Development of potentially probiotic and synbiotic pumpkin frozen desserts

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ABSTRACT
The aim of this work was the development of novel potentially probiotic and synbiotic sorbets using one variety of pumpkin in laboratory conditions and estimation of products quality during storage. Five kinds of fermented pumpkin sorbets were produced with one probiotic strain Lactobacillus rhamnosus Lock 0900, concentrations of 17% added sugar with or without pineapple juice addition. The fruit-pumpkin sorbets contained 1%, 2%, 3% of inulin addition. Products were frozen and stored for 6 months at −30°C. On the basis of the research carried out, the hypothesis assumed was proved: it was possible to use a probiotic bacterial strain and conditions of fermentation of pumpkin pulp, which is a semi-finished product, for sensory acceptable sorbet, containing an appropriate number of probiotic bacteria. Developed pumpkin sorbets, after storage at −30°C were characterized by good sensory quality and contained an appropriate probiotic bacterial number, therefore could be considered as potentially probiotic products.

1. Introduction
Functional foods have been used for centuries to prevent disease throughout the world, and they are actually foods that can alter disease in addition to providing nutrition. The rapid growth in the development of functional food products is associated with increased consumer awareness of the influence of food on human health. The role of food producers is not limited only to providing consumers with a wide choice of new products, the composition of these products is also not without significance. According to Bornkessel, Broring, Omta (Onno), and van Trijp (2014), among different factors, consumers’ health motivation seems to have the highest relevance in explaining consumer awareness.

Probiotic food is one type of functional food. Probiotic products have become the primary choice for consumers because of their health benefits (Leandro, Araujo, Conceicao, Moreaes, & Carvalho, 2013). Probiotic microorganisms are defined as “live microorganisms that when administered in adequate dose confer beneficial effects on the host’s health beyond inherent basic nutrition” (FAO & WHO, 2001). Probiotic food should contain at least $10^6–10^7$ cfu/g of probiotic bacteria at the end of shelf life (Ertem & Cakmakci, 2018).

Synbiotics refers to foods or dietary supplements combining probiotics and prebiotics in a form of synergism (Pandey, Naik, & Vakil, 2015). According to the report by FAO&AGNS (2007), a prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota (Śliżewska, Nowak, Barczyńska, & Libudzisz, 2013).

Progress in science and medicine has enabled the discovery, isolation of new probiotic bacterial strains and their use in food production on an industrial scale. Spontaneous fermentation can be replaced by fermentation which uses selected probiotic bacterial strains. In addition, it can achieve higher stability of these cultures in the product using different solutions such as the microencapsulation process (Ephrem, Najjar, Charcosset, & Greige-Gerges, 2018; Schoina, Terpou, Bosnea, Kanellaki, & Nigam, 2018). Also, other methods are used such us immobilization, use of prebiotics, coacervates, etc. (Bosnea, Moschakis, & Biladeris, 2014; Terpou, Bekatorou, Kanellaki, Koutinas, & Nigam, 2017).
The probiotic food market is dominated by dairy products. But there is a growing interest to develop a new segment of functional foods – non-dairy probiotic products (Blana, Polymeneas, Tassou, & Panagou, 2014; Ranadheera, Baines, & Adams, 2010). The use of new food matrices for probiotic bacterial strain delivery is especially beneficial for consumers who are lactose-intolerant or require a low-cholesterol diet, or people on vegan or vegetarian diets (Rodríguez-Gómez, Romero-Gil, García-García, Garrido-Fernández, & Arroyo-López, 2014).

There is a tendency on the market for consumers to look for raw products rich in most beneficial nutrients as far as their health-promoting properties are concerned. In turn, consumers often are more eager to choose vegetable products of high nutritional and biological value. Pumpkin is one of the vegetables with high biological and high nutritional value. It is still underrated by consumers as well as food growers. Its fruit can be a healthy and valuable component of a number of dishes and fruit products (Nawirska-Olszańska, Biesiada, Kucharska, & Sokół-Lętowska, 2012) because of high content of fibre and carotenoids, including β-carotene, lutein, and violaxanthin, or considerable content of vitamin C and low calorific value (Biesiada, Nawirska, Kucharska, & Sokół-Lętowska, 2009, 2011; De Carvalho et al., 2012).

An important issue related to probiotic products is the kind of food matrix used for the delivery of probiotic bacterial strains. An incorrect selection of this factor may be the cause of a decrease in the viability of bacteria and effect on their properties (Yeo & Liong, 2010). Apart from dairy products, other novel food matrices are also recommended for the delivery of probiotics, such as fruit juices, sorbets, etc. (Fernandes Pereira & Rodrigues, 2018; Mantzourani et al., 2018; Semjonovs et al., 2013). The results of many studies indicate also that ice-cream may be a good carrier of probiotic bacteria (Akalin, Kesenkas, Dinkci, Unal, & Kınik, 2018; Ferraz et al., 2012; Marinho, da Silva, Mazzocato, Tulini, & Favaro-Trindade, 2017; Matias, Padilha, Bedani, & Isay Saad, 2016; Ozturk, Demirci, & Akin, 2018).

In this context, the aim of this study was the development novel potentially functional food products – frozen pumpkin desserts and estimation of their quality during storage.

2. Materials and methods

2.1. Preparation of probiotic bacterial strain

Freeze cultures containing probiotic bacterial strain Lactobacillus rhamnosus Lock 0900 (patent number 209988) were obtained from a pure culture maintained at the Laboratory of Microbiology, Łódź University of Technology, Poland (Alekandrzaik-Piekarczyk, Koryszewska-Bagińska, & Bardowski, 2013). The bacteria added to the pasteurized pumpkin pulps in the fermentation process had been grown prior to that for 24 h in the MRS broth (Biokar Diagnostics, France) at 37°C. The culture was centrifuged, washed in distilled water and re-suspended in distilled water to its original volume. The probiotic bacteria count in the preparation starter cultures was approximately 9.00 log cfu/ml. The bacterial strain was added in the amount of 1% of the pumpkin pulp volume.

