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# A concept of mould spoilage prevention and acrylamide reduction in wheat bread: Application of lactobacilli in combination with a cranberry coating

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ABSTRACT

The current study evaluated the technological and antifungal properties of the newly isolated lactic acid bacteria (LAB) strains (*P. pentosaceus* LUHS183, *P. acidilactici* LUHS29, *Lactobacillus paracasei* LUHS244, *Lactobacillus brevis* LUHS173, *Lactobacillus plantarum* LUHS135 and *Leuconostoc mesenteroides* LUHS242) and determined the influence of different LAB sourdoughs and their quantities (10, 15 and 20%) on wheat bread quality, including acrylamide content. In order to prolong the final products shelf life, LAB fermentation was combined with coating of the bread surface using cranberry preparations. All the tested LAB strains showed antifungal activities against *Aspergillus nidulans*, *Penicillium funiculosum* and *Fusarium poae*. *L. brevis* LUHS173 and *Leu. mesenteroides* LUHS242 strains showed weak antifungal activities, but good technological and acrylamide-lowering properties (the lowest acrylamide content (5.21 $\mu g\,kg^{-1}$) in bread with 20% of LUHS173 sourdough was achieved). However, by increasing the sourdough content, the bread quality decreased (except *Leu. mesenteroides* LUHS242), therefore, additional experiments were undertaken, using a cranberries-based bread surface coating. This approach showed antifungal activities against *Aspergillus fischeri*, *Penicillium oxalicum*, *P. funiculosum*, *F. poae*, *Alternaria alternata* and *Fusarium graminearum*. Finally, selected LAB, in combination with an antimicrobial coating, leads to wheat bread with improved quality, safety and extended shelf life.



## 1. Introduction

Sourdough fermentation is the most natural and best-performing process to ensure optimal sensory and functional characteristics of bread (Pontonio et al., 2016). Most of the useful and valuable features attributed to the sourdough fermentation are mainly related to its dominant microflora, and the choice of the starter cultures has a critical impact on the palatability, processability, nutritional attributes and safety of bread. Fermentation processes by lactic acid bacteria (LAB) reduce the acrylamide content in bread. Our previous studies focused on lowering the acrylamide in rye-wheat, wheat-barley, wheat bread and biscuits, by using *Pediococcus acidilactici* KTU05-7, *Pediococcus pentosaceus* KTU05-9 and *Lactobacillus sakei* KTU05-06 strains (Bartkiene et al., 2013b, 2013c, 2015, 2016, 2017a, 2013a). The studies showed that fermentation with selected LAB strains is more effective for decreasing the acrylamide in bread compared to acidification with L(+) lactic acid (Bartkiene et al., 2017b). Therefore, selection of LAB possessing particular characteristics, for example, with an acrylamide-lowering effect, could be very promising for the baking industry.

Despite the significant progress made in this field, LAB display unique properties, even within the same species. These attributes

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influence bread quality in different ways, including not only the main quality parameters of bread (e.g., specific volume, porosity, overall acceptability) but also its safety characteristics, such as resistance to mould spoilage. The selection of different LAB (or their combinations) with a wide spectrum of antifungal activities and good technological properties is an important issue for improving bread safety. Sourdough LAB strains have the potential to be used for bread bio-preservation because they are safe to the consumers. These LAB naturally dominate in sourdough, and they produce metabolites that can inhibit fungi growth under the native growth conditions (Hassan, Zhou, & Bullerman, 2015). Mould spores are typically killed by the thermal treatment during bread baking yet remain a huge problem, requiring a multifaceted approach. In this context, surface treatment with agents showing antifungal activities becomes very relevant. Antifungal compounds of plant origin, for example, cranberry products, could be attractive candidates for natural preservation against fungal growth (Antolak & Kregiel, 2017, chap. 3). It has been noted that a concentrate of cranberry juice can inhibit the growth of *Penicillium* spp., *Absidia glauca*, *Penicillium brevicompactum*, *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* (Ermis et al., 2015). However, there is no prior literature about the effect of coating a wheat bread surface with cranberry products for preventing fungal spoilage, to prolong the final products shelf life.

The current study evaluated the technological and antifungal properties of the newly isolated LAB strains (*P. pentosaceus* LUHS183, *P. acidilactici* LUHS29, *Lactobacillus paracasei* LUHS244, *Lactobacillus brevis* LUHS173, *Lactobacillus plantarum* LUHS135 and *Leuconostoc mesenteroides* LUHS242) and determined the influence of different LAB sourdoughs and their quantities (10, 15 and 20%) on wheat bread quality, including acrylamide content. In order to prolong the final products shelf life, LAB fermentation was combined with coating of the bread surface using cranberry preparations.

