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The influence of lactic acid fermentation on biogenic amines and volatile compounds formation in flaxseed and the effect of flaxseed sourdough on the quality of wheat bread

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ABSTRACT

The aim of the study was to investigate if differences in acidification, biogenic amines (BA) and volatile compounds (VC) occurred when *Lactobacillus sakei KTU05-6*, *Pediococcus acidilactici KTU05-07* and *Pediococcus pentosaceus KTU05-8* are used as starters in solid state fermentation of flaxseed and the impact of flaxseed sourdough on the wheat bread making process assessed.

Results have shown that in SSF flaxseed with *P. acidilactici* and *P. pentosaceus* produced more acids than with *L. sakei*. A correlation between pH and moisture content ($R = 0.9783$; $P = 0.0002$) and between moisture content and total titratable acidity ($R = 0.7150$; $P = 0.0339$) was noticed. BA content in fermented flaxseed is far below those levels causing a health risk. Also, the use of lactobacillus for flaxseed fermentation has an influence on VC formation and the biggest influence on flavor formation had such compounds as: acetic acid, acetyl methyl carbinol and benzaldehyde. The main flavor component of flaxseed is ρ-cymene. Addition of fermented flaxseed to a standard wheat bread formula doesn't show big changes of the bread crumb structure. We conclude that fermentation allows to enrich wheat bread with 10% of partially defatted flaxseed without affecting the quality in a negative way.

1. Introduction

Solid state fermentation (SSF) has built up credibility in recent years in biotech industries due to its potential applications in the production of biologically active secondary metabolites. Composition of microbiota and its development are important factors influencing fermentation and final product quality (Rodriguez et al., 2009). Also, bacteriocin production by lactic acid bacteria (LAB) strains used as starter cultures for food fermentations has been largely proposed as one of their most desirable traits (Cotter, Hill, & Ross, 2005). Food-fermenting LAB's are generally considered as to be non-toxic and non-pathogenic (Spano et al., 2010). However, important food safety factors in fermented products are biogenic amines (BA), especially when for fermentation used raw materials are rich in proteins. The consumption of food containing large amounts of BA can have toxicological consequences (Simpson, Danquah, Benjakul, & Simpson, 2012). Although there is no specific legislation regarding BA content in many fermented products, it is generally assumed that they should not be allowed to accumulate. Therefore, BA should be eliminated or minimized in food products.

Current consumers needs have led to a food production with high nutritional quality and health benefits properties (Roccia, Ribotta, Ferrero, Perez, & Leon, 2012). Flaxseed has been identified as a potential functional food because of its high content of

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phytochemicals, alpha-linolenic (α-linolenic) acid and lignans (Conforti & Cachaper, 2009). Most valuable flaxseed products from a nutrition point of view are dried and defatted flaxseed, because during the baking process 50–60% of fatty acids are transferred to *trans*-isomers (Bartkiene et al., 2009).

The use of fermented products, such as sourdough for bread making, affect bread properties in different ways (Ravyts & De Vuyst, 2011). Besides yeasts, it contains a wide diversity of LAB that produce lactic acid and acetic acid, which results in a sour taste of the final products (De Vuyst & Neysens, 2005). Also, the sourdough LAB have an effect on the rheological and organoleptic properties of the dough, in turn influencing bread volume, flavor, texture, and staling. As it has been shown that certain metabolic products are LAB-strain-dependent, the flavor of the final sourdough products will depend on flavor compounds or precursors made available by the microbiota present (Thiele, Gänzle, & Vogel, 2002). However, studies on industrial starter cultures of LAB are scarce.

The aim of the present study is therefore to investigate if differences in acidification, BA and volatile compounds production can be obtained when different LAB strains are used as starter cultures for flaxseed sourdough SSF. Additionally, the impact of flaxseed sourdoughs from selected LAB strains on bread making will be assessed.

2. Materials and methods

2.1. Flours and lactic acid bacteria

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). Partially defatted flaxseed (*Linum usitatissimum* L.) ("BioFlax", producer Institute of Natural Fibres and Medicinal Plants, Poland; protein content 40 g/100 g; fat content 9 g/100 g; carbohydrates content 3 g; cellulose content 36 g/100 g; sodium content 0.05 g/100 g) flours were purchased at a pharmacy network in Kaunas (Lithuania) and used as the starting material for flaxseed sourdough production.

The *Lactobacillus sakei KTU05-6*, *Pediococcus acidilactici KTU05-7* and *Pediococcus pentosaceus KTU05-8*, strains previously isolated from spontaneous rye sourdough (Digaitiene, Hansen, Juodeikiene, & Josephsen, 2005) were cultured at 30 °C; 32 °C and 35 °C temperatures, respectively, which are optimal for each strain, for 48 h in MRS broth (CM0359, Oxoid Ltd, Hampshire, UK) with the addition of 40 mmol/l fructose and 20 mmol/l maltose prior to be used.

