

# The Impact of Novel Fermented Products Containing Extruded Wheat Material on the Quality of Wheat Bread

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## Summary

*Lactobacillus sakei* MI806, *Pediococcus pentosaceus* MI810 and *Pediococcus acidilactici* MI807, able to produce bacteriocin-like inhibitory substances, were originally isolated from Lithuanian spontaneous rye sourdough and adapted in the novel fermentation medium containing extruded wheat material. The novel fermented products (50 and 65 % moisture content) were stored at the temperatures used in bakeries (15 days at 30–35 °C in the summer period or 20 days under refrigeration conditions at 0–6 °C). The number of lactic acid bacteria (LAB) was determined during the storage of fermented products for 15–20 days. Furthermore, the effect of novel fermented products stored under different conditions on wheat bread quality was examined. Extruded wheat material was found to have a higher positive effect on LAB growth compared to the control medium by lowering the reduction of LAB populations in fermented products with the extension of storage time and increase of temperature. During storage, lower variation and lower decrease in LAB count were measured in the novel fermented products with a moisture content of 65 % compared to those with 50 %. Furthermore, this humidity allows for the production of a product with higher moisture content in continuous production processes. The addition of the new fermented products with 65 % humidity to the wheat bread recipe (10 % of the quantity of flour) had a significant effect on bread quality: it increased the acidity of the crumb and specific volume of the bread, and decreased the fractal dimension of the crumb pores and crumb firmness. Based on the microbiological investigations of fermented products during storage and baking tests, the conditions of LAB cultivation in novel fermentation media were optimized (time of cultivation approx. 20 days at 0–6 °C and approx. 10 days at 30–35 °C).

*Key words:* lactic acid bacteria, extruded wheat material, fermented products, wheat bread

## Introduction

Sourdough is a very complex biological system based on lactic acid and alcoholic fermentation that depends on the microflora and fermentation conditions (1–5). Sourdough fermentation is considered to play a key role in improving the flavour, texture, nutritional and shelf-life properties of bakery products. Moreover, its application

enables the stabilization or increased levels of various bioactive compounds, the retardation of starch and the improvement of mineral bioavailability. It has been observed that when sourdough is added, changes in the fundamental rheological properties of wheat dough occur, making it soft, less elastic and therefore easily exten-

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sible (6). Microbial spoilage limits the shelf life of food-stuffs, therefore sourdough addition is the most promising application to control mould growth and ropiness in wheat bread products (7,8).

In sourdough ecosystems, lactic acid bacteria (LAB) (9) and yeasts constitute the dominant microflora and contribute not only to the development of the desired sensorial properties of the final product but also to its microbiological safety, either as the natural microflora or as starter cultures added under controlled conditions. The initial counts of LAB in rye or wheat sourdoughs are up to  $3 \cdot 10^9$  colony forming units (CFU) per g or  $5 \cdot 10^8$ – $1 \cdot 10^9$  CFU/g, respectively (10). However, the number of LAB in rye and wheat sourdough depends on the properties of dough, starter cultures and fermentation conditions (4,10). Sourdough fermentation is evaluated by the following parameters: pH, acidity and microflora composition (11,12). The main factor regulating acidification is the amount of fermentable carbohydrates. The production of acids also depends on the fermentation temperature, time and dough yield: higher temperature, higher water content of sourdough and the use of wholemeal flour enhance the production of acids in the sourdough (13,14). Acidification of the dough, proteolysis of gluten and moderate hydrolysis of starch are LAB activities that vary among sourdough strains and may affect the physicochemical changes throughout the shelf life of bread (15). Analysis of structures such as bread cell networks by means of fractal geometry may provide useful information for finding relationships between structure functionality and processing variables (16,17).

Until now, the main focus has been given to the natural antimicrobials produced by LAB such as organic acids, hydrogen peroxide,  $\text{CO}_2$ , diacetyl, acetaldehyde, D-isomers of amino acids and reuterin. Over the last few years, there has been an explosion of basic and applied research on extracellularly released and ribosomally synthesized proteinaceous compounds – bacteriocins, produced by LAB and exhibiting bactericidal and/or bacteriostatic modes of action against closely related species and even foodborne pathogens. The highly promising results of these studies underline the potential application of bacteriocinogenic LAB strains in the food industry as starter cultures, cocultures, or bioprotective cultures to improve food quality and safety. Nevertheless, the literature lacks updated reviews dealing with the antimicrobial activity of bacteriocins or bacteriocin-producing LAB against ropiness caused by *Bacillus subtilis* and other bacteria in order to increase the stability of sourdough. The structural genes of LAB are responsible for the antimicrobial activity of the strains. Furthermore, according to the literature, the production of LAB metabolites may be intensified by applying the appropriate fermentation media. In the Baltic States, a traditional process of scalding (a process of heat treatment of flour matrix with hot water or steam that brings it to starch gelatinization) is applied in the production of bread. However, scalded bread preparation is a long, complicated and economically inefficient process. The alternative technological means for the intensification of sourdough production, its higher stability and improved bread quality are still being sought. To attain these goals,

