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Protein isolates from two Mediterranean legumes: *Lathyrus clymenum* and *Lathyrus annuus*. Chemical composition, functional properties and protein characterisation

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ABSTRACT

Protein isolates were analysed from two Mediterranean legumes, *Lathyrus clymenum* and *L. annuus*. Protein isolates were prepared by alkaline extraction, including sodium sulphite and acid precipitation of *Lathyrus* proteins at their isoelectric point (pH 4.5). The percentage of proteins recovered from *L. annuus* and *L. clymenum* flours during the preparation of the protein isolates was around 60%. Chemical composition, nutritional parameters, main functional properties and protein composition of *Lathyrus* protein isolates were studied. *L. annuus* and *L. clymenum* protein isolates contained 81.07% and 82.4% of proteins, respectively, and they have a balanced content of essential amino acids, except for sulphur amino acids, with respect to the FAO pattern. The *in vitro* protein digestibility increased in the protein isolates to 93% and 95% in *L. annuus* and *L. clymenum*, respectively. Functional properties were similar to those observed in other legumes protein isolates. These results confirm the interest of local crops as sources of high value protein products obtained after convenient protein extraction procedures and the removal of antinutritional components. These high added value protein isolates are of interest for the food industry and for the revalorisation of *L. annuus* and *L. clymenum* favouring the bioconservation of *Lathyrus*.

1. Introduction

Plant protein isolates have increasing applications in the food industry as consumers concerns regarding animal food products increases. Protein isolates production is also a useful tool for the exploitation of proteins that otherwise could not be used in human nutrition. For example, plant protein isolates have been produced from industrial by-products such as defatted rapeseed (Vioque et al., 1999), or sunflower meal (Sánchez-Vioque, Clemente, Vioque, Bautista, & Millán, 1999; Villanueva et al., 1999). These proteins cannot be used directly for human nutrition, but after convenient extraction methods protein isolates may be produced and employed in different food products. Another example are pulses that cannot be directly consumed because they are damaged, of small size or hard to cook. These pulses may also be an important substrate for the generation of protein isolates (Sánchez-Vioque et al., 1999).

Legumes constitute a large family of plants, many of them cultivated. The nutritional value of legumes is related to the high protein, mineral and vitamin content of the seeds. Hence, legumes are consumed in many parts of the world as an essential component of the diet. The *Lathyrus* genus is a legume belonging to the tribe *Fabeae* and represents an ancient crop cultivated broadly in the past for human and animal consumption. Thus, *L. sativus* is probably the oldest crop cultivated in Europe (Kislev, 1989). Since the "green revolution" a large amount of the world's phytodiversity has been lost because agriculture has substituted local crops with others with higher yields and which are genetically uniform. Legumes in general and the genus *Lathyrus* in particular are not an exception to this problem and many of the cultivated species have seen their cultivated areas reduced dramatically in the last century. In spite of the potential interest of *Lathyrus* species as a source of high quality proteins, studies on their proteins are limited to the most cultivated species, such as *L. sativus* or *L. cicera*. In the present work, we have studied the production and characterisation of protein isolates from two *Lathyrus* species widely distributed in the Mediterranean Region. *L. annuus* has been locally cultivated for forage and weed, while *L. clymenum* is a wild specie not cultivated in the past.

These high added value protein isolates are of interest for the food industry and for the revalourisation of *L. annuus* and *L. clymenum* favouring the bioconservation of *Lathyrus*.

2. Materials and methods

2.1. Materials

Trypsin, chymotrypsin and peptidase were acquired from Sigma (Tres Cantos, Madrid, Spain). Diethyl ethoxymethylenemanolate

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was purchased from Fluka. All other chemicals were of analytical grade. The samples of *Lathyrus* seeds were taken from wild populations. Voucher specimens of the populations studied are deposited in the Herbarium of the Department of Plant Biology and Ecology of the University of Seville.

2.2. Preparation of protein isolates

2.2.1. Isolate A

Previously to alkaline protein extraction, *Lathyrus* flours were washed with methanol, ethanol and water at pH 4.5 to remove polyphenols and neurotoxic amino acids such as β-ODAP.

Lathyrus flours were suspended in 0.2% NaOH solution pH 12 in 1/10 (w/v) proportion, and extracted by stirring for 1 h. After centrifugation at 8000*g* and recovery of the supernatant, two additional extractions were carried out. The supernatants were pooled and analysed for nitrogen content. The pellet was dried in an oven at 50 °C, weighed and analysed for nitrogen content. The pH of the soluble proteins was adjusted to the isoelectric point (pH 4.5) and the precipitate formed was recovered by centrifugation at 8000*g*. The precipitate was washed with distilled water adjusted to pH 4.5 and freeze-dried.

