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## ACID WHEY-BASED SMOOTHIES WITH PROBIOTIC STRAINS\*

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#### Abstract

The aim of study was to determine potential applications of acid whey in the production of whey-based beverages containing plant-based ingredients in equal proportions to acid whey (1:1): beverage 1 with the addition of carrot, rosehip and sea-buckthorn purée; beverage 2 with addition of pear purée and sour cherry juice; beverage 3 with the addition of parsley and banana purée. The composition and mineral content of acid whey were determined. Whey-based beverages containing fruit and vegetables were analysed for mineral content, sensory attributes and the survivability of Lactobacillus rhamnosus HN001 probiotic bacteria. Acid whey is characterised by a low content of major milk components, namely fat (0.441 g kg<sup>-1</sup>), protein (2.978 g kg<sup>-1</sup>), especially of whey protein in native form  $(0.052 \text{ g kg}^{-1})$ , lactose  $(42 \text{ g kg}^{-1})$  and lactic acid (6.304 g kg<sup>-1</sup>). Acid whey is abundant in mineral nutrients, including calcium (1199 mg kg<sup>-1</sup>), magnesium (87.1 mg kg<sup>-1</sup>) and potassium (1533 mg kg<sup>-1</sup>). The mineral analysis revealed that the addition of fruit and vegetables increased the iron content of beverages 2 and 3, and the content of potassium and magnesium in beverage 3. Beverage 3 (100 g) met the Recommended Dietary Allowances (RDA) for potassium and magnesium in 25% and 15%, respectively, in children aged 4 to 7. Whey-based beverages had uniform colour and consistency. The predominant taste and aroma were those associated with the fruit and vegetables, followed by some sweet flavour and aroma, whereas the acidic flavour was moderately discernible. All beverages containing fruit and vegetables received very high scores in a sensory evaluation. The survival of the probiotic strain was monitored for two weeks during which bacterial counts remained stable at 7 log cfu ml<sup>-1</sup>. Smoothies based on acid whey with the addition of fruit and vegetables and the probiotic strain are functional foods that are recommended for people with nutrient deficiencies.

**Keywords:** acid whey, mineral salts, sensory analysis, *Lactobacillus rhamnosus HN001*, probiotics, smoothie, beverages.

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## INTRODUCTION

Whey is a by-product of cheese production. Its composition and properties are determined by the applied processing technology, including the thermal processing of milk and the protein precipitation method. Two types of whey are produced in the dairy industry: sweet whey obtained during the production of rennet cheese, and acid whey which is a by-product derived from the manufacture of unripened cheese (NISHANTHI et al. 2017). Enzymatic and acid coagulation of milk during curd processing produces whey with different physicochemical properties. Rennet coagulation relies on the selective action of rennet enzymes, where the glycomacropeptide is separated from the  $\kappa$ -casein fraction and transferred to rennet whey. Casein deprived of the protective  $\kappa$ -casein fraction is aggregated in the presence of calcium ions (DALGLEISH 1993). In the production of tvorog cottage cheese, milk undergoes acid coagulation where casein micelles are aggregated at the isoelectric point. The resulting decrease in pH transforms colloidal minerals, mainly calcium and phosphate salts, into water-soluble forms (GAUCHERON 2005).

Milk and dairy products contain mineral salts, which play an important role in nutrition and regulation of physiological functions. Mineral salts contribute to bone health, in particular to the development, strength and density of bones in children, and the prevention of bone loss and osteoporotic fractures in the elderly (CASHMAN 2006). The phosphoproteins produced during the hydrolysis of casein as well as whey proteins and lactose are natural ingredients that increase the bioavailability of calcium (GUEGUEN, POINTILLART 2000).

