, 2004). Dry matter content was evaluated only after one day of refrigerated storage by drying at temperature of 130°C (PN-75/A-86130). Microbiological analysis was carried out according to the Polish Norm (PN-A-86034:1993). It involved a determination of *L. acidophilus* or *B. animalis* cells in beverages using MRS Agar Medium of BTL Company. According to the literature (Drgalić et al., 2005), both bacteria cultures were kept at temperature of 37°C for 72 hours, *L. acidophilus* strain was kept in microaerophilic and *B. animalis* in anaerobic conditions.

Physico-chemicals characteristics of acid whey were also tested. Analysis of density using aerometric method, titratable acidity in °SH, dry matter content (PN-A-86364:1996) and active acidity (pH) using pH--meter were performed.

All assays were conducted in 3 or 5 replicates. Obtained results were statisticaly analysed at the significance level $p = 0.05$ using Microsoft Office Excel 2007. It included t-test and two-factor analysis of variance.

## RESULTS AND DISCUSSION

Physico-chemical characteristics of acid whey used for fermented beverages production are shown in Table 1. Raw whey had high acidity and low dry matter content. Liquid consistency sour taste and smell cause that consumption of unprocessed acid whey is unattractive for consumers. Another disadvantage of whey is its perishability.

The beverages obtained in present study had sensory characteristic similar to fermented milk. After 21 days of storage, beverages did not show symptoms of spoilage, however their quality changed. The sensory acceptance and shelf life are important factors, thus the beverages can be a good method to incorporate whey into a diet. Results of appearance, taste, smell and consistency of beverages as indicators of organoleptic quality are shown in Table 2.

**Table 1.** Physico-chemical parameters of acid whey used for beverages manufacture

| Parametrs | Values |
|---|---|
| Density, g·cm$^{-3}$ | 1.025 |
| Titrable acidity, °SH | 23.1 |
| Active acidity, pH | 4.34 |
| Dry matter content, % | 6.74 |

**Table 2.** Results of sensory evaluation of beverages during refrigerated storage

| Variant of beverage | Storage time, days | | | | |
|---|---|---|---|---|---|
| | 1 | 4 | 7 | 14 | 21 |
| | Appearance | | | | |
| La-B | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| La-W | 3.4 | 3.4 | 3.5 | 3.8 | 3.8 |
| Bb-B | 4.2 | 3.6 | 3.6 | 4.0 | 3.9 |
| Bb-W | 3.9 | 3.1 | 3.0 | 3.0 | 2.6 |
| | Taste | | | | |
| La-B | 2.7 | 3.1 | 3.1 | 2.5 | 2.2 |
| La-W | 3.6 | 3.4 | 2.8 | 2.3 | 1.8 |
| Bb-B | 2.4 | 2.6 | 2.5 | 2.2 | 2.1 |
| Bb-W | 3.4 | 3.2 | 3.3 | 2.4 | 2.1 |
| | Smell | | | | |
| La-B | 4.8 | 4.8 | 4.9 | 4.7 | 4.4 |
| La-W | 4.7 | 4.7 | 4.5 | 4.0 | 4.1 |
| Bb-B | 4.7 | 4.4 | 4.2 | 4.1 | 3.8 |
| Bb-W | 4.8 | 4.5 | 4.5 | 4.1 | 3.9 |
| | Consistency | | | | |
| La-B | 3.6 | 3.5 | 3.3 | 3.4 | 3.6 |
| La-W | 2.8 | 3.2 | 3.0 | 2.5 | 2.7 |
| Bb-B | 3.4 | 3.4 | 3.4 | 3.5 | 3.3 |
| Bb-W | 2.7 | 2.9 | 2.8 | 2.3 | 2.6 |

Explanatory notes: La-B – beverage with La-5 strain and buttermilk powder addition, La-W – beverage with La-5 strain and whey powder addition, Bb-B – beverage with Bb-12 strain and buttermilk powder addition, La-W – beverage with Bb-12 strain and whey powder addition.