2.2. Preparation of SSF flaxseed products (sourdough)

Partially defatted flaxseed, tap water, and LAB cell suspension (5 mL), containing of 8.9 $\log_{10}$ colony-forming units (cfu) per mL of the above individual LAB strains were used to prepare flaxseed sourdough following the fermentation at 30 °C temperature – *L. sakei*; at 32 °C temperature – *P. acidilactici*; at 35 °C temperature – *P. pentosaceus* for 48 h. Final colony number in the sourdoughs was on average of 7.28 $\log_{10}$ cfu/g. Water content was calculated with reference to moisture content of the raw materials, water absorption capacity and required humidity of the end product. Final moisture of SSF products was 47 g/100 g. Prepared sourdoughs were used for wheat-flaxseed bread production.

Also, flaxseed was fermented by using *Saccharomyces cerevisiae* at 30 °C temperature for 48 h. Prepared sourdough was used for volatile compounds analysis.

2.3. pH and total titratable acidity (TTA) of the SSF flaxseed

The pH value of SSF flaxseed was measured and recorded by a pH electrode (PP – 15, Sartorius, Goettingen, Germany).

Total titratable acidity (TTA) was determined on 10 g of sample homogenized with 90 mL of distilled water and expressed as the amount (mL) of 0.1 M NaOH to get a pH of 8.2.

2.4. BA analysis in flaxseed sourdough

Extraction of samples and determination of BA were carried out according to the procedures developed by Ben-Gigirey, De Sousa, Villa, and Barros-Velazquez (1999) and are described by Bartkiene, Juodeikiene, Vidmantiene, Viskelis, and Urbonaviciene (2011).

2.5. Analysis of volatile compounds by GC–MS method

Samples for gas chromatography analysis were prepared by using solid phase microextraction. For samples preparation was used a solid phase microextraction device with Stableflex (TM) fiber coated with 65 μm PDMS-DVB layer (Supelco, USA). For headspace extraction 0.01 g of sample in the 10 mL extraction vial sealed with tetrafloroethylene septa was thermostated at 25 °C for 1 h exposing the fiber in the headspace.

For gas chromatography–mass spectrometry GCMS-QP2010 (Shimadzu, Japan) gas chromatograph with a mass spectrometer was used. The ionization of analytes was performed using electron ionization mode at 70 eV. For separation of volatile compounds a low polarity RTX-5MS column (Restek, USA) (length 30 m, coating thickness 0.25 μm, the inner diameter of 0.25 mm) was used. The following method conditions were used for analysis: injector temperature 230 °C, ion source temperature 220 °C, interface temperature 260 °C. Sample injection was carried out for 1 min on order to ensure full desorption of volatiles from the SPME fiber. Temperature gradient was from 30 °C till 200 °C (5 °C/min) up to 280 °C 20 °C/min and maintained for 2 min.

The carrier gas helium (99.999% detector purity, AGA, Lithuania) pressure of 15 psi (10.3 kPa) at the column head was used, at the column flow of 1.6 ml/min. The compounds were identified according to the mass spectra library v.8.0 (NIST, USA). For solid phase micro extraction – gas chromatography mass spectrometry method repeatability was measured by extracting and injecting the same sample 5 times. The relative standard deviation according peak area was 5.15%.

2.6. Bread-making procedure

Experimental bread-making was done according to the traditional procedure used for bread making in Lithuania. The recipe, based on 2 kg of flour, was: salt 1.5 g/100 g, fresh compressed yeast 2 g/100 g, water 56 g/100 g (control bread). The wheat flour was substituted by SSF with different LAB flaxseed sourdough at a level of 10 g/100 g. Water content was calculated with reference to moisture content of the raw materials, water absorption capacity and required humidity of the end product (45 g/100 g). The dough was then mixed for 8 min, fermented at 32 °C for 45 min (85 g/100 g relative humidity). Then the dough shaping and proofing at 35 °C temperature for 45 min (80 g/100 g relative humidity) were performed. Dough balls (each of 500 g) were baked in a deck MIWE Condo oven (MIWE Michael Wenz GmbH, Arnstein, Germany) at 230 °C for 25 min. Determination of the quality and acceptability of the control (wheat) and wheat-flaxseed breads were performed 12 h after baking.

2.7. Dough texture analysis methods

Doughs with a final mass of 17.5 g were mixed to peak dough development in a 10 g prototype Mixograph for extension testing