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SENSORY QUALITY OF NEW PROBIOTIC BEVERAGES BASED ON CHEESE WHEY AND SOY PREPARATION

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Four fermented probiotic beverages, three based on a mixture of cheese whey and soy preparation, and one on a soy preparation solely were evaluated. The products were fermented with selected probiotic strains of *Bifidobacterium breve*, *B. infantis*, *B. animalis/lactis*, *Lactobacillus plantarum*, *L. casei*, and *Streptococcus thermophilus*, used in the form of synergistic sets. After fermentation, the beverages were supplemented with processed fruits, and subsequently, their sensory properties were evaluated by Quantitative Descriptive Analysis (QDA). Eighteen attributes for the investigated products, describing odour, appearance, taste, and consistency, were defined. The results obtained showed that during fermentation and subsequent fruit supplementation non-desired attributes of the soy preparation, like beany taste and flavour as well as aftertaste disappeared, and pleasant fruity, slightly acidic and sweet tastes appeared. All beverages were appraised with relatively high overall score (7.07–7.82 out of 10) over the control non-fermented soy preparation (score 1.13 out of 10). The final products, as new probiotic beverages, were characterised with good sensory properties, and in addition a high cell number of the probiotic bacteria (about 10^9 cells/mL).

INTRODUCTION

The health benefits of probiotic bacteria, mainly bifidobacteria and lactobacilli, are numerous, including: prevention and treatment of diarrhoeal diseases, systemic infections, and atopic diseases, enhancement of the specific parameters of the immune system, lowering the blood cholesterol level, and improving the lactose intolerance conditions [Gomes & Malcata, 1999; Gorbach, 2000; Gill & Guarner, 2004]. To exert beneficial actions, the probiotic bacteria should be supplied to the human intestine in a high number. The best way to achieve this is probiotics to be prepared in the form of probiotic foods. As such, they must possess appropriate sensory properties in order to be accepted adequately by the consumer. Nowadays, probiotics are being produced mainly in the form of beverages based on milk fermented with dairy starter cultures. Probiotic bacteria added together with the starter culture only survive the technological process or their growth is negligible. Thus, in the final products the live cell number of probiotic bacteria is usually at the same level as the introduced number, *i.e.* $\sim 10^6$ cfu/mL. When compared with approximately 10^9–10^{10} of these bacteria per gram of gut content, there is no base to expect any significant effect.

Whey is a byproduct from the cheese and casein production and its volumes are on the raise worldwide [McIntosh *et al.*, 1998]. Whey contains the whole amount of lactose, and thus could be a suitable medium for the cultivation of most of the lactic acid bacteria. Furthermore, whey is a rich source of proteins with diverse nutritive value, which makes it a valuable component of food [Smithers *et al.*, 1996]. Soymilk (soy preparation, SP) is a substitute of milk with postulated several health benefits [Gardner *et al.*, 2001; Bazzoli *et al.*, 2002; Stauffer, 2002; Tonstad *et al.*, 2002]. It contains raffinose and stachyose [Naczk *et al.*, 1997], two oligosaccharides which are readily utilised by bifidobacteria.

In the last 30 years, the development of various beverages on the basis of different substrates has raised considerable interest and attempts have been undertaken continuously at producing fermented probiotic beverages on the basis of cheese whey and soymilk solely or in mixtures [Rossi *et al.*, 1999; Chou & Hou, 2000; Wang *et al.*, 2002, Shimakawa *et al.*, 2003]. However, despite the fact that most of the researchers take care of important parameters as fermentation conditions, cell number in the product, contents of metabolites, not much study has been devoted to the sensory properties of the products. Those who take into account the sensory properties of the final products, often supplement them with different fruit additives [Lin *et al.*, 2004] or extra-flavour [Macedo *et al.*, 1999]. The assessment of the sensory properties of new food products by means of

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modern standard techniques is a crucial step for the development of acceptable and attractive foods.

The aim of this study was to evaluate the sensory properties of new developed beverages fermented with probiotic bacteria. The sensory properties are assumed as one of the most important characteristics of food for the consumers.

