DEVELOPMENT OF OAT-BANANA FERMENTED BEVERAGE WITH BETA-GLUCAN ADDITIVE

Running title: Oat-banana fermented beverage

Lubomiła Owczarek, Urszula T. Jasińska, Sylwia Skąpska

Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology, Department of Fruit and Vegetable Technology, 36 Rakowiecka str., 02-532 Warsaw, Poland

lubomila.owczarek@ibprs.pl

Summary

The aim of the study was to create a pro-healthy, tasteful, fermented, milkless beverage for people showing an allergy to milk proteins and/or lactose intolerance.

An oat-banana matrix for fermentation, in versions fortified with beta-glucan preparations, PromOat, Betaven, OatFibre, was soured with starter variants, *Streptococcus thermophilus*, *Lactobacillus paracasei*, *Lactobacillus plantarum*. The fermentation processes were characterized within kinetic parameters. *Streptococcus thermophilus* selected for fermentation of the oat-banana matrix fortified with a PromOat beta-glucan preparation, assured a highly acceptable sensory profile for the fermented beverage. Beverage was characterized according to pH, viscosity, titratable acidity, lactic acid enantiomers, beta-glucan content, sensory features and the LAB number. The beverage's sensory profile subsisted alongside the LAB number above 7 lg cfu g^{-1} during 4 weeks of cold storage; beta-glucan content was stable thus ensuring health benefits for consumers. All lactic acid formed and maintained in the product was L-lactate, assuring the beverage's appropriateness for children.

Practical applications. The developed functional fruit-cereal fermented beverage, fortified with beta-glucan additive, containing high number of LAB and prevalence of L-isomer of lactic acid can be an attractive alternative for people with allergy or intolerance for milk, including children. The developed technology enables to obtain product of high sensory and nutrition quality, stable up to 4 weeks of cold storage.

Key words: milkless fermented beverage, lactic acid bacteria (LAB), L-lactate, beta-glucan, sensory profile, functional food

INTRODUCTION

Pursuant to scientific prognosis (Lee and Salminen 1995), in recent years increased consumer demands for plant products with high sensory acceptance and functionality have been observed. New, functional food has often been developed using lactic acid (LA) fermentation, e.g. oatmeal based non-dairy milk substitute (Mårtensson et al. 2001/a) and oatmeal soup for hospital nutrition used in clinical *in vivo* study of colonization of human intestinal mucosa by lactic acid bacteria (LAB) cultures (Johansson et al. 1993).

The consumption of oat groats or flakes has always been highly deemed in nutrition but recent studies evaluating the nutritional effects of oats are founded on clinical experiments. According to current knowledge, the total dietary fibre in oat flakes, representing 6-9 per cent of its weight is of the greatest worth in nutrition. The most precious part of oat dietary fibre, ca. one half of its total, is the soluble fraction; a linear polysaccharide representing $(1\rightarrow3)$ $(1\rightarrow4)$ β-D-glucan which acts as a prebiotic and causes the most important, physiological effects in the human body (Mälkki and Virtanen 2001). The break up products of beta-glucans can selectively promote the growth of beneficial bacteria thus being able to use the beta-glucotetraose and/or beta–glucotriose as nutrients on a competitiveness basis with pathogens (Jaskari et al. 1998). As has been concluded from medical experiments, the consumption of oat foods containing the soluble fraction of dietary fibre, beta-glucan, can diminish the hypercholesterolemic symptoms and cause a decrease in postprandial hyperglycaemia (Bartnikowska and Lange 2000).

Officially, the beneficial effect of beta-glucan consumption was confirmed in Commission Regulation (EU) *on the authorization and refusal of authorization of certain health claims made on foods and referring to the reduction of disease risk*) permitting the following health claim: "Oat beta-glucan has been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease". [Commission Regulation (EU) No 1160/2011 of 14 November 2011].

Bananas (*Musa paradisiaca* L.) are an important crop – the fruit grows widely in tropical and subtropical areas, in the zone corresponding to the $20^{O}C$ isotherm, however encompassing Israel. Numerous processed banana products are available on the world market, i.e. purée, pulp, juice, chips, flour, canned slices, jam, vinegar and wine.

Bananas containing high amounts of saccharides, especially saccharose, glucose and fructose, were the subject of studies on lactic acid (LA) fermentation focusing on prolonging the product shelf live and ameliorating bananas pro-health effects. It was found, however, that banana purée reveals two characteristics which are unfavourable for LA fermentation, i.e.

pectin content and rather low pH level (Adams and Moss 2000). In particular, the banana's pH value does no promote LA fermentation (Tsen et al. 2004). But bananas contain fructooligosaccharides which have been shown to exhibit good pro-health effects on consumers through stimulation the growth of LAB, in parallel with putrefactive pathogens, in the colon as well as a reduction in the serum cholesterol level. Beneficial phenomena, such as the prebiotic effects of some plant components, have been observed and acknowledged as the basis for the concept of prebiotics (Gibson and Roberfroid 1995).

Besides, bananas are a source of minerals, e.g. potassium and in particular selenium, an indispensable microelement but one which rarely occurs in the soil (Ebert et al. 1984). Hozyasz (2009) has confirmed the excellent iron absorption from banana meals in infants (after Fomon et al. 1989) and formation of proper stools after adding bananas to infant milk formula (after Linseisen et al. 1998) – probably thanks to the water binding capacity of the insoluble fraction of fruit dietary fibre and the high viscosity level resulting from the presence of pectin. Meals with a banana taste are well perceived by both healthy and sick adults and children; even those averse to eating – suffering from diarrhoea and dehydration. The pleasant odour of bananas soothes patients and lowers their arterial tension as well as sensitivity to pain (Knaapila et al. 2007). Bananas are not cariogenic, and despite their high complex carbohydrate content, are a moderate glycemic index food (Lako et al. 2004).

The aim of the present study was to develop an oat-banana (OB) matrix fortified with different beta-glucan preparations, as a basis for LA fermentation which could provide a sensorily well-perceived, pro-health, milkless beverage, analogous to yoghurt, with the desired nutritional and physicochemical properties, e.g. with a possibly high number of LAB in the beverage biotum and an exclusive or highly dominant content of L-LA over D-LA enantiomer; from the children' nutritional needs point of view. The presupposition for the study was maintaining the beverage's good quality during four weeks of cold storage.

MATERIALS AND METHODS

Oat beta-glucan preparations

Oat beta-glucan preparations, PromOat, Betaven and OatFibre, were used as pro-health, texture-improving additives to the fermentation matrix. PromOat, containing 353 g kg^{-1} of beta-glucan, was kindly provided by BioVelop (Sweden); Betaven and OatFibre containing 280 g kg^{-1} and 210 g kg^{-1} of soluble fibre, respectively, were kindly supplied by Microstructure (Poland).

Fermentation starters

Starter cultures from the IAFB Culture Collection of Industrial Microorganisms: *Lactobacillus plantarum* KKP2025p, *Lactobacillus paracasei* ssp. *paracasei* JCM2769 KKP2027p and *Streptococcus thermophilus* T_KM_3 KKP2030p − were pre-selected for the plant material fermentations (Bielecka et al. 2002). Stock cultures were stored at -70°C using Viabank system (Abtek Biological Ltd., Liverpool, United Kingdom). Depending on the strain species, the cultures were grown in MRS broth (Merck, Darmstadt, Germany) or LAB medium (Davies et al. 1971) without agar; 2 times for ca. 24 h at 37°C.

