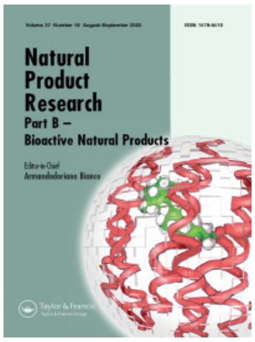

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Polyphenols of white lupin (*Lupinus albus* L.) seeds cultivated in Southern Italy by a LC-HRMS method

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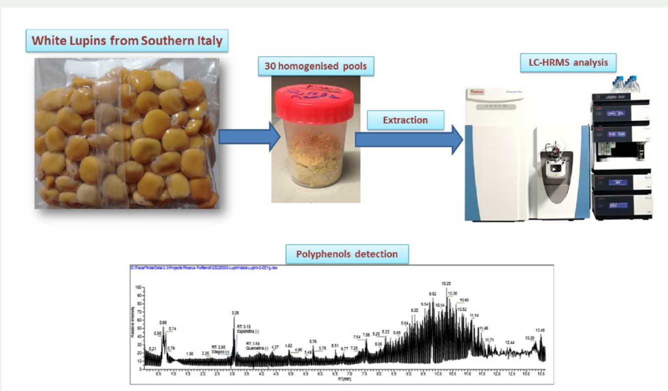
ABSTRACT

In this work we examined the contents of 14 polyphenols in white lupin (*Lupinus albus* L.) samples cultivated in Southern Italy by the optimisation and validation of a LC-HRMS method. The validation of the LC-HRMS method showed linearity results $r^2 > 0.989$ and recovery values between 71 and 119% for a very wide range of concentrations. Ellagic acid was the most abundant polyphenol, with mean concentrations of 16271.86 ± 19798.53 µg/Kg, followed by apigenin (2749.51 ± 889.95 µg/Kg). A significant variability in ellagic acid contents was found between the areas of cultivation examined ($p < 0.05$). As far as we know, this work provides the first data on the polyphenols contents of white lupins cultivated in Italy. The comparison with other study confirms the role of the cultivation area for the determination of the polyphenol's contents. The study also confirms white lupins as a promising source of antioxidant and anti-inflammatory substances in a balanced diet.



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1. Introduction

The chickpea is an annual herbaceous plant belonging to the *Fabaceae* family, which can be erected, semi-prostrated or prostrated, with a height that can reach 1 m. Among the *Lupinus* genus, white lupins (*Lupinus albus* L.) are the most consumed and produced in the world, with a high production in Europe (Huyghe 1997). The seeds of *Lupinus albus* have globular or angular-rostrate shapes; a smooth or rough surface and different colours (whitish, cream, green, red, brown, black).

The cultivation and production of this legume is still increasing, especially in Eastern Europe countries and Southern Italy, where it was previously used exclusively in crop rotation during intensive grain production (Fan et al. 2002). The seeds of this species are characterised by high protein content (average content of up to 36%) and also by the presence of many biologically active substances such as polyphenols and other phenolic compounds (Sujak et al. 2006; Siger et al. 2012).

Polyphenols are considered the major responsible of a wide range of biochemical activities in foods of plant and animal origin (Karamać et al. 2018; Lo Dico et al. 2019). There is a significant correlation between antioxidant activity, polyphenol content, and inhibition of human serum lipoprotein oxidation *in vitro* (Pérez-Gregorio et al. 2014; Calabrese et al. 2023).

The presence of polyphenols in lupin seeds has been reported in many studies. The most abundant phenolic compounds detected in lupin seeds belong to the subclasses of phenolic acids, flavones and isoflavones (Ranilla et al. 2009; Siger et al. 2012). However, the polyphenols contents of lupin seeds have not been fully explored, especially in those European countries which have a large production, such as Italy.

Given that the chemical composition of legumes and other vegetables may vary based on the cultivar, geographical belonging, and growth conditions (Zelalem and Chandravanshi 2014; Pantano et al. 2016; Cammilleri et al. 2022), the aim of this work was to evaluate the concentration of 14 polyphenol compounds in white lupins cultivated and commercialised in Italy by a LC-HRMS method to have information on the biologically active compounds responsible for health-promoting properties of white lupin seeds. This study has also the aim of verifying differences in polyphenols contents between areas of production.