Appearance notes estimated as very good had only variant La-B. Other variants had lower assessment because of syneresis that is whey separation on the surface of beverages which occurred during all storage period. Castro et al. (2013) claims, that replacing part of milk with whey in production of fermented beverages leads to decreasing the strength of the gel and viscosity what cause occurring of syneresis. Similarly to appearance, higher consistency notes had variants fortified with buttermilk powder. These beverages had thicker and more homogenous consistency compared to variants with sweet whey powder. Better appearance and consistency of samples with buttermilk powder can be caused by a higher content of casein which occurs in buttermilk. This allows to obtain a stronger gel structure, syneresis reduction and consistency improvement. In case of taste, it was higher assessed in samples supplemented with whey powder than with buttermilk powder. These beverages had more distinct whey taste and smell regarded as positive. However, all batches of fermented beverages had too intensive sour taste. Smell was the highest marked factor in all variants and it had notes. varied from good (4) to very good (5). Samples with La-5 strain had slightly better smell. After 2 and 3 weeks of storage, deterioration of taste and smell in all beverage variants was observed. Increase of sour taste and smell is common within milk fermented products and it is the effect of post-acidification during the product shelf life. Taking into account used bacteria strain, beverages with *Bifidobacterium animalis* Bb-12 had poorer organoleptic qualities than these with *Lactobacillus acidophilus* La-5. Over the entire period of study, beverages fermented by La-5 and supplemented with buttermilk powder had the highest assessment.

Probiotic whey drinks were also produced by Drgalić et al. (2005). In that study, reconstituted sweet whey was inoculated with following strains: *Lactobacillus acidophilus* La-5, *Lactobacillus casei* Lc-01 and *Bifidobacterium animalis* Bb-12. Sensory evaluation showed that drink with La-5 had higher acceptability than with Bb-12 which was referred as drink with poor taste and odour. Those results are consistent with our study. Moreover, as well as in our research, there occurred gradually deterioration of odour and taste.

In case of dry matter content (Table 3) in fermented probiotic beverages, there were no significant differences between both variants with La-5 strain and variant with Bb-12 strain and buttermilk powder. Content of dry matter in these samples was about 12.50%. Slightly higher dry matter content had beverage Bb-W (12.85 ±0.17%).

**Table 3.** Dry matter content in fermented probiotic beverages

| Variant of beverage | Dry matter content, % | |
|---|---|---|
| | $x$ | $\sigma$ |
| La-B | 12.54 | 0.1350 |
| La-W | 12.54 | 0.1137 |
| Bb-B | 12.50 | 0.0755 |
| Bb-W | 12.83 | 0.1677 |
| Significant differences* | La-B:Bb-W, La-W:Bb-W, Bb-B:Bb-W | |

Explanatory notes: $x$ – mean value; $\sigma$ – standard deviation; La-B – beverage with La-5 strain and buttermilk powder addition; La-W – beverage with La-5 strain and whey powder addition; Bb-B – beverage with Bb-12 strain and buttermilk powder addition; La-W – beverage with Bb-12 strain and whey powder addition.
*Significance level $p = 0.05$.

Texture is important factor affecting perception and attractiveness of product (Surmacka Szczesniak, 2002). Over the entire period of study, the highest hardness (0.190–0.226 N) had variant La-B, which also was characterized as product with the best appearance and consistency in sensory evaluation. Hardness of variant Bb-B varied between 0.138–0.175 N. Variants with whey powder had distinctly lower hardness than those with buttermilk powder. Hardness of beverage La-W was 0.117–0.136 N and of Bb-W 0.109–0.125 N. The statistical evaluation of obtained results revealed that in case of this texture parameter, all studied variants were different (Table 4).

