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The influence of lactic acid fermentation on functional properties of narrow-leaved lupine protein as functional additive for higher value wheat bread



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ABSTRACT

The effect of solid state fermentation (SSF) with *Pediococcus pentosaceus* KTU05-9 on narrow-leaved lupine (*Lupinus angustifolius* L.) protein functional properties was evaluated. Analysis of the texture and sensory characteristics of bread produced by wheat flour supplementation with non-fermented (NLF) and fermented (FLF) lupine flours was performed. For the assessment of wheat-lupine bread acceptability the professional software for analysis of facial expressions was used. The SSF treatment of lupine flour resulted in an increase of soluble protein content and improvement of lupine protein functional properties at pH 8. NLF reduced the quality and acceptability of wheat-lupine bread compared to control. The supplementation of wheat flour with 3 g/100 g of FLF resulted in a reduction of bread hardness and chewiness on average by 46%, reduction in resilience by 21%, and slight increase in springiness (by 5%). Higher amount of FLF had no significant influence on bread texture, but gave the most intensive colour, flavour and acidity. Sensory analysis showed significant differences between facial expressions elicited by the different samples. The measurement reflected the introspective liking ratings, especially “happy” and “sad” showed a high correlation with liking and were good indicators for liked and disliked samples, respectively.

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1. Introduction

Plant-derived protein plays an essential role in healthy human diet and the formulation of food products. The consumption of plant protein is recommended by most of health organizations, since it is known that it may reduce serum cholesterol levels, the risk of coronary heart diseases and diabetes (Martínez-Villaluenga, Frías, & Vidal-Valverde, 2006). They are important as effective food ingredients to trigger feelings of satiety, either fast or slowly (Van Kleef, Van Trijp, Van Den Borne, & Zondervan, 2012).

Lupine is increasingly used as a protein source in European countries as a replacement for potentially genetically modified soy products. Lupine seeds are rich in protein and have a good nutritional balance of essential amino acids: high lysine and low

methionine content and albumins and globulins in an approximate 1 to 9 ratio (Arnoldi, Boschin, Zanoni, & Lammi, 2015). The dietary value of lupine protein is higher than that of beans or peas (Bartkiene, Krungleviciute, Juodeikiene, Vidmantiene, & Maknickiene, 2015). Besides, lupine does not contain gluten thus it could be used as a functional ingredient in gluten-free foods (Scarafoni, Ronchi, & Duranti, 2009). Several authors have incorporated lupine protein to the formulation of bread (Doxastakis, Zafiriadis, Irakli, Marlani, & Tananaki, 2002; Paraskevopoulou, Provatidou, Tsotsiou, & Kiosseoglou, 2010). Addition of non-wheat flours from cereal and grain legumes, e.g. lupine, to bread formula helps to improve its nutritional value and meet consumer demands for healthier bread (Villarino et al., 2015), but also improves protein quality of the final product and changes rheological properties of the dough (Duodu, Minnaar, Preedy, Watson, & Patel, 2011, pp. 193–203). Composite doughs of wheat with legumes tend to have higher water absorption and lower strength and stability. Quality and technological usability of food proteins are determined

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by their nutritional value and functional properties. When the protein is considered as one of the constituents, its functionality can be a criterion of more importance than the nutritional value. Of all the different types of functional properties, emulsifying properties and foaming properties are among the most important quality determinants in food formulations. Protein is amongst the most effective emulsifiers commonly applied in the food industry. Their behaviour in food systems are affected by amino acid compositions, their sequence and molecular weight, which change the secondary structure of proteins, its hydrophobicity, flexibility of the molecule (Lampart-Szczapa et al., 2006). However, extrinsic factors, such as temperature and pH also have influence on functionality of protein.

The nutritional quality of lupine can be modified by various methods and treatments, such as heating and fermentation. Lactic acid bacteria (LAB), which have a GRAS (Generally Recognized As Safe) status, play a key role in food fermentation. LAB fermentation is beneficial for development of desired sensory properties or removal of undesirable “beany” flavour in legumes (Lampart-Szczapa et al., 2006). Enlargement of product biosafety can also be achieved using lactic acid fermentation. Solid-state fermentation (SSF) with LAB is defined as the fermentation involving solids in absence (or near absence) of free water and it is more economical compared to the traditional methods (Bartkiene et al., 2014, 2015; Hölker & Lenz, 2005; Juodeikiene et al., 2013). SSF with LAB of legume protein leads to variety of changes, which induce diverse modifications of protein functionality. Changes in protein physical properties might be attributed to the action of both proteases of LAB and proteolytic enzymes naturally occurring in plant raw material. During sourdough fermentation, as a result of acidity increase, significant increase in positive net charge and electrostatic repulsion and reduction in disulfide bonds lead to an increase in the protein solubility (C.I. Clarke, Schober, Dockery, O’Sullivan, & Arendt, 2004; Thiele, Gänzle, & Vogel, 2002).