2.2. Sorbet formulation

Pineapple juice (Hortex, Poland), Still water (Żywic Zdrój, Poland), Inulin (Fruta Fit Tex, The Netherlands, importer: Hortimek), Gelatine (Gellwe, Poland), Sucrose (Diamant, Dostyn, Poland) were purchased in the local market in Warsaw, Poland.

This study used the Cucurbita maxima Duchesne pumpkin variety, Polish name – ‘Justynka F1’, according to the Development of Polish Official Variety Testing. The fruit of this cultivar bred at the Department of Plant Genetics, Breeding and Biotechnology of the Warsaw University of Life Sciences, Poland, were characterized by a high total carotenoid content 0,548 mg/g (Chilczuk, Perucka, Materska, & Buczkowski, 2014).

The preparation of pumpkin pulp and the conditions of fermentation process have been established in previous studies (Szydlowska & Kołożyn-Krajewska, 2010). A cube of pumpkin was pasteurized at the temperature of 90°C for 25 min. Afterward, the pumpkin was blended to obtain homogenous vegetable pulp, then cooled and packaged into plastic pots and stored at the temperature of −30°C. The defrosted pumpkin pulp was fermented using one probiotic bacterial strain Lactobacillus rhamnosus Lock 0900 at the temperature of 32°C for 26 h. All of the fermented samples of pumpkin pulp contained a concentration of 17% added sugar. Sucrose was added before the fermentation process.

The pumpkin sorbets were produced as five formulations based on the author’s Polish patent PL 213822 B1 (2010), using laboratory ice-cream maker (Philips HR/2304/70) and Table 1 shows sample characteristics.

According to the formulations shown in Table 1, five pumpkin sorbet mixes (3 kg each) were manufactured, each in triplicate. After combining all ingredients from the recipe, the mass was placed in an ice-cream machine where it underwent the mixing process, followed by the simultaneous

| Compounds                        | PS1 Pumpkin | PS2 Pumpkin-pineapple | PS3 Pumpkin-pineapple, 1% inulin | PS4 Pumpkin-pineapple, 2% inulin | PS5 Pumpkin-pineapple, 3% inulin |
|----------------------------------|-------------|-----------------------|----------------------------------|----------------------------------|----------------------------------|
| 1. Pumpkin pulp fermented by Lactobacillus rhamnosus Lock 0900 | +           | +                     | +                                | +                                | +                                |
| 2. Still water                   | +           | +                     | -                                | -                                | -                                |
| 3. Sucrose                       | +           | +                     | +                                | +                                | +                                |
| 4. Pineapple juice               | -           | +                     | +                                | +                                | +                                |
| 5. Gelatine                      | +           | +                     | +                                | +                                | +                                |
| 6. Inulin                        | -           | -                     | +                                | +                                | +                                |
cooling of the mass. After that pumpkin sorbets were packaged into plastic pots (300 g each) and stored for 24 weeks at the temperature of −30°C. Finally, there were five kinds of frozen desserts: pumpkin as control sample (PS1), pumpkin-pineapple (PS2) and pumpkin-pineapple with 1% (PS3), 2% (PS4), 3% (PS5) of inulin addition (Table 1).

Samples were analyzed regarding pH, viability of probiotic cells and sensory characteristics during freezing storage for 6 months. The initial analysis was performed 24 h after the manufacturing process.

2.3. Microbiological analysis of products

The counts of probiotic bacteria were enumerated on MRS agar (Biokar Diagnostic, France). The plates were incubated aerobically at 37°C for 72 h (PN-ISO:15214:2002). The colony counts were calculated by converting to log cfu/g.

2.4. pH measurement

The pH of the potentially probiotic and symbiotic sorbets tested was determined with the use of a calibrated pH meter (ELMETRON CP551, Poland). The measurements were performed in triplicates.

2.5. Sensory evaluation

The sensory quality of five different kinds of pumpkin sorbets was assessed as fresh products and after 1, 2, 3, 4, 5 and 6 months of storage at the temperature of −30°C using QDA (Quantitative Descriptive Analysis; ISO 13299: 2016). Nine attributes of the probiotic and symbiotic pumpkin sorbets were described: taste characteristics: bitter taste, pungent taste, burning taste, pumpkin flavor, sweet flavor, acid flavor, other flavour, thickness and smoothness, as well as overall quality. The panelists’ aim was to determine the intensity of each of the quality attributes mentioned and to conduct their assessment on an appropriate scale (linear graphical scale 0–10 conventional units [c.u.]). On the basis of the evaluation of the features mentioned, the overall sensory quality of the products was additionally determined on a separate scale. The evaluations were conducted with the participation of 10 members of staff of the Department of Food Gastronomy and Food Hygiene. The panelists were trained in the methodology of the analyses performed and conducted examinations in terms of sensory sensitivity. The assessment was performed in triplicates.

2.6. Chemical analysis of pumpkin sorbets

The content of dry matter, carbohydrates and D and L form of lactic acid were analyzed in sorbets, 24 h after the manufacturing process and during storage at the temperature of −30°C.

Dry matter content was determined using the gravimetric method with loss of mass on drying (PN-EN 12145:2001). The sample was dried to a constant weight under certain pressure and temperature conditions and a percentage of the dried residues of the sample before drying was calculated.

Carbohydrates were evaluated according to the Lane-Eynon method (PN-90A-75101/07). This method consisted of a colorimetric quantification using a copper sulfate solution and methylene blue indicator. A calibration curve was used beforehand, with a series of standard solutions of known sugar concentration.

The content of D and L form of lactic acid was determined using the spectrometric method with NAD (PN-EN 12631:2002). The method consisted in the measurement of the amount of NADH formed, produced as a result of the oxidation of L and D lactic acid, measured as the increase in absorbance at 365 nm, which corresponds to the amount of D and L-lactic acid in the sample.

2.7. Statistical analysis

The statistical analysis was performed using Statistica 13.1 (StatSoft). A one-way analysis of variance (Anova) was used for the statistical evaluation of results of the pH measurements and bacterial determination (the significance level p = 0.05 was assumed), and sensory quality (the significance level p = 0.01 was assumed).