new prospects have become available by developing this technology for the industrial production of fermented products with antimicrobial properties. Recently, considerable interest has arisen in the application of new fermentation media such as extruded products possessing specific physical properties (higher amounts of dietary fibre). Extrusion cooking causes gelatinization of starch among other physicochemical and functionality changes the grain components undergo; moreover, this results in the enhanced amount of dietary fibre and the elimination of the bacterial contamination of cereal material. However, there is lack of literature data on the effect of extruded wheat material on lactic acid bacteria cultivation processes under different conditions for the industrial sourdough production and the influence of fermented products on wheat bread quality.

The objective of this study is to adapt selected LAB with antimicrobial properties into novel fermentation media prepared with extruded wheat material. Further essential point of the study is the determination of the impact of novel fermented products on wheat bread quality.

## Materials and Methods

### *Microorganisms and culture conditions*

*Lactobacillus sakei* MI806, *Pediococcus pentosaceus* MI810 and *Pediococcus acidilactici* MI807, able to produce bacteriocin-like inhibitory substances (BLIS) designated as sakacin 806, pediocin 810 and pediocin Ac807, respectively, were used throughout the study. They were originally isolated from Lithuanian sourdough (18) and their bacteriocin activity was confirmed (19). Strains were stored at  $-70^\circ\text{C}$  in a Microbank™ system (Pro-Lab Diagnostics, Bromborough, Wirral, Merseyside, UK) and were later propagated in DeMan, Rogosa and Sharpe (MRS) broth (CM 0359, Oxoid Ltd, Basingstoke, Hampshire, UK) at  $30^\circ\text{C}$  (MI806) or  $35^\circ\text{C}$  (MI810 and MI807) for 72 h. Sourdough starter and fermented products (sourdough) for wheat bread baking were made using a single culture from the three selected LAB.

### *Fermentation medium*

The extruded wheat material, obtained from Ustuki Malunas Ltd., Lithuania, was produced using a single-screw extruder with a  $1 \times 3$  mm nozzle. The extrusion parameters during the experiment were constant: screw speed 470 rpm, feed rate 150 kg/h, water dosage 10 kg/h and temperature in the individual zones 45, 65 and  $135^\circ\text{C}$ . Moisture content, water absorption index (WAI) and water solubility index (WSI) of the extruded samples were determined following the method described previously (20,21). Wheat flour (type 550C, ash content 0.51–0.57 %, falling number 350 s and moisture content 14.5 %) was obtained from Kauno Grudai Ltd., Lithuania, and was used as control for bread-baking experiments. The extruded wheat flour had the following characteristics: moisture content 8.6 %, WAI 2.2 g/g, and WSI 10.8 %.

### Preparation of sourdough starter and fermented products

A four-stage fermentation process for the inoculation of lactic acid bacteria cultures into extruded wheat material was used (Fig. 1). A continuous propagation (after 4, 20 and 44 h) was adjusted in order to keep the microorganisms in an active metabolic state. The fermentation process during sourdough starter production was carried out at temperatures optimal for the strains used: *L. sakei* at 30 °C, *P. pentosaceus* and *P. acidilactici* at 35 °C.

Fermented products were prepared by adding sourdough starter, using a single culture of LAB, to the extruded wheat material mass (50 or 65 % moisture content) (Fig. 1, 5th stage). The fermentation process was carried out for 24 h at an optimal temperature for the LAB. In this stage, fermented products can be used for bread production. In the case of industrialized production of fermented products, further microbiological processes and their effect on wheat bread quality depend on bakery storage conditions (room temperature in the summer or in the refrigerator). Based on this elaborate procedure, prepared fermented products were stored at two different temperature regimes: at 30–35 °C for 10 days or at 0–6 °C up to 20 days during the experiment.

### Microbiological analysis

The effect of a novel fermentation medium on the growth of LAB was determined by estimating their number in the newly prepared fermented products and after storage for 5, 10, 15 and 20 days. LAB counts in fermented product samples were determined on MRS agar (Liofilchem, Roseto degli Abruzzi, Teramo, Italy) using plate count techniques. Plates were incubated for (48±4) h under anaerobic conditions. The number of LAB was expressed as decimal logarithmic value of colony forming units per gram (log CFU/g). The experiment was run in triplicate.

