LWT - Food Science and Technology 83 (2017) 157–164

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Application of *Pediococcus acidilactici* LUHS29 immobilized in apple pomace matrix for high value wheat-barley sourdough bread

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ARTICLE INFO

Article history:
Received 7 December 2016
Received in revised form
8 May 2017
Accepted 10 May 2017
Available online 11 May 2017

Keywords:
P. acidilactici
Immobilization
Apple pomace
Fermentation
Bread
Acrylamide

ABSTRACT

The aim of this study was to evaluate the potential use of *Pediococcus acidilactici* LUHS29 immobilized in apple pomace in case to apply in barley sourdough fermentation for functional bread production. The strain was phenotypically characterized by the growth and acidification rate, carbohydrate metabolism and resistance to acidic conditions. The effect of immobilized bacterial cells on antioxidant properties of barley sourdough and on the acrylamide content in wheat-barley bread was analyzed. The phenotypic and molecular testing indicates the *P. acidilactici* having a versatile carbohydrate metabolism and acid resistance, showing 42.7% of viable cells surviving after incubation at low pH as compared to initial number (7.5 $\log_{10}$ CFU/g). Fermentation with immobilized strain increased by 15.3% the production of LA compared to spontaneous fermentation (24.2 g/kg), and the ability to produce L-lactic acid contents up to 92.7% from the total LA. The use of *P. acidilactici* for barley sourdough fermentation increased β-glucan solubility by 1.3–5.1%, moreover, the total phenolic compounds (TPC) content and radical scavening activity were found higher up to 34.6% and 79.7%, respectively. Addition of barley sourdough at a level of 10% could reduce acrylamide content in bread up to 44% and retard bread staling process. The application of immobilized in apple pomace bacterial cells could have the future impact for the food industry due to the bioactive potential.

Chemical compounds studied in the article

Acrylamide
PubChem CID: 6579; MF: C_3H_5NO; MW: 71.077 g/mol
IUPAC Name: prop-2-enamide
DL-Asparagine
PubChem CID: 236; MF: $C_4H_8N_2O_3$; MW: 132.117 g/mol
IUPAC Name: 2,4-diamino-4-oxobutanoic acid
Phenolphthalein
PubChem CID: 4764; MF: $C_{20}H_{14}O_4$; MW: 318.322 g/mol
IUPAC Name: 3,3-bis(4-hydroxyphenyl)-2-benzofuran-1-one
Beta Glucan
PubChem CID: 92024379; MF: $C_{18}H_{32}O_{14}$; MW: 472.438 g/mol
IUPAC Name: 2-[3,5-dihydroxy-2-(hydroxymethyl)oxan-4-yl]oxy-4-[3,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-6-(hydroxymethyl)oxane-3,5-diol

(*continued*)

DPPH radical
PubChem CID: 15911; MF: $C_{18}H_{12}N_5O_6^+$; MW: 394.317 g/mol
IUPAC Name: diphenyl-(2,4,6-trinitrophenyl)iminoazanium
D-Glucose
PubChem CID: 5793; MF: $C_6H_{12}O_6$; MW: 180.155 g/mol
IUPAC Name: (3R,4S,5S,6R)-6-(hydroxymethyl)oxane-2,3,4,5-tetrol
Gallic acid
PubChem CID: 370; MF: $C_7H_6O_5$; MW: 170.119 g/mol
IUPAC Name: 3,4,5-trihydroxybenzoic acid
D-Galactose
PubChem CID: 6036; MF: $C_6H_{12}O_6$; MW: 180.155 g/mol
IUPAC Name: (3R,4S,5R,6R)-6-(hydroxymethyl)oxane-2,3,4,5-tetrol

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http://dx.doi.org/10.1016/j.lwt.2017.05.010
0023-6438/

1. Introduction

A major challenge of the food industry in the world today is to make their products more sustainable, competitive and healthier for consumers. For these reason, lactic acid bacteria (LAB) are including to the food production chain in order to produce high value food. To ensure strain viability during the storage and technological processes, different technologies are used. The immobilization for protection of bacterial cells have resulted in an enhanced viability of LAB as a useful tool to incorporate living cells into foods to extend their storage life (Mozuriene et al., 2016). Therefore, materials used for design of immobilization must be food-grade, biodegradable and safe. These characteristics meet pectin, which high content could be found in apples processing by-products. The processing of apples generates large amounts of residues, apple pomace, which could be utilized either for direct extraction of useful compounds, or for the production of value added products (Bhushan, Kalia, Sharma, Singh, & Ahuja, 2008).

Vialability of LAB is very important in fermented foods production. Sourdough is widely used in the bread production to improve the organoleptic and technological properties of bread as well as its shelf life, nutritional value and healthy aspects (De Angelis et al., 2006).