The Principal Component Analysis (PCA) was used to interpret the sensory evaluation results of products during the 6-month storage. In this research, 10 principal components were identified, of which the total of the first four components explains 77% of the total variance of variables, and the total of the first two principal components ~ 57% of variance of variables. In addition, the coefficients of simple correlation were calculated between the overall sorbets quality and the assessed distinguishing features.

3. Results and discussion

3.1. Microbiological quality

During 6 months storage of sorbets at the temperature of −30°C, the count of bacteria Lactobacillus rhamnosus Lock 0900 was measured at four-week intervals. As shown in Table 2, in the case of pumpkin and pineapple sorbets and sorbets with a 2% and 3% inulin addition, a significant influence of the time of storage on the count of Lactobacillus rhamnosus Lock 0900 bacteria was observed.

After 5 and 6 months of storage, the count of the probiotic bacterial strain in the products analyzed decreased significantly. The count of Lactobacillus rhamnosus Lock 0900 bacteria remained at the highest level in the stored sorbets with 3% (9.40 log cfu/g) and with a 1% of prebiotic addition (9.14 log cfu/g). The other probiotic and symbiotic sorbets with a 2% addition of inulin, after the indicated storage period, contained the lactic acid bacteria count below 9 log cfu/g. The lowest count of probiotic bacterial strain in own study was observed after 6 months in pumpkin sorbets (8.77 log cfu/g) and pumpkin-pineapple sorbets with a 2% inulin addition (8.75 log cfu/g).

Other researchers, e.g., Heenan, Adams, Hosken, and Fleet (2004) noted the number of the Lactobacillus paracasei spp. Lp 01 bacteria in frozen soy desserts after 7 months of storage of products at the temperature of −20°C at the level of 6.8 log cfu/ml. Akalin et al. (2018) reported that addition of wheat fiber to probiotic milk ice-cream can achieve a high level of probiotic starter cultures of Lactobacillus acidophilus (6.88 log cfu/ml), while maintaining good overall quality, and improve rheological and textural characteristics during storage at −18°C for 180 days. Ozturk et al. (2018) notified that dark-blue and white Myrtus communis fruit pulps added to probiotic goat milk ice-cream
formulations containing probiotic bacterial strain *Lactobacillus casei* 431 can work as prebiotics in the ice-cream media. The count of *Lactobacillus casei* 431 bacteria in ice-cream samples decreased during the freezing process and this probiotic bacterial strain showed high viability during storage at −20°C for 8 weeks. At the end of the storage of products, the numbers of *Lactobacillus casei* 431 were detected as 7 log cfu/g. But the temperature of storage was higher and time of storage shorter than in this study. An important fact is also that pumpkin sorbets were manufactured using a vegetable base fermented by probiotic bacteria and prebiotic (inulin) was added. It could have influenced the higher count of probiotic bacterial strains in the frozen pumpkin dessert after storage, compared to probiotic goat milk ice-cream.

It should be emphasised that results of own research indicate a very high number of *Lactobacillus rhamnosus* Lock 0900 bacteria (over 8.7 log cfu/g) in potentially probiotic and synbiotic pumpkin sorbets during the entire period of storage at the temperature of −30°C, although its statistically significant reduction was noted after 6 months of product storage. Between samples of fresh and stored pumpkin-pineapple sorbets with 1% and 2% inulin addition, there were no statistically significant differences in terms of the number of lactic acid bacteria. A question arises – what is the reason for such high number of lactic acid bacteria being maintained in the food medium environment?

One of the hypotheses that we propose concerns the mechanism of response of lactic acid bacteria to cold stress. The rapid reduction of the temperature of environment may result in a number of changes in the physiology of microbes and expression of genes, i.e., reduction of liquidity of cell membranes or stability of secondary RNA and DNA structures. In order to reduce the possibility of occurrence of these unfavorable effects, bacterial cells develop the so-called transitory adaptive system. In such case, they start to synthesize cold-induced proteins (CIPs) which maintain the liquidity of membranes through the increase in concentration of unsaturated fatty acids and lipids. Additionally, bacterial cells sustain transcriptions and translations necessary to initiate adaptive mechanisms and retain the DNA particle structure (Phadtare, Yamanaka, & Inouye, 2000). The shock reduction of the temperature of environment in which LAB bacteria exist allows the maintenance of a high number of microorganisms in the finished product, in this case, the pumpkin sorbet.

The positive role of the inulin addition on the survival of bacteria in sorbets should also be pointed out. The addition of inulin to pumpkin sorbets has been shown to improve the viability of *Lactobacillus rhamnosus* Lock 0900 bacteria which maintained the count of about 9.00 log cfu/g over 6 months of storage at the lower temperature of −30°C. According to Ahmadi et al. (2014), a similar effect can be obtained through the microencapsulation process which can also enhance the survival of probiotic bacteria through freezing during ice-cream manufacture. According to Ranadheera et al. (2010), products of plant origin, fermented with probiotic starters, often contain prebiotic compounds which attract and stimulate the growth of probiotics. The appropriate combination of prebiotics and probiotics manifest higher potential for a synergistic effect. The addition of prebiotics before fermentation improves the viability of probiotic bacterial strains in products during the process, and stability of cultures during the shelf life of functional food (Farinha et al., 2015; Yeo & Liong, 2010).

On the other hand, Marinho et al. (2017) developed synbiotic sorbets with jussara pulp. The products were not fermented as in our study. The probiotic bacterial strains (*Lactobacillus acidophilus* LA3; *Lactobacillus paracasei* BG1) were incorporated into sorbets at the end of the manufacturing process. These authors reported that probiotic bacteria were kept viable during the entire time of storage, 120 days at the temperature of −18°C, reaching about 8.8 log cfu/g.

### 3.2. Physicochemical quality

The total carbohydrates content in fresh products and after 6 months of storage at the temperature of −30°C was assessed during own research. In the case of pumpkin sorbets (PS1), the statistically significant highest of total carbohydrates content in was recorded among all fresh products (85 g per 100 g of dry mass) (Table 3). However, after storage, a small but significant reduction in the quantity of this ingredient was observed in all samples tested, nevertheless.
Table 3. Physical–chemical properties of manufactured pumpkin sorbets.