Preparation of oat-banana matrix versions for fermentation

The ingredients of the OB matrix were: oat meal from the common oat (*Avena sativa* L.) ground by Polskie Zakłady Młynarskie, Poland and aseptic, non-acidified, seedless banana (*Musa paradisiaca* L.) purée made by Frutucorp S.A., Poland.

The basal matrix, OBReference, was prepared by suspending 20 g of oat meal and 100 g of banana purée in a water solution containing 45 g kg^{-1} of saccharose and 2 g kg^{-1} of food grade sodium citrate dihydrate (Sigma-Aldrich), in a quantity with a total matrix weight of up to 1 kg.

Beta-glucan preparations, PromOat, Betaven, OatFibre were incorporated into the OBReference formula, each in a quantity assuring 4.0 g kg^{-1} of beta-glucan in each studied matrix version: OBPromOat, OBBetaven and OBOatFibre.

The OB matrix versions were prepared by mixing dry ingredients (oatmeal, beta-glucan preparation, saccharose, sodium citrate dihydrate), diluting the mix with water and pasting the suspended starch at 80°C for 5 min while stirring. Banana purée was then introduced into the oat starch paste. The matrix versions were sterilized for 10 min at 118°C, cooled to fermentation temperature and inoculated with 6-7 lg cfu g^{-1} of active culture.

Physicochemical determinations

Valuation of the chemical composition and energy content of the developed matrix/beverage − was calculated basing on the matrix formula and the average chemical composition of the primary ingredients (Kunachowicz et al. 1998) − as the sum of basic nutrients energy. as well as taking into account the average conversion factors for: proteins (17 kJ/4 kcal); fat (37 kJ/9 kcal); carbohydrates (17 kJ/4 kcal) and fibre (8 kJ/2 kcal) (Regulation (EU) No 1169, 2011). The dietary fibre and beta-glucan contents in the OB matrix were evaluated based on published data (Bartnikowska and Lange 2000; Mälkki and Virtanen 2001) and the producers' certificates of beta-glucan preparations.

The pH measurements – conducted with Mettler Toledo digital MP235 pH-meter, at 20^oC.

Total titratable acidity (TTA) – measured according to the AOAC method n° 947.05 (AOAC methods 2000) and expressed as lactic acid (LA) [g kg^{-1}].

Content of L-LA and D-LA enantiomers – analyzed using a Boehringer Mannheim Roche enzymatic kit; Cat. No. 11112821035.

Mixed-linkage beta-glucan content – determined using a Megazyme International Ireland Limited enzyme kit following the AOAC method n° 995.16 (AOAC methods, 2000).

Viscosity – measured with a Mettler Toledo RM180 Rheomat. The measurement system, consisting of a tube, filled with beverage, coupled with the rotating bob, was immersed in the bath of an F12 refrigerated/heating circulator (Julabo Labortechnik GmbH, Seelbach, Germany). The temperature applied 20^OC; shear rate used 1291 s^{-1}.

Dry matter content – determined at 105^OC with a Mettler Toledo HB43 moisture analyzer.

Microbiological analysis

Enumeration of LAB, rod- or cocci- shaped bacteria, in the beverage was - effectuated by plating the sample dilutions in sterile buffered 1g kg^{-1} pepton water in triplicate on MRS-agar (MerckKGaA Darmstadt, Germany; de Man, Rogosa and Sharpe) or LAB medium (Davies et al. 1971), respectively.

Acidifying activity of LAB strains

The acidifying activity of preselected LAB strains in the OBReference matrix was evaluated by taking pH measurements: the OB matrix and an appropriate, MRS or LAB (Davies et al. 1971) broth as control were inoculated with ca. 6 lg cfu g^{-1} of each of 24-h culture, incubated at 37^oC up to pH reduction to the level 4.5. The fermented OBReference was further stored at 4ºC. The pH value was controlled in ca. 2-h intervals during fermentation and weekly at cold storage.

Sensory analysis

The sensory profile and overall sensory quality (SQ) of the fermented beverage were evaluated in a laboratory meeting ISO requirements (ISO 8589:1988), by a trained panel (ISO 8586-1:1993) of six assessors using the Quantitative Descriptive Analysis (QDA) method (Stone et al. 1974) to describe the sensory notes perceived as being the most essential. The sensory note descriptors were proposed, discussed and agreed by the panel during training sessions. The list of descriptors, established by the assessors as the most typical for fermented OB beverage, comprised: texture notes: homogeneity, fluidity, smoothness, oral cavity coating (mouthfeel); odour notes: sour, cereal, fruity-banana, and taste notes: sour, sweet, cereal, fruity-banana.

The note intensities and SQ factors of samples were determined using scales according to ISO requirements (ISO 4121:1987): a non-structured scale with the border restraints denominated as "slight/none"-"high" and numeral ordinal, 10-unit scale. The evaluation of the length of segments marked by the assessors on unstructured scales, corresponding to the note intensities or SQ values, was performed by an apposition of numeral ratio scale to the segments.

Fermented beverage was served in 30 mL portions in white glass cups of 100 mL, labelled randomly with selected codes. Beverage portions were served at ca. 16ºC as this temperature favoured the panellists' sensory perception of the beverage. Each assessor received 2 portions of beverage during one session.

The sensory analyses of the beverage were carried out in 5 terms: on a fresh sample and in one week intervals on the samples stored at 4ºC.

Statistical analysis

Physicochemical and microbiological analyses were performed in triplicate; sensory evaluations – in twenty four repetitions. The relevant means and standard deviation values were calculated. The significance of differences between mean values was searched using 1-Way Analysis of Variances ANOVA and Duncan tests ($P \leq 0.05$) using Statistica 7.1 StatSoft.

RESULTS AND DISCUSSION

Nutritional value of oat-banana matrix

The OB matrix valuation was carried out in accordance with the principles given in the Methods' Section. All the matrix versions had an approximate equal nutritive value. Their energy (37-42 kcal/153-176 kJ) were similar to the average energy of 100 g buttermilk or grapefruit/apple juice, but lower than typical, milk, banana yoghurt (1.5% fat, 3.7% protein) containing 70 kcal/292 kJ in 100 g.

Versions of OB matrix fortified with beta-glucan preparations of PromOat, Betaven or B.Owsiany has been formulated as a satisfying source of dietary fibre (11-12 g kg^{-1}) and beta-glucan (4 g kg^{-1}) for human beings. One single serving of 250 g of each developed oat-banana beverage version contained 1 g of oat beta-glucan – thus assuring the accomplishment of the EU condition for health claim use (Commission Regulation (EU) No 1160, 2011).

Acidifying activity of studied LAB strains in the OB matrix

The acidifying activity of the strains: *L. paracasei* ssp. *paracasei* JCM2769 KKP2027p; *L. plantarum* KKP2025p and *S. thermpohilus* T_KM_3 KKP2030p, was checked against the

OBReference matrix. This was composed as a milkless medium for the growth of LAB strains, to obtain a fermented beverage analogous to yoghurt, with high sensory acceptability, with a proper texture and distinct banana flavour notes. Important criteria for acceptation of the technology, comprised fast matrix fermentation to pH 4.5 at which the LAB population, reaching a stationary growth phase, would possibly gain a high population number in the beverage biotum. The effects of the study, alongside initial sensory recognition of the soured product profiles, can be seen in Table 1.

Table 1. Growth and acidifying activity of LAB strains in the oat-banana matrix. Hedonic sensory evaluation of fermented beverage.