The content of acetaldehyde was carried out to estimate the ability of used bacterial strains to produce flavour compounds in tested samples. Acetaldehyde is one of main aroma constituents of milk fermented beverages. The results of measurement showed that beverages with Bb-12 strain had a higher content of acetaldehyde than beverages with La-5 strain. It was also noted that addition of buttermilk powder caused more intensive

**Table 4.** Physico-chemical properties and probiotic bacteria content in beverages during refrigerated storage

| Variant of beverage | Storage time, days | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 4 | | 7 | | 14 | | 21 | |
| | *x* | σ | *x* | σ | *x* | σ | *x* | σ | *x* | σ |
| Hardness, N | | | | | | | | | | |
| La-B | 0.19 | 0.011 | 0.24 | 0.0045 | 0.198 | 0.0404 | 0.22 | 0.0194 | 0.226 | 0.0276 |
| La-W | 0.127 | 0.0136 | 0.125 | 0.0108 | 0.128 | 0.0089 | 0.125 | 0.0105 | 0.117 | 0.0056 |
| Bb-B | 0.138 | 0.0113 | 0.151 | 0.014 | 0.156 | 0.0219 | 0.175 | 0.0165 | 0.161 | 0.0203 |
| Bb-W | 0.109 | 0.0053 | 0.124 | 0.0086 | 0.115 | 0.0064 | 0.125 | 0.0095 | 0.112 | 0.008 |
| Significant differences* | La-B:La-W, La-B:Bb-B, La-B:Bb-W, La-W:Bb-B, La-W:Bb-W, Bb-B:Bb-W | | | | | | | | | |
| Acetaldehyde content, mg/dm$^3$ | | | | | | | | | | |
| La-B | 1.026 | 0.0373 | 0.952 | 0.0728 | 0.742 | 0.0186 | 0.419 | 0.0576 | 0.348 | 0.0195 |
| La-W | 0.612 | 0.0236 | 0.521 | 0.0112 | 0.405 | 0.0186 | 0.269 | 0.0214 | 0.380 | 0.0447 |
| Bb-B | 1.616 | 0.0565 | 1.569 | 0.0901 | 1.448 | 0.0261 | 1.191 | 0.0449 | 1.194 | 0.0307 |
| Bb-W | 0.969 | 0.032 | 1.078 | 0.1019 | 0.893 | 0.0236 | 0.811 | 0.0261 | 0.757 | 0.0117 |
| Significant differences* | La-B:La-W, La-B:Bb-B, La-B:Bb-W, La-W:Bb-B, La-W:Bb-W, Bb-B:Bb-W | | | | | | | | | |
| Titratable acidity, °SH | | | | | | | | | | |
| La-B | 50.0 | 0 | 49.3 | 0.2309 | 49.9 | 0.2309 | 46.3 | 0.4619 | 45.3 | 0.2309 |
| La-W | 43.9 | 0.6110 | 42.5 | 0.2309 | 41.6 | 0 | 40.9 | 0.2309 | 39.9 | 0.2309 |
| Bb-B | 34.7 | 0.2309 | 35.3 | 0.2309 | 35.5 | 0.2309 | 36.0 | 0.4 | 34.8 | 0 |
| Bb-W | 28.7 | 0.2309 | 30.0 | 0 | 30.8 | 0.4 | 30.7 | 0.2309 | 30.1 | 0.2309 |
| Significant differences* | La-B:La-W, La-B:Bb-B, La-B:Bb-W, La-W:Bb-B, La-W:Bb-W, Bb-B:Bb-W | | | | | | | | | |
| Active acidity, pH | | | | | | | | | | |
| La-B | 3.91 | 0.0208 | 3.80 | 0.0208 | 3.85 | 0.0361 | 4.22 | 0.0635 | 4.28 | 0.0603 |
| La-W | 3.90 | 0.01 | 3.92 | 0.01 | 3.94 | 0.0557 | 4.22 | 0.0173 | 4.26 | 0.0058 |
| Bb-B | 4.49 | 0.0058 | 4.43 | 0.0252 | 4.42 | 0.0404 | 4.72 | 0.0473 | 4.61 | 0.0404 |
| Bb-W | 4.60 | 0.0153 | 4.62 | 0.0231 | 4.61 | 0.0265 | 4.80 | 0.0321 | 4.83 | 0.0436 |
| Significant differences* | La-B:La-W, La-B:Bb-B, La-B:Bb-W, La-W:Bb-B, La-W:Bb-W, Bb-B:Bb-W | | | | | | | | | |
| Count of viable bacteria cells, log cfu/ml | | | | | | | | | | |
| La-B | 8.81 | 0.0462 | – | – | 8.99 | 0.0252 | 8.95 | 0.0153 | 8.02 | 0.0819 |
| La-W | 8.85 | 0.0252 | – | – | 8.99 | 0.01 | 8.92 | 0.0603 | 8.49 | 0.1415 |
| Bb-B | 9.16 | 0.0451 | – | – | 9.32 | 0.0212 | 8.92 | 0.0473 | 8.75 | 0.0709 |
| Bb-W | 9.34 | 0.5572 | – | – | 8.86 | 0.0551 | 8.60 | 0.04 | 8.13 | 0.1997 |
| Significant differences* | La-B:Bb-B, La-W:Bb-B | | | | | | | | | |

