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Alkaloids in the valorization of European *Lupinus* spp. seeds crop

Sara C.Q. Magalhães[a], Fátima Fernandes[b], Ana R.J. Cabrita[a], António J.M. Fonseca[a], Patrícia Valentão[b], Paula B. Andrade[b,*]

[a] *REQUIMTE/LAQV, ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua Jorge Viterbo Ferreira, n° 228, 4050-313 Porto, Portugal*
[b] *REQUIMTE/LAQV, Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, R. Jorge Viterbo Ferreira, n° 228, 4050-313 Porto, Portugal*

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ABSTRACT

In this study, alkaloids from lupin varieties with commercial interest in Europe were identified and quantified. Additionally, the anti-inflammatory and antioxidant potential of some rich-alkaloid lupin extracts was assessed by 5-lipoxygenase (LOX) inhibition and nitric oxide radical (•NO) scavenging assays, respectively. Relationships between extracts activity and those of pure standards were stablished.

Nine alkaloids belonging to quinolizidine, indole and piperidine classes were identified by means of GC-IT/MS and quantified by GC-FID using a validated method. Lupanine was the most abundant alkaloid in white and narrow-leafed lupins (*Lupinus albus* L. and *Lupinus angustifolius* L., respectively) and sparteine in most yellow lupins (*Lupinus luteus* L.), but their proportions were cultivar-dependent. Gramine, smipine, angustifoline and lupanine derivatives were also identified. Five lupin varieties (Amiga, Estoril, Lumen, Dukat and Mister) were characterized as sweet (<0.5 g alkaloids/kg, dry matter basis) and two of them respected the safety limit imposed by the European health authorities for human consumption (≤0.2 g/kg, dry matter basis). Despite the weak effect on •NO, a dose-dependent response towards LOX was found for all the studied extracts, which followed the order Taper > Estoril > Multitalia-PT > Dukat > Azuro > Multitalia-IT > Nacional.

To our knowledge, the alkaloids composition of some of the varieties, as well as the study of the anti-inflammatory and antioxidant potential of rich-alkaloid lupin extracts are here reported for the first time. The results presented are a source of easily available data for producers, nutritionists and geneticists, and add biological knowledge on a major class of compounds in lupins.

1. Introduction

Lupins (*Lupinus* spp.) are low cost and non-genetic modified legume seeds widely known for their high protein content and overall interesting nutritional value for human food and animal feeding. They provide 30–40% dietary protein, ca. 28% fiber, healthy fatty acids (e.g., linoleic and linolenic acids), vitamins and minerals (Sbihi et al., 2013).

In Europe, white lupins (WL; *Lupinus albus* L.), are usually consumed as snack food (whole seed) and, together with narrow-leafed lupins (NLL; *Lupinus angustifolius* L.), have been gaining interest also as food ingredients (flour) for the manufacture of bread, pasta, biscuits, gluten-free cakes or dairy products (Kohajdova et al., 2011; Villarino et al., 2015). Yellow lupins (YL; *Lupinus luteus* L.) are more appreciated by the intensive livestock industries (Sweetingham and Kingwell, 2008).

Besides nutrients, lupins contain several phytochemicals (e.g. polyphenols, carotenoids, alkaloids, phytosterols) that result from the plant secondary metabolism, being produced in response to diverse biotic and abiotic stresses (e.g. UV radiation, pathogens, herbivores). Phytochemicals are of pharmacological interest as they may positively impact humans and animals' health by providing therapeutic benefits; nonetheless, adverse effects on health are also associated to these compounds, limiting nutrients digestibility and bioavailability and inducing pathological changes in different organ tissues, with impacts on metabolism (Bernhoft, 2010; Khan et al., 2015).

Alkaloids are major phytochemicals in lupins that function as natural agrochemicals (Muzquiz et al., 1994b). However, they deserve extra attention: a safe consumption of lupins presupposes an alkaloid level in the seed as low as possible (Lucas et al., 2015). Acute toxicity of lupin alkaloids in humans comprises neurologi-

* Corresponding author.
E-mail address: pandrade@ff.up.pt (P.B. Andrade).

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cal, cardiovascular and gastrointestinal disturbances, children, in particular, being more sensitive (Koleva et al., 2012). In feedstuffs, lupins bitter taste, highly related to the seed alkaloids content (Dupont et al., 1994), may decrease diet palatability, affecting feed intake and body weight gain (Pastuszewska et al., 2001; Pilegaard and Gry, 2009). The safety limit fixed by the health authorities of UK, France, Australia and New Zealand for the total amount of alkaloids in lupin flours and derived products is of 0.2 g/kg dry matter (DM; Pilegaard and Gry, 2009).

Although alkaloids may be toxic when ingested at high concentrations, several biological properties were already described for rich-alkaloid lupin extracts, such as antimutagenic, antibacterial, antifungal and anticancer, a topic recently reviewed by Khan et al. (2015). As far as we are aware, the anti-inflammatory and antioxidant potential of these lupins secondary compounds has not been studied yet.

The present work aimed, firstly, at determining the alkaloids composition of several European lupin varieties of commercial interest by means of advisable chromatographic techniques. Furthermore, aiming to broaden the knowledge on the biological potential of these matrices, the anti-inflammatory and antioxidant potential of rich-alkaloid lupin extracts (at non-toxic concentrations for consumption) was also screened in a cell-free system, by evaluating the 5-lipoxygenase (LOX) inhibitory capacity and the nitric oxide radical (•NO) scavenging activity, respectively. In an attempt to relate the effect observed in the extracts with their composition in alkaloids, pure compounds were also evaluated individually.