The composition and properties of acid whey and rennet whey (DARADE, GHODAKE 2012, NISHANTI et al. 2017, CHANDRAJITH, KARUNASENA 2018), including fermented whey (ZNAMIROWSKA et al. 2017), have been analysed in the literature to determine their suitability to produce beverages. Acid whey is more abundant in mineral salts than rennet whey, which makes it a valuable ingredient in the production of novel beverages for consumers with specific preferences and dietary needs. The applicability of acid whey in the production of dairy beverages and fermented foods has been discussed by many authors (Lievore et al. 2015, Skryplonek, Jasińska 2015, Flinois et al. 2019). Whey-based beverages can also be enhanced with probiotic bacteria. Selected strains of lactic acid bacteria (LAB) deliver health benefits by exerting immunomodulatory effects, decreasing serum cholesterol levels, alleviating the symptoms of lactose intolerance, reducing the risk of cancer, increasing resistance to gut pathogens, promoting the treatment of rheumatoid arthritis, preventing or alleviating the symptoms of atopic dermatitis, regulating metabolic processes, and aiding in the treatment of candidiasis and urinary tract infections (FAJSAL et al. 2017). Commercial strains of Lactobacillus spp. with clinically proven probiotic properties include L. rhamnosus GG, L. casei Shirota, L. fermentum ME3, L. acidophilus NCFM and *L. plantarum* Lp-115 (Collado et al. 2007). This group of strains includes *Lactobacillus rhamnosus* HN001 (DR20 <sup>TM</sup>), which has been found to improve the immune system function in humans (GILL ET AL. 2000). In addition to strengthening humoral and cellular immunity (GILL 2000), *L. rhamnosus* HN001 enhances gut health in children (DEKKER et al. 2009) and promotes the absorption of minerals such as calcium and magnesium (KRUGER et al. 2009). It exhibits antibacterial properties (EPHRAIM et al. 2012, INTURRI et al. 2016, BERTUCCINI et al. 2017), prevents eczema in young children (WICKENS et al. 2012), and alleviates the symptoms of post-partum depression and anxiety (SLYKERMAN et al. 2017). Whey-based smoothies can be enhanced with *L. rhamnosus* HN001 to deliver health benefits for consumers.

The aim of this study was to determine the composition of acid whey, and to describe its potential applications in the production of whey-based beverages such as smoothies.

## MATERIALS AND METHODS

#### **Materials**

Acid whey was obtained during the production of traditional semi-fat acid tvorog from cow's milk pasteurised at a temp. of 83°C for 15 seconds. A schematic diagram of the experiment is presented in Figure 1. The experiment was conducted in three replicates.



Fig. 1. Schematic diagram of the experiment

The tested probiotic strain was *Lactobacillus rhamnosus* HN001 (DuPont, Poland) which was added to smoothie-type beverages at a minimum concentration of 7.0 log cfu ml<sup>-1</sup>. The strain has well-established probiotic properties (GILL et al. 2000).

#### Smoothie-type beverages containing acid whey

Acid whey was used to produce smoothie-type beverages with different ingredients. Beverage 1 was composed of acid whey (50%), carrot purée (25%), rosehip purée (12.5%) and sea-buckthorn jam (12.5%); beverage 2 contained acid whey (50%), pear purée (37.5%) and concentrated sour cherry juice (12.5%); beverage 3 was composed of acid whey (50%), blended parsley (25%) and banana purée (25%). Non-plant additives, citric acid and xylitol, were used in the production process. The produced beverages contained 50% of acid whey and 50% of the remaining ingredients.

#### **Chemical composition**

The chemical composition of acid whey was determined in a proximate analysis. The content of total solids was determined according to AOAC guidelines (AOAC International, 2007; method 990.20; 33.2.44). Fat content was determined by the Gerber method according to standard IDF 238 (ISO 19662, 2018), and lactose content was determined according to standard ISO 5765-1:2002 (IDF 79-1:2002; IDF 79-2:2002). The content of total nitrogen (TN), non-casein nitrogen (NCN) and non-protein nitrogen (NPN) was determined with the use of AOAC methods 991.20 (33.2.11), 998.05 (33.2.64) and 991.21 (33.2.12), respectively (AOAC International, 2007). Nutrient concentrations were calculated as follows: 1) total protein = TN  $\cdot$  6.38; 2) true protein = (TN - NPN)  $\cdot$  6.38; 3) casein = (TN - NCN)  $\cdot$  6.38; 4) serum protein = (NCN - NPN)  $\cdot$  6.38.

#### **Macronutrients and micronutrients**

Macronutrient and micronutrient levels were determined in acid whey and in beverages containing plant-based ingredients. Nutrient concentrations were determined by dry mineralisation. The analysed samples were incinerated in quartz crucibles in a muffle furnace. The resulting white-grey ash was dissolved in 5M HNO<sub>3</sub> (Merck, Germany) and analysed. The Ca content of ash was determined by adding 10%  $Cl_3La \cdot 7H_2O$  (Merck, Germany) in an amount required to obtain La<sup>+3</sup> with a final concentration of 1%.