Strain	Control pH 6.4-6.6			Oat-Banana matrix pH 6.1			First established descriptors of texture, colour, odour, taste Overall SQ factor
	pH	time (h)	lg cfu·g^{-1}	pH	time (h)	lg cfu·g^{-1}	
***L. paracasei* ssp. *paracasei* JCM2769** KKP2027p	4.36 ± 0.02	7.0 ± 0.76	8.97 ± 0.03	4.60 ± 0.04	8.0 ± 0.76	8.64 ± 0.08	**texture:** cereal paste **colour:** beige **odour:** banana, lightly sour **taste:** intensively banana and sweet-sour **SQ GOOD**
L. plantarum KKP2025p	4.51 ± 0.03	7.0 ± 1.00	9.23 ± 0.10	4.59 ± 0.02	11.0 ± 1.00	8.36 ± 0.08	**texture:** cereal paste **colour:** beige **odour:** banana, sour **taste:** sweet-sour **SQ GOOD**
***S. thermpohilus* T$_K$M$_3$** KKP2030p	4.51 ± 0.04	6.0 ± 0.30	9.10 ± 0.02	4.60 ± 0.05	7.0 ± 1.00	7.78 ± 0.01	**texture:** cereal paste/cream with intensive mouthfeel/ oral cavity coating **colour:** plainly beige **odour:** sour, banana, **taste:** harmonized sour-sweet, banana. **SQ VERY GOOD**

Fermentation tests have shown the comparable acidifying activity of *L. paracasei* and *S. thermophilus* cultures in the OB matrix and in the respective control media – proper acidification of the matrix and controls occurred during 6-8 h. The *L. plantarum* culture took a few hours longer to acidify the OB matrix. All the strains studied showed poorer growth in the oat-banana than the control media. Depending on the strain, the studied cultures resulted

in oat-banana beverages of a GOOD/VERY GOOD sensory quality. The acidifying activity of the three LAB strains during OB matrix fermentation and cold storage of the fermented product is presented in Figure 1.
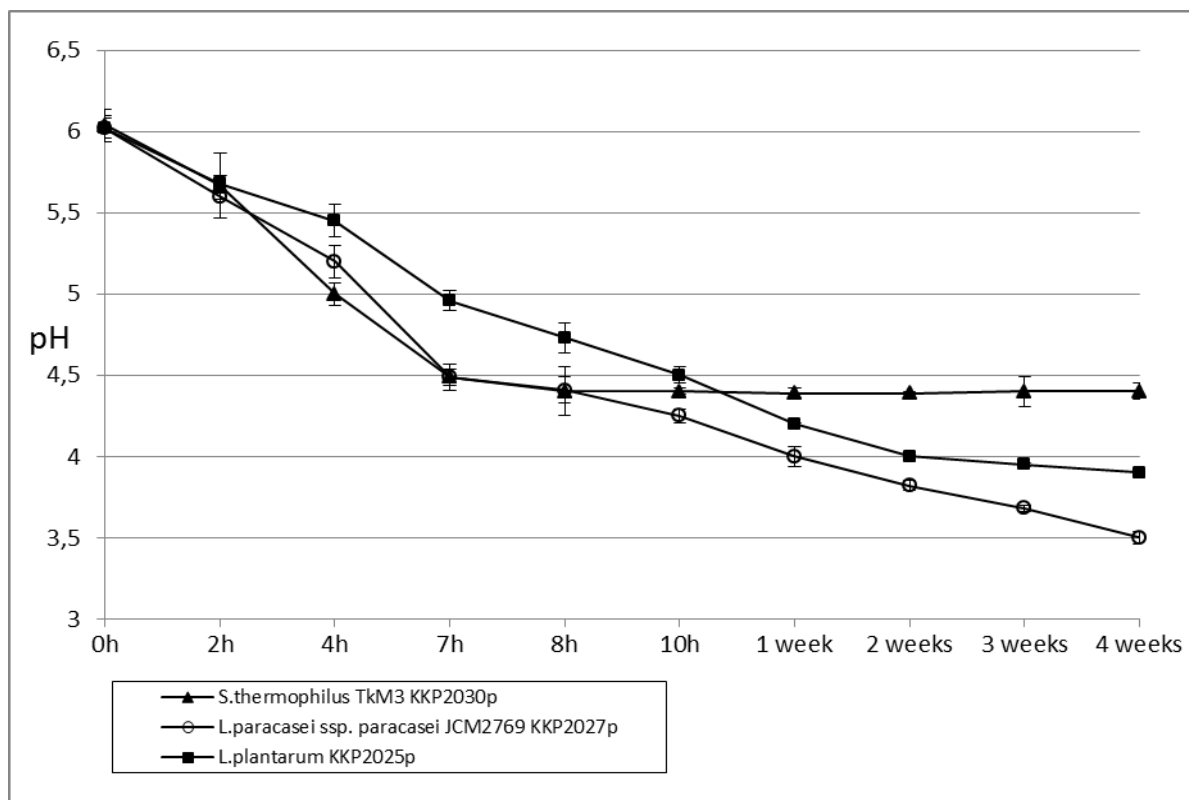

Figure 1. Acidifying activity of preselected LAB strains during fermentation of OBReference matrix and four weeks' storage of products' variants at 4°c.

Data are the means of three independent experiments. Bars of SD are presented in the figure.

This study has confirmed the previously stated different abilities of three LAB strains for matrix acidifying during fermentation and elucidated their acidifying dispositions with regard to storage (Figure 1). It was found that the rod-shaped bacteria belonging to *L. plantarum* or *L. paracasei,* contrary to the coccus-shaped *S. thermophilus*, revealed the post-acidification phenomenon manifested in the continuous lowering of the pH value of the fermented beverage during storage. The pH value of the stored product fermented with LAB strains belonging to the *Lactobacillus* genus dropped from 4.5 to 3.5-3.9 after four weeks of storage. In the same period, the pH value 4.4 of *Streptococcus thermophilus* $T_K M_3$ KKP2030p fermented product, was stable (Figure 1). The best abilities of the starters, according to the Authors' expectation as to the developed beverage properties, relied on their fast fermentation (proper acidification) and possible lack of post-acidification activity. The drop in the pH value

of the fermented beverage during storage could potentially lead to faster unfavourable changes in the product's sensory quality (Quero et al. 2014).

<u>Characteristics of the studied beverage versions within variants</u>

The most profitable beta-glucan additive to the OB matrix, was searched among three preparations: PromOat, Betaven, OatFibre, based on the fermentation tests of the OB matrix versions: OBPromOat, OBBetaven, OBOatFibre compared with the OBReference. The selected criteria for the best technology and highest quality product, apart from fast initial acidification and possibly lack of post-acidification effects, were the following: well-perceived sensory profile of the fermented product, high rate of bacterial population growth and highly prevalent L- over D-lactate content in the beverage. The characteristics of the fermentation processes, as well as different OB product versions, depending on the beta-glucan additive, are presented within the three starter strain variants studied (Table 2).

The technological tests proved that the single factor influencing the fermentation time of the OB matrix was the starter strain. All three studied LAB strains grew in all OB matrix versions similarly, giving higher numbers of bacterial populations in the fermented beverage biota than the expected minimal value in common fermented food, i.e. 7.0 lg cfu g^{-1}. Generally product versions, with different beta-glucan preparations added revealed viscosity which did not differ significantly ($P \leq 0.05$) from one another within the variants − with the exception of a significantly ($P \leq 0.05$) lower result for OBBetaven compared with the OBPromOat and OBOatFibre versions in the *S. thermophilus* variant. The viscosities of all product versions with beta-glucan added in all variants (64.0-67.0; 63.7-65.5; 66.5-73.0 mPa·s) were significantly ($P \leq 0.05$) higher than the viscosity values for the OBReference (17.33-21.00 mPa s), irrespective of the product variant (Table 2).