As the 68th United Nations General Assembly declared 2016 as the International Year of Pulses (United Nations, 2014), we consider of interest to study a major group of phytochemicals in lupins from a nutritional and pharmacological perspective.

2. Material and methods

2.1. Materials and reagents

(-)-Sparteine (97%), angustifoline (>95%) and lupanine (>95%) were purchased from ChemFaces (Wuhan, Hubei, China). (-)-Lupinine (only for qualitative purposes, as indicated by the supplier) and quercetin were obtained from Extrasynthese (Lyon Nord, France). Gramine (99%), trichloroacetic acid, dichloromethane, the *n*-alkane series (C8-C40), potassium dihydrogen phosphate, sulphanilamide, lipoxidase from *Glycine max* (L.) Merr. (type V-S; EC 1.13.11.12), cis-9,12-octadecadienoic acid (≥99.0%) and ethanol were obtained from Sigma (St. Louis, MO). Sodium hydroxide was purchased from VWR (Radnor, PA). *N*-(1-Naphthyl)-ethylenediamine dihydrochloride was obtained from Acros Organics (Waltham, MA,). Sodium nitroprusside dihydrate (SNP) was from Riedel-de Haën (St. Louis, MO). Fosforic acid and di-sodium hydrogen phosphate dihydrate were acquired from Merck (Darmstadt, Germany). Water was treated in a Milli-Q (Millipore, Bedford, MA) water purification system.

2.2. Sampling

Eleven varieties (included in the European Plant Variety Database (PVD, 2015)) and one Portuguese ecotype of lupins, corresponding to mature raw seeds of 5 white lupins (*L. albus*), 2 narrow-leafed lupins (*L. angustifolius*) and 5 yellow lupins (*L. luteus*) (Table 1), were analyzed. Seeds were dried in a forced-air oven (65 °C, 24 h) and grounded (1 mm). Dry matter content of lupin flours was determined after drying the powdered samples at 103 °C overnight (AOAC, 2000).

Table 1
Characterization of the studied lupins samples.

Material	Sample code	Sample origin	Breeder country[a]
White lupin			
Amiga	WL-A	France	Czech Republic, France
Lumen	WL-L	France	France
Estoril	WL-E	Portugal	Portugal
Multitalia-PT	WL-M-PT	Portugal	Italy
Multitalia-IT	WL-M-IT	Italy	Italy
Yellow lupin			
Dukat	YL-D	Poland	Poland
Taper	YL-T	Poland	Poland
Mister-PT	YL-M-PT	Portugal	Poland
Mister-PL	YL-M-PL	Poland	Poland
Nacional	YL-N	Portugal	Portugal
Narrow-leafed lupin			
Azuro	NLL-A	Portugal	Denmark
Sonet	NLL-S	Poland	Denmark, Poland

[a] According to the European Plant Variety Database (PVD, 2015).

2.3. Alkaloids extraction

Alkaloids were extracted as previously described by Muzquiz et al. (1994a) and Gresta et al. (2010), with slight modifications. Briefly, 2.0 g of seed flour (1 mm) were added to 20 mL of trichloroacetic acid (5%, w/v), homogenized in a magnetic stirrer for 30 min at 400 rpm and then centrifuged at 4000 rpm for 15 min. The extraction procedure was repeated twice and the supernatants were decanted while the solid residue was discarded. 4 mL of 10 M sodium hydroxide were added to the supernatant. The alkaloids fraction was separated with dichloromethane (3 × 20 mL). The resulting dichloromethane extract was evaporated to dryness under reduced pressure (40 °C) and stored at −20 °C protected from light, until analysis. The yields (g extract/kg seed DM) obtained were 2.64 for WL var. Estoril (WL-E), 1.63 for WL var. Amiga (WL-A), 71.64 for WL var. Multitalia from Italy (WL-M-IT), 22.19 for WL var. Multitalia from Portugal (WL-M-PT), 8.59 for WL var. Lumen (WL-L), 23.09 for YL ecotype Nacional (YL-N), 0.37 for YL var. Mister from Portugal (YL-M-PT), 1.02 for YL var. Mister from Poland (YL-M-PL), 2.49 for YL var. Taper (YL-T), 0.29 for YL var. Dukat (YL-D), 33.11 for NLL var. Azuro (NLL-A) and 3.00 for NLL-Sonet (NLL-S).

2.4. GC-IT/MS qualitative analysis of alkaloids

Rich-alkaloids extracts were redissolved with dichloromethane, filtered (0.45 μm) and analyzed by GC-IT/MS. Stock solutions of alkaloids were prepared individually in dichloromethane, filtered (0.45 μm) and kept at −20 °C until analysis.

GC-IT/MS analysis was performed following a previously established method (Gresta et al., 2010). Samples extracts (1 μL) were analyzed using a Varian CP-3800 gas chromatograph (USA) equipped with a Varian Saturn 4000 ion trap mass detector (USA) and a Saturn GC–MS workstation software version 6.8. Analysis were carried out using a capillary column VF-5 ms (30 m × 0.25 mm × 0.25 μm) from Varian. The oven temperature was set at 150 °C for 1 min, then increased at a rate of 5 °C/min to 235 °C (held for 15 min). High purity helium C-60 (Gasin, Portugal) was the carrier gas at a constant flow rate of 1.0 mL/min. The injector port was heated to 240 °C and the injections performed in a split mode, with a ratio of 1/10. All mass spectra were acquired in electron impact (EI) mode. Ionization was maintained off during the first 3 min to avoid solvent overloading. The detection was performed using an Ion Trap detector set as follows: the transfer line, manifold and trap temperatures were 280, 50 and 180 °C, respectively. The mass range was 50–1000 *m*/*z*, with a scan rate of 6 scan/s. The emission current was 50 μA, and the electron multiplier was

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