### Wheat bread making

Wheat bread samples were baked on the 1st, 5th, 10th, 15th and 20th day after the storage of the fermented product under different temperature conditions. Wheat bread samples containing fermented product (moisture content of 65 %) 10 % of the quantity of flour were prepared using a laboratory baking device (automatic Germatic BM-2 oven, AFK, Hamburg, Germany). Bread samples were baked under the following program: mixing for 10 min, first proofing for 25 min at 25 °C, mixing for 15 min at 30 °C, second proofing for 35 min at 32 °C, third proofing for 70 min at 38 °C, and baking for 55 min at 121 °C. Fermented products made from wheat flour with LAB, and baked goods produced without the addition of extruded material (using only wheat flour) were analyzed as control samples. The bread quality was evaluated 17 h after baking.

### Bread quality evaluation

Total titratable acidity (TTA) of the fermented products and bread crumbs was measured according to the method described in AACC (22). The TTA value was expressed in milliliters of 0.1 M NaOH solution used per 10 g of sample to obtain pH=8.5. The specific volume of the bread samples was evaluated as described in AACC (22).

### Fractal and image analysis of wheat bread bubble distribution

The analysis was performed following the method described by Calderón-Domínguez *et al.* (16). Twenty-four bread loaves were sliced using an electrical cutter in three pieces for each sample. The image and fractal analysis were performed using image analysis system and image public domain software (National Institutes of Health, Bethesda, MD, USA). Images of bread slices were scanned in RGB colour and saved in a bmp for-

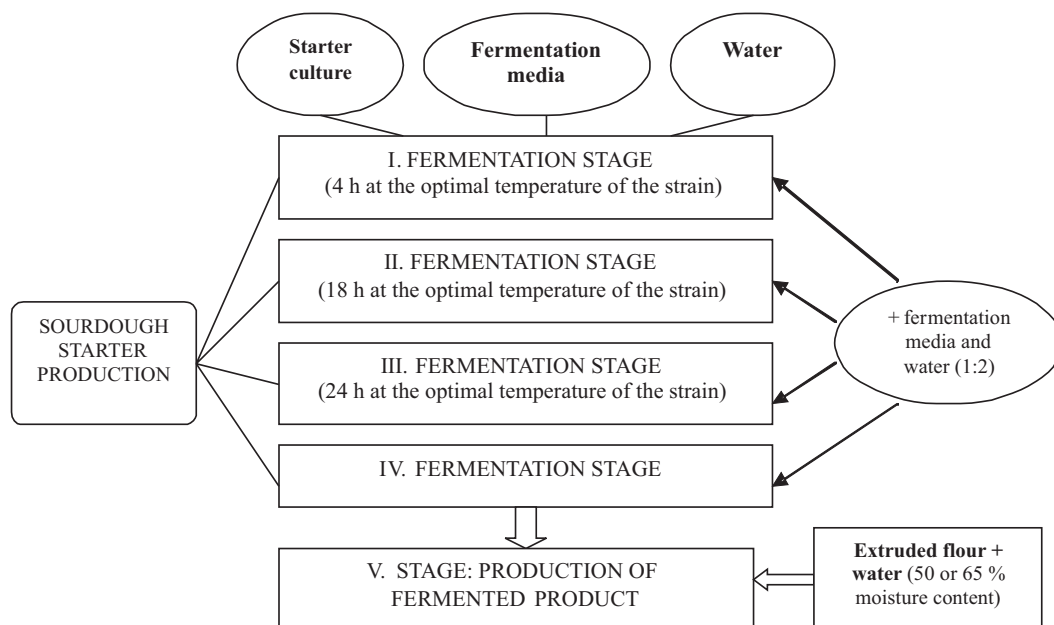


Fig. 1. Scheme of production of sourdough starter and fermented products

mat. The RGB colour images were converted to grey level and binary images using the ImageJ software. The characteristics of crumbs selected from each image field were: number of cells/cm<sup>2</sup>, cell maximal perimeter (P) defined as the length of all pixels along the boundaries of a specified image object, and the area of single cells (A) in pixels. Cell shape for each pore was analyzed using fractal dimension (FD<sub>cell</sub>) as follows (23):

$$FD_{cell} = 2 \cdot \ln(P/4) / \ln(A) \quad /1/$$

individual P and A values were used to evaluate FD<sub>cell</sub> and were reported as the average value of individual dimensions of the measured pores.

Bread crumb firmness was determined as a maximum compression force (60 % compression with 10-mm diameter plunger, compression rate 2 mm/s) using a Stevens LFRA Texture Analyzer (Leatherhead Food Research Association, Leatherhead, Surrey, UK).

