Food Research International 72 (2015) 82–90

Contents lists available at ScienceDirect

Food Research International

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

Aqueous fractionation yields chemically stable lupin protein isolates

J.A.M. Berghout, C. Marmolejo-Garcia, C.C. Berton-Carabin, C.V. Nikiforidis, R.M. Boom, A.J. van der Goot *

Food Process Engineering Group, Wageningen University, Bornse Weilanden 9, 6708 WG Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 16 December 2014
Accepted 24 March 2015
Available online 28 March 2015

Keywords:
Lupin protein
Lipid oxidation
Protein oxidation
Aqueous fractionation

ABSTRACT

The chemical stability of lupin protein isolates (LPIs) obtained through aqueous fractionation (AF, i.e. fractionation without the use of an organic solvent) at 4 °C or 20 °C was assessed. AF of lupin seeds results in LPIs containing 2 wt.% oil. This oil is composed of mono- and poly-unsaturated fatty acids and the isolate may thus be prone to lipid and protein oxidation. Lipid and protein oxidation marker values of LPIs obtained at 4 °C and at 20 °C were below the acceptability limit for edible vegetable oils and meat tissue protein; the level of lipid oxidation markers was lower at 20 °C than at 4 °C. The fibre-rich pellet and the protein-rich supernatant obtained after AF also had lower levels of oxidation markers at 20 °C than at 4 °C. This is probably the result of a higher solubility of oxygen in water at lower temperature, which could promote lipid oxidation. The differences between fractions can be explained by the differences in their composition; the fibre-rich pellet contains polysaccharides that potentially have an anti-oxidative effect, while the protein-rich supernatant is rich in sulphur-rich proteins that may scavenge metal ions and free radicals from the aqueous phase. Additionally, the differences in solubility of metal ions and metal-chelating properties of protein at pH 4.5 and pH 7.0 explain the higher level of oxidation in the LPI at pH 4.5 compared with the LPI at pH 7.0. The application of a heat treatment to reduce oxidation decreased the protein and oil recovery values, and increased oxidation values above the acceptability limit. Therefore, AF at 20 °C is the most suitable process to obtain chemically stable LPIs.

1. Introduction

Lupin seeds are rich in protein, which makes this crop a promising candidate for plant-based, high-protein foods. Dehulled lupin seeds of *Lupinus angustifolius* L. contain about 39–44 wt.% protein, about 7–10 wt.% oil and are further composed of dietary fibres, sugars, minerals and water (Bähr et al., 2014). *L. angustifolius* L. is a sweet lupin, implying that the alkaloid levels are well below the critical value of 200 mg/kg for lupin-based foodstuffs, and thus not toxic to humans (Small, 2012). Lupin seeds are an attractive alternative to soybean because the seeds have similar protein content, nutritive value and both oils are rich in unsaturated fatty acids (Cerletti & Duranti, 1979; Duranti, Consonni, Magni, Sessa, & Scarafoni, 2008; Sbihi, Nehdi, Tan, & Al-Resayes, 2013). An advantage of lupin is that it can be grown in moderate climates and on poor soils (Sujak, Kotlarz, & Strobel, 2006).

Lupin protein isolation is generally performed through wet fractionation processes (Lqari, Vioque, Pedroche, & Millan, 2002; Wäsche et al., 2001), in which aqueous fractionation is preceded by defatting using an organic solvent. In a previous paper we introduced an alternative fractionation process, referred to as purely aqueous fractionation (AF), which is a sustainable alternative to conventional wet fractionation because the oil extraction step is omitted and thereby the use of organic solvents (Berghout, Boom, & van der Goot, 2014). It is worth mentioning that the solubility and water holding capacity of the lupin protein isolate (LPI) obtained with AF was similar to that obtained with conventional wet fractionation (Berghout et al., 2014). However, a consequence of AF is the presence of some lupin oil during the fractionation process and in the resulting products. The composition of lupin oil has already been studied (Sbihi et al., 2013; Schindler et al., 2011), and includes a substantial amount of polyunsaturated fatty acids (PUFAs), which is an asset from a nutritional point of view, but also makes the oil sensitive to oxidation. Hence, the presence of oil could give rise to increased oxidation of both oil and protein in the obtained fractions. Oil in lupin seeds is present in the form of oil bodies that have a size around 0.1–0.5 μm (Tzen, 1992), which are stabilized by phospholipids and a dense protein complex. This outer structure provides physical and chemical protection against environmental stresses, such as moisture variation, temperature fluctuation and the presence of oxidative reagents (Chen, McClements, Gray, & Decker, 2012; Gray, Payne, McClements, Decker, & Lad, 2010; Karkani, Nenadis, Nikiforidis, & Kiosseoglou, 2013).