MATERIALS AND METHODS

Probiotic beverages. Probiotic beverages were prepared with cheese whey and a soy preparation (soymilk) as an equivolume mixture and a soy preparation solely. These natural base substrates were fermented by selected probiotic strains: *Bifidobacterium breve* ATCC 15700, *B. infantis* ATCC 15697, *B. animalis*/*lactis* J38, *Lactobacillus plantarum* W42, *L. plantarum* IB, *L. casei* LcY and *Streptococcus thermophilus* TkM_3, applied in the form of synergistic two or three strain sets. The fermentation was carried out at 37°C for 8–24 h depending on the set used, until pH was reduced to a level of 4.4–4.6. After fermentation, the beverages were cooled and supplemented with processed fruits. The ready products were assigned coded names: Product B, Product C, Product D, and Product E, and subjected to the sensory evaluation. The raw, non-fermented soy preparation, as the control, was assigned coded name Product A. The fermented probiotic beverages contained approximately 10^9 of live probiotic cells per mL.

Sensory evaluation. The sensory quality of the products tested was assessed with Quantitative Description Analysis (QDA) [Stone & Sidel, 1993]. The procedure used in the study was in agreement with requirements of the International Standard [ISO/DIS 13299:1998]. In the first part of QDA, a procedure was run to select sensory descriptors (attributes) for products' evaluation. The panelists received samples varying in sensory properties and individually generated a set of descriptors for odour, appearance, taste/flavour and consistency of the products. After generating and agreeing the descriptors, the QDA was used for the evaluation of the samples. Definitions of descriptors are presented in Table 1. The assessments were marked on 10-cm non-structural line scales shown on the monitors. Each scale was appropriately marked at both sides (low intensity – high intensity), showing the continuum being measured. The results were converted to numerical values (from 0 to 10 units) by a computer. The analysis was performed in two replications. Results were expressed in conventional units (whole scale divided into 10 units). The overall sensory quality for products in hedonic rating was evaluated using the same type of scale as above.

Sensory panel. The panel of sensory assessors was trained and monitored according to ISO standards [1993]. Sensory evaluation of the samples was performed by 6 panelists (4 females, 2 males), all with at least one year of experience in discrimination and descriptive tests on different food products.

Prior to their participation in the experiments, the subjects were trained to rate the perceived intensity of the following different sensations: sweetness, saltiness, sourness, bitterness and astringency using aqueous solutions of sucrose, NaCl, citric acid, quinine sulphate, caffeine and tannic acid.

TABLE 1. Descriptors (attributes) used in sensory analysis of the products and their definitions.

Descriptor	Definition
	Odour descriptors
1. Soy milk odour	typical odour of market soy milk
2. Cereal odour	odour note of cereal mix (Muesli):oats, barley, corn
3. Fermented odour	typical note of fermented milk products such as yoghurt
4. Milky odour	odour note of fat pasteurised milk
5. Strawberry odour	specific note reminding of fresh strawberries
6. Fruity odour	odour characteristic reminding of fruits (non-citrus)
	Appearance descriptors
7. Colour	visual evaluation of colour of product
	Taste descriptors
8. Soy milk taste	typical taste of marked soy milk
9. Cereal taste	taste note of cereal mix (Muesli):oats, barley, corn
10. Fermented taste	taste characteristic of fermented milk products such as yoghurt
11. Acid taste	basic taste typical of citric acid diluted in water (1%)
12. Strawberry taste	specific note reminding of fresh strawberries
13. Fruity taste	taste characteristic reminding fruits (non-citrus)
14. Sweet taste	basic taste illustrated by sucrose diluted in water (1–5%)
15. Astringent taste	taste sensation when tasting tannic acid (0.2%)
16. Bitter taste	basic taste typical of caffeine in water (0.5%)
17. Aftertaste	after-taste of soy milk which continues after the removal of sample
	Consistency descriptors
18. Density	visual evaluation of density of products
	Overall quality
the general impression that covers all estimated attributes of quality	

Evaluation conditions. For testing 20 mL volume individual samples were prepared from each product, coded and presented at random to each panel member to avoid carry-over effect. One minute break was taken between samples, during which the panelists were asked to eat unsalted biscuits and rinse thoroughly their mouth with water. Each sample of products was evaluated in three replications. The analysis was performed in a standardised test room, provided with 6 test booths [ISO 8589:1998] and with a computerised system ANALSENS for data collection and processing [Baryłko-Piekielna, 1992]. The room temperature varied between 20 and 22°C. Testing sessions were held in the morning, between 10 and 12 a.m.