### Statistical analysis

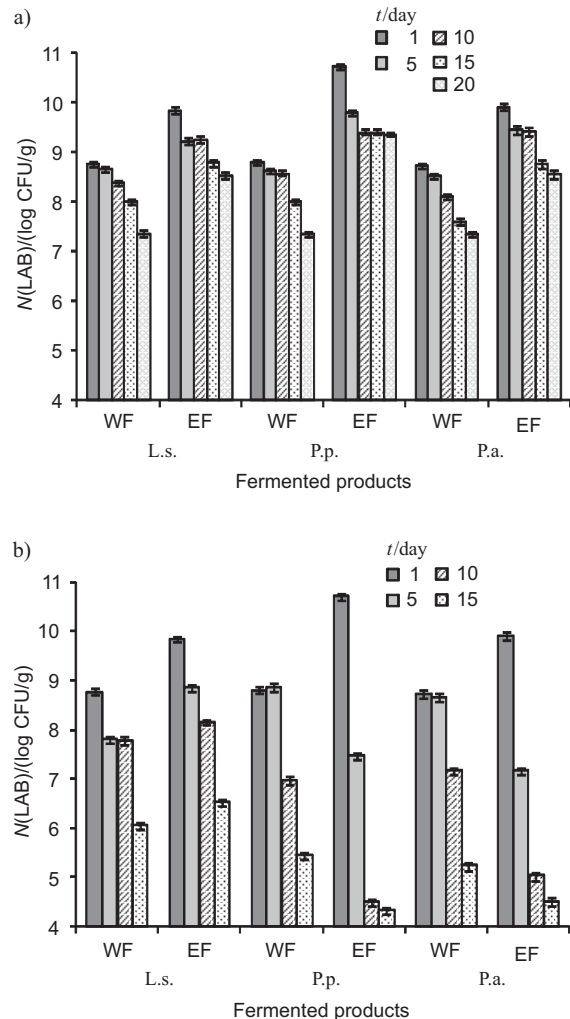
All the experiments were carried out in at least three independent experiments. The mean calculations for the obtained raw data were calculated and indicated together with the standard deviation. LAB counts were converted into decimal logarithmic values to closely match the assumption of a normal distribution. The obtained data were analyzed using the statistical package SPSS v. 15.0 for Windows (IBM, Armonk, NY, USA), for correlation coefficient and regression analyses. The significance of each instrumental measurement/descriptive attribute in discriminating between the samples was analyzed using an analysis of variance (ANOVA). The significance of differences was evaluated using Tukey's multiple range test at a 5 % level.

## Results and Discussion

### Microbiological analysis of fermented product

The microbiological analysis of fermented product samples made under laboratory conditions revealed that *L. sakei*, *P. pentosaceus* and *P. acidilactici* counts ranged from 6.05 to 9.85, from 4.32 to 10.72 and from 4.51 to 9.88 log CFU/g, respectively (Fig. 2). The fermentation medium was found to have a significant effect ( $p < 0.05$ ) on the LAB growth. The *L. sakei*, *P. pentosaceus* and *P. acidilactici* counts were detected higher on average by 16 and 8 % after 1 day of storage at 0–6 and 30–35 °C, respectively, in extruded wheat material than in wheat flour medium.

The tendency of the reduction in the LAB populations with the extension of storage time of the prepared fermented products was noticed. The decrease of LAB count on average by 17 % was measured in *L. sakei*, *P. pentosaceus* and *P. acidilactici* in wheat flour medium with a moisture content of 50 % stored for 15 days at 30–35 °C. In the case of the extruded flour medium compared to the control, a considerable reduction in LAB count of *L. sakei*, *P. pentosaceus* and *P. acidilactici*, on average by 40 %, was detected. According to the adaptation in the medium containing extruded wheat material, the best results were obtained with *L. sakei* since the lowest decrease (34 %) in LAB count during 15 days of storage



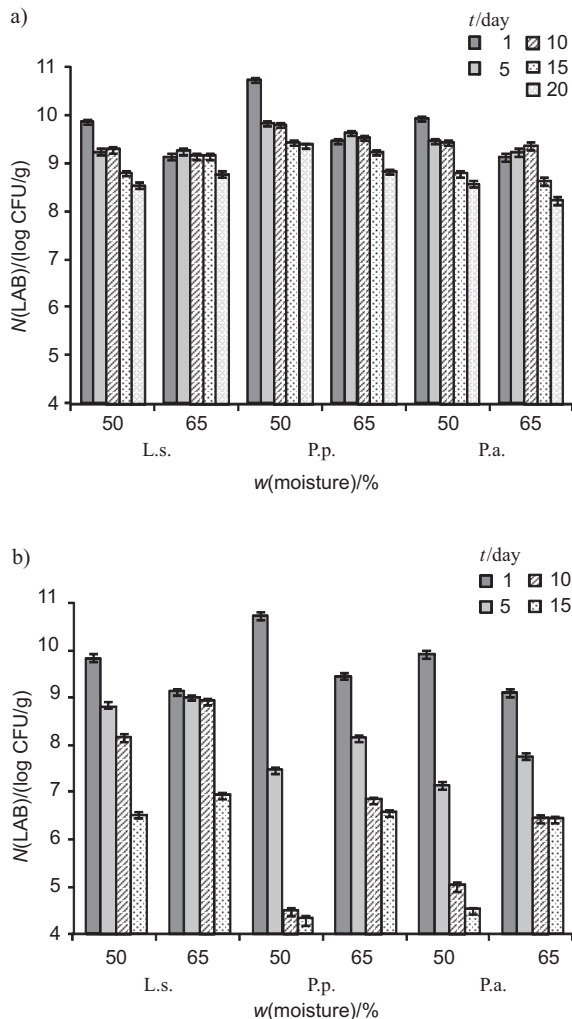
**Fig. 2.** The effect of fermentation media (50 % moisture content) on the change of the number of *L. sakei* (L.s.), *P. pentosaceus* (P.p.) and *P. acidilactici* (P.a.) during storage of fermented products at: a) 0–6 °C and b) 30–35 °C; WF – wheat flour (control), EF – extruded flour