Table 2. Physicochemical and microbial properties of the fermented ob beverage versions depending on the starter strain variant. (Mean±SD)

Fermented OB beverage		The pH	LAB population number	Total titratable acidity TTA (as lactic acid)	Total lactic acid (LA)	L-LA enantio-mer	L-LA contribution to the total LA	Dynamic viscosity[*]	Dry matter
Starter strain	Matrix version		$[\text{lg cfu g}^{-1}]$	$[\text{g kg}^{-1}]$	$[\text{g kg}^{-1}]$	$[\text{g kg}^{-1}]$	[%]	[mPa s]	$[\text{g kg}^{-1}]$
***Lactobacillus. paracasei* ssp *paracasei* JCM2769 KKP2027p** Fermentation time: 8.0 h	Reference	4.54±0.03	8.72 ± 0.05	2.22 ± 0.08	1.73±0.05	1.65±0.40	95.38±0.49	17.33±0.58	90.6±0.60
	PromOat	4.50±0.02	8.87 ± 0.04	1.76 ± 0.03	2.04±0.08	1.95±0.08	95.59±0.17	67.00±0.00[a]	102.6±0.40
	Betaven	4.54±0.05	8.92 ± 0.06	2.10 ± 0.02	2.14±0.21	2.04±0.02	95.33±0.39	64.00±1.73[a]	106.0±0.20
	OatFibre	4.52±0.03	8.93 ± 0.05	2.14 ± 0.04	2.19±0.01	2.09±0.02	95.43±0.03	67.00±2.31[a]	111.1±0.10
***Lactobacillus plantarum* KKP2025p** Fermentation time: 13.5 h	Reference	4.53±0.02	8.38 ± 0.05	2.20 ± 0.05	1.88±0.02	1.00±0.02	53.19±0.08	18.50±0.71	90.8±0.50
	PromOat	4.57±0.04	8.44 ± 0.08	2.16 ± 0.01	1.84±0.01	1.02±0.01	55.43±0.07	65.00±0.00[a]	103.1±0.70
	Betaven	4.58±0.04	8.43 ± 0.02	2.30 ± 0.04	1.96±0.01	1.09±0.01	55.61±0.26	63.00±0.71[a]	105.8±0.60
	OatFibre	4.54±0.03	8.48 ± 0.04	2.54 ± 0.08	1.88±0.01	1.01±0.02	53.72±0.68	65.05±1.41[a]	111.6±1.00
***Streptococcus thermophilus* $T_K M_3$ KKP2030p** Fermentation time: 8.5 h	Reference	4.49±0.04	7.66 ± 0.01	1.82 ± 0.01	1.62±0.01	1.61±0.02	99.38±0.56	21.00±1.00	90.6±0.60
	PromOat	4.50±0.02	7.84 ± 0.01	1.92 ± 0.04	1.61± 0.01	1.60±0.01	99.38±0.55	72.25±2.75[b]	102.6±0.80
	Betaven	4.42±0.03	7.86 ± 0.01	1.94 ± 0.04	1.75±0.10	1.74±0.10	99.43±0.02	66.50±1.91[a]	104.9±1.60
	OatFibre	4.40±0.03	8.02 ± 0.03	1.94 ± 0.08	1.81±0.04	1.80±0.40	99.45±0.21	73.50±0.71[b]	111.0±0.60

[*]Shear rate of measuring bob 1291 s⁻1; [a, b] Means marked with the same letters do not differ significantly at $P \le 0.05$

The LAB strains studied showed a weak ability to form acid during the fermentation of each OB matrix version, gaining TTA values of ca. 2.0 g kg^{-1}. In the case of *S. thermophilus* and *L. paracasei* strains, almost the whole LA production presented the desired L-LA enantiomer (*S. thermophilus* 99.38-99.45%; *L. paracasei* 95.33-95.59%). On the contrary, the *L. plantarum* starter produced L- and D-lactates in a proportion close to 1.2:1.0 without regard to the OB matrix version, i.e. the beta-glucan preparation added to the matrix (Table 2). Taking into account the slow acidifying activity of *L. plantarum* KKP 2025p (Table 1), its post-acidification effect (Figure 1) and the metabolism character resulting in the production of similar amounts (about 1.2:1.0) of L- and D-lactic acid in the OB matrix (Table 2), this strain was eliminated from more in-depth study.

The sensory profiles of fermented product versions within the variants of accepted monocultures were analysed. Texture, odour and taste notes, as well as the hedonic SQ factor were determined. The histograms of the sensory features, as well as the overall sensory quality factors of the OB beverage versions, generally showed no statistically significant (*P*≤0.05) differences among most of the sensory relevant parameters within the two studied variants (Figure 2).