Table 3. Propiedades físicoquímicas de sorbetes de calabaza elaborados.

| Sample | pH    | Dry matter content (%) | Total carbohydrates content (%) | Lactic acid content (g/100g dry weight basis)* |
|--------|-------|-------------------------|--------------------------------|-----------------------------------------------|
| PS1 (0)| 5.46 ± 0.06 a | 24.10 ± 0.03 a | 85.00 ± 0.06b | 1.30 ± 0.05a b | 0.62 ± 0.03a b | 0.68 ± 0.04a b |
| PS1 (6) | 5.34 ± 0.01c | 23.90 ± 0.01a | 77.00 ± 0.13a | 1.60 ± 0.04a | 0.71 ± 0.07a | 0.89 ± 0.05a |
| PS2 (0) | 5.45 ± 0.06 b | 24.03 ± 0.04 a | 80.00 ± 0.11a | 1.60 ± 0.07b | 0.82 ± 0.05b | 0.78 ± 0.08a |
| PS2 (6) | 5.43 ± 0.02 c | 23.80 ± 0.04 a | 78.00 ± 0.04ab | 1.80 ± 0.03b | 0.84 ± 0.11b | 1.06 ± 0.05ab |
| PS3 (0) | 5.44 ± 0.03 b | 24.15 ± 0.02 ab | 81.00 ± 0.08a | 1.50 ± 0.12b | 0.71 ± 0.07a | 0.79 ± 0.04a |
| PS3 (6) | 5.36 ± 0.07 b | 23.85 ± 0.03a | 76.00 ± 0.05a | 1.92 ± 0.06c | 0.96 ± 0.05b | 0.96 ± 0.05a |
| PS4 (0) | 5.45 ± 0.05 b | 24.39 ± 0.03ab | 80.00 ± 0.18a | 1.30 ± 0.06a | 0.60 ± 0.05b | 0.70 ± 0.07a |
| PS4 (6) | 5.37 ± 0.06 b | 24.03 ± 0.03ab | 76.00 ± 0.11a | 1.80 ± 0.06b | 0.81 ± 0.04ab | 0.99 ± 0.09a |
| PS5 (0) | 5.25 ± 0.04 ab | 24.40 ± 0.03 ab | 81.00 ± 0.09a | 1.60 ± 0.08b | 0.80 ± 0.06b | 0.80 ± 0.07a |
| PS5 (6) | 5.19 ± 0.01 a | 24.05 ± 0.02ab | 76.00 ± 0.08a | 1.90 ± 0.12c | 0.85 ± 0.09ab | 1.05 ± 0.15ab |

Explanatory notes: Formulations: PS1- pumpkin sorbet; PS2-pumpkin-pineapple sorbet; PS3-pumpkin-pineapple sorbet with 1% of inulin addition, PS4-pumpkin-pineapple sorbet with 2% of inulin addition; PS5- pumpkin-pineapple sorbet with 3% of inulin addition. The results are expressed as the mean ± standard deviation (n = 3). Mean values denoted by the same letters aren’t significantly different (p > 0.05). Upper index means significantly difference between two periods of storage (0 and 6 months) for one kind of sorbet. (0) Results obtained for sorbets on day first storage at –30°C. (6) Results obtained for sorbets after 6 months storage at –30°C. * on dry weight basis.

without any statistically significant differences in this respect between products.

On the basis of our research conducted on storage of sorbets from fermented pumpkin pulp (Table 3), a statistically significant impact (p < 0.05) of the time of storage on the pH value was found only in the case of potentially synbiotic sorbets. The pH value of products underwent a slight though statistically significant reduction during 6 months of storage at the temperature of –30°C. No statistically significant correlations between the number of probiotic bacteria Lactobacillus rhamnosus Lock 0900 and the pH value of sorbets during storage were noted. Other researchers, e.g., Favoro – Trindade, de Carvalho, Felix Dias, Amaral Sanino, and Boschini (2007), did not observe statistically significant changes in the pH of ice-cream made of fermented “ubos” fruit after 105 days of storage at the temperature of −18°C. Also, Basylit, Kuleašan, and Karahan (2006) did not note significant changes in the pH value of probiotic milk ice-cream with added sucrose or asparagine during 180 days of storage at the temperature of −20°C.

The results obtained in our research indicate that, after 6 months of storing sorbets at the temperature of −30°C, a small though statistically significant increase in the lactic acid content took place, up to around 1.6–1.9 g/100 g of dry mass of the product (Table 3). Also, a slight but statistically significant reduction of the dry mass content was recorded (Table 3). No statistically significant differences were observed in the dry mass content in individual products. The results of this study showed that the dry mass content of evaluated pumpkin sorbets is about 24%.

One of the important characteristics of lactic acid bacteria is the ability to produce lactic acid as the major end product during the fermentation of carbohydrates. Lactic acid has an asymmetric carbon and it naturally occurs as two optical isomers, D and L lactic acid. The proportion of each isomer confers different physical properties to the final product. The D-lactic acid isomer in high doses is considered harmful to humans and can cause for example de-calciﬁcation or acidosis. For this reason, the L-lactic acid isomer is used especially by two industries, pharmaceutical and food (Datta, Tsai, Bansignore, Moon, & Frank, 1995; Hofvendahl & Hahn-Hägerdal, 2000; Oliveira, Maciel Filho, & Rossell, 2016; Salminen, Von Wright, & Ouwehand, 2004).

In all sorbets studied statistically significant reduction of the total content of carbohydrates was observed after 6 months of storage at the temperature of −30°C. At the same time, in the sample of pumpkin-pineapple sorbets (PS2) and samples of pumpkin-pineapple sorbets with different levels of inulin addition (PS3, PS4, PS5), after the appointed storage time, a statistically significant higher level of lactic acid was noted in comparison with the control sample PS1. The fact that the lactic acid content increased is difficult to explain. The authors have formulated a hypothesis that the reason behind this phenomenon may also be the share of cold-induced proteins (CIP) in the process of metabolic transformation of sugars. During the analysis of the process of CIP synthesis in the Lactobacillus lactis bacterial cells, it was found that among other things they take part in the sugar metabolism as enzymes (phosphoglucose mutase-PMGs) (Wouters et al., 2000a, 2000b).