at 30–35 °C was noticed compared to *P. pentosaceus* (60 %) or *P. acidilactici* (55 %). The results indicate that in most cases higher LAB count remained in the fermentation medium stored at 0–6 °C temperature in that time range.

The decrease in LAB counts on average by 16 % was noted in control medium (50 %) after 20 days of storage at 0–6 °C, while in extruded wheat medium the LAB levels decreased by 13 %.

Furthermore, the effect of moisture content of the novel fermentation media on the changes in different LAB counts during storage of fermented products at different temperature ranges was analysed (Fig. 3). The study revealed a significant effect ( $p < 0.05$ ) of moisture content of the media on LAB count. The highest levels of *L. sakei*, *P. pentosaceus* and *P. acidilactici* after one day of storage were detected in extruded wheat flour medium (9.85, 10.72 and 9.92 log CFU/g, respectively) with a moisture content of 50 %, while in the case of a higher moisture content (65 %), the detected LAB levels were lower by 0.81, 1.27 and 0.72 log CFU/g, respectively.





**Fig. 3.** The effect of moisture content of extruded wheat media on the changes of the number of *L. sakei* (L.s.), *P. pentosaceus* (P.p.) and *P. acidilactici* (P.a.) during storage of fermented products at: a) 0–6 °C and b) 30–35 °C

However, during the storage of fermented products, it was noticed that the more liquid medium (65 %) generally showed lower decrease in LAB count. The reduction in LAB count in 50 % extruded wheat material within 15 days (30–35 °C) and 20 days (0–6 °C) of storage was found on average by 50 and 13.5 %, respectively, while in the 65 % fermentation medium, this parameter decreased by 28 and 7 %, respectively. Moreover, the lower variation of LAB count was measured in the fermented products with the higher moisture content.

In this study most of the samples contained LAB counts within the range reported by other authors (24–26). The LAB count during fermentation was reported to increase to some level, followed by a decrease afterwards (27,28). This can be explained by four different bacterial growth phases: lag phase, exponential or log phase, stationary phase and death phase. There is some controversy about the effect of fermentation medium moisture content on LAB growth. Wang *et al.* (29) reported that lactic acid bacteria survive better at low water activity, while Hansen and Schieberle (2) stated that a medium with high water content was more proper for the LAB

cultivation due to the solubility of nutrients in the medium. Nevertheless, microorganisms are found to be very sensitive to the changes in the water content and nutrient concentration. The direct addition of selected starter cultures to raw materials is the highlight of the production of fermented foods, helping to control the overall standardization of the fermentation process and quality of the end product (24).

