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Lipoxygenase inactivation kinetics and quality-related enzyme activities of narrow-leafed lupin seeds and flakes

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ABSTRACT

Lupin seeds are promising sources for the production of vegetable proteins. However, these proteins are associated with green and bean-like flavour, which is attributed to endogenous enzyme activities. Therefore, the objective was to develop a suitable process for the thermal inactivation of quality-affecting enzymes for lupin (*Lupinus angustifolius* cv. Boregine) seeds and de-oiled flakes. After monitoring the specific activities of lipoxygenase (LOX), peroxidase (POD) and lipase during seed maturation, LOX was selected as the most appropriate indicator enzyme to assess inactivation as lipase and POD activities extensively declined at full maturity. Effective reduction of LOX activity was achieved by various hydrothermal treatments at 70–100 °C for up to 30 min and 100% relative humidity. The calculation of kinetic parameters revealed a first-order reaction, whereby the k values increased from 0.09 to 0.58 min^{-1} and 0.06 to 0.62 min^{-1} for seeds and flakes, respectively. The activation energies were significantly lower for inactivation in seeds (60.5 kJ mol^{-1}) than in de-oiled flakes (78.0 kJ mol^{-1}), suggesting the application of whole seeds for LOX inactivation. Based on these results, a gentle inactivation process at 80 °C for 7 min for lupin LOX is proposed.

1. Introduction

In recent years, lupin proteins (*Lupinus* L.) have gained importance as promising alternative to soy proteins as they comprise a similar nutritious amino acid composition, some health-promoting effects as well as good functional properties (Villarino et al., 2015; Wäsche, Müller, & Knauf, 2001). Despite improved processing techniques and the utilisation of low-alkaloid varieties, green and bean-like odour and bitter taste is characteristic for lupin seed fractions (Bader, Czerny, Eisner, & Büttner, 2009; Schindler et al., 2011). Earlier studies also have shown such off-flavours in soybeans (Kumar, Rani, Tindwani, & Jain, 2003; Rackis, Sessa, & Honig, 1979) and their formation has been associated with the activities of lipoxygenase (LOX) and other endogenous enzymes like lipase or peroxidase (POD) (Barrett & Theerakulkait, 1995; Robinson, Wu, Domoney, & Casey, 1995; Wolf, 1975). Therefore, knowledge on the behaviour of these enzymes during seed maturation might be crucial for the development of an appropriate inactivation process for the production of lupin ingredients with improved sensory properties. For soybeans, the activities of lipase, POD and LOX have been reported to change during seed maturation (Attia, Aman, Shehata, & Hamza, 1996; Rackis, Sessa, Honig, & Moser, 1972), whereas literature data about enzyme activities in maturing lupin seeds are unavailable to date.

LOX enzymes (EC 1.13.11.12) catalyse the dioxygenation of certain polyunsaturated fatty acids into reactive hydroperoxides, which are rapidly converted into odour-active compounds by enzymatic or non-enzymatic reactions (Axelrod, Cheesbrough, & Laakso, 1981; Mandal, Dahuja, Kar, & Santha, 2014). In contrast to other legumes, LOX from narrow-leafed lupin (*Lupinus angustifolius* L.) seeds has been reported to be specific for free fatty acids as substrates (Stephany, Bader-Mittermaier, Schweiggert-Weisz, & Carle, 2015), which might imply the involvement of endogenous lipase enzymes (triacylglycerol lipase, EC 3.1.1.3) in lupin flavour formation, catalysing the release of free fatty acids from triacylglycerols. In addition to lipase and LOX, POD enzymes (EC 1.11.1.7) are also involved in off-flavour formation in legumes,

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catalysing the reduction of fatty acid hydroperoxides into odour-active aldehydes and alcohols (Lee & Hammes, 1979). In order to provide high-quality products with improved sensory properties, different thermal and non-thermal processes, aiming at the inactivation of those enzymes, have been reported for soybeans, peas and beans (Indrawati, Van Loey, Ludikhuyze, & Hendrickx, 1999; Reyes De Corcuera, Cavalieri, & Powers, 2004). While the industrial use of non-thermal processes like pulsed electric fields is limited, thermal conditioning of seeds or toasting of flakes and flours at 110 °C-165 °C are commonly applied in soybean processing (Monari, 1990). However, the application of such high temperatures resulted in diminished protein functionality and losses of vitamins and nutrients (Xie & Fretzdorff, 1992). For beans, peas and other vegetables, blanching with hot water is a common pre-treatment to inactivate enzymes prior to further processing. Due to the effective heat transfer, water blanching can be performed at lower temperatures and shorter treatment duration: For instance, LOX in green beans has been suggested to be inactivated during 2 min of blanching at 70 °C, while blanching at 90 °C for 3 min is required to inactivate 90% of the initial POD activity (Bahceci, Serpen, Gökmen, & Acar, 2005). However, water blanching may lead to leaching of proteins and other nutrients as well as an increased water activity of seeds, which hereinafter may result in growth of bacteria and mould during subsequent storage (Reyes De Corcuera et al. 2004). These problems might be avoided using steam blanching (hydrothermal treatment) at temperatures up to 100 °C (Reyes De Corcuera et al. 2004). According to Rodriguez-Saona, Barrett, and Selivonchick (1995), for instance, complete inactivation of POD in sweet corn may be achieved by 15 min of steam blanching at 100 °C. Crude LOX from *L. angustifolius* and *Lupinus albus* has been reported to be stable up to 80 °C (Yoshie-Stark & Wäsche, 2004); however, studies on LOX inactivation in intact lupin seeds or flakes are missing.