The content of minerals (Ca, Mg, Na, K, Fe, Zn) was analysed by flame atomic absorption spectrometry (acetylene flame/air) with the iCE 3000 Series Atomic Absorption Spectrometer (Thermo Scientific, Hertfordshire, UK) equipped with a GLITE data station, a deuterium lamp for background correction, and the appropriate cathode lamps.

#### Acidity

The pH of acid whey and beverages containing plant-based ingredients was determined with the inoLab Level 1 pH-meter (WTW, Weilheim, Germany). The lactic acid content of acid whey was determined according to AOAC guidelines (AOAC International, 1990; method 947.05).

#### Sensory analysis

The sensory profiles of the evaluated beverages were determined on a five-point descriptive scale according to ISO Standard (ISO 13299, 2016). The evaluation was conducted by a panel of eight trained specialists with confirmed taste sensitivity according to ISO Standard (ISO 8586, 2014). Each panellist received a product sheet and a score sheet. The following quality descriptors were taken into account in the evaluation: appearance (4 descriptors), aroma (6 descriptors), consistency (6 descriptors), mouthfeel (5 descriptors) and taste (7 descriptors). Sensory attributes were evaluated on a five-point descriptive scale (1 to 5 points). The intensity of each sensory attribute was determined (1 – absence of descriptor, 5 – very intense). The overall acceptability of the product was determined based on all descriptors and their balance (1 – low quality, 5 – very high quality).

#### Microbial analysis

The analysed beverages and acid whey were inoculated with the *Lacto-bacillus rhamnosus* HN001 probiotic strain and stored in a refrigerator for 14 days. Bacterial counts in whey and beverages were determined immediately after inoculation and after 2, 7 and 14 days of cold storage. Bacteria were plated on MRS agar (Merck, Poland) and incubated at a temperature of 37°C for 48 h under anaerobic conditions. Several bacterial colonies were identified with the MaldiTOFF Vitek MS Plus microbial identification system (bioMerieux, Poland) to confirm the species of the cultured bacteria.

#### Statistical analysis

The results were analysed by one-way ANOVA, and the significance of differences between means was determined in the Duncan's test at 0.05. Data were processed statistically in StatSoft Inc. Statistica v. 13.1 software (Tulsa, Oklahoma, USA).

## **RESULTS AND DISCUSSION**

The chemical composition analysis revealed a low content of non-fat solids in the colloidal and emulsion suspensions of acid whey. The dry matter content of acid whey was determined at 67.43 g kg<sup>-1</sup>±0.200, including

0.441 g kg<sup>-1</sup> ±0.052 of fat, 5.156 g kg<sup>-1</sup> ±0.026 of total protein, 2.978 g kg<sup>-1</sup> ±0.079 of true protein, and 0.341 g kg<sup>-1</sup> ±0.009 of non-protein nitrogen. The content of casein or the casein / denatured whey protein complex reached 2.927 g kg<sup>-1</sup> ±0.068, and the content of non-denatured native whey proteins was determined at 0.052 g kg<sup>-1</sup> ±0.011. The low content of whey proteins, mainly casein and the casein / whey protein complex, as well as very low quantities of native whey proteins can probably be attributed to specific milk treatments during tvorog production. Tvorog is produced from pasteurized milk, and denatured whey proteins (mainly β-lactoglobulin) interact with casein during the production process (VASBINDER, DE KRUIF

2003), which is why casein and denatured whey proteins undergo acid coagulation at the isoelectric point. Pasteurized milk undergoes acid coagulation, and the resulting tworog contains casein as well as whey proteins. At lower pasteurization temperature, whey proteins could be less denatured and therefore retained in whey. The lactose content of acid whey was also low (42.00 g kg<sup>-1</sup> ±0.150), which can be attributed to the partial fermentation of lactose into lactic acid. The lactic acid content of acid whey was determined at 6.304 g kg<sup>-1</sup> ±0.015.