Among the <u>texture</u> notes within the variants, the OBPromOat was the closest to the OBReference. Exclusively some version pairs in the variants differed significantly (*P*≤0.05) as to fluidity and oral cavity coating from one another; the product versions with lower fluidity consistently showed higher mouthfeel.
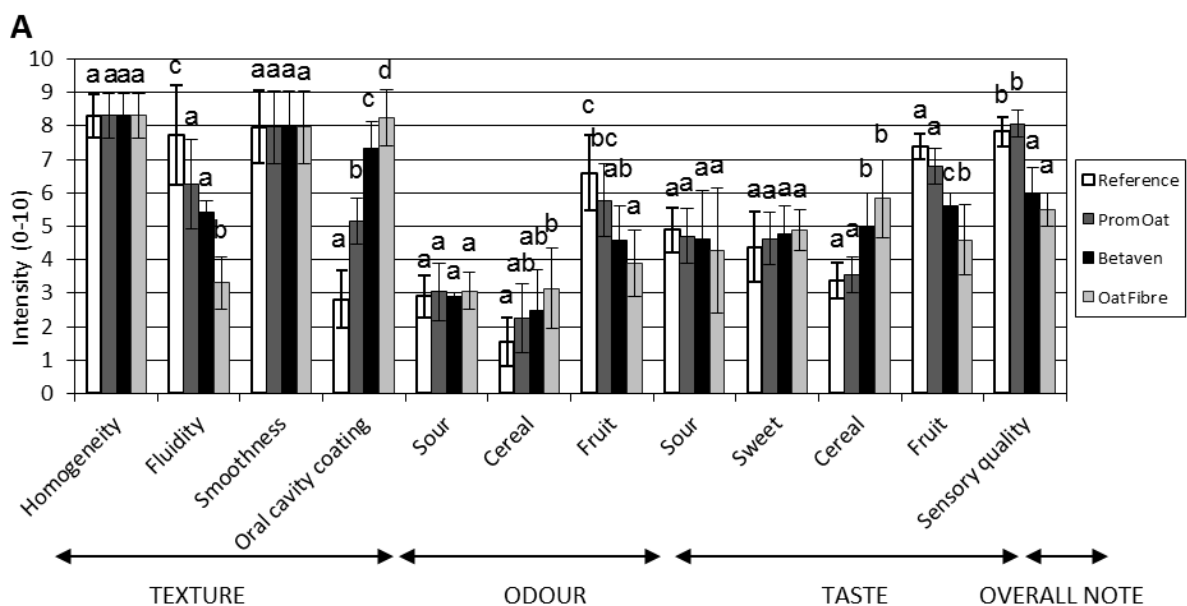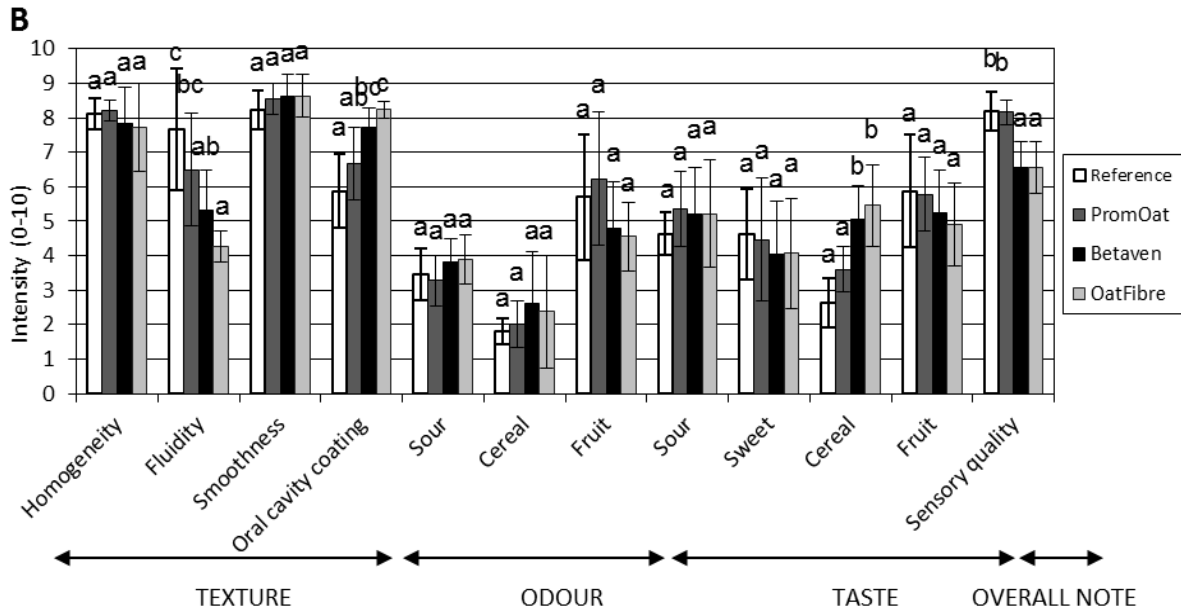
Figure 2. Sensory profiles and SQ factors of fermented beverage versions: OBReference, OBPromOat, OBBetaven, OBOatFibre in variants:

a) *S. thermophilus* $T_K M_3$ KKP2030P;

b) *L. paracasei* ssp. *paracasei* JCM2769 KKP2027p.

Based on three independent experiments. Bars of SD are presented and different lowercase letters on each pillar indicates a significant difference ($P \leq 0.05$).

The harmony of sour-sweet taste impressions was confirmed in all beverage versions within the variants. The fruit-banana notes, being the most intensely perceived among all the product's taste notes, did not differ significantly ($P \leq 0.05$) within the pair OBReference-OBPromOat in both variants. A stronger cereal taste was perceived in the beverage versions OBBetaven and OBOatFibre in both variants. No significantly ($P \leq 0.05$) different cereal taste notes were observed within the pairs OBReference-OBPromOat and OBBetaven-OBOatFibre.

Comparing the odour of all product versions within the variants, there were no significant ($P \leq 0.05$) differences among the sour notes reflecting the fermentation effect. The best sources of banana odour were the OBPromOat and OBReference beverage versions (Figure 2). In both product variants, the cereal note odour was strongly damped, with no essential differences among the versions. There were no significant ($P \leq 0.05$) differences among the fruit notes in all versions in the *L. paracasei* variant.

Taking into consideration the results of the sensory study of the OB versions of beverages fermented with *S. thermophilus* and *L paracasei*, there were no outstanding dissimilarities

among the respective sensory notes within the variants of the fresh beverage. The overall SQ factors of the fermented beverage versions, independently of the starter, pointed to the PromOat and Reference versions as the products with the best sensory quality (assessed for ca. 8.0 points), not differed significantly ($P \leq 0.05$) from one another. Taking into consideration the assumed pro-health applying of the developed product, with the need, above all, for a good sensory character, it should be noted that the OBPromOat version of the matrix was the best fermentation medium for two starter strains, enabling a fermented product to be obtained with a highly-desired sensory profile.

Run of fermentation and storage of the beverage in the selected variants

The growth kinetics of selected LAB strains during OBPromOat matrix fermentation as well as their survival in the fermented product stored at 4ºC – in the context of beta-glucan subsistence – were studied (Figure 3).

Tests confirmed 8.0 hour period for completing the fermentation process regardless of the starter. It was shown that both studied cultures grew well in the OBPromOat matrix, attaining population numbers in the fermented beverage of 8.99±0.1 or 7.55±0.04 lg cfu g^{-1} for *S. thermophilus* or *L. paracasei,* respectively, and maintained acceptable population numbers, more than 7.0 lg cfu g^{-1}, until the fourth week of storage.
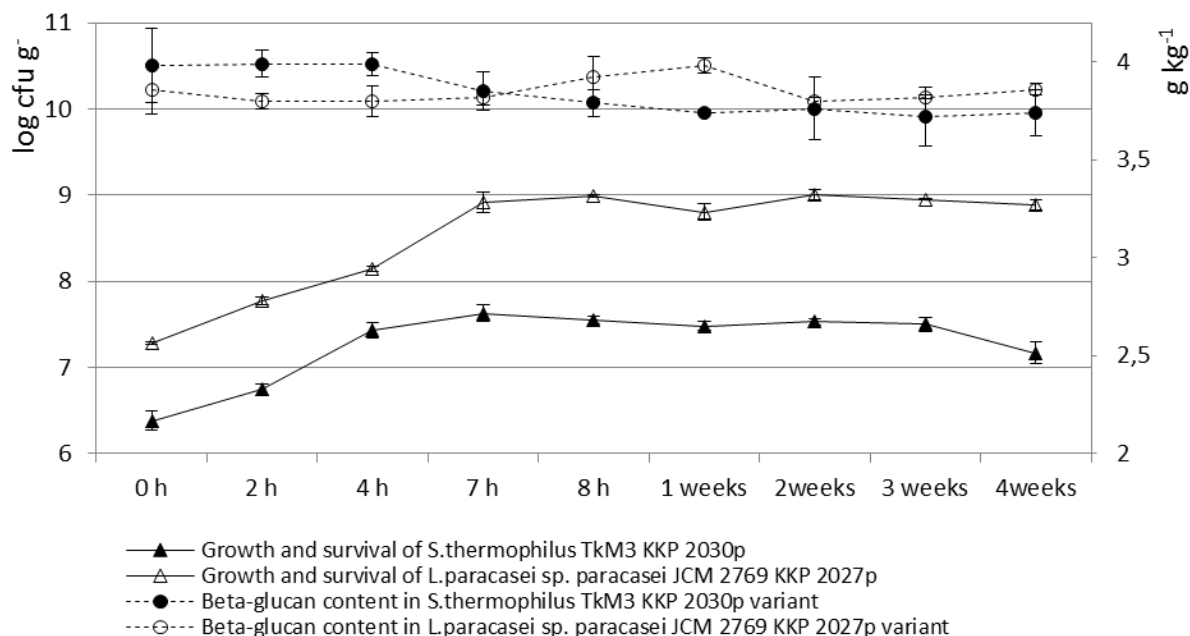

Figure 3. Growth and survival of selected starter strains in view of beta-glucan content in the OBPromOat matrix during fermentation and in fermented beverage variants stored at 4ºC. The fermented beverage variants: *S. thermophilus* T$_K$M$_3$ KKP2030P (solid symbols); *L. paracasei* ssp. *paracasei* JCM2769 KKP2027P (open symbols).

