SUPPLEMENTARY MATERIAL

Essential and non-essential elements in white lupin (*Lupinus albus* L.) cultivated in Southern Italy

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Abstract

We assessed the presence of V, Cr, Ni, Cu, Zn, As, Se, Sb, Cd and Pb in white lupin samples cultivated in Southern Italy by the validation of an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) method. The ICP-MS method validation showed satisfactory values of linearity (r^2>0.999), recovery (87.4-100.7%), repeatability and reproducibility. Zinc was the most abundant element, showing mean concentrations of 0.778±0.09 mg/Kg wet weight (w.w.) and a maximum of 1.013 mg/Kg w.w., followed by copper (0.191±0.05 mg/Kg w.w.). Among the non-essential elements, important levels of cadmium were found (0.017±0.004 mg/Kg w.w.) with 28% exceeding the limits set by the EU Regulation. The results of this work confirm the role of white lupins and other legumes in reducing the pH of the soil, increasing the exchangeable forms of Cd. This work also provides first data on nutritional and antinutritional properties of white lupins cultivated in Italy.

Keywords: white lupin; legumes; essential elements; non-essential elements; ICP-MS

Experimental

Reagents and gases

Ultrapure deionized water was obtained by Milli-Q® Integral water purification system (Millipore, Bedford, MA, USA). Ultrapur nitric acid (60% V/V) was purchased from Merck KgaA (Darmstadt, Germany). The multielement calibration solutions were of ICP-MS grade and purchased from VWR International LTD (Randon, Pennsylvania, USA). A tuning solution for ICP-MS, capable of covering a wide range of masses (Ce, Co, Li,Mg, Tl and Y 1 µg/l) was purchased from Agilent Technologies, Santa Monica, CA, USA) to optimize the ICP-MS performances. Ultrapure carrier (Ar, 99.9995% pure) and dilution (He, 99.9995% pure; H_2, 99.9995% pure) gases were purchased from SOL S.P.A. (Monza, Italy).

Sample collection

Processed white lupins (soaked in salt water) were collected from local markets of Sicily (Southern Italy). The lupins were cultivated and processed in Southern Italy (Apulia region) as stated on the label. 25 pools of 100±0.5 g of white lupin samples from were collected and homogenised by a vertical mixer B-400 (Büchi, Flawil, Switzerland).

Samples extraction and essential and non-essential elements determination

The sample extraction for essential and non-essential elements detection was carried out by a digestion procedure using a Ultrawave digestor (Milestone, Sorisole, Italy) according to protocols reported before (Parrino et al. 2021; Cammilleri et al. 2022). The digestion conditions are listed in table S1. The samples digested were made up to a volume of 50 ml with Millipore deionised water until the ICP-MS analysis. V, Cr, Ni, Cu, Zn, As, Se, Sb, Cd and Pb levels were quantified by a 7700x series ICP-MS (Agilent Technologies, Santa Monica CA, USA). The operating conditions and instrumental settings are shown in table S2. The analysis were carried out on the basis of

calibration curves, constructed by the linear interpolation of at least 7 points corresponding to the readings of 7 standard solutions and white calibration, admitting a maximum error of 5% on the reading of the single standards and a correlation coefficient $r^2 > 0.999$. The repeatability limit was calculated by adding to 15 digested samples spiked 1.00 - 2.00 to 5.00 µg / L of all analytes. The repeatability and reproducibility within-laboratory was calculated according to Cammilleri et al. (2019); all the two values must be smaller than 2.

The validation of the analytical method allowed us to identify all the uncertainty contributions in order to calculate the expanded uncertainty.

Data analysis

The elements concentrations were expressed as mg/Kg wet weight (w.w.). All the concentrations below the LOQ of the method were considered as the LOQ values for the statistical analysis, according to Helsel (2005).

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Ph (Fase)	POWER (Watts)	RAMP (minuti)	HOLD (minuti)	FAN
1	450	10:00	40:00	1
2	0	0	25	3

Table S1. Microwave digestion conditions.

PARAMETER	VALUE
RF-Power (W)	1500
Carrier gas flow (mL/min)	2
Plasma gas flow (mL/min)	15
Auxiliary gas flow (mL/min)	1.0
Spray chamber	Water cooled double pass
Spray chamber temperature (°C)	2
Lens voltage	4.5
Mass resolution	0.7
Integration time points/ms	3
Points per peak	3
Replicates	4

Table S2. ICP-MS instrumental conditions.