The content of total solids, total protein, lactose and lactic acid in acid whey was similar to that reported by CHANDRAPALA et al. (2015). However, the analysed acid whey was characterised by lower fat content (0.441 g kg<sup>-1</sup>), probably due to the low fat content of milk used in the production process. NISHANTHI et al. (2017) analysed various whey concentrates and found that acid whey concentrate had a lower content of total solids, protein and lactose and higher lactic acid content than sweet whey concentrate.

The tested additives decreased the pH of the produced beverages. Acid whey had a pH of  $4.36 \pm 0.02$ , whereas the pH of beverages 1, 2 and 3 was similar at  $3.76 \pm 0.04$ ,  $3.68 \pm 0.03$  and  $3.96 \pm 0.05$ , respectively.

The potassium, calcium, sodium, magnesium, zinc and iron content of the analysed acid whey (Table 1) was similar to that determined in

Table 1

| Mineral                          | Acid whey             | Beverage                  |                           |                       |
|----------------------------------|-----------------------|---------------------------|---------------------------|-----------------------|
|                                  |                       | 1                         | 2                         | 3                     |
| Potassium (mg kg <sup>-1</sup> ) | 1533±26.20°           | $1879 \pm 78.82^{b}$      | $1309 \pm 9.989^{d}$      | $2750 \pm 46.96^{a}$  |
| Calcium (mg kg <sup>-1</sup> )   | $1199 \pm 14.90^{a}$  | $747.0 \pm 2.380^{b}$     | $647.5 \pm 1.060^{d}$     | 680.8±2.240°          |
| Sodium (mg kg <sup>-1</sup> )    | $318.0 \pm 7.700^{a}$ | $196.1 \pm 9.430^{b}$     | $150.9 \pm 1.920^{\circ}$ | $134.3 \pm 5.640^{d}$ |
| Magnesium (mg kg <sup>-1</sup> ) | $87.10 \pm 1.300^{b}$ | $90.30 \pm 0.450^{b}$     | $67.74 \pm 6.070^{\circ}$ | $196.8 \pm 1.570^{a}$ |
| Zinc (µg kg <sup>-1</sup> )      | $240 \pm 30^{a}$      | $141.9 \pm 7.532^{\circ}$ | $144.8 \pm 21.75^{\circ}$ | $184.7 \pm 3.689^{b}$ |
| Iron (µg kg <sup>.1</sup> )      | $400 \pm 100^{c}$     | $1468 \pm 18.81^{b}$      | $434.3 \pm 16.00^{\circ}$ | $2976 \pm 6.143^{a}$  |

Mineral content of acid whey and beverages

Explanatory notes: beverage 1 - whey, carrot, rosehip and sea-buckthorn purée; beverage 2 - whey, pear and sour cherry juice; beverage 3 - whey, parsley and bananas. Means in rows with different superscripts are significantly different ( $P \le 0.05$ ).

cow'smilk (PARK et al. 2007), which indicates that acid whey is an equally valuable source of minerals. Minerals and other components in milk and dairy products are characterized by high nutritional, energy and bioactive value (GÓRSKA-WARSEWICZ et al. 2019). In view of the above, changes in the content of selected macronutrients and micronutrients resulting from the application of plant-based ingredients were determined.

The evaluated beverages differed significantly in the potassium content (Table 1). The potassium concentration was the highest in beverage 3 containing parsley and banana purée, followed by beverage 1. The lowest potassium content was noted in beverage 2, which was less abundant in potassium than acid whey ( $P \le 0.05$ ).

Calcium, sodium and zinc levels were significantly lower in beverages containing plant-based ingredients than in acid whey ( $P \le 0.05$ ). The magnesium content was more than twice as high in beverage 3 as in whey. The addition of plant-based ingredients did not influence the magnesium content of beverage 1 and decreased the magnesium content of beverage 2. The iron content was higher in beverage 1, and it was considerably higher in beverage 3 than in acid whey ( $P \le 0.05$ ). The addition of plant-based ingredients did not induce significant differences in the iron content of beverage 2 (P > 0.05).

The results of the analysis indicate that beverage 3 most effectively meets the Recommended Dietary Allowances (RDA) for magnesium in children aged 4-6 (15.1%), but it is a less abundant source of magnesium for the remaining age groups (4.7% to 6.8%) – Table 2. All of the tested beverages (100 g) meet the RDA for calcium in 5.0-7.5%, and the RDA for sodium in only 0.9-2.0%. For the group of children aged 4-6, the RDA for potassium is met in 17.1%, 11.9% and 25.0% by beverages 1, 2 and 3, respectively (100 g). The RDA for iron is met in 1.0-1.5% by beverage 1, in 0.3-0.4% by beverage 2, and in 2.0-3.0% by beverage 3. All beverages meet the daily requirements for zinc in less than 0.2%.