TABLE 2. Mean values of intensity of sensory attributes in products A, B, C, D and E (0–10 scale).

Product / Attribute	A	B	C	D	E	Significance of variability among samples
			Odour descriptors			
1. Soy milk odour	6.36	0.01	0.02	0.03	0.43	***
2. Cereal odour	4.96	0.02	0.00	0.06	0.09	***
3. Fermented odour	0.00	4.36	4.27	3.59	1.18	***
4. Milky odour	0.43	1.03	0.72	0.59	2.32	**
5. Strawberry odour	0.00	5.47	6.27	4.71	0.08	***
6. Fruity odour	0.00	3.10	3.10	2.71	4.39	ns
			Appearance descriptors			
7. Colour	0.25	8.22	8.64	7.94	0.71	***
			Taste descriptors			
8. Soy milk taste	7.26	0.00	0.00	0.03	0.02	***
9. Cereal taste	5.72	0.10	0.04	0.14	0.54	***
10. Fermented taste	0.00	4.29	4.44	4.17	2.02	*
11. Acid taste	0.02	5.12	5.38	4.42	1.61	***
12. Strawberry taste	0.00	5.44	5.41	5.12	0.03	***
13. Fruity taste	0.00	3.87	3.68	3.72	6.56	*
14. Sweet taste	0.28	1.66	2.33	2.73	7.33	***
15. Astringent taste	1.07	2.77	2.55	2.12	1.14	ns
16. Bitter taste	1.60	0.50	0.04	0.28	0.01	ns
17. Aftertaste	4.47	0.41	0.03	0.09	0.03	***
			Consistency descriptors			
18. Density	0.29	5.31	2.48	2.38	2.83	*
			Overall quality			
19. Overall quality score	1.13	7.07	7.82	7.36	7.65	***

A – Unfermented soya preparation (control); B – Fermented soya preparation, set: *Bifidobacterium breve* ATCC15700 and *Lactobacillus casei* LcY; C – Fermented equivolume mixture of cheese whey and soya preparation, set: *Bifidobacterium infantis* ATCC15697 and *Streptococcus thermophilus* TkM_3; D – Fermented equivolume mixture of cheese whey and soya preparation, set: *Bifidobacterium animalis* J38 and *Lactobacillus plantarum* W42; E – Fermented equivolume mixture of cheese whey and soya preparation, set: *Bifidobacterium breve* ATCC15700, *Lactobacillus plantarum* IB and *Streptococcus thermophilus* TkM_3

*$p<0.05$; **$p<0.01$; ***$p<0.001$; ns – not significant

Statistical analysis. Analysis of variance (ANOVA) was used to check the significance of obtained differences in separate attribute of intensity among products.

Principal component analysis (PCA) was applied for general assessment of similarity-dissimilarity of the evaluated samples and for description of their attributes. Statistical analyses were processed by Statgraphics Plus 5.1 (Statistical Graphics corp., USA, 2001).

RESULTS

For sensory evaluation, a quantitative descriptive analysis (QDA) method was used. This methodology provides quantitative descriptions of products based on the perceptions of qualified subjects. It is distinctive from other sensory testing methods in that, it seeks to profile a product on all of its perceived sensory characteristics. This means that descriptive tests make it possible to obtain a descriptions of all sensations that are perceived when evaluating a product, such as: odour, appearance, taste, flavour, consistency/texture and aftertaste. The descriptive analysis is not only the one of the most sophisticated methodologies in sensory evaluation, but also one of the most appreciated methods, due to its significant value in the product's development. That is why this particular method was used in the study.