Data are the means of three independent experiments. Bars of SD are also presented.

During matrix fermentation and fermented beverage storage, no significant ($P \leq 0.05$) changes in the beta-glucan content of the analyzed samples of *S. thermophilus* and *L. paracasei* beverage variants in the successive terms were found. Despite the fact that beta-glucans can provide growth substrates – prebiotics (Gibson and Roberfroids 1995) for some probiotic LABs and e.g. the intestinal strain *Clostridium* difficile (Jaskari et al. 1998) – it became evident that *S. thermophilus* $T_K M_3$ KKP2030p and *L. paracasei* ssp. *paracasei* JCM 2769 KKP2027p were unable to utilize the beta-glucan of OBPromOat (Figure 3). A similar observation was made by Mårtensson et al. (2001b) for developed, oat-based, fermented beverage made with commercial yoghurt cultures. Regardless this effects it should, however, be underlined that both these fermented products could provide valuable sources of beta-glucans for consumers; clinical trials have demonstrated that the beta-glucooligomers of oat products have hypocholesterolemic and hypoglycaemic effects (Dubois et al. 1995; Jenkins et al. 1978).

The measured acidity conditions during OBPromOat fermentation and beverage storage comprised: TTA and L-LA contents in samples during fermentation and storage phases as well as the per cent contribution of L-LA to the total LA (Figure 4). In the course of acidity formation in the OBPromOat matrix, the L-LA enantiomer was progressively produced until fermentation completion by *S. thermophilus* – from 0.38±0.11 to 1.47±0.05 g kg^{-1}; by *L. paracasei* from 0.37±0.01 to 2.38±0.08 g kg^{-1} (Figure 4). In each analytical term of matrix fermentation with *S. thermophilus*, the determined quantity of L-LA enantiomer was not significantly ($P \leq 0.05$) different than the whole quantity of LA generated in the process medium; the per cent contribution of L-LA to total lactic acid in each analytical term was 98.9±0.18→99.8±0.01. With regards to L-LA generation by *L. paracasei* in the OBPromOat matrix, the L-LA contribution to total lactic acid content during fermentation, presenting significantly ($P \leq 0.05$) lower values (95.2±2.99→95.95±0.28 per cent), did not change significantly ($P \leq 0.05$) during storage.

Legend:
- —▲— L-LA content in S.thermophilus TkM3 KKP 2030p variant
- —△— L-LA content in L.paracasei sp. paracasei JCM 2769 KKP 2027p variant
- —◆— TA of S.thermophilus TkM3 KKP 2030p variant
- —◇— TA of L.paracasei sp. paracasei JCM 2769 KKP 2027p variant
- – ●– L-LA % of sum in L- and D-LA of S.thermophilus TkM3 KKP 2030p variant
- – ○– L-LA % of sum in L- and D-LA of L.paracasei sp. paracasei JCM 2769 KKP 2027p variant
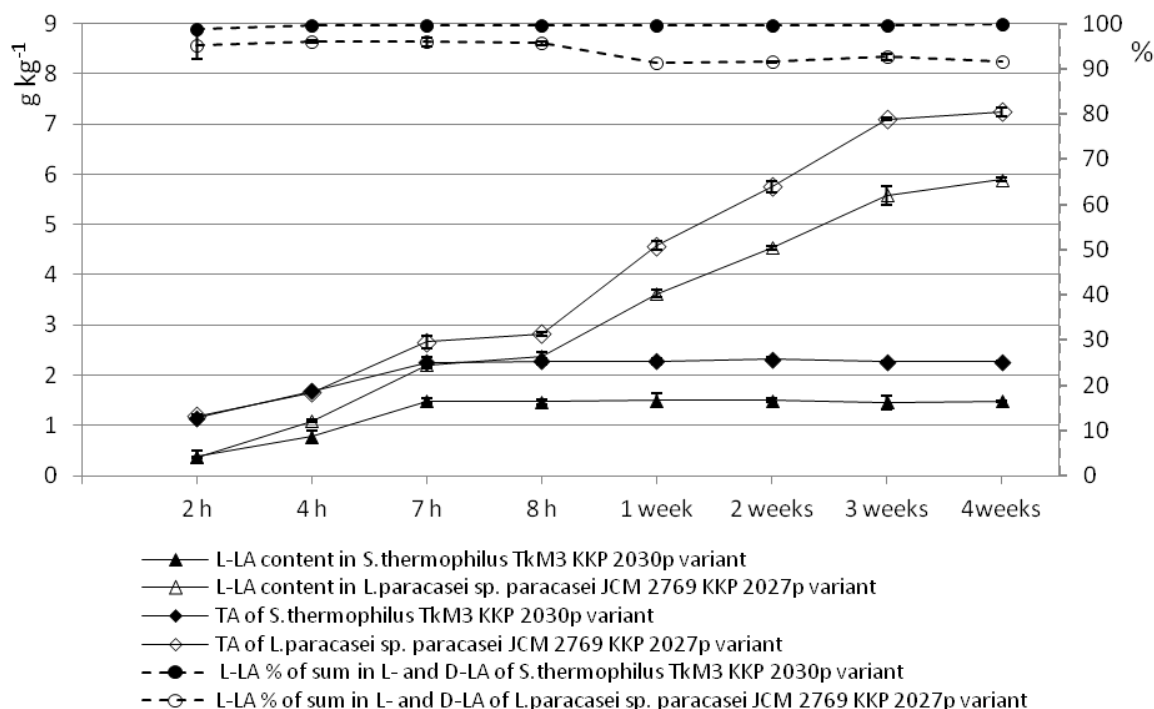
Figure 4. Changes of L-lactic acid enantiomer (L-LA) content with the TTA (titratable acidity and percent contribution of L-LA to the total LA content in the OBPromOat matrix under fermentation and in fermented beverage variants: *S. thermophilus* T_KM_3 KKP2030P (solid symbols); *L. paracasei* ssp. *paracasei* JCM2769 KKP2027P (open symbols).

Data are the means of three independent experiments. Bars of SD are also presented.

In terms of the analyses, the stored fraction of L-LA subsisted in the beverage of the *S. thermophilus* variant at levels which did not differ significantly ($P ≤ 0.05$), from $1.47±0.05$ to $1.50±0.13$ g kg^{-1}, and contributed from $99.53±0.01$ to $99.84±0.01$ percent of L-LA to the product's total LA content. These results are analogical to the results obtained during the fermentation step. In the *L. paracasei* variant (Figure 4), revealing a post-acidification effect, the L-LA content of the product rose during storage from $2.38±0.08$ to $5.89±0.04$ g kg^{-1} but the percentage of L-LA to total LA content of the product was maintained from $91.51±0.07$ to $91.65±0.06$, i.e. not significantly ($P ≤ 0.05$) differing among themselves; but significantly ($P ≤ 0.05$) differing from the values determined in the fermentation step ($95.15±2.99$ do $95.95±0.28$).