The studied beverages would be most effective in meeting the demand for the analysed minerals in children aged 4-6 due to lower macronutrient requirements in this age group.

Five groups of attributes were analysed in the sensory evaluation of whey-based beverages: appearance, aroma, consistency, mouthfeel, and taste (Table 3). Beverages 1, 2 and 3 containing plant-based ingredients were characterised by intense orange, red and green colour, respectively. Plantbased ingredients did not induce differences in colour intensity or uniformity (P>0.05). Undesirable appearance attributes such as separation or non-homogeneity were not observed. The beverages were characterized by an intense aroma characteristic of the added fruit and vegetables, which was most pronounced in beverage 3 (P≤0.05). The second most distinctive olfactory attribute was the sweet aroma, which was more intense in beverage 2 than in the remaining beverages (P≤0.05). A weak acidic aroma was detected

|             |                        | RDA* (mg/nerson)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Beverage                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |      |      |
|-------------|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|------|
| Mineral     | Sex /age (years) group | AI ** (mg/day)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Beverage           1         2           17.1         11.9           6.3         4.4           5.4         3.7           6.3         4.4           5.4         3.7           7.5         6.5           5.7         5.0           7.5         6.5           5.7         5.0           7.5         6.5           5.7         5.0           6.2         5.4           2.0         1.5           1.3         1.0           1.2         1.1           1.3         1.0           1.4         1.1           6,9         5.2           2.2         1.7           2.2         1.6           2.5         1.9           2.8         2.1           0.3         0.3           0.1         0.1           0.2         0.2           0.2         0.2 | 2    | 3    |
|             | children aged 4-6      | 1100                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 17.1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 11.9 | 25.0 |
|             | boys aged 13-15        | 3000                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 6.3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 4.4  | 9.2  |
| Potassium** | men aged 51-65         | 3500                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 5.4                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 3.7  | 7.9  |
|             | girls aged 13-15       | 3000                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 6.3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 4.4  | 9.2  |
|             | women aged 51-65       | 3500                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 3.7  | 7.9  |
|             | children aged 4-6      | 1000                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 7.5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 6.5  | 6.8  |
|             | boys aged 13-15        | P         RDA* (mg/person)<br>AI ** (mg/day)           1100         3000           3500         3           3500         1           3500         1           3500         1           3500         1           3000         1           3000         1           3500         1           1000         1           1000         1           1000         1           1000         1           1000         1           1000         1           1000         1           11000         1           1200         1           1300         1           1400         1           1400         1           130         1           410         1           420         3           320         3           320         1           111         1           9         8           10         12           10         12           10         15           10         10 | 5.7                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 5.0  | 5.2  |
| Calcium*    | men aged 51-65         | 1000                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 7.5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 6.5  | 6.8  |
|             | girls aged 13-15       | 1300                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 5.7                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 5.0  | 5.2  |
|             | women aged 51-65       | 1200                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | $\begin{array}{c c c c c c c c c c c c c c c c c c c $                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 5.4  | 5.7  |
|             | children aged 4-6      | 1000                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 2.0                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1.5  | 1.3  |
|             | boys aged 13-15        | 1500                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1.0  | 0.9  |
| Sodium**    | men aged 51-65         | 1400                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1.1  | 1.0  |
|             | girls aged 13-15       | 1500                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1.0  | 0.9  |
|             | women aged 51-65       | 1400                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.1  | 1.0  |
|             | children aged 4-6      | 130                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 6,9                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 5.2  | 15.1 |
|             | boys aged 13-15        | 410                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 2.2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1.7  | 4.8  |
| Magnesium*  | men aged 51-65         | 420                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.6  | 4.7  |
|             | girls aged 13-15       | 360                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 2.5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1.9  | 5.5  |
|             | women aged 51-65       | 320                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 2.8                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 2.1  | 6.2  |
|             | children aged 4-6      | 5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 0.3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.3  | 0.4  |
|             | boys aged 13-15        | 11                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.1  | 0.2  |
| Zinc*       | men aged 51-65         | 11                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.1  | 0.2  |
|             | girls aged 13-15       | 9                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 0.2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.2  | 0.2  |
|             | women aged 51-65       | 8                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 2.8         2.1           0.3         0.3           0.1         0.1           0.2         0.2           0.2         0.2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 0.2  |      |
| Iron*       | children aged 4-6      | 10                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1.5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.4  | 3.0  |
|             | boys aged 13-15        | 12                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1.2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.4  | 2.5  |
|             | men aged 51-65         | 10                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1.5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.4  | 3.0  |
|             | girls aged 13-15       | 15                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1.0                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.3  | 2.0  |
|             | women aged 51-65       | 10                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 1.5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.4  | 3.0  |