Explanatory notes: like in Table 3.

acetaldehyde production compared to the beverage with sweet whey powder (Table 4). The highest content of the analysed metabolite was assessed in beverage Bb-B (1.191–1.616 mg/dm$^3$). The lowest amount of this compound occurred in the La-W and ranged from 0.269 to 0.612 mg/dm$^3$. These results are contrary to sensory evaluation (Table 2), in which beverages with La-5 bacteria had more attractive smell. It indicates that acetaldehyde is not a crucial determinant of aroma quality. In all analysed variants, a gradual decrease of tested aroma-compound content was observed. A slight increase was noticed in La-W and Bb-B between 14 and 21 days of storage and in the Bb-W between 1 and 4 days. Total reduction of acetaldehyde content in all 21--days analyzed period was distinctly lower in beverages with Bb-12. It was 21% and 26% reduction respectively for Bb-W and Bb-B. In case of beverages with La-5 it was respectively 38% (for La-W) and 66% reduction (La-B). Reduction of acetaldehyde content is consistent with declination of smell notes in organoleptic assessment (Table 2). Statistical evaluation confirmed that differences noted in acetaldehyde content between all beverage variants were significant.

According to literature, concentration of this essential component of fermented milk flavour can reach level of 10–15 mg/dm$^3$ (Libudzisz, 1998) and minimal content perceptible by human smell is 0.415 mg/dm$^3$ (Lees and Jago, 1978). As a product of bacteria metabolism, the level of acetaldehyde and other flavour compounds is a result of composition of beverage, conditions during fermentation process and storage and properties of used strains of bacteria (Libudzisz, 1998; Dzwolak et al., 2000). Low content of that compound in studied beverages can be caused by using probiotic bacteria which do not have typical capacities to produce aroma compounds during fermentation (Zaręba et al., 2008).

Assessment of acidity is one of the most important quality indicators of fermented milk. The study included measurement of titratable and active (pH) acidity. In case of both kind of acidity all beverage variants differ significantly (Table 4). During three weeks of storage, the higher acidity was recorded in the La-B (45.3–50.0°SH, pH 3.80–4.28) and the lowest in Bb-W (28.7–30.8°SH, pH 4.60–4.83). Active acidity was noticeably higher in beverages with *L. acidophilus* La-5 than with *B. animalis* Bb-12. These results are consistent with presented in study of Drgalić et al. (2005) higher fermentation capabilities of La-5 strain. Moreover, titratable acidity varied between beverages with different additions. Higher value of this parameter than with sweet whey powder had variants with buttermilk powder. This is due to a higher protein level in beverages with buttermilk, responsible for the increase in potential acidity. Considering acidity changes during all analysed storage period, titratable acidity of beverages with La-5 strain gradually declined, whilst in case of Bb-12 strain, this parameter was more stable and there were observed slight increase between the first and the last day of study. Active acidity decreased in all samples. Increase of pH value was more distinct in case of variants with La-5 strain.