Using QDA method, 18 attributes for the investigated products describing odour, appearance, taste and consistency were selected and defined (Table 1). The mean values of intensity of these attributes and the analysis of variance are presented in Table 2. The results show, that among 18 attributes only the intensity of two of them was not statistically significant. Highly significant differences ($p<0.001$) were observed in the intensity of attributes such as: soy milk odour, cereal odour, fermented odour, strawberry odour, soy milk taste, cereal taste, acid taste, strawberry taste, sweet taste, and aftertaste. In order to observe the abovementioned differences in the analysed products more clearly, sensory profiles were displayed as spider diagrams in Figure 1. The profile of product A differs profoundly from those of B, C, and D products which show high similarity to one another. In the profile of Product A the dominating attributes were as follows: soy milk odour, cereal odour, soy milk taste, cereal taste and aftertaste. These notes were absent in the other products. However, the products B,C and D presented the following attributes: fermented odour, strawberry odour, fermented taste, acid taste, strawberry taste and relatively weak astringency. In contrast, in the sensory profile of Product E sweet taste and fruity tastes were dominating. The results of the overall quality of the investigated products show that Product A obtained the lowest score – just 1.13 unit, whereas the other products – B, C, D, and E obtained 7.07, 7.82, 7.36 and 7.65 units, respectively (Table 2, Figure 2).

The data obtained from QDA were subjected to PCA employing statistical software. Two principal components, PC1 and PC2, were extracted which accounted for 98.31% of the total data variance (Figure 3). The projection of data points ascribed to the samples and to input attributes on the