Daily nutrient intake per 100 g of the analysed beverages (%)

Source: own elaboration based on JAROSZ et al. (2017) and WOJTASIK et al. (2017).

\* RDA – Recommended Dietary Allowances,

\*\* AI – Adequate Intake.

For the remaining explanatory notes, refer to Table 1.

Table 3

| Descriptor    |                    | Beverage                                                                                                                                                                                                                                                                                                                                                                                                                           |           |           |
|---------------|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|-----------|
|               |                    | 1                                                                                                                                                                                                                                                                                                                                                                                                                                  | 2         | 3         |
| Appearance    | colour intensity   | 4.8                                                                                                                                                                                                                                                                                                                                                                                                                                | 4.7       | 4.4       |
|               | colour uniformity  | 4.8                                                                                                                                                                                                                                                                                                                                                                                                                                | 4.9       | 4.7       |
|               | separation         | 1.0                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.0       | 1.0       |
|               | homogeneity        | 4.9                                                                                                                                                                                                                                                                                                                                                                                                                                | 4.7       | 4.7       |
|               | tvorog             | 1.3                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.1       | 1.1       |
| Aroma         | acidic             | $2.1^{a}$                                                                                                                                                                                                                                                                                                                                                                                                                          | $1.5^{b}$ | $2.0^{a}$ |
|               | fruit or vegetable | $4.4^{b}$                                                                                                                                                                                                                                                                                                                                                                                                                          | $4.5^{b}$ | $5.0^a$   |
|               | sweet              | $3.5^b$                                                                                                                                                                                                                                                                                                                                                                                                                            | $4.5^{a}$ | $3.3^{b}$ |
|               | astringent         | 1.1                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.4       | 1.3       |
|               | foreign            | 1.0                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.0       | 1.0       |
| Consistency   | smooth             | 4.9                                                                                                                                                                                                                                                                                                                                                                                                                                | 5.0       | 4.9       |
|               | lumpy              | 1.1                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.1       | 1.1       |
|               | sticky             | 2.0                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.9       | 2.4       |
|               | firm               | $3.0^a$                                                                                                                                                                                                                                                                                                                                                                                                                            | $1.5^{b}$ | $2.6^a$   |
|               | liquid             | $2.8^{b}$                                                                                                                                                                                                                                                                                                                                                                                                                          | $4.6^{a}$ | $2.4^{b}$ |
|               | uniform            | 4.8                                                                                                                                                                                                                                                                                                                                                                                                                                | 4.8       | 4.8       |
|               | smooth             | 4.9                                                                                                                                                                                                                                                                                                                                                                                                                                | 4.9       | 4.7       |
|               | firm               | ntensity $4.8$ $4.4$ niformity $4.8$ $4$ ion $1.0$ $1$ neity $4.9$ $4$ $1.3$ $1$ $2.1^a$ $1$ vegetable $4.4^b$ $4$ $3.5^b$ $4$ $ant$ $1.1$ $1$ $1.0$ $1$ $1$ $1.0$ $1$ $1$ $1.0$ $1$ $1$ $1.0$ $1$ $1$ $1.0$ $1$ $1$ $2.0$ $1$ $1$ $2.0$ $1$ $1$ $2.0$ $1$ $1$ $2.0$ $1$ $1$ $2.0$ $1$ $1$ $1.1$ $1$ $1$ $2.8^b$ $4$ $4$ $4.9$ $4$ $4$ $1.9$ $1$ $1$ $1.1$ $1$ $1$ $1.3$ $1$ $1$ $2.9^a$ $1$ $1.4$ $1.0$ <td>2.3</td> <td>2.5</td> | 2.3       | 2.5       |
| Mouthfeel     | sticky             | 1.9                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.4       | 1.4       |
|               | lumpy              | 1.1                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.3       | 1.4       |
|               | dense              | $3.5^a$                                                                                                                                                                                                                                                                                                                                                                                                                            | $1.3^{b}$ | $3.4^a$   |
|               | tvorog             | 1.3                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.1       | 1.0       |
| Taste         | acidic             | $2.9^{a}$                                                                                                                                                                                                                                                                                                                                                                                                                          | $1.9^{b}$ | $2.1^{b}$ |
|               | sweet              | $3.5^b$                                                                                                                                                                                                                                                                                                                                                                                                                            | $4.4^{a}$ | 2.9°      |
|               | bitter             | 1.0                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.0       | 1.0       |
|               | fruit or vegetable | 4.5                                                                                                                                                                                                                                                                                                                                                                                                                                | 4.3       | 4.9       |
|               | astringent         | 1.4                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.1       | 1.1       |
|               | foreign            | 1.0                                                                                                                                                                                                                                                                                                                                                                                                                                | 1.0       | 1.0       |
| Overall score |                    | 4.9                                                                                                                                                                                                                                                                                                                                                                                                                                | 4.5       | 4.9       |