2. Results and discussion

The results of the LC-HRMS method validation are shown in Table S3. The linearity test of the LC-HRMS method for all the analytes examined gave satisfactory results (r^2 > 0.993). The recovery studies showed values between 71% and 119%. All the results were satisfactory for the limit of repeatability. The mean contents of the phenolic compounds examined are shown in Table S4. None of the pools of samples analysed showed Gallic acid, Catechin, Syringic acid, Rutin, Ferulic acid, Naringenin and Kaempferol contents. Only 7 pools (23.33%) showed Chlorgenic acid contents, with a maximum value of 1547.73 μg/Kg. The mean contents of the phenolic compounds found followed the order Ellagic acid > Apigenin > Myricetin > Chlorgenic acid > Hesperidin > Quercetin > Caffeic acid. Ellagic acid was the most abundant compound, showing a high variability between pools of samples (16271.86 ± 19798.53 μg/Kg). The high variability was confirmed by the Kruskal-Wallis test, showing significant differences between areas of production ($p < 0.05$). Ellagic acid is a dimeric derivative of gallic acid that forms part of ellagitannins (polymeric molecules; (Larrosa et al. 2006)). Ellagitannins are the bioactive polyphenols present in pomegranate; however, they are not absorbed intact by the human gut, but they can be hydrolysed to ellagic acid by colonic gastrointestinal flora. It was proved that ellagic acid has anti-inflammatory effects in acute or chronic models of ulcerative colitis and is considered a promising agent for the treatment of chronic diseases such as Cronh's disease, Alzheimer's disease, and diabetes. Therefore, the data obtained in this study suggest a possible role of white lupins seeds as dietary supplements for the prevention of chronic diseases.

Conversely to ellagic acid, caffeic acid was the less abundant compound, with mean values of 441.41 ± 58.74 µg/Kg, significantly lower than what was found by Siger et al. (2012) in *L. luteus* seeds from Poland. The comparison with our results suggests a variability in the contents of phenolic acids in seeds between lupin species. Among the flavonoids, myricetin and quercetin were the most abundant, with very high values in 13.3% of the samples examined and maximum values of 4012.92 µg/Kg and 722.98 µg/Kg for myricetin and quercetin, respectively. The flavonoids found in the white lupin seeds could be modified quantitatively and qualitatively by the germination, with a significant increase during this process (Siger et al. 2012).

No kaempferol was detected in all the samples examined, in accordance with studies reported before (Vollmannova et al. 2021). Caffeic acid was present among hydroxycinnamic acids in the *L. albus* seeds examined in this work, in contrast to what was found in *L. albus* seeds from Turkey (Karamać et al. 2018). Nevertheless, it should be noted that most of the study reported in literature used a method based on the Folin–Ciocalteu protocol; this assay has several disadvantages, including a low specificity for phenols, a lack of relevance to biological oxidative processes and interferences with other compounds. It measures the reducing capacity of phenols or other reducing agents present in samples that can react with the Folin–Ciocalteu reagent. Furthermore, the high amount of alkaloids present in this plant may also have an appreciable effect on the results obtained. The processing by soaking to decrease alkaloids of the quinolizidine group could improve the reliability of the results but it decreases the nutritive value, because all the water-soluble compounds are often lost with the steeping medium and rinsing procedure (Zelalem and Chandravanshi 2014).

In white lupins, the polyphenols are primarily found in the seeds (Siger et al. 2012; Karamać et al. 2018). Some of the specific polyphenols found in this work include flavonoids, such as quercetin and myricetin, phenolic acids, such as chlorogenic acid and caffeic acid, can bind to proteins and other compounds in the body. In addition, polyphenols from white lupins have been shown to have anti-inflammatory effects. In a study published in the Journal of Agricultural and Food Chemistry, researchers found that a polyphenol-rich extract from white lupin seeds was able to reduce inflammation in human colon cells (Khan et al. 2015).

As far as we know, this is the first report on the detection of polyphenols in white lupin by the application of a LC-HRMS method, suggesting that further studies based on the application of reliable procedures are needed to have a comprehensive evaluation on the nutritional properties of this important legumes. Overall, while more studies are needed to fully understand the health benefits of polyphenols from white lupins, there is evidence to suggest that they may have antioxidant, anti-inflammatory, and cardiovascular benefits. Incorporating white lupin seeds or flour into your diet may be a way to increase the intake of these beneficial phytochemicals.

3. Conclusions

As far as we know, this was the first report on the determination of polyphenols in white lupin cultivated in Southern Italy. The method carried out provided satisfactory results in a wide range of concentrations. Ellagic acid was the most abundant polyphenol in the white lupin seed samples analysed suggesting a possible role of this product in the prevention of pancreatitis, as confirmed by Suzuki et al. (2009).

4. Experimental

See the Experimental section in the supplementary files

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Disclosure statement

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