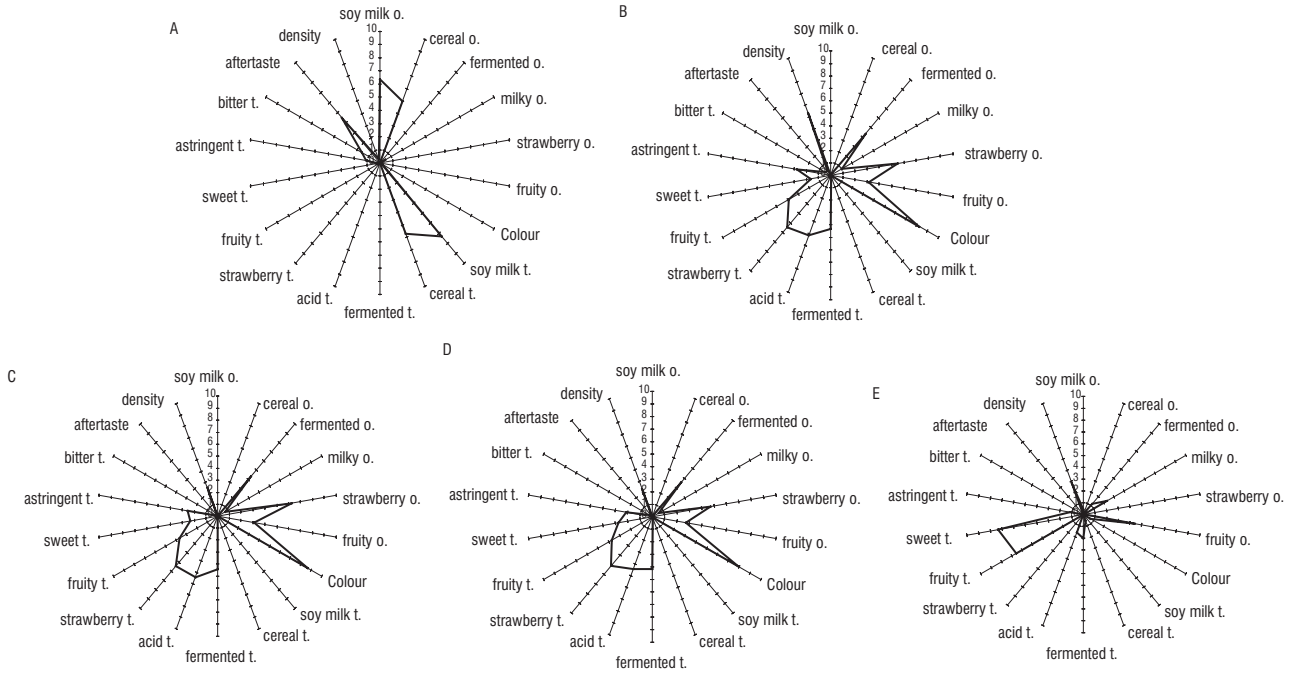

FIGURE 1. Graphical chart sensory profile (QDA) of products A, B, C, D, E.
A – Unfermented soya preparation (control); B – Fermented soya preparation, set: *Bifidobacterium breve* ATCC15700 and *Lactobacillus casei* LcY; C – Fermented equivolume mixture of cheese whey and soya preparation, set: *Bifidobacterium infantis* ATCC15697 and *Streptococcus thermophilus* TkM3; D – Fermented equivolume mixture of cheese whey and soya preparation, set: *Bifidobacterium animalis* J38 and *Lactobacillus plantarum* W42; E – Fermented equivolume mixture of cheese whey and soya preparation, set: *Bifidobacterium breve* ATCC15700, *Lactobacillus plantarum* IB and *Streptococcus thermophilus* TkM_3

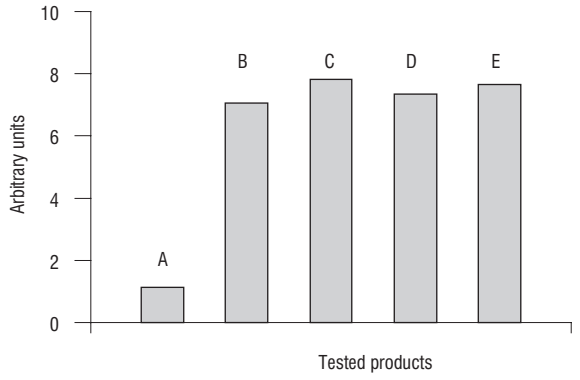

FIGURE 2. Overall quality of investigated products A, B, C, D, and E.
A – Unfermented soy preparation (control); B – Fermented soy preparation, set: *Bifidobacterium breve* ATCC15700 and *Lactobacillus casei* LcY; C – Fermented equivolume mixture of cheese whey and soya preparation, set: *Bifidobacterium infantis* ATCC15697 and *Streptococcus thermophilus* TkM_3; D – Fermented equivolume mixture of cheese whey and soya preparation, set: *Bifidobacterium animalis* J38 and *Lactobacillus plantarum* W42; E – Fermented equivolume mixture of cheese whey and soya preparation, set: *Bifidobacterium breve* ATCC15700, *Lactobacillus plantarum* IB and *Streptococcus thermophilus* TkM_3

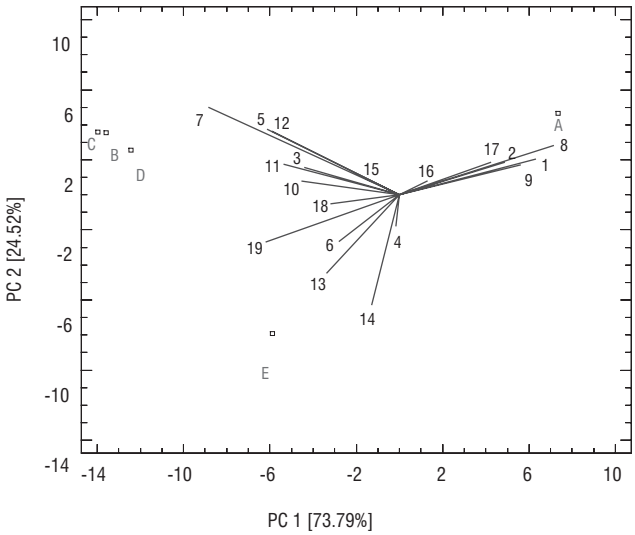

FIGURE 3. PCA plot of sensory profiling results of investigated products A, B, C, D, E.
Vectors: 1 – soy milk odour, 2 – cereal odour, 3 – fremented odour, 4 – milky odour, 5 – strawberry odour, 6 – fruity odour, 7 – colour, 8 – soy milk taste, 9 – cereal taste, 10 – fermented taste, 11 – acid taste, 12 – strawberry taste, 13 – friuty taste, 14 – sweet taste, 15 – astringent taste, 16 – bitter taste, 17 – aftertaste, 18 – density, 19 – overall quality.
A – Unfermented soy preparation (control); B – Fermented soy preparation, set: *Bifidobacterium breve* ATCC15700 and *Lactobacillus casei* LcY; C – Fermented equivolume mixture of cheese whey and soya preparation, set: *Bifidobacterium infantis* ATCC15697 and *Streptococcus thermophilus* TkM_3; D – Fermented equivolume mixture of cheese whey and soya preparation, set: *Bifidobacterium animalis* J38 and *Lactobacillus plantarum* W42; E – Fermented equivolume mixture of cheese whey and soya preparation, set: *Bifidobacterium breve*ATCC15700, *Lactobacillus plantarum* IB and *Streptococcus thermophilus* TkM_3