Average values of sensory descriptors in whey-based smoothies

For the remaining explanatory notes, refer to Table 1. Means in rows with different superscripts are significantly different (P≤0.05).

in the whey-based beverage containing sour cherry juice and pear purée (beverage 2), whereas the beverages containing carrots, rosehip, sea-buckthorn (beverage 1), parsley and bananas (beverage 3) were characterized by a moderately discernible acidic aroma ( $P \le 0.05$ ). The analysed whey-based beverage were characterised by a tvorog aroma and an astringent aroma of very low intensity (P>0.05). Foreign aromas were not detected in any of the evaluated products (P>0.05).

All samples were characterised by smooth (P>0.05) and uniform (P>0.05) consistency without the presence of lumps (P>0.05). Stickiness was evaluated as moderate, and it was more pronounced in beverage 3 than in the remaining samples (P>0.05). An assessment of consistency descriptors revealed the greatest variations in liquidity and density. Beverage 2 containing pear purée and sour cherry juice was characterised by the highest liquidity and the lowest density and firmness ( $P \le 0.05$ ). The statistical analysis revealed differences in one of the five mouthfeel descriptors. Beverage 2 was characterised by lower density and firmness than the remaining samples ( $P \le 0.05$ ). The mouthfeel of all beverages was described as very smooth (P>0.05), moderately firm (P>0.05) and sticky (P>0.05) with barely detectable lumpiness (P>0.05). The taste of the added fruit and vegetables predominated in the analysed beverages, but its intensity was not determined by the addition plant-based ingredients (P>0.05). The sweet taste was the second most distinctive sensory descriptor whose intensity varied subject to the added plantbased ingredients. The analysed beverages were arranged in the following ascending order based on their sweetness: beverage 2 < beverage 1 < beverage 3 ( $P \le 0.05$ ). The perception of acidity was moderate, and it was highest in beverage 1 of all the samples ( $P \le 0.05$ ). Plant-based ingredients masked the tworog and astringent flavour more effectively in beverages 2 and 3 than in beverage 1 (P>0.05). Bitter and foreign taste was not detected in any of the analysed samples. All beverages received very high scores in the sensory evaluation (P > 0.05).

The *L. rhamnosus* HN001 probiotic strain, added in the amount of 7.0 log cfu ml<sup>-1</sup>, was characterised by high survival rates in refrigerated whey (control) and in whey-based beverages (Table 4).