The bread produced from white wheat flour is the most popular product in human diet, but its functional value is low because of low dietary fiber content. The incorporation of barley into wheat-based foods is aimed at increasing the content of β-glucans and arabinoxylans (Izydorczyk, Chornick, Paulley, Edwards, & Dexter, 2008), the consumption of which is associated of their physiological functions in the gastro-intestinal tract and lowering risk of diseases (Threapleton et al., 2013). Therefore, the major problem associated with fiber in baked goods is its detrimental effect on consumer acceptance due to a reduction of bread quality parameters (Rosell, Santos, & Collar, 2009). Also, flour with higher extraction rates could increase acrylamide formation in bread (Helou et al., 2016). According to Bartkiene et al. (2013), acrylamide content in bread could be reduced by using LAB strains excreting high proteolytic activity in sourdough medium. Lacto-fermentation of flour could decrease the reduced saccharides contents and led to a decrease of pH value, thus allow to reduce the acrylamide content in bread (Ciesarov et al., 2014).

In case to improve wheat-barley bread functional and quality parameters, the implementation of appropriate starter culture in sourdough production as flavour, texture-improving, and health-promoting dough ingredient is of great importance (De Vuyst, Vrancken, Ravyts, Rimaux, & Weckx, 2009).

The aim of this study was to evaluate the potential application of immobilized in apple pomace *P. acidilactici* in barley sourdough fermentations in case to improve wheat bread functional value and quality parameters, and to reduce the acrylamide content.

2. Materials and methods

2.1. Materials and enzymes

Wheat flour (type 550D, falling number 350 s, gluten 27 g/100 g, ash 0.68 g/100 g) was obtained from Kauno Grudai Ltd. mill (Kaunas, Lithuania). Wholemeal barley (*Hordeum vulgare* L., var. Bambina) flour (moisture 10.7 g/100 g, protein 12.82 g/100 g, ash 1.47 g/100 g) was obtained from the local agricultural company (Trakai, Lithuania). By-product after apple processing, apple pomace (moisture content 55 g/100 g), was obtained from MV Group Production wine factory (Anyksciai, Lithuania).

Hemicellulase preparation Cellustar XL (*Aspergillus niger* cellulase, Dyadic International Inc., FL, USA), containing cellulase (>20000 AU/g), xylanase (>15000 AU/g) and β-glucanase (>9000 AU/g) activities, was used for barley wholemeal polysaccharide hydrolysis. For the experiment enzyme powder (1 g) was dissolved in the 100 mL of distilled water to obtain the 200 AU/mL cellulase solution.

2.2. Microorganisms

Pediococcus acidilactici LUHS29 was previously isolated from spontaneous rye sourdough after 48 h fermentation at 30 °C and identified by phenotypic and molecular techniques (Manini et al., 2016). Carbohydrate metabolism of the strain was determined by using API 50 CH Kits (BioMerieux, Marcy-l'Etoile, France) according to the manufacturer's instructions. Gas production was detected by Durham tube method in MRS broth (Oxoid CM361, Basingstoke, Hampshire, England) for 24 h at 30 °C. The growth performance of strain was monitored at 10, 30, 37 and 45 °C for 24 h in a MRS broth using a Thermo Bioscreen C automatic turbidometer (Labsystems, Helsinki, Finland). The ability of the strain to survive at low pH was evaluated in acidified (final pH 2.5) MRS broth according to Lee et al. (2011). All analyses were carried out in triplicate.

2.3. Cell immobilization

Apple pomace (1 kg) for strain immobilization was mixed with the 2 L of water and heated for 1 h at 70 °C. Then, the content was filtered through a 1 mm diameter mesh filter and was autoclaved for 15 min at 121 °C. Pure cell suspension (9.2 $\log_{10}$ CFU/mL) was added to sterilized and cooled to 30 °C apple pomace (3/97; v/w) following the incubation for 24 h at 30 °C.

For stabilization of immobilized bacteria cells, the dehydration in a spray-drying system (SD-06, Keison, GB) was used. For spray-drying immobilized cells product was injected into the spray-dryer in a peristaltic way (inlet temperature +60 °C, inlet air temperature +150 °C, outgoing air temperature +80 °C, air flow 200 m^3/h).

2.4. Barley sourdough preparation

Barley wholemeal (100 g) and tap water (150 mL) were mixed in a glass vessel (500 mL) and saccharified by adding different amounts of Cellustar XL (20, 30, 40, 50, 60 AU/kg flour) at 55 °C for 30 min in a water-bath. After enzymatic treatment sourdough sample was cooled to 30 °C, and immobilized cell powder (2 g/100 g flour) was added following the fermentation for 48 h at 30 °C. The control sourdough sample was prepared using same formulation following spontaneous barley flour fermentation under the same conditions as the test sourdoughs without addition of immobilized cell culture. Sourdough samples (SB – spontaneous barley sourdough; S1 – sourdough fermented with immobilized cell starter without cellulase treatment; S2, S3; S4, S5, S6 – barley sourdoughs treated with different amounts of cellulase (20, 30, 40, 50, 60 AU/kg flour, respectively) and fermented with immobilized cell starter were used for wheat-barley sourdough bread production.

Samples were collected on the 24th and/or 48th h of the fermentation process for the determination of the pH and total titratable acidity, viable cells, lactic acid concentration, β-glucan content, and antioxidant activity.

2.5. pH, total titratable acidity (TTA) and lactic acid determination

For acidity determination, a sample (10 g) was homogenized with the 90 mL of distilled water and filtered. The pH value of