2.2.2. Isolate B

Lathyrus flours were extracted as earlier with 0.2% NaOH solution but adding also 0.25% Na_2SO_3 at pH 10.5 to avoid the darkening of the final product.

2.3. Analytical methods

Moisture and ash contents were determined using AOAC (1999) 945.39 and 942.05 approved methods, respectively. Total nitrogen was determined by the microKjeldahl method according to AOAC (1999) 960.52 approved method. Crude protein content was estimated using a conversion factor of 6.25. Total fibre was determined according to the procedure described by Lee, Prosky, and De Vries (1992). Lipids associated with the flour and protein isolates were extracted and measured following the method of Nash, Eldridge, and Woolf (1967). Soluble sugars and polyphenols were measured using standard curves of glucose (Dubois, Gilles, Hamilton, Reber, & Smith, 1956) and chlorogenic acid (Moores, Demott, & Wood, 1948), respectively.

2.4. Determination of the isoelectric point (pI)

For the determination of the pI, 1.5 g of *Lathyrus* flour were extracted twice with 30 ml of 0.2% NaOH solution and centrifuged for 20 min at 10,000*g*. Aliquots (4 ml) of the supernatant were titrated with 0.5 N HCl to various pH values, ranging from 3 to 6. The precipitate formed was separated by centrifugation as above. The percentages of nitrogen in the supernatants in relation to the total nitrogen extracted were plotted vs. pH to determine the pI.

2.5. Determination of β-ODAP

β-ODAP was determined colourimetrically according to Hussain, Chowdhurry, Haque, Wouters, and Campbell (1994).

2.6. Amino acid analysis

Samples (10 mg) were hydrolysed with 4 ml of 6 N HCl. The solutions were sealed in tubes under nitrogen and incubated in an oven at 110 °C for 24 h. Amino acids were determined after derivatisation with diethyl ethoxymethylenemalonate by high-performance liquid chromatography (HPLC), according to the method of Alaiz, Navarro, Giron, and Vioque (1992), using D, L-α-aminobutyric acid as an internal standard.

2.7. In vitro protein digestibility (IVPD)

In vitro protein digestibility was determined according to the method of Hsu, Vavak, Satterlee, and Miller (1977).

2.8. Determination of nutritional parameters

The amino acid composition of *Lathyrus* flour and protein isolates was used for the determination of several nutritional parameters:

(a) Amino acid score (chemical score) was calculated as:
% sample essential amino acids contents/% recommended essential amino acids (FAO/WHO/UNU, 1985).

(b) Protein efficiency ratio values (PER) were calculated from the amino acid composition of *Lathyrus* seeds based on the following three equations (Alsmeyer, Cunningham, & Happich, 1974):

$$PER_1 = -0.684 + 0.456 \times Leu - 0.047 \times Pro$$
$$PER_2 = -0.468 + 0.454 \times Leu - 0.105 \times Tyr$$
$$PER_3 = -1.816 + 0.435 \times Met + 0.78 \times Leu + 0.211 \times Hys - 0.944 \times Tyr$$

(c) Protein digestibility corrected amino acid score (PDCAAS) (FAO/WHO, 1989) was calculated as:
Lowest individual amino acid score × IVPD.

(d) Predicted biological value (BV) was calculated according to Morup and Olesen (1976) using the following equation:

$$BV = 10^{2.15} \times Lys^{0.41} \times (Phe + Tyr)^{0.60} \times (Met + Cys)^{0.77} \times Thr^{2.4} \times Trp^{0.21}$$

where each amino acid symbol represents:

% amino acid/% amino acid FAO pattern (1985), if % amino acid $\leqslant$ % amino acid FAO pattern or:
% amino acid FAO pattern (1985)/% amino acid, if % amino acid $\geqslant$ % amino acid FAO pattern.

2.9. Protein determination

For SDS–PAGE, method described by Bradford (1976) was used for protein determination, using bovine serum albumin as standard, in *Lathyrus* protein extracts.

2.10. Gel filtration chromatography

Gel filtration was carried out in a AKTA purifier system equipped with a Superose 12 HR 10/30 column from GE as previously described (Lqari, Vioque, Pedroche, & Millán, 2002). The approximate molecular masses were determined using blue dextran 2000 (2000 kDa), β-amylase (200 kDa), bovine serum albumin (67 kDa) and ribonuclease A (13.7 kDa) as molecular weight standards. With these standards, the resulting calibration curve is:

$$V/Vo = 0.78 \log(MW) + 5.66$$

2.11. Electrophoresis

Lathyrus seed proteins from the flour and protein isolate B were extracted with 0.2% NaOH (1:10 w:v) by shacking the flour at 1000 rpm during 30 min at room temperature. Extracted proteins were recovered by centrifugation at 12,000 rpm during 15 min.