After 14 days of storage, the highest increase in L. *rhamnosus* counts was noted in whey (0.5 log), and the relevant increase in whey-based bever-

Table 4

| Time (days) | Colony counts of <i>L. rhamnosus</i> (log cfu ml <sup>-1</sup> ) |                 |                 |                 |
|-------------|------------------------------------------------------------------|-----------------|-----------------|-----------------|
|             | Acid whey                                                        | beverage 1      | beverage 2      | beverage 3      |
| 0           | 7.20±0.40                                                        | 7.31±0.50       | 7.34±0.53       | 7.35±0.49       |
| 2           | 7.28±0.43                                                        | $7.44{\pm}0.47$ | 7.48±0.53       | $7.48 \pm 0.55$ |
| 7           | $7.56\pm0.49$                                                    | $7.58 \pm 0.44$ | $7.56\pm0.42$   | $7.58 \pm 0.54$ |
| 14          | $7.70{\pm}0.57$                                                  | $7.69 \pm 0.52$ | $7.63 \pm 0.54$ | 7.74±0.51       |

Survival rates of the *L. rhamnosus* HN001 probiotic strain in acid whey and smoothie-type beverages

For the remaining explanatory, refer to Table 1. Mean values in rows and columns do not differ significantly at 0.05.

ages reached 0.29-0.39 log. Parsley and banana purée offered the most supportive environment for the growth of the probiotic strain, and colony counts in beverage 3 reached 7.74 log cfu ml<sup>-1</sup> after 14 days of storage. In an analysis conducted after 14 days of storage, colony counts were lowest in the beverage enhanced with pear purée and sour cherry juice (7.63 log cfu ml<sup>-1</sup>), which can probably be attributed to the low pH of the added fruit. No significant differences in *L. rhamnosus* counts were observed between the examined beverages (P>0.05). Storage time had no significant influence on colony counts (P>0.05).

These results indicate that whey-based smoothies offer a supportive environment for the survival of *L. rhamnosus* HN001 probiotic bacteria. All bacterial isolates identified with the Vitek MS system were characterized by 99% species identity with *Lactobacillus rhamnosus*. SADY et al. (2017) produced fruit beverages with the addition of acid whey. In their study, orange, apple and black current juice was combined with acid whey and probiotic strains, including *L. rhamnosus* HN001. The resulting whey-based beverages were characterized by high survival rates of probiotic bacteria. SKRYPLONEK and JASIŃSKA (2015) also reported on the high survival rates of probiotic strains in whey-based beverages and satisfactory sensory properties. Like in the present study, the cited authors observed a minor increase in the abundance of *Lactobacillus acidophilus* La-5 (approx. 0.1 log cfu ml<sup>-1</sup>) and high survival rates of the applied probiotic strain in whey-based beverages after 14 days of refrigerated storage. Probiotic bacteria deliver additional health benefits by improving the bioavailability of macronutrients.

Whey-based beverages containing natural ingredients and probiotic bacteria can be consumed on a daily basis to enhance physiological functions and improve the nutritional status of consumers. The results of this study indicate that acid whey can be used to develop a new range of functional foods.

### CONCLUSIONS

Acid whey was characterised by: 1) the presence of lactic acid, 2) low lactose content, 3) high content of macronutrients and micronutrients, 4) high survival rates of *Lactobacillus rhamnosus* probiotic bacteria. The content of minerals in beverages depended on plant ingredients. The addition of carrot purée, rosehip purée and sea-buckthorn jam (beverage 1), and parsley and banana purée (beverage 3) contributed to an increase in potassium and iron levels, whereas parsley and banana purée increased the magnesium content of the produced beverages. The content of the remaining minerals in smoothies was similar to or lower than in acid whey. Beverage 3 most effectively catered to the recommended daily amount of dietary minerals. The RDA values for minerals vary across age groups. Beverage 3 met the RDA for potassium and magnesium in 25% and 15%, respectively, in children aged 4 to 7, but it was a less abundant source of the remaining minerals. The intake of dietary minerals from 100 g of beverage 3 was lower in the remaining age groups. Whey-based beverages containing plant-based ingredients were characterised by satisfactory sensory properties. They had uniform colour and consistency; their taste and aroma were determined mainly by the added ingredients, and the characteristic flavour and aroma of acid whey were barely detectible. Whey-based beverages offer a supportive environment not only for the survival, but also for the proliferation of probiotic bacteria, although no significant differences in colony counts were found in the analysed beverages after 14 days of storage. Bacterial counts remained stable in excess of 7 log cfu ml<sup>-1</sup>. Acid whey, the low-cost by-product of the cheese-making process, can be widely used as a base of smoothie-type beverages with probiotic bacteria to enhance their nutritional value and health-promoting properties.

#### **Conflict of interest**

Katarzyna Kiełczewska and Renata Pietrzak-Fiećko contributed equally to this paper.

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