Lipid oxidation is one of the prime mechanisms of quality deterioration in foods, as it leads to the formation of unpleasant flavours and odours and to the loss of nutritional value (Velasco, Dobarganes, & Márquez-Ruiz, 2010). Mono- and especially poly-unsaturated fatty acids, are related with health benefits (Siriwardhana, Kalupahana, & Moustaid-Moussa, 2012) and it is thus important that these remain non-oxidized. Due to physical–chemical changes during storage and fractionation (high moisture content) and the presence of oxygen,

* Corresponding author. Tel.: +31 317 480852.
E-mail address: atzejan.vandergoot@wur.nl (A.J. van der Goot).

http://dx.doi.org/10.1016/j.foodres.2015.03.039
0963-9969/

chemically active compounds can be oxidized, but the stability of lupin flour against oxidation has not yet been the subject of study. The initiation mechanism of lipid oxidation occurs through photo-oxidation, enzymatic oxidation, and auto-oxidation (Berton-Carabin, Ropers, & Genot, 2014; Kolakowska & Bartosz, 2014; Skibsted, 2010). While oxidation through direct (sun) light (photo-oxidation) is not of concern for AF of lupin seeds, the other mechanisms are relevant. The enzyme lipoxygenase (LOX) enhances lipid oxidation and is naturally present in lupin seeds. LOX in lupin seeds is reported to have an optimum pH of about 7.5 (Stephany, Bader-Mittermaier, Schweiggert-Weisz, & Carle, 2014; Yoshie-Stark & Wäsche, 2004) and its activity is suppressed at temperatures below 20 °C and above 80 °C (Yoshie-Stark & Wäsche, 2004). De-hulling, flaking and protein isolation from de-oiled lupin flakes result in about 10 times lower LOX activity (Stephany et al., 2014; Yoshie-Stark & Wäsche, 2004). The activity of LOX in full fat (non-de-oiled) LPIs has not been reported. The free radicals formed through lipid oxidation can oxidize proteins in the aqueous phase as well, leading to the formation of protein carbonyls, peptides, and protein cross-linking, which deteriorates the protein's functional properties (Baron, 2014; Skibsted, 2010). Boatright and Hettiarachchy (1995) studied protein oxidation in different types of soy protein isolates (SPIs) and found that reduced-lipid SPI had significantly lower levels of protein oxidation than full-fat and commercial SPIs.

The aim of the work reported here was to investigate the chemical stability of LPIs containing oil, during aqueous processing. The initial level of oxidation of full fat lupin flour at storage temperatures of 4 °C and 20 °C was determined. The effect of storage time on oxidation values was not part of this study. Full fat lupin flour was then subjected to AF in various conditions. First, to suppress the potential activity of LOX, we performed the AF process under chilled conditions (4 °C). For comparison, the process of AF was also conducted at ambient temperature (20 °C). Finally, the influence of a heat treatment (80 °C) during AF was evaluated regarding protein and oil recovery, and regarding lipid and protein oxidation.

2. Materials and methods

2.1. Materials

Untoasted, full fat lupin seeds (*L. angustifolius* L.) were purchased from L.I. Frank (Twello, The Netherlands) in October, 2012 and stored at 4 °C in dark, air-tight containers (8 wt.% water). The reagents and chemicals used were of analytical grade and obtained from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA) except for the ethanol, ethyl acetate, n-hexane, and petroleum ether that were obtained from Merck (Merck, Germany). The bicinchoninic acid-assay kit was obtained from Sigma Aldrich. Tap water was used throughout, unless stated otherwise.