In turn, these enzymes connect carbohydrate catabolism and anabolism routes. The B-PGM enzyme catalyzes the transformation of glucose β-1-phosphoate into 6-phosphate, whereas α-PGM catalyses the transformation of glucose 6-phosphate into α-1-phosphoate. Depending on the composition of the substrate, glucose α-1-phosphate may be formed in a different metabolic route. If fructose is present in the substrate (as in our experiment), Lactobacillus bulgaricus transports sugar into the cell using the PEP-fructose-PTS (phosphoenolpyruvate: fructose phosphotransferase) system. Sugar is transformed into fructose-1-phosphate. Next, in the reaction catalysed by α-phosphofructokinase, fructose-1,6-bisphosphate is formed, which can participate in the glycolysis process and production of lactic acid (Grobben, Smith, Sikkema, & de Bond, 1996).

Samples of sorbets PS2, PS3, PS4, PS5 constituted an environment with high content of fructose for the Lactobacillus rhamnosus Lock 0900 bacteria, which is the result of the addition of pineapple juice and prebiotic – inulin to the fermented...
pumpkin base in the form of various lengths of fructose chain. This fact could be the reason behind the higher intensity of carbohydrate transformations.

### 3.3. Sensory Quality

The overall sensory quality of fresh sorbets produced was good. The sorbet with 3% addition of inulin (PS5) (2.79 c.u.) was characterized by the highest intensity of the sour taste among fresh products, whereas pumpkin sorbet (PS1) (1.55 c.u.) (Table 4) – the lowest. In pumpkin-pineapple sorbets, a significantly lowest intensity of the pumpkin flavor in comparison with other fresh products was noted. The overall sensory quality of fresh pumpkin-pineapple sorbet (PS2) was significantly higher than the quality of fresh sorbet with 2% (PS4) and 3% of inulin added (PS5). However, no statistically significant difference was noted in terms of overall sensory quality of fresh pumpkin sorbet (PS1), pumpkin-pineapple (PS2) and pumpkin-pineapple with 1% inulin added (PS3).

The results of a univariate variance analysis concerning the impact of the type of sorbets on selected characteristics of the sensory quality of products stored for a period of 6 months at the temperature of ~30°C indicate the lack of difference between sorbets in terms of intensity of the sour taste. The significantly highest intensity of bitter taste was noted in the case of pumpkin sorbet, and the lowest in the case of pumpkin-pineapple sorbet with 2% inulin added (PS4). It was observed that the intensity of the pungent taste was the highest in the case of pumpkin sorbet (PS1) and pumpkin-pineapple sorbet with 3% inulin added (PS5), the lowest in the case of sorbet with 1% prebiotic addition (PS3). After 6 months of storage of sorbets, the highest intensity of the pumpkin flavor was found in the case of the pumpkin-pineapple sorbet (PS2) and pineapple sorbet (PS1). Sorbets with the addition of inulin were not diversified in terms of intensity of this taste characteristic. The highest notes of intensity of the burning taste among all samples stored were observed for the sorbet with 3% inulin addition (PS5). In sorbets with the inulin addition (PS4; PS5), the longer the storage time, the higher was the feeling of “grittiness”. No significant differences were noted in terms of the consistency characteristic – smoothness – between samples of sorbets with 2% (PS4) and 3% inulin addition (PS5).

The results of the sensory evaluation of sorbets using the QDA method prove that from among nine quality characteristics evaluated, four were positively correlated with the overall sensory quality of sorbets (Table 5). Smoothness and intensity of pumpkin flavor displayed the strongest positive correlation with the overall sensory quality. Other five characteristics were negatively correlated with the overall sensory quality of sorbets tested, of which such taste characteristics as tart, burning and other flavor decreased the quality of products stored to the highest degree.

Results of evaluation of the sensory quality of sorbets stored obtained by the profiling method have been subjected to the principal component analysis (PCA) (Figure 1) which enables to observe which sensory attributes are significantly correlated and thus obtain the full characteristic of the sensory quality, allowing for only several from a large number of characteristics evaluated.

The analysis of the results obtained suggests that the pumpkin flavor, thickness and smoothness had the greatest positive impact on the overall sensory quality. On the other hand, distinguishing attributes such as “other” flavor, a pungent taste and a burning taste had the greatest negative impact on the overall sensory quality (Figure 1).

The first group, uniform in terms of quality, were samples of pumpkin sorbets (samples No 1–7), pumpkin-pineapple sorbets (samples No 8–14) and sorbets with 1% inulin addition (samples No 15–21) (Figure 1). These products were characterized by detectable pumpkin taste and smooth consistency, and the highest overall sensory quality notes. On the other hand, samples of sorbets with 2% (samples No 22–28) and 3% inulin addition (samples No 29–35) were on the opposite “quality pole”. Particularly after 24 weeks of storage, sorbet sample 35 is considerably removed on the graph from other samples, it has a negative value of the first factor coordinate and is characterized by much poorer overall sensory quality in comparison with other sorbet samples. This is also confirmed by the results of the univariate analysis of variance concerning the impact of different types of sorbets on selected characteristics of sensory quality of fresh products and products stored for a period of 6 months (Table 4).

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**Table 4.** The selected results of sensory evaluation of manufactured pumpkin sorbets in fresh products and after 6 months storage at temperature ~30°C.