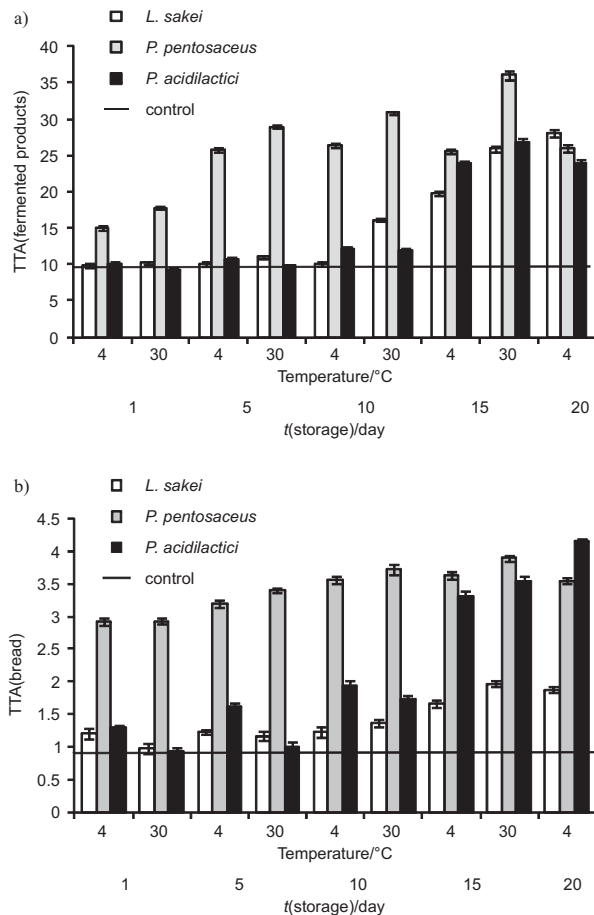
### Bread quality evaluation

#### Total titratable acidity (TTA)

The results of total titratable acidity showed that TTA values of fermented products ranged from 9.3 to 27.7 on the first day of the experiment (Fig. 4a). In all samples, the TTA values increased with increased storage time and temperature. The application of extruded material in the preparation of the products fermented with *L. sakei*, *P. pentosaceus* and *P. acidilactici*, stored for 20 days at 0–6 °C, influenced the increase in TTA values by an average of 39 % as compared to the control samples. The TTA profile of the fermented products after 15 days of storage at 30–35 °C was higher by an average of 22 % (starters continued to produce acids more intensively) than TTA values measured in the samples stored at 0–6 °C. A considerable increase in TTA by 50 and 55 % was noticed in the samples with *P. acidilactici* from day 10 to day 15 of storage at temperatures ranging from 0–6 °C and 30–35 °C, respectively.

In parallel, a comparable TTA of wheat bread baked with fermented products was evaluated (Fig. 4b) and commensurate trends were noticed. The highest TTA levels (2.9–3.9) were measured in the bread samples baked with novel *P. pentosaceus*-fermented products stored at 0–6 °C. Bread samples containing the products fermented with *L. sakei* and *P. acidilactici* were found to have lower acidity levels, ranging from 1.2 to 1.97 and from 1.3 to 3.53, respectively, as compared to the bread samples prepared with the *P. pentosaceus*-fermented products. A slight increase in TTA values with the increasing storage time, and the highest TTA values of samples stored at 30–35 °C were observed. The following statements can be inferred according to the TTA results of bread samples with the fermented products prepared with the extruded material and *L. sakei*, *P. pentosaceus* or *P. acidilactici*. The TTA values of bread produced using the products fermented with *L. sakei*, *P. pentosaceus* or *P. acidilactici* and stored for 20 days at 0–6 °C increased by 36, 17 and 69 %, respectively, while in bread samples with fermented products stored for 15 days at 30–35 °C they increased by 39, 25 and 74 %, respectively.

The acidification activity of LAB contributes to the beneficial properties of fermented products. Typical TTA values of acidic wheat sourdough were reported to be 8–13 (2,30), while in wholemeal and rye sourdough the TTA values were in the range of 16–22 and 15–26, respectively (31). As reported by other authors, higher temperature, higher water content of fermented products and the utilization of whole meal flour enhance the production of acids in wheat sourdough (4,32). Katina (27) reported about the significant interaction of fermentation time and temperature on the formation of acidity in LAB-fermented sourdough, indicating that high levels of



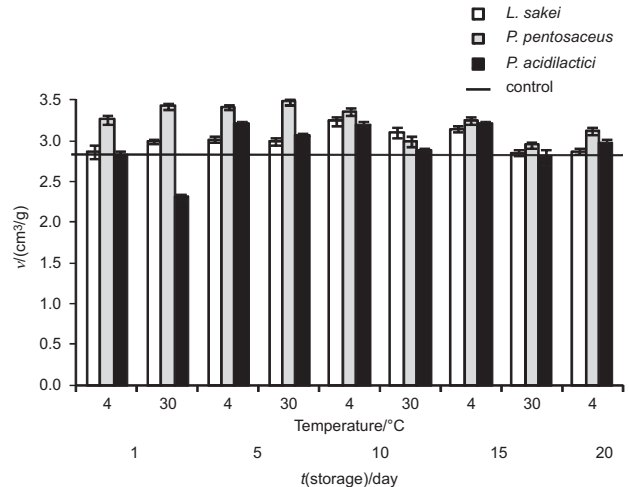
**Fig. 4.** The influence of storage time and temperature on TTA of: a) fermented products and b) bread, fermented with *L. sakei*, *P. pentosaceus* and *P. acidilactici*

acidity in sourdough required both elevated temperature levels and longer fermentation time, and preferably the use of flour with a high ash content. The main factor regulating acidification is the amount of fermentable carbohydrates (33), although the production of acids depends also on other things such as fermentation temperature, time and dough yield (27). The results obtained in the microbiological study confirmed that extruded material can be effective in a beneficial modification of the fermentation process by intensification of the growth of LAB and increase of the acidity level in bread samples.

#### Specific volume

In all the tested bread samples a decrease in specific volume with the increasing storage time and higher temperature of fermented products was noticed (Fig. 5). The bread samples with novel fermented products made using *P. pentosaceus* stored for 15 days at 0–6 °C or 10 days at 30–35 °C were found to have the highest specific volume ((2.95–3.41) cm<sup>3</sup>/g) (Fig. 5).