Enzyme inactivation has been described to follow first-order reaction kinetics (Gökmen, Bahceci, Serpen, & Acar, 2005; Morales-Blancas, Chandia, & Cisneros-Zevallos, 2002). The temperature dependence of the reaction rates can be described using the empirical Arrhenius equation, which allows the determination of activation energies (E_a values). For water blanching and dry heating of vegetables and legumes, E_a values for LOX inactivation typically range between 100 and 200 kJ mol^{-1}, whereby rate constants, k (min^{-1}), of 0.005–0.252 were determined for pea flour at 90–130 °C (Henderson, Blank, & Sustackova, 1991; Indrawati et al., 1999; Kermasha, Bisakowski, Ramaswamy, & Van de Voort, 1993).

The objective of the present study was to develop an appropriate process for the gentle inactivation of deteriorative enzymes in lupin seeds. Concomitantly, an enzyme indicating sufficient thermal treatment is identified. For this purpose, changes in lipase, LOX and POD activities in lupin seeds during maturation are first investigated to identify an appropriate indicator enzyme for succeeding thermal inactivation. Secondly, different thermal treatments of lupin seeds and de-oiled flakes are applied, and residual enzyme activities are determined. Furthermore, inactivation rate constants (k), activation energy (E_a) values and decimal reduction times (D_T) are calculated at different temperatures, and the values obtained are compared to soybeans and other legumes.

2. Materials and methods

Fig. 1 displays the design of the current study. First of all, lipase, POD and LOX activities were determined during lupin seed maturation starting 36 days prior to harvest. Based on these results, LOX was selected as indicator enzyme for the thermal inactivation trials and a suitable enzyme assay was evaluated to monitor hydroperoxide formation of heat treated samples. Subsequently, lupin seeds and de-oiled flakes were thermally treated at different temperatures for up to 40 min. Kinetic parameters (k, E_a and D_T values) of LOX inactivation were calculated in order to deduce a suitable process for the production of raw materials with low LOX activities.

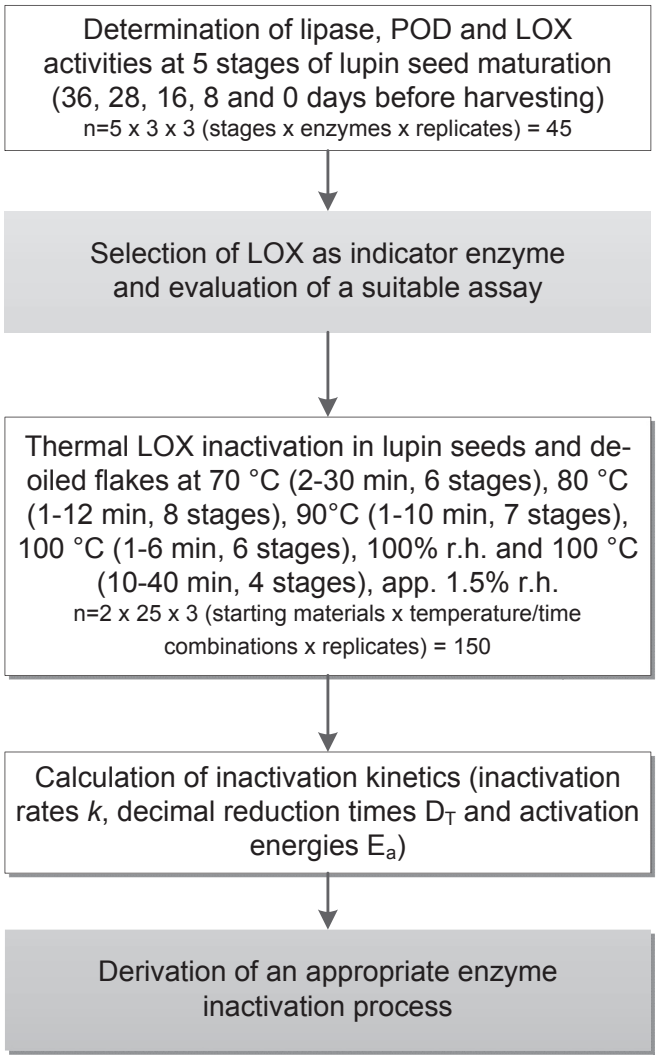

Fig. 1. Design of the study.

2.1. Materials

2.1.1. Chemicals

All reagents used were of analytical grade and obtained from Merck (Darmstadt, Germany) with the exception of polyvinylpolypyrrolidone (PVPP), Triton X-100 and Tween 20, which were purchased from Sigma–Aldrich (Munich, Germany). Reagents used for the protein assay (Dye reagent concentrate and Protein Assay Standard) were from Bio-Rad Laboratories (Munich, Germany).

2.1.2. Sample collection and preparation

Narrow-leafed lupin seeds (*L. angustifolius* cv. Boregine) were obtained from Saatzucht Steinach (Steinach, Germany). To assess the specific activities of lipase, LOX and POD of maturing lupin seeds, samples were collected 36, 28, 16 and 8 days before harvest as well as at the day of harvest. The samples were lyophilised prior to the preparation of crude enzyme extracts.