**Tabla 4. Resultados seleccionados de la evaluación sensorial de sorbetes de calabaza valorados en productos frescos y después de 6 meses de almacenamiento a una temperatura de ~30°C.**

| Sample | Storage (months) | Smoothness | Acid flavor | Bitter taste | Pungent taste | Burning taste | Pumpkin flavor | Overall quality |
|--------|------------------|------------|-------------|-------------|--------------|--------------|----------------|----------------|
| PS1    | 0                | 6.73 ± 1.01ab | 1.55 ± 1.21 a | 1.17 ± 1.20 a | 1.06 ± 0.85 a | 1.04 ± 1.10 a | 7.20 ± 1.48 b | 7.31 ± 0.95 ab |
| PS2    | 6                | 7.52 ± 1.40 d | 2.19 ± 1.33 ab | 1.07 ± 1.20 a | 1.19 ± 1.56 a | 0.96 ± 0.99 a | 6.18 ± 0.76 a | 7.61 ± 0.79 ab |
| PS3    | 6                | 6.25 ± 1.10 bc | 2.34 ± 1.20 ab | 1.07 ± 0.89 a | 1.34 ± 0.96 a | 0.92 ± 1.22 a | 6.82 ± 1.16 ab | 7.34 ± 1.10 ab |
| PS4    | 6                | 5.46 ± 0.90 ab | 2.33 ± 1.05 ab | 1.04 ± 1.60 a | 1.01 ± 1.13 a | 0.83 ± 1.32 ab | 6.57 ± 0.86 ab | 7.08 ± 0.96 ab |
| PS5    | 6                | 5.24 ± 0.85 a | 2.79 ± 0.78 b | 0.98 ± 0.87 a | 1.18 ± 0.86 a | 1.09 ± 1.11 ab | 6.82 ± 0.95 ab | 6.82 ± 1.31 a |

**Note:** The results are expressed as the mean ± standard deviation (n = 3). Mean values denoted by the same letters are not significantly different (p > 0.01).

**Formulations:** PS1– pumpkin sorbet; PS2- pumpkin-pineapple sorbet; PS3- pumpkin-pineapple sorbet with 1% of inulin addition, PS4- pumpkin-pineapple sorbet with 2% of inulin addition; PS5- pumpkin-pineapple sorbet with 3% of inulin addition.

**Formulaciones:** PS1- sorbete de calabaza; PS2- sorbete de calabaza y piña; PS3- sorbete de calabaza-piña con 1% de adición de inulina; PS4- sorbete de calabaza-piña con 2% de adición de inulina; PS5- sorbete de calabaza-piña con un 3% de adición de inulina.

Los resultados se expresan como la media ± desviación estándar (n = 3). Los valores medios expresados por las mismas letras no son significativamente diferentes (p > 0.01).
In the course of storing samples of sorbets with 2% and 3% inulin addition (PS4, PS5) at the temperature of −30°C, the volume of products decreased, hollow spaces were also observed on the surface of the sorbets, which could be the consequence of ice sublimation on internal walls of containers. The appearance of gritty structure of the products tested in the course of the storage process could be explained by the sugar crystallization process.

Similar as in the research by El-Nagar, Clowes, Tudorica, and Kuri (2002), on the basis of results of the sensory evaluation based on the QDA method it was found that control samples of low-fat ice-cream without prebiotic addition were hard, rough, with discernible ice crystals, whereas the texture of control samples of ice-cream with high-fat content received higher notes, as more delicate and smooth. The addition of inulin (with high level of polymerization) at the level of 5%, 7%, 9% to products with reduced fat content significantly improved their texture; however, the viscosity of the product became decreased. The authors explain this fact by inulin’s ability to combine water and create gel structure which may be modified by adding other components of the ice-cream mix. Also, Cruz et al. (2010) reported that the substitution with prebiotic ingredients has a greater influence on texture.

The results of our sensory evaluation show that all formulations of pumpkin sorbets were accepted in terms of sensory characteristics. However, the potentially probiotic pumpkin sorbet (PS1), pumpkin-pineapple sorbet (PS2) and pumpkin-pineapple sorbet with 1% inulin addition received the highest scores from the panelists among all sorbet samples evaluated that had been stored for 6 months at the temperature of −30°C. The probiotic frozen desserts described can constitute an alternative to probiotic dairy products, the most popular among types of probiotic food.
4. Conclusions
The potentially probiotic and synbiotic pumpkin and pumpkin-pineapple sorbets, manufactured on the basis of fermented pumpkin pulp, showed promising results, indicating possibilities for adding probiotic bacterial strains into recipes of dessert recipes manufactured using fresh vegetables. It is a highly advantageous technological solution, considering that the process of fermentation of sour material makes food safer and richer in health benefits because it contains many antioxidant components such as carotenoids, vitamins.

First and foremost, it should be emphasized that the number of probiotic bacteria in sorbets remained at a high level, which is in all likelihood related to the share of cold-induced proteins – CIP. They are probably responsible also for the transformation of sugars into lactic acid the content of which in products increased during the storage of sorbets. Also, inulin added to sorbets in the quantity of 1% had a positive impact on the survival of bacteria and the sensory quality during the entire period of storage.

Disclosure statement
No potential conflict of interest was reported by the authors.

References
Ahmadi, A., Milani, E., Madadlou, A., Mortazavi, A., Morakam, R., & Salabarshi, D. (2014). Symbiotic yogurt-ice cream produced via incorporation of microencapsulated Lactobacillus acidophilus la-5) and fructooligosaccharide. Journal of Food Science and Technology, 51, 1568–1574.

Akalin, A. S., Keskenas, H., Dinkci, N., Unal, G., & Kinik, O. (2018). Enrichment of probiotic ice cream with different dietary fibers: Structural characteristics and culture viability. Journal of Dairy Science, 101, 37–46.

Aleksandrzak-Piekarczyk, T., Koryszewska-Bagińska, A., & Bardowski, J. (2013). Genome sequence of the probiotic strain Lactobacillus rhamnosus (Formerly Lactobacillus casei) LOCK 900. Genome Announcements, 1(4), doi:10.1128/genomeA.00640-13.

Başyığit, G., Kuleaşan, H., & Karahan, A. G. (2006). Viability of human-derived probiotic lactobacilli in ice cream produced with sucrose and aspartame. Journal of Industrial Microbiology & Biotechnology, 33(9), 796–800. doi:10.1007/s10295-006-0128-x.

Biesiada, A., Nawirska, A., Kucharska, A. Z., & Sokół-Lętowska, A. (2011). Chemical composition of pumpkin fruit depending on cultivar and storage. Ecological Chemistry and Engineering, 18, 9–18.

Biesiada, A., Nawirska, A., Kucharska, A. Z., & Sokół-Lętowska, A. (2009). The effect of nitrogen fertilization methods on yield and chemical composition of pumpkin (Cucurbita maxima) fruits before and after storage. Vegetable Crops Research Bulletin, 70, 202–211.