plane reflects a comprehension of graphical manner of the similarities and dissimilarities among them. It can be seen that Product A was separated from the other products. The vectors describing the sensory attributes of this product are opposite to the vector of the overall quality. It indicates that there is a negative correlation between the overall quality and the sensory notes of Product A. The remaining prod-

ucts B, C, D and E are located on the opposite side of the chart and two clusters are observable among them. The samples B, C, D situated close to each other (which indicated their similar sensory characteristics) and far from product E. The vectors describing the attributes of the above-mentioned four products were mainly characterised by positive attributes which influenced the positive correlation with the overall quality.

DISCUSSION

In the present work four probiotic beverages fermented with lactic acid bacteria and subsequently supplemented with processed fruits were evaluated. The preliminary (after fermentation and before supplementation) sensory evaluation of the products (data not shown) demonstrated that the products possess fresh and pleasant taste and flavour. However, despite the acceptable sensory quality these products were characterised with new, unknown components of the taste and flavour for a market drink which could repel the consumer. The supplementation of the taste and the flavour with well known and well accepted components such as fruit taste, sweet taste is an approach with a potential positive effect on the overall sensory quality of the beverages. The positive effect from the supplementation was confirmed by the QDA procedure. The need of supplementation can be seen in a study conducted by Rossi *et al.* [1999]. The authors developed four products by fermentation of soymilk, fortified with whey powder, with *Enterococcus faecium* in combinations with *Lactobacillus jugurti* or *L. acidophilus* and in single culture as well as with *L. delbrueckii* ssp. *bulgaricus* with *Streptococcus thermophilus* without any supplementation. In a scale from 0 to 9 the products were rated with 5.90, 2.70, 5.88 and 2.10 points after descriptive evaluation of the overall flavour quality. Indeed in this scale two of the ratings are relatively high, but the other two are quite low. In another trial, Lin *et al.* [2004] supplemented a milk-soymilk mixture with 5, 10, 15, and 20% Lycium chinese Miller juice before the fermentation with *L. paracasei* ssp. *paracasei* and *B. longum*. Results of the sensory evaluation showed that the supplementation with 5% Lycium chinese Miller juice improved the acceptability of the fermented milk-soymilk mixture. Macedo *et al.* [1999] fermented a mixture of 35% skimmed cow milk, 35% cheese whey and 30% soymilk with a mixed culture of *L. casei* and *B. adolescentis* and subsequently additionally sweetened and flavoured the product. A sensory evaluation was conducted by the following characteristics: appearance and consistency, soy flavour, acid flavour, total flavour, soy taste, acid taste, and total taste in ten-point unstructured scale. The total quality score of the product was 7.35, which excels the rating received by Rossi *et al.* [1999]. This is one more confirmation of the superiority of flavour and taste supplemented fermented products of this type over the non-supplemented ones.

The fermented products based on SP, have had little application, which is in sharp contrast with several fermented and non-fermented products made of cow's milk, containing assumed probiotic bacteria such as *Streptococcus* sp., *Lactobacillus* sp., and *Bifidobacterium* sp. [Scalabrini *et al*., 1998]. Soy milk has been used for the production of yoghurt-like products, but their poor organoleptic properties were responsible for low consumer acceptance compared to milk derivatives. The principal objection to those soy milk derivatives was their beany flavour due to the unpleasant off-flavour of *n*-hexanal and pentanal, naturally present in soy milk as products of the breakdown of unsaturated acids [Arai *et al.*, 1966; Matoba *et al.*, 1985 cited after by Scalabrini]. Indeed, it was confirmed in this work as high scores of the unpleasant/astringent attributes of beany odour and soy milk taste (6.36 and 7.26, relatively) were noted for the non-fermented SP. The aftertaste attribute received also a relatively high score (4.47) which was also considered as an unpleasant attribute. However, in the fermented final products these three attributes received negligible scores varying from 0.00 to 0.43, which could be a sign of the improved organoleptic properties of beverages following fermentation and fruit supplementation. Scalabrini *et al.* [1998] examined the ability of *B. breve* to metabolise *n*-hexanal and pentanal in soymilk. The authors reported on the presence of these two undesirable components in soymilk (16.5 and 8.9 ppb, respectively) and found that *n*-hexanal was reduced to 4 ppb whereas pentanal was not detected after 24 h of fermentation with *B breve*. and no further change in the *n*-hexanal content could be detected within next 24 h. Disappearance of the beany flavour and taste of the soy milk in the examined new beverages could probably be related to the metabolising of *n*-hexanal and pentanal by bifidobacteria as well as due to the masking effect of the new pleasant attributes, emerging after fermentation and fruit supplementation.