Acidity is an important factor affecting the number of bacteria. According to the literature, increase in acidity causes decrease in the number of *L. acidophilus* and *Bifidobacterium*. Growth of the former species ceases when a pH 4.0–3.6 is reached. The latter organism is less acid tolerant and its growth is retarded at pH 5.0–4.5 (Shah, 2007). pH of both La beverages fell below 4.0 in period from 1 to 7 day, but it was higher than pH 3.6 throughout all storage. Acidity of Bb samples was lower than pH 5.0 during all experiment. Moreover, in case of Bb-B, it exceeded the limit of pH 4.5.

The titratable and active acidity of probiotic sweet whey drinks were studied also by Drgalić et al. (2005). In that research, titratable acidity increased during 28-days of storage in samples with both La-5 and Bb-12 strain. pH value decreased in beverages with La-5 while in beverages with Bb-12 there was almost no pH decrease.

According to FAO/WHO (2001) guidelines, the minimum content of probiotic bacteria required for functional product and ensuring the achievement of a therapeutic effect is 6 log cfu/ml. Count of viable probiotic bacteria cells during all three weeks storage period highly exceeded mentioned level and it ranged from 8.02 to 9.34 log cfu/ml (Table 4), thus the beverages can be classified as functional products. Amount of viable bacteria cells was assessed after 1 day and 1, 2 and 3 weeks of refrigerated storage. Exceeding the level of 9 log cfu/ml was noticed in beverages with Bb-12 strain after 1 day (Bb-W) and after 1 and 7 days (Bb-B) of storage. It was found that level of *L. acidophilus* in both beverage variants and of *Bifidobacterium* in variant with buttermilk powder increased during first week of study and decreased in further period. Amount of *B. animalis* in the Bb-W decreased during all study. More intensive bacteria reduction in Bb-W than Bb-B can be caused by higher acidity of the former sample. From 1 to 7 day of storage, pH in Bb-W was below value of 4.5 and as it was said, in that conditions *Bifidobacterium* does not growth. Statistical evaluation shows that there were no significant differences in viable cells count between variants with buttermilk and sweet whey powder fermented with the same bacterial culture.

In the study of Drgalić et al. (2005) count of La-5 decreased throughout all 28 days of storage and it was correlated with acidity increase, although amount of this bacteria maintained at level 7 log cfu/ml. Content of Bb-12 cells was stable during all period and it was about 8 log cfu/ml. In the research on commercial yoghurts with *L. acidophilus* and *Bifidobacterium* cultures, during 5 weeks period of refrigerated storage, there was observed more rapid decline for *Bifidobacterium* as compared to *L. acidophilus* (Shah et al., 1995).

Hardness, acidity and content of viable bacteria are significant quality determinants of probiotic fermented beverages. Results of two-factor analysis of variance revealed that both type of beverage (used probiotic strain and additives) and storage time had significant influence on studied properties (Table 4).

**Table 5.** Results of two-factor analysis of variance of physico-chemical properties and probiotic bacteria content in beverages

| Feature and factor | F | P | Test F |
|---|---|---|---|
| **Hardness** | | | |
| Storage time | 5.232 | 0.000853* | 2.486 |
| Type of beverage | 190.181 | 2.64E-36* | 2.719 |
| Interactions | 2.511 | 0.007462* | 1.875 |
| **Acetaldehyde content** | | | |
| Storage time | 198.298 | 8.48E-26* | 2.606 |
| Type of beverage | 1 197.305 | 3.53E-39* | 2.839 |
| Interactions | 14.101 | 8.29E-11* | 2.003 |
| **Titratable acidity** | | | |
| Storage time | 101.500 | 2.18E-20* | 2.606 |
| Type of beverage | 11 990.560 | 4.2E-59* | 2.839 |
| Interactions | 71.569 | 3.97E-23* | 2.003 |
| **Active acidity** | | | |
| Storage time | 243.913 | 1.63E-27* | 2.606 |
| Type of beverage | 1 475.131 | 5.66E-41* | 2.839 |
| Interactions | 9.696 | 1.9E-08* | 2.003 |
| **Count of bacteria cells** | | | |
| Storage time | 51.873 | 2.17E-12* | 2.901 |
| Type of beverage | 11.170 | 3.57E-05* | 2.901 |
| Interactions | 5.848 | 8.36E-05* | 2.189 |

*Statistically significant differences ($p = 0.05$).