Blana, V. A., Polymeneas, N., Tassou, C. C., & Panagou, E. Z. (2014). Inoculated fermentation of green olives with potential probiotic Lactobacillus pentosus and Lactobacillus plantarum starter cultures isolated from industrially fermented olives. Food Microbiology, 38, 208–218.

Borkmessel, S., Broring, S., Omata (Onno), S. W. F., & van Trijp, H. (2014). What determines ingredient awareness of consumers? A study on ten functional food ingredients. Food Quality and Preference, 32, 330–339.

Bosnea, L. A., Moschakis, T., & Billaderis, C. G. (2014). Complex coacervation as a novel microencapsulation technique to improve viability of probiotics under different stresses. Food and Bioprocess Technology, 7(10), 2767–2781.

Chlaczuk, B., Perucka, I., Materska, M., & Buczkowska, H. (2014). Content of lutein, zeaxanthin, and beta-carotene in lyophilized fruits of selected cultivars of Cucurbita maxima D. Zyznosc. Nauka. Technologia. Jakosc/Food. Science Technology. Quality, 21(2), 139–150.

Cruz, A. G., Cadena, R. S., Walter, E. H. M., Mortazavian, A. M., Granato, D., Faria, A. F., & Bolini, H. M. A. (2010). Sensory analysis: Relevance for probiotic, probiotic, and synbiotic product development. Comprehensive Reviews in Food Safety, 9, 358–373.

Datta, R., Tsai, S., Bonsignore, P., Moon, S., & Frank, J. R. (1995). Technological and economical potential of poly(lactic acid) and lactic acid derivatives. FEMS Microbiology Reviews, 16, 221–231.

De Carvalho, L. M. J., Games, P. B., Godoy, R. L. D. O., Pacheco, S., da Motta, P. H. F., de Carvalho, J. L. V. … Ramos, S. R. R. (2012). Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (Cucurbita moschata Duch): A preliminary study. Food Research International, 47(2), 337–340.

El-Nagar, G., Clowes, G., Tudorica, C. M., & Kuri, V. (2002). Rheological quality and stability of yog-ice with added inulin. International Journal of Dairy Technology, 55(2), 89–93.

Ephrem, E., Najjar, A., Charcosset, C., & Greige-Gerges, H. (2018). Encapsulation of natural active compounds, enzymes, and probiotics for fruit juice fortification, preservation, and processing: An overview. Journal of Functional Foods, 48, 56–84.

Ertmem, H., & Calmakiç, S. (2018). Shelf life and quality of probiotic yogurt produced with Lactobacillus acidophilus and God. In International Journal of Food Science & Technology, 2018, doi:10.1111/jifs.13653.

FAO & WHO. (2001, October 1–4) Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic bacteria. Córdoba, Argentina.

FAO/BAGNS. 2007. September 15-16. Report of a FAO technical meeting on prebiotics. Rome, Italy.

Farinha, L. R. L., Sabo, S. S., Porto, M. C., Souza, E. C., Oliveira, M. N., & Oliveira, R. P. S. (2015). Influence of probiotic ingredients on the growth kinetics and bacteriocin production of Lactococcus lactis. Chemical Engineering Journal, 43, 313–318.

Favaro-Trindade, C. S., de Carvalho, J. C., Felix Dias, P., Amaral Sanino, F., & Boschni, C. (2007). Effects of culture, ph and fat concentration on melting rate and sensory characteristics of probiotic fermented yellow mombin (spondias mombin l) ice creams. Food Science and Technology International, 13(4), 285–291. doi:10.1177/1082013207082387.

Fernandes Pereira, A. L., & Rodrigues, S. (2018). Chapter 15 - turning fruit juice into probiotic beverages. In G. Rajauria & B. K. Tiwari (Eds.), Fruit juices (pp. 279–287). San Diego: Academic Press.

Ferraz, J. L., Cruz, A. G., Cadena, R. S., Freitas, M. P., Qinto, U. M., Carvalho, C. C., … Bolini, H. M. (2012). Sensory acceptance and survival of probiotic bacteria in ice cream produced with different overrun levels. Journal of Food Science, 77(1), 24–28.

Grobben, G. J., Smith, M. R., Sikkerma, J., & de Bond, J. A. (1996). Influence of fructose and glucose on the production of exopolysaccharides and the activities of enzymes involved in the sugar metabolism and the synthesis of sugar nucleotides in Lactobacillus delbrueckii subsp. Bulgaricus NFCB 2772. Applied Microbiology and Biotechnology, 46, 279–284.

Heenan, C. N., Adams, M. C., Hosken, R. W., & Fleet, G. H. (2004). Survival and sensory acceptability of probiotic microorganisms in a non-fermented frozen vegetarian dessert. Lwt- Food Science and Technology, 37, 461–466. doi:10.1016/j.lwt.2003.11.001.

Hofvendahl, K., & Hahn-Hägerdal, B. (2000). Factors affecting the fermentative lactic acid production from renewable resources. 1. Enzyme and Microbial Technology, 26, 87–107.

ISO 13299:2016. Sensory analysis. Methodology. General guidance for establishing a sensory profile.

Leandro, E. S., Araujo, E. A., Conceicao, L. L., Moreaes, C. A., & Carvalho, A. F. (2013). Survival of Lactobacillus delbrueckii UFV H2620 in ice cream produced with different fat levels and after submission to stress acid and bile salts. Journal of Functional Foods, 5, 503–507.

Manztourani, I., Nouska, C., Tserpo, A., Alexopoulos, A., Bezirtzoglou, E., Panagiotidou, M., & Pliass, S. (2018). Production of a novel functional fruit beverage consisting of cornelian cherry juice and probiotic bacteria. Antioxidants, 7(11), 163.

Marinho, J. F. U., da Silva, M. P., Mazzocato, M. C., Tulini, F. L., & Favaro-Trindade, C. S. (2017). Probiotic and synbiotic sorbets produced with Jussara (Euterpe edulis) pulp: Evaluation throughout the storage period and effect of the matrix on probiotics exposed to simulated gastrointestinal fluids. Probiotics and Antimicrobial Proteins. doi:10.1007/s12602-017-9346-y.
Matias, N. S., Padilha, M., Bedani, R., & Isay Saad, S. M. (2016). In vitro gastrointestinal resistance of Lactobacillus animalis Bb-12 in soy and r milk based symbiotic apple ice creams. International Journal of Food Microbiology, 234, 83–93.