The L-lactate content highly prevalent over D-lactate in the beverage developed was expected as a valuable pro-health/functional effect of the product. Within this study the elimination-

limitation of D-lactic acid content in the beverage was successful as a result of the metabolic behaviour of selected starters grown in the OBPromOat matrix.

The use of *S. thermophilus* or *L. paracasei* as a starter for OBPromOat fermentation has ensured obtaining beverages characterized by an outstandingly predominant content of L-over D-lactate and therefore proper for nutrition in conformity with the general recommendation of FAO/WHO (FAO/WHO, 1974) limiting the consumption of D-lactate to a max 100 mg per day for adults or eliminating it from infant/child formulae. This recommendation results from the recognized facts of poor utilization of the D-LA by a child's organism and the risk of D-acidosis; particularly in populations of children with short bowel syndrome and bacterial overgrowth (Vanderhoof et al. 1998; Connolly et al. 2005). The phenomenon of lactic acid production of the special composition of enantiomers could be explained as the metabolic abilities of strains belonging to the different LAB species that do or do not possess appropriate lactate dehydrogenase. There are LAB species which produce exclusively L-lactate (e.g. here *S. thermophilus*), exclusively D-lactate, predominantly one form of lactic acid with measurable amount of the other (e.g. here *L. paracasei*) or almost equal amounts of L- and D-lactic acid enantiomers (i.e. *L.plantarum*; Table 2). In the case of *L. paracasei*, a possible second reason for obtaining a lower ratio of L- to D-lactate than in the *S. thermophilus* variant, could be the fact that initially generated L-lactate might induce the racemase generation which converted L-lactate to D-lactate. Besides, the phenomenon of decreasing the L-LA contribution to total lactic acid content in the fermented product with time could be explained as follows: generally L-lactic acid is the major form produced by LABs in the early-growth phase whereas D-LA is formed in the late to stationary phase (Axelsson 2004).

The assessment of the sensory profile of fermented beverage variants, as fresh and after 4 weeks' cold storage, is given in Table 3. Some descriptors used here have already been used by others (Ott et al. 2000; Majchrzak et al. 2010) for sensory characterization of yoghurts. The common notes for OBPromOat beverage and yoghurt, attesting to the common, in some aspects, characteristics of both product categories, were: within texture: homogeneity/heterogeneity, mouthfeel, smoothness; odour: sourness; taste: sourness, sweetness.

Table 3. Comparison of the sensory evaluation of OBPromOat beverage fermented in the *S. thermophilus* and *L.paracasei* variants - as fresh and after storage at 4°C (Mean±SD).

SENSORY ATTRIBUTES/ Notes	Analyzed variants of OBPromOat beverage			
	Streptococcus thermophilus		*L. paracasei* ssp. *paracasei*	
	Fresh	After 4 weeks' storage	Fresh	After 4 weeks' storage
1	2	3	4	5
TEXTURE				
Homogeneity	8.3 ± 0.58a	7.9 ± 0.62a	8.2 ± 0.31a	7.8±0.80a
Fluidity	6.2 ± 1.13a	6.7 ± 0.35ab	6.5 ± 1.64a	8.2±0.58b
Smoothness	8.1 ± 0.88a	8.2 ± 0.82a	8.5 ± 0.46a	8.0±0.50a
Oral cavity coating	6.1 ± 1.57a	8.1 ± 0.67b	6.7 ± 1.06ab	5.3±0.58a
ODOUR				
Sour	3.6 ± 1.13a	4.0 ± 0.71a	3.3 ± 0.74a	6.3±1.01b
Cereal	2.5 ± 0.55a	2.6 ± 0.97a	2.0 ± 0.67a	0.9±0.30b
Fruity	6.3 ± 1.26a	6.8 ± 0.47a	6.2 ± 1.93a	2.6±1.13b
TASTE				
Sour	5.0 ± 0.77a	5.4 ± 1.48a	5.4 ± 1.08a	8.1±0.40b
Sweet	4.8 ± 0.70a	4.6 ± 0.51a	4.5 ± 1.79a	1.6±0.60b
Cereal	3.5 ± 0.92a	3.2 ± 0.92a	3.6 ± 0.65a	1.5±0.06b
Fruity	7.2 ± 0.96a	7.0 ± 0.93a	5.8 ± 1.07a	2.2±1.11b
SENSORY QUALITY				
SQ	8.4 ± 0.65a	8.7 ± 0.63a	8.2 ± 0.36a	5.1±0.10b

Different lowercase letters indicate significant differences ($P \leq 0.05$).

Studying the sensory differences between the *S. thermophilus* and *L. paracasei* variants of fresh OBPromOat beverage, it was confirmed that both sensory profiles were analogous because of no significantly differring ($P \leq 0.05$) the overall SQ factors and all corresponding notes within the sensory attributes. After four weeks of cold storage no significant ($P \leq 0.05$) changes were stated in the sensory note intensities and SQ factor for the *S. thermophilus* variant of the beverage; with one exception – the oral cavity coating note changed significantly ($P \leq 0.05$) but without essentially differentiating the SQ value of the stored product. Contrary to the sensory stability of the stored *S. thermophilus* variant, the *L. paracasei* variant was evidently unstable on storage with regard to its odour and taste notes, which influenced the product's overall sensory quality. So all odour and taste notes as

well as the SQ factor of this beverage variant after 4 weeks storage were significantly ($P \leq 0.05$) changed compared with its fresh counterpart. This observation has proved the essential worsening of the sensory quality of the *L. paracasei* beverage variant which underwent a post-acidification phase during storage (Figure 1). Taking into account the beverage's whole chemical and sensory profile, *L. paracasei* is not recommended for fermentation practices.

It was shown (data not presented) that the OBPromOat matrix revealed the highest viscosity at the inoculation stage (*S. thermophilus* 75.5±3.53 mPa s; *L. paracasei* 70.5±0.71 mPa s). The viscosities of product variants decreased to 61.2±2.06 mPa s; 65.5±0.71 mPa s, respectively; at the storage end the variant products gained values 58.0±0.05 mPa s; 56.5±0.71 mPa s, which did not differ significantly ($P \leq 0.05$).

CONCLUSIONS

The developed oat-banana matrix, fortified with a PromOat, the BioVelop beta-glucan preparation, was successfully used as the milkless basis for a pro-health, highly sensory acceptable, fermented beverage analogous to yoghurt revealing proper texture and distinct notes of banana flavour. The technology developed, with the use of a carefully selected LAB strain, *S. thermophilus* $T_K M_3$ KKP2030p, assured fast matrix acidification and a LAB population number in the beverage biotum higher than this one met in common fermented products. The fermented beverage contained LA exclusively in the L-lactate form recommended by WHO for child nutrition as well as the required 1 g of oat beta-glucan per beverage portion, thus complying the EU requirement permitting an appropriate health claim on the label. The sensory, physicochemical and microbial properties of the beverage developed, were maintained during four weeks of cold storage. The high sensory quality of the OBPromOat fermented beverage during its whole shelf life represented valuable functional feature for the pro-health product. Thus the beverage, enabling the willing consumption of a tasteful plant yoghurt for people with an allergy to milk or lactose intolerance could at the same time also help prevent hypercholesterolemia and postprandial hyperglycaemia.

ACKNOWLEDGEMENTS

REFERENCES

1. Adams M.R., Moss M.O. Food microbiology: Microbiology of primary food commodities. Cambridge, The Royal Society of Chemistry; 2000. pp. 148-160.