The most considerable reduction in specific volume, by 16 and 19 % on average, was measured in bread samples prepared with the control products (without extruded products) stored for 15 days at 30–35 and 0–6 °C, respectively. With the increasing storage time of fer-



**Fig. 5.** The influence of storage time and temperature on the products fermented with *L. sakei* (L.s.), *P. pentosaceus* (P.p.) and *P. acidilactici* (P.a.) on the specific volume of bread samples

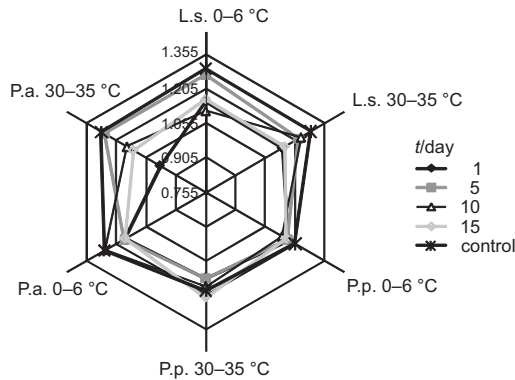
mented products containing extruded material, a less negative effect (decrease by 7 %) on the quality of bread was noticed, as compared to the reference samples. Bread samples produced with novel fermented products made from extruded wheat material fermented by *L. sakei* and *P. acidilactici* were found to have the most stable quality. These results are in agreement with the previous data that extruded wheat material had a higher positive effect on LAB growth compared to the control medium (Fig. 2) as well as on the increase of the acidification profile in novel fermentation medium (Fig. 4).

Sadeghi *et al.* (34) published contrary results where by increasing fermentation time and temperature, bread specific volume was increased. The improvement of the bread specific volume by the application of sourdough was reported (3,35,36); nevertheless, the decrease in specific volume by using fermented products was indicated in a few reports (37). The contradictory results might be explained by the different acidification rate and by the bacterial strains used.

#### Fractal analysis

In this study binary images were used to evaluate the fractal dimension of crumb pores ( $FD_{cell}$ ) with regard to the applied fermented products stored under different conditions. This criterion was characterized as a function of storage time and temperature of different fermented products (Fig. 6).  $FD_{cell}$  increased with the storage time of fermented products and decreased after reaching the maximum value.  $FD_{cell}$  was found to be the highest on the fifth day of storage at 30–35 °C and on the tenth day of storage at 0–6 °C, followed by the decrease when storage time of the fermented products was prolonged.

In all the cases, the storage of fermented products at lower temperatures (0–6 °C) contributed to the most homogeneous structure of the end products with the smallest values of  $FD_{cell}$ . Furthermore,  $FD_{cell}$  decreased when the fermented products prepared with extruded wheat material were applied for the bread production. The application of extruded material in the fermented products



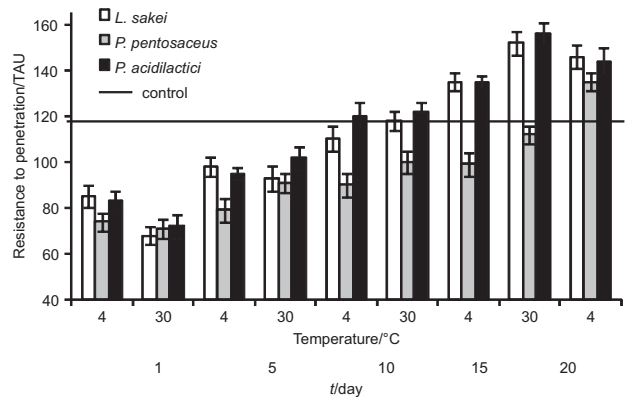
**Fig. 6.**  $FD_{cell}$  values as a function of storage time and temperature of the bread prepared with *L. sakei* (L.s.), *P. pentosaceus* (P.p.) and *P. acidilactici* (P.a.) fermented products after storage at different temperatures

prepared with *L. sakei*, *P. pentosaceus* and *P. acidilactici* reduced the  $FD_{cell}$  value in bread samples by 1.4, 3.9 and 2.8 %, respectively, as compared to the control samples (Fig. 6). This may contribute to the formation of smaller and more homogeneous pores, which may be due to the change in the dough viscoelastic properties. As dough becomes more resistant to extension, cells better withstand the progressively increasing internal pressure, enlarging their size but keeping a homogeneous cell form, reflected in low  $FD_{cell}$  (16).

Calderón-Domínguez *et al.* (16) and Crowley *et al.* (38) reported that pore density was mainly affected by fermentation time; moreover, the cell distribution of materials was related to the rheological properties. In general, the irregularity of bread crumb texture and the increase in the regularity of pores are associated with fermentation time. However, there is no literature data about the effect of lactic acid bacteria or extruded material applied for the sourdough production on the fractal dimension of bread crumb pores.