## 2. Materials and methods

### 2.1. Materials used for sourdough and bread preparation

Wheat flour (type, 550D; falling number, 350 s; wet gluten, 27%; ash, 0.68%) obtained from Kauno Grudai Ltd. mill (Kaunas, Lithuania) was used for the preparation of wheat sourdough and wheat bread. For sourdough fermentation, newly isolated *P. pentosaceus* LUHS183, *P. acidilactici* LUHS29, *L. paracasei* LUHS244, *L. brevis* LUHS173, *L. plantarum* LUHS135 and *Leu. mesenteroides* LUHS242 (from the collection of Lithuanian University of Health Sciences, Kaunas, Lithuania) strains were used. The strains were stored at −80 °C in a Microbank system (Pro-Lab Diagnostics, UK), and before the experiment, were propagated in a De Man−Rogosa−Sharpe (MRS) broth (CM 0359, Oxoid Ltd., Hampshire, UK) at 30 ± 2 °C for 48 h.

### 2.2. Evaluation of LAB carbohydrate metabolism, gas production, tolerance to temperature and low pH conditions

Carbohydrate fermentation of the strains was determined (API 50 CH system, BioMérieux, Marcy-l'Etoile, France). Each pure strain was further characterised by the Durham tube method in MRS broth at 30 °C for 24 h, for detecting gas evolution. The growth performance was monitored at 10, 30, 37 and 45 °C for 24 h in MRS broth, using a Thermo Bioscreen C automatic turbidimeter (Labsystems, Helsinki, Finland). The ability of the strains to survive at low pH was evaluated in triplicate, as described by Lee et al. (2011), in acidified MRS broth (final pH 2.5).

### 2.3. Evaluation of LAB antifungal activity

The antifungal activities of the LAB were determined against *Aspergillus fischeri*, *Aspergillus nidulans*, *Penicillium oxalicum*, *Penicillium funiculosum*, *Fusarium poae*, *Alternaria alternata* and *Fusarium graminearum*. These fungi were previously isolated from grain-based food and were obtained from the collection of the Lithuanian University of Health Sciences (Kaunas, Lithuania). All fungi were cultured on yeast extract, peptone and dextrose (YEPD) medium at 25 °C. The antifungal activity of LAB strains was tested by an agar well diffusion assay (Cizeikiene, Juodeikiene, Paskevicius, & Bartkiene, 2013).

### 2.4. Sourdoughs with different starters preparation and analysis methods

Wheat flour, tap water and LAB cell suspension (5 mL), containing on average of 8.5 $\log_{10}$ colony-forming units (CFU) $mL^{-1}$ of the individual LAB strain, were used to prepare sourdough. The mixture was fermented at 30 °C for 48 h (sourdough moisture was 75%). For the evaluation of the LAB count, 10 g sourdough was homogenised with 90 mL saline (9 g $L^{-1}$ NaCl solution). Serial dilutions of $10^{-4}$–$10^{-8}$ with saline, were used for sample preparation. Sterile MRS agar (CM0361, Oxoid) of 5 mm thickness was used for bacterial growth on Petri plates. The plates were separately seeded with the sample suspension, using sowing in surface and were incubated under anaerobic conditions at 30 °C for 72 h. The number of bacterial colonies was calculated and expressed as CFU per gram of sample (CFU $g^{-1}$).

The pH values of sourdoughs were measured (Sartorius PP-15 pH electrode, Göttingen, Germany). The total titratable acidity (TTA) was determined for a 10 g sample of sourdough homogenised with 90 mL distilled water and expressed as millilitres of 0.1 mol $L^{-1}$ NaOH required to achieve a pH of 8.2. The amylase activity was determined by the starch-iodine method, as described by Nguyen, Rezessy-Szabó, Claeyssens, Stals, and Hoschke (2002). The mode of action of protease enzymes was determined by a non-specific protease assay (Cupp-Enyard, 2008).

### 2.5. Bread preparation

The wheat bread recipe consisted of 1 kg flour (100%), 2% salt, 3% fresh compressed yeast and 56% water (control bread). The dough was mixed (3 min at a low-speed regime and 8 min at a high-speed regime) in a mixer (Diosna SP25, Osnabrück, Germany), shaped and proofed at 30 °C and 80% relative humidity for 45 min. Dough loaves of 350 g were formed and baked in a deck oven (MIWE Michael Wenz GmbH, Germany) at 210 °C for 25 min. The wheat bread samples were prepared with the addition of different quantities of sourdough (10, 15 and 20%) and sourdough starters (*P. pentosaceus* LUHS183, *P. acidilactici* LUHS29, *L. paracasei* LUHS244, *L. brevis* LUHS173, *L. plantarum* LUHS135, *Leu. mesenteroides* LUHS242). Bread without sourdough was used as the control.

### 2.6. Bread quality parameters evaluation

Wheat bread samples were analysed for TTA, moisture content, specific volume, porosity, overall acceptability and acrylamide content. Also, the mould spoilage of bread samples during 10 days was monitored. The TTA was determined for a 10 g sample homogenised with 90 mL distilled water and expressed as the volume (mL) of 0.1 M NaOH solution required to reach pH 8.2. Moisture content was established by drying the sample at 103 ± 2 °C to constant weight. Crumb porosity was ascertained according to the Lithuanian standard method (LST 1442, 1996). Bread volume was