The main objectives of our study were to determine the effect of solid state fermentation with bacteriocins producing *Pediococcus pentosaceus* KTU05-9 on narrow-leaved lupine (*Lupinus angustifolius* L.) protein functionality and to evaluate the effect of non-fermented and fermented lupine wholemeal sourdough on quality parameters of wheat-lupine bread.

2. Materials and methods

2.1. Materials

The narrow-leaved lupine (*Lupinus angustifolius* L., hybrid line No. 1682) seeds were supplied by the Voke Branch (Lithuanian Institute of Agriculture, Lithuania) after harvest in 2015. Lupine seeds were ground in a laboratory mill (Bühler-Miag, Brunswick, Germany) to pass a 0.5–2 mm screen. The final moisture content of wholemeal lupine flour was 8.9 g/100 g.

The *Pediococcus pentosaceus* KTU05-9 (Pp9) strain was used for lupine wholemeal fermentation. Strain was previously isolated from Lithuanian rye sourdoughs and stored at –80 °C in a *Microbank* system (Pro-Lab Diagnostics, Neston, Cheshire, UK). Bacteria were cultured in a MRS broth (CM 0359, Oxoid Ltd, Basingstoke, Hampshire, UK) at optimal temperature (+35 °C) until further use.

2.2. Chemicals

Kjeldahl tablets (for protein determination) were obtained from FOSS Analytical (Hillerød, Denmark), concentrated sulphuric acid, sodium chloride (powder), hydrochloric acid (0.1 mol/L and 1 mol/L), sodium hydroxide (1 mol/L) and hexane (anhydrous) were obtained from JSC Eurochemicals (Vilnius, Lithuania).

2.3. Fermentation of lupine

Lupine wholemeal was defatted with an organic solvent (hexane) using Soxhlet apparatus before solid state fermentation (SSF). For solid state fermentation, a freshly prepared *P. pentosaceus* cell suspension (5 mL), containing 9.2 log₁₀ colony-forming units (CFU) per mL, was mixed with flour, and sterile water was added to obtain final moisture content of 45 g/100 g. After mixing lupine wholemeal flour was incubated for 72 h at 35 °C. Fermented lupine wholemeal flour was further used for the protein isolation and bread making.

2.4. pH determination

A sample (5 g) was blended with 50 mL of sterile distilled water and filtered through a Whatman’s filter paper No. 1. The pH was then measured directly by using a pH meter (PP-15, Sartorius, Goettingen, Germany).

2.5. Protein analysis

Total nitrogen was determined by using the Kjeldahl method and was multiplied by a factor of 5.7 to determine the protein content in lupine. For soluble protein determination, the sample (5 g) was mixed with 20 mL of distilled water, after the NaCl solution (10 g/L) was added and mixture was stirred for 10 min. The solution was filtered through cotton. The residue, which was left in the flask, was extracted again with 20 mL of distilled water and filtered. The filtrates were collected and 10 mL was used for nitrogen determination.

2.6. Protein isolation

Protein isolation from non-fermented and fermented lupine flour was performed according Jayasena, Chih, and Nasar-Abbas (2010), with some modifications. The flour was mixed with the distilled water (1:10), and after adjusting the pH to 9.0 with NaOH (1 mol/L) was stirred for 1 h. Following centrifugation at 805g for 20 min supernatant was collected. The described step was repeated with the residue and obtained supernatants were combined and the pH was adjusted to 4.5. The proteins were precipitated by centrifugation at 805g for 20 min at 8 °C. The protein isolate was freeze-dried in a freeze dryer (Sublimator 3 × 4 × 5 Zirbus technology, GmbH, Germany) at –40 °C temperature and further used for assessment of functional properties.

2.7. Determination of protein functional properties

The modified method employed by Jayasena et al. (2010), was used to evaluate the emulsifying activity (EA), emulsion stability (ES), foaming capacity (FC) and foam stability (FS) of lupine proteins. For protein emulsifying activity and emulsion stability determination three protein suspensions of 1 g/100 mL were prepared in distilled water. The pH of the each suspension was adjusted to 4, 6 and 8 with 0.1 mol/L NaOH or 0.1 mol/L HCl, and homogenized (IKA T 25, Staufen, Germany) for 10 min. After 5 min, canola oil (100 mL) was added gradually to the suspension with continuous stirring. The emulsion was later centrifuged at 805g for 10 min. Volume of the emulsified layers was recorded to calculate EA based on the following formula:

$$EA (\%) = \frac{\text{volume of emulsified layer (mL)}}{\text{total volume of suspension (mL)}} \times 100 \quad (1)$$

Emulsion stability was determined in a similar way as EA. After

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