The lactic acid fermentation of a mixture of cheese whey and a soy preparation as well as a soy preparation solely with selected probiotic bacteria, and subsequent supplementation with processed fruits is a feasible way for the production of probiotic beverages with good sensory properties. The beverages, evaluated in this study, are new products with a high organoleptic quality and a high number of live cells of probiotic bacteria – over 100-times higher as compared to dairy probiotic products available on the market.

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JAKOŚĆ SENSORYCZNA NOWYCH NAPOJÓW PROBIOTYCZNYCH PRZYGOTOWANYCH NA BAZIE SERWATKI SEROWARSKIEJ I PREPARATU SOJOWEGO

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Oceniono jakość sensoryczną czterech nowych napojów probiotycznych, przygotowanych na bazie serwatki serowarskiej i preparatu sojowego, fermentowanych z zastosowaniem wyselekcjonowanych probiotycznych szczepów *Bifidobacterium breve*, *B. infantis*, *B. animalis*/*lactis*, *Lactobacillus plantarum*, *L. casei* i *Streptococcus thermophilus*, zastosowanych w formie synergicznych zestawów dwu- lub trzyszczepowych. Po kontrolowanej fermentacji (pH, liczba żywych komórek kultur bakterii) napoje były suplementowane odpowiednio przetworzonymi owocami. Ocenę sensoryczną wykonano metodą ilościowej analizy opisowej (QDA-Quantitative Descriptive Analysis), zgodnie z procedurą ISO/DIS 13299:1998. W odróżnieniu od innych metod oceny sensorycznej, metoda ta umożliwia profilowanie wszystkich dostrzeganych sensorycznie charakterystyk produktu, takich jak: zapach, wygląd, smak/aromat, konsystencja/tekstura oraz ogólną ocenę na podstawie wszystkich ocenianych atrybutów jakości. Charakterystyki te zostały wyselekcjonowane i zidentyfikowane na podstawie 18 wyróżników (tab. 1). Intensywność każdego wyróżnika oceniano na ciągłej skali liniowej i wykonano dodatkowo ogólną ocenę próbek podsumowującą jakość sensoryczną wszystkich produktów w oparciu o uwzględnione wyróżniki. Kontrolę stanowił materiał wyjściowy (substrat) przed inokulacją i fermentacją. Ocenę sensoryczną wykonał 6-osobowy zespół, przygotowany metodycznie i doświadczony w ocenie sensorycznej oraz spełniający wymagania określone normą ISO 8589:1998. Do przygotowania ocen oraz rejestracji i opracowania wyników zastosowano skomputeryzowany system wspomagania analiz sensorycznych ANALSENS. Statystyczną analizę wariancji (ANOVA) wykonano z zastosowaniem pakietu STATGRAPHICS 5.1, USA. Badane produkty uzyskały ocenę 7.07-7.82 pkt. w 10 punktowej skali, stosunkowo wysoką w porównaniu z preparatem sojowym niefermentowanym (jako kontrolą), który oceniono na poziomie 1.13 pkt (tab. 2; rys. 1, 2, 3). Oceniane fermentowane napoje probiotyczne charakteryzowały się dobrymi cechami sensorycznymi i dodatkowo wysoką liczbą żywych komórek probiotycznych bakterii (ok. 10^9 jtk/mL).