2.2. Processing methods

The seeds were pre-milled to grits with a pin mill (Condux-Werk LV 15 M, Wolfgang bei Hanau, Germany). The grits were then milled into full fat flour with an impact mill ZPS50 (Hosokawa-Alpine, Augsburg, Germany), by setting the classifier wheel at 1000 rpm and the air flow at 80 m^3/h. The screw feeder speed was 2 rpm (0.75 kg/h), the impact mill speed was 8000 rpm and the batch size was 1 kg. One-tenth of the full fat flour was stored in six separate containers: three containers were kept at 4 °C and three containers were kept at 20 °C in the dark for 2 weeks to analyse the oxidation levels. The flour for aqueous fractionation was stored in separate containers; each container had 30 g flour for triplicate experiments.

Aqueous fractionation (AF) was performed as described in Berghout et al. (2014) at 4 °C and at 20 °C. Additionally, AF was performed with the inclusion of a heat treatment. An overview of the AF processes is shown in Fig. 1. The additional heating step was applied after the solubilization step at pH 9. The flour was solubilized in tap water and adjusted to pH 9 with 1 mol/L NaOH. After 1 h, the samples were heated to 80 °C in a water bath while shaking. The temperature of the sample reached 80 °C after about 40 min and the samples were kept at this temperature for 1 min. Subsequently, the samples were cooled to about 30 °C in 20 min and centrifuged at 11,000 ×*g* at 4 °C for 30 min. The protein-rich supernatant was decanted and then adjusted to pH 4.5 with 1 mol/L HCl. After stirring at 4 °C for 1 h, the samples were centrifuged at 11,000 ×*g* at 20 °C for 30 min and the supernatant was discarded. The protein pellet was washed twice with distilled water (Millipore, Merck, Germany) and then either kept at pH 4.5 or neutralized to pH 7 with 1 mol/L NaOH. The fibre-rich pellets, protein-rich supernatants and LPIs at pH 4.5 and pH 7 were frozen at −20 °C and then freeze-dried. Each AF process was performed in triplicate.

2.3. Oil extraction

The oil for oxidation measurements was extracted by performing Standard Soxhlet (Dobarganes Nodar, Molero Gomez, & Martinez de la Ossa, 2002; Özcan & Al Juhaimi, 2014) on a fully automated Büchi B-811 extractor (Flawil, Switzerland) according to AACC method 30-25 (AACC, 1983). Petroleum ether was used as extraction solvent. The extraction was performed for 3 h and the solvent was evaporated on the Büchi extractor until a small layer of solvent remained in the beaker. The beakers were then removed from the Büchi extractor, covered in aluminium foil and left in the fume hood so that the solvent could evaporate. Evaporation was considered to be complete when the weight of the beakers remained constant. Longer extraction times (up to 10 h) resulted in 18% higher oil recoveries. Since lipid oxidation values increased with longer waiting times after evaporation of the solvent, it was chosen to have slightly lower oil recoveries to avoid the higher oxidation values.

2.4. Chemical composition

The dry matter content of each fraction obtained with AF was determined by weighing every fraction before and after freeze-drying. The moisture contents of the full fat flours stored at 4 °C and at 20 °C were determined by drying 2 g flour at 105 °C in an oven until constant weight. The protein content was determined by measuring the nitrogen content with the Dumas combustion method on a Flash EA 1112 Series NC Analyser (CE Instruments Ltd., Wigan, UK). The protein content was calculated by multiplying the nitrogen content with a nitrogen-to-protein conversion factor of 5.7, a value which is commonly used for seed storage proteins (FAO, 2002). Methionine was used as a standard. Samples of about 10 mg were measured in duplicate. The amount of sulfhydryl groups for each fraction was determined with the sulfhydryl reactivity method as described by Berghout, Boom, and van der Goot (2015). Briefly, protein samples reacted with Ellmann's reagent (DTNB) and the absorbance of the samples was measured at 412 nm with a spectrophotometer UV–vis Beckman Coulter DU-720 (Woerden, The Netherlands), after which the number of sulfhydryl groups was calculated. The iron and copper content of the flour, the fibre-rich pellet and the LPI were determined by the Chemical Biological Laboratory Bodem (soil) (Wageningen University, The Netherlands). The samples (about 300 mg) were dried at 70 °C and subsequently destructed using HNO_3–HCl–H_2O_2 in a microwave (MARS-Xpress, CEM, USA). The iron and copper content were then measured on an Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) (Varian Vista Pro Radial, Varian Inc., USA) according to SWV E1362.

2.5. Water activity measurements

The water activity of the full fat flour that was stored at 4 °C and at 20 °C was measured with an Aqualab water activity meter (Decagon