#### Crumb firmness

The crumb texture of sourdough bread, characterized as crumb firmness, is an important quality attribute. Initial texture analysis, performed after baking, revealed a softer crumb (in accordance with the resistance to penetration) in wheat bread prepared with fermented extruded material stored at 0–6 and 30–35 °C with the values of  $(124 \pm 2)$  and  $(145 \pm 6)$  texture analyzer units (TAU), respectively, as compared to the reference samples (Fig. 7). The tendency of increasing crumb firmness has been noticed in the fermented products stored for a longer time. The higher (30–35 °C) temperature range for the storage of fermented products increased the bread crumb hardness by 5.4 % on the 5th day of storage and by 12.1 % until the 15th day of storage, as compared to the lower (0–6 °C) temperature range. The application of extruded wheat material in bread production was found to have a higher positive effect on the crumb firmness of novel fermented products during the extended storage time at higher temperatures in comparison with the control medium. According to the different effects of fermented products on crumb firmness, their storage time can be divided into two periods (up to and more than 10 days of storage). During the first



**Fig. 7.** Influence of storage time and temperature on the products fermented with *L. sakei*, *P. pentosaceus* and *P. acidilactici* on the resistance to penetration of bread

period of storage of novel fermented product made using *P. pentosaceus*, the firmness of the crumb decreased by 25 % (0–6 °C) and by 16.7 % (30–35 °C) as compared to the initial value, while the increase of firmness by 11 % (0–6 °C) and 18 % (30–35 °C) was noticed only in the second period (20 days of storage). Contrary to the above-mentioned results, the positive effect in the reduction of crumb hardness by 18.3 and 20.8 % (0–6 °C) and by 22 and 15 % (30–35 °C) was noticed in the novel products fermented with *L. sakei* and *P. acidilactici*, respectively, resulting in a softer crumb after 10 days of storage.

It was reported that the addition of fermented products to wheat bread reduced crumb firmness and slowed down the process of hardening as compared to bread samples prepared without the addition of fermented products (39,40). A soft crumb is associated with the dough acidity that reduces its elasticity and resistance to extension (41,42).

In this study, the use of sourdough with the extruded material generally improved the overall characteristics of bread. In addition, the irregularity of bread crumb texture and the increase in the regularity of pores are associated with fermentation time and storage conditions. Both experiments (microbiological and technological) confirmed that the effect of fermentation on wheat bread quality depends on the LAB fermentation medium systems (adaptation of LAB in the medium with extruded wheat material). LAB in these systems could be very important. One possible explanation for the different effects of selected LAB strains is their enzymatic effect on the fermentation media (in this case on extruded material) and produced metabolites. Positive effect of sourdough was reported to be due to the formation of exopolysaccharides during fermentation, leading to the delayed staling upon the increased volume (43). The improvement in quality might be explained by the solubilisation of arabinoxylans during long fermentation or by the production of dextrins, which have the ability to interfere with starch retrogradation during extended fermentation periods (40). A soft crumb is associated with the dough acidity, which reduces its elasticity and resistance to extension (41). From another point of view, the extruded material is partly gelatinized, which can have an impact on the rheological properties of dough



and the texture of wheat bread. This is in agreement with the results reported by Calderón-Domínguez *et al.* (16) and Crowley *et al.* (38) that pore density is mainly affected by fermentation time; moreover, the cell distribution of materials was related to the rheological properties.

## Conclusions

In this study three lactic acid bacteria (LAB) strains with antimicrobial properties (*Lactobacillus sakei* MI806, *Pediococcus pentosaceus* MI810 and *Pediococcus acidilactici* MI807), previously isolated from spontaneous Lithuanian sourdough, were applied in a novel fermentation medium of extruded wheat material for wheat bread production with a perspective of industrial production of fermented products stored at different temperatures and period in bakeries. Extruded wheat material was found to have a higher positive effect on LAB growth compared to the control medium by lowering the reduction of LAB populations in fermented products with the extension of storage time and increase of temperature. Lower variation of LAB counts and lower decrease in LAB counts were measured in the novel fermented products with a moisture content of 65 % during storage. Based on the microbiological investigations of fermented products during storage and the baking tests, the conditions of LAB cultivation in novel fermentation media have been optimized (time of cultivation of <20 days at 0–6 °C and <10 days at 30–35 °C). The addition of the new fermented products with 65 % humidity to the wheat bread recipe (10 % of the quantity of flour) had significant effects on bread quality by increasing the specific volume of the bread and decreasing the fractal dimension of crumb pores and crumb firmness. This study highlights the possibility of industrial production of the novel fermented products with the aim to increase the efficiency of bread production and improve its quality characteristics.

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