Nawirska-Olszańska, A., Biesiada, A. Z., Kucharska, A., & Sokół-Lętowska, A. (2012). Wpływ sposobu przygotowania i warunków przechowywania przeciów, soków przecierowych i soków miętnych z owoców dyni obłazem oraz przeniesieniem, efekt produkcji méthodes and storage conditions on the phsyicochemical properties of purees, puree juices and cloudy juices obtained from pumpkin enriched with japanese quince and cornelian cherry. Food Science, Technology and Quality, 3(82), 168–178.

Oliveira, R. A., Maciel Filho, R., & Rossell, C. E. V. (2016). High lactic acid production from molasses and hydrolysed sugarcane bagasse. Chemical Engineering Transactions, 50, 307–312.

Ozturk, H. I., Demirci, T., & Akin, N. (2018). Production of functional probiotic ice creams with white and dark blue fruits of Myrtus communis: The comparison of the prebiotic potentials on Lactobacillus casei 431 and functional characteristics. LWT- Food Science and Technology, 90, 339–345.

Pandey, K. R., Naik, S. R., & Vakil, B. V. (2000a). Probiotics, prebiotics and synbiotics—A review. Journal of Food Science and Technology, 52(12), 7577–7587. doi:10.1007/s11319-015-1921-1

Phadtare, S., Yamanaka, K., & Inouye, M. (2000). The cold shock response. In G. Storz & R. Hengge-Aronis (Eds.), Bacterial stress responses (pp. 33–45). Washington, DC: ASM Press.

PL. (2010). Patent 213822 B1. “Frozen dessert and its preparation”. Poland: Polish Patent Office.

PN-EN 12145:2001. Soki owocowe i warzywne – Oznaczanie całkowitej suchej substancji – Metoda gravimetryczna oznaczania ubytku masy w wyniku suszenia (Fruit and vegetable juices - Determination of total dry matter - Gravimetric method with loss of mass on drying). PN-EN 12631:2002. Soki owocowe i warzywne – Oznaczanie enzymatycznych zawartości kwasu D- i L-mleczanu (meleczanu) – Metoda spektrometryczna z NAD (Fruit and vegetable juices - Enzymatic determination of D- and L-lactic acid (lactate) - Spectrometric method with NAD).

PN-ISO 15214:2002. Mikrobiologia żywności i pasz. Horyzontalna metoda oznaczania liczby mezofilnych bakterii fermentacji mlekojowej [Metoda płytkowa w temperaturze 30 stopni C. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of mesophilic lactic acid bacteria — Colony-count technique at 30 degrees C].

Ranadheera, R. D. C. S., Baines, S. K., & Adams, M. C. (2010). Importance of food in probiotic efficacy. Food Research International, 43(1), 1–7.

Rodríguez-Gómez, F., Romero-Gil, V., García-García, P., Garrido-Fernández, A., & Arroyo-López, F. N. (2014). Fortification of table olive packing with the potential probiotic bacteria Lactobacillus pentosus TOMC-LAB2. Frontiers Microbiology, 5, 467.

Salminen, S., Von Wright, A., & Ouwehand, A. (2004). Lactic acid bacteria: Microbiological and functional aspects (3rd ed.). New York: Marcel Dekker, Inc.

Santos Cruzen, C. E., Hoffmann, J. F., Zandonà, G. P., Fiorentini, A. M., Rombaldi, C. V., & Chaves, F. C. (2017). Probiotic butia (Butia odorata) ice cream: Development, characterization, stability of bioactive compounds, and viability of Bifidobacterium lactis during storage. LWT- Food Science and Technology, 75, 379–385.

Schoina, V., Terpou, A., Bosnea, L., Kanellaki, M., & Nigam, P. S. (2018). Entrapment of Lactobacillus casei ATCC393 in the viscus matrix of Pistacia terebinthus resins for functional myzithra cheese manufacture. LWT - Food Science and Technology, 89(Supplement C), 441–448.

Semjonovs, P., Denina, I., Fomina, A., Sakirova, L., Auzina, L., Patetko, A., & Upide, D. (2013). Evaluation of Lactobacillus reuteri strains for pumpkin (Cucurbita pepo L.) juice fermentation. Biotechnology, 12(5), 202–208.

Sliżewska, K., Nowak, A., Barczyńska, R., & Libudzisz, Z. (2013). Prebiotics—definition, properties and applications in industry. Żywność. Nauka. Technologia. Jakość/Food. Science Technology. Quality, 1(86), 5–20.

Szydłowska, A., & Kołodzyn-Krajewska, D. (2010). Applying potentially probiotic bacterial strains to pumpkin pulp fermentation. Żywność. Nauka. Technologia. Jakość/Food. Science Technology. Quality, 17(6), 109–119.

Terpou, A., Bekatorou, A., Kanellaki, M., Koutinas, A. A., & Nigam, P. (2017). Enhanced probiotic viability and aromatic profile of yogurts produced using wheat bran (Triticum aestivum) as cell immobilization carrier. Process Biochemistry, 55, 1–10.

Wouters, J. A., Kamphuis, H. H., Hugenholtz, J., Kuipers, O. P., de Vos, W. M., & Abeel, T. (2000a). Changes in glycolytic activity of Lactococcus Lactis induced by low temperature. Applied and Environmental Microbiology, 66, 3686–3691.

Wouters, J. A., Mailhes, M., Rombouts, F. M., de Vous, W. M., Kuipers, O., & Abeel, T. (2000b). Physiological and regulatory effects of controlled overproduction of five cold shock proteins of Lactococcus lactis MG 1363. Applied and Environmental Microbiology, 66, 3756–3763.

Yeo, S. K., & Liang, M. T. (2010). Effect of prebiotics on viability and growth characteristics of probiotics soy milk. Journal of the Science of Food and Agriculture, 90(2), 267–275.