2. AOAC Official Method 2000.947.05 Acidity of milk. Tritimetric method. Gaithersburg, MD; USA: AOAC International; 2000.

3. AOAC Official Method 2000.995.16 β-D-glucan in Barley and Oats. Gaithersburg, MD, USA: AOAC International; 2000.

4. Axelsson L. Lactic Acid Bacteria: Classification and Physiology. In: Salminen S., Wright von A., Ouwehand A., editors. Lactic Acid Bacteria. Microbial and Functional Aspects. New York, Marcel Dekker Inc; 2004. pp. 1-66.

5. Bartnikowska E., Lange E.. Dietetic significance of oat products: their influence on cholesterol concentration in plasma and postprandial glycaemia. Żywność Nauka Technol. Jakość. 2000; 22:18-36 (Polish).

6. Bielecka M., Owczarek L., Grzybowski R., Majkowska A., Biedrzycka E., Markiewicz L., Jasińska U. Selection of lactic acid bacteria strains for fermentation of cereal and soybean preparations. Pol. J. Food Nutr. Sci. 2002; 11/52; SI 2**:**59-64.

7. Commission Regulation (EU) No 1160/2011 on the authorization and refusal of authorization of certain health claims made on foods and referring to the reduction of disease risk. ANNEX I, OJ L 296, 15.11.2011. p.26.

8. Connolly E., Abrahamsson T., Björkstén B. Safety of D(-)-Lactic Producing Bacteria in the Human Infant. J. Pediatr. Gastroenterol. Nutr. 2005;41:489-492.

9. Davies J.G., Ashton T.R., McCaskill M. Enumeration and viability of *L. bulgaricus* and *Str. thermophilus* in yogurts. Dairy Industries. 1971;6:569-573.

10. Dubois C., Armand M., Senft M., Portugal H., Bernard P.M., Lafont H., Lairon D. Chronic oat bran intake alters postprandial lipemia and lipoproteins in healthy adults. Am. J. Clin. Nutr. 1995;61:325-333.

11. Ebert K.H., Lombeck I., Kasperek K., Feinendegen L.E., Bremer H.J. The selenium content of infant food. Z. Ernährungswiss. 1984;23:230-236.

12. FAO/WHO. Toxicological evaluation of certain food additives with a review of general principles and of specifications. In WHO Technical Report Series (1-40). Geneva, FAO and WHO; 1974.

13. Fooks L.J., Fuller R., Gibson G.R. Prebiotics, probiotics and human gut microbiology. Int. Dairy J.,1999; pp. 53-61.

14. Gibson G.R., Roberfroid M.B. Dietary modulation of human colonic microbiota: introducing the concept of prebiotics. J. Nutrition 1995; 125:1401-1412.

15. Hozyasz K.K. Banana in nutrition of pediatric patients. Pediatr. Pol. 2009;84:256-264 (Polish).

16. ISO 4121:2003. Sensory Analysis - Guidelines for the use of quantitative response scales. International Organization for Standardization. Geneva, Switzerland; 2003.

17. ISO 8586:2012. Sensory Analysis - General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors. International Organization for Standardization. Geneva, Switzerland; 2012.

18. ISO 8589:2007. Sensory Analysis − General guidance for the design of test rooms. International Organization for Standardization. Geneva, Switzerland; 2007.

19. Jaskari J., Kontula P., Siitonen A., Jousimies-Somer H., Mattila-Sandholm T., Poutanen K. Oat β-glucan and xylan hydrolysates as selective substrates for *Bifidobacterium* and *Lactobacillus* strains. Appl. Microbiol. Biotechnol.1998;49:175-181.

20. Jenkins D.J.A., Wolever T.M.S., Leeds A.R., Gassul M.A., Haisman P., Dilwari J., Goff D.V., Metz G.L., Alberti K.G.M.M. Dietary fibers, fiber analogues and glucose tolerance: importance of viscosity. Br. Med. J. 1978;1:1392-1394.

21. Johansson M.L., Molin G., Jeppson B., Nobaek S., Ahrné S. and Bengmark S. Administration of different *Lactobacillus* strains in fermented oatmeal soup: In vivo colonisation of human intestinal mucosa and effect of the indigenous flora. Appl. Environm. Microbiol. 1993;59:15-20.

22. Knaapila A., Keskitalo K., Kallela M., Sammalisto S., Hiekkalina T., Palotie A., Peltonen L., Tuorila H., Perola M. Genetic components of identification, intensity and pleasantness of odours: a Finnish family study. Eur. J. Hum. Genet. 2007;15:596-602.

23. Kunachowicz H., Nadolna I., Przygoda B., Iwanow K. Food Composition Tables. National Food and Nutrition Institute, Warsaw, Poland; 1998.

24. Lako J., Sotheeswaran S., Aalbersberg W., Sreekumar K.P. The glycemic index (GI) and glycemic load (GL) of five commonly consumed foods of the South Pacific. Pac. Health Dialog. 2004;11:47-54.

25. Lee Y.-K., Salminen S. The coming age of probiotics. Trends Food Sci. Technol. 1995;6:241-245.

26. Majchrzak D., Lahm B., Dürrschmid K. Conventional and probiotic yogurts differ in sensory properties but not in consumers' preferences. J. Sensory Studies, 2010;25:431-446.

http://dx.doi.org/10.1111 / j.1745-459X.2009.00269.x

27. Mälkki Y., Virtanen E.. Gastrointestinal effects of oat bran and oat gum – a review. Lebensm. Wiss. u-Technol. 2001;34:337-347.

28. Mårtensson O., Andersson C., Andersson K., Öste R., Holst O. Formulation of an oat-based fermented product and its comparison with yoghurt. J. Sci. Food Agric. 2001b;81:1314-1321.

29. Mårtensson O., Öste R., Holst O. Lactic acid bacteria in an oat-based non-dairy milk substitute: fermentation characteristics and exopolysaccharide formation. Lebensm. Wiss. Technol. 2001a;29:525-530.

http://dx.doi.org/10.1002/jsfa.947

30. Ott A., Hugi A., Baumgartner M., Chaintreau A. Sensory investigation of yogurt flavour perception: Mutual influence of volatiles and acidity. J. Agric Food Chem. 2000;48:441-450.

31. Quero G.M., Fusco V., Cocconcelli P.S., Owczarek L., Borcakli M., Fontana C., Skąpska S., Jasińska U., Ozturk T., Morea M. Microbial, physico-chemical, nutritional and sensory characterization of traditional Matsoni: Selection and use of autochthonous multiple strain cultures to extend its shelf-life. Food Microb. 2014;38:179-191.

http://dx.doi.org/10.1016/j.fm.2013.09.004

32. Regulation (EU) No 1169/2011 of the European Parliament and of the Council on the provision of food information to the consumers. OJ L 304, 22.11.2011. p.18.

33. Stone H., Sidel J., Oliver S., Woolsey A., Singleton R.C. Sensory evaluation by quantitative descriptive analysis. Food Technology. 1974;28:24-33.

34. Tsen J.-H., Lin Y.-P., King V.A.-E. Fermentation of banana media by using κ-carrageenan immobilized *Lactobacillus acidophilus*. Int. J. Food Microb. 2004;91:215-220.

35. Vanderhoof J.A., Young R.J., Murray N., Kaufmann S.S. Treatment strategies for small bowel bacterial overgrowth in short bowel syndrome. J. Pediatr. Gastroenterol. Nutr. 1998;27:155-160.