## CONCLUSIONS

The results of the experiment show that acid whey can be used as a raw material to manufacture probiotic fermented beverages containing commonly used strains *Lactobacillus acidophilus* La-5 or *Bifidobacterium animalis* Bb-12. Obtained products made of acid whey combined with milk and fortified with buttermilk powder or sweet whey powder, are a good medium for growth and survival of the examined probiotic bacteria strains. Throughout 3 weeks storage period the content of viable cells of La-5 and Bb-12 exceeded 8 log cfu/ml. This level is sufficient to provide health benefits to consumers, because according to FAO/WHO, minimal level of probiotic bacteria required for functional product is 6 log cfu/ml.

The effect of different probiotic strain and additives on characteristics of the obtained beverages was significant. Quality properties of the beverages depended also on time of refrigerated storage. The beverage with *L. acidophilus* enriched with buttermilk powder had the best sensory scores. It also had the highest hardness which is one of texture measurement. Content of acetaldehyde, one of fermented milk flavour compound was low and it gradually reduced in all samples. Considering acidity of beverages, it was found that samples with *B. animalis* had lower acidity than samples with *L. acidophilus*. In all the examined beverages, pH acidity decreased. It can indicate poor ability of the studied cultures to carry out a post-fermentation process.

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## PROBIOTYCZNE NAPOJE FERMENTOWANE OTRZYMANE NA BAZIE SERWATKI KWASOWEJ

### STRESZCZENIE

**Wstęp.** Produkcja probiotycznych napojów fermentowanych może być dobrym sposobem na zagospodarowanie serwatki kwasowej. Napoje te łączą wysoką wartość żywieniową serwatki i prozdrowotne właściwości bakterii probiotycznych. Celem badań było określenie cech jakościowych probiotycznych napojów fermentowanych otrzymanych ze świeżej serwatki kwasowej z dodatkiem maślanki w proszku oraz serwatki podpuszczkowej w proszku.

**Materiał i metody.** Próby doświadczalne przygotowano z wykorzystaniem dwóch komercyjnych kultur probiotycznych: *Lactobacillus acidophilus* La-5 oraz *Bifidobacterium animalis* Bb-12. Po procesie fermentacji napoje przechowywano w warunkach chłodniczych. Po 1, 4, 7, 14 i 21 dniach przechowywania przeprowadzono ocenę organoleptyczną napojów, wykonano analizę twardości, zawartości aldehydu octowego, kwasowości miareczkowej, pH oraz zmierzono zawartość komórek bakterii probiotycznych.

**Wyniki.** W całym 3-tygodniowym okresie trwania doświadczenia, zawartość bakterii probiotycznych we wszystkich otrzymanych wariantach napojów przekraczała wartość 8 log jtk/ml. Napoje ze szczepem La-5 odznaczały się większą twardością i kwasowością, natomiast napoje zawierające kulturę Bb-12 miały wyższą zawartość aldehydu octowego. Napoje wzbogacone w maślankę w proszku zostały ocenione wyżej pod kątem właściwości organoleptycznych niż napoje zawierające serwatkę podpuszczkową w proszku.

**Wnioski.** Napoje otrzymane na bazie serwatki kwasowej połączonej z mlekiem i wzbogaconej o maślankę lub podpuszczkową serwatkę w proszku są dobrą pożywką pozwalającą na zadowalającą przeżywalność badanych szczepów bakterii probiotycznych. Duża zawartość bakterii we wszystkich otrzymanych wariantach napojów spełnia wymagania stawiane produktom probiotycznym.

**Słowa kluczowe:** serwatka kwasowa, maślanka w proszku, serwatka podpuszczkowa w proszku, probiotyczne napoje fermentowane, właściwości fizykochemiczne, *Lactobacillus acidophilus*, *Bifidobacterium animalis*

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