



EVALUATION OF TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT CAPACITY OF DIFFERENT VARIETY LUPIN SEEDS

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ABSTRACT

Legumes, including lupins, beans, lentil and chickpea, are one of the most important crops in the world because of their nutritional quality. Lupin seeds have been used as human food and animal feed since ancient times. It was known that antioxidant photochemical in foods have many health benefits including prevention of various diseases associated with oxidative stress such as cancer, cardiovascular disease, neuro-degeneration and diabetes. Lupin grains are rich sources of complex carbohydrates, protein, vitamins and minerals. Antioxidants can be found naturally in foods. Total polyphenols content and antioxidant activity were measured in four varieties of lupin, namely in white lupin, blue lupin, yellow lupin and Mutabilis lupin species. A majority of antioxidants naturally present in foods occur in phenolic structures and especially in flavonoid structures. The content of the total polyphenols was determined by using the Folin-Ciocalteu reagent (FCR). Antioxidant activity was measured by using a compound DPPH[•] (2,2-diphenyl-1-picrylhydrazyl). In the present experiment according to the average contents of total polyphenols (TPC) in dry matter of lupin seeds there was the following line: *L. Angustifolius* (blue) lupin (696.212 mg GAE.100g⁻¹) > *L. Albus* (white) lupin (614.13 mg GAE.100g⁻¹) > *L. Luteus* (yellow) lupin (467.78 mg GAE.100g⁻¹) > *L. Mutabilis* (pearl) lupin (367.36 mg GAE.100g⁻¹). Based on the measured values of total antioxidant capacity (TAC) of lupin samples can be classified as follows: *L. Albus* (white) lupin (43.44%) > *L. Angustifolius* (blue) lupin (38.27%) > *L. Luteus* (yellow) lupin (22.29%) > *L. Mutabilis* (Pearl) lupin (20.80%). The relationship of antioxidant capacity with total polyphenolic was discussed. According to used statistical analyzes. Correlation between the phenolic contents and antioxidant capacity was significantly positive ($r = 0.88$). Our results confirmed that legumes can be a good source of bioactive compounds in the human nutrition. The main objective of the present work was to evaluate the content of total polyphenols and an antioxidant capacity of four Lupine species.

Keywords: total polyphenols; antioxidant capacity; variety lupine seeds

INTRODUCTION

Legumes used by humans are commonly called food legumes or grain legumes. The food legumes can be divided into two groups, the pulses and the oilseeds. Grain legumes can offer ecosystem services such as renewable N input, the improvement of soil fertility and the diversification of cropping systems, Jensen et al. (2012). Animal proteins being more expensive, especially people in developing countries depend largely on plant to fulfill their protein requirements. Moreover, it is also a good source of minerals (Kirmizi and Guleryuz, 2007). Leguminous seeds present one of the most promising alternative protein sources for the nutritional supplementation and technological improvement of traditional foods (Martinez-Villaluenga et al., 2009). Lupin is a representative of legume family which includes over 450 species are known, from which only four are of agronomic interest Reinhard et al. (2006). *L. Albus* L.: white lupin, *L. Angustifolius* L.: blue or narrow-leafed

lupin, *L. Luteus* L.: yellow lupin and *L. Mutabilis* L.: pearl or Tarrwi lupin (Reinhard et al., 2006; Uzun et al., 2007). The first three species originate from the Mediterranean area, including Turkey, while *L. Mutabilis* belongs to South America (Mulayim et al., 2002). *Lupinus Albus* native to Mediterranean area is agriculturally important (Kirmizi and Guleryuz, 2007). white lupine and spring wheat or spring triticale, was most successful in yield and protein content. Also, harvest dates are as crucial as seeding rates for lupin/cereal forage because time of harvest determines the stage of maturity and therefore, forage quality. Harvesting between 116 – 130 days is recommended (Azo et al., 2012). Lupin seeds have been used as human food and animal feed since ancient times. White lupine can be a phosphorus efficient plant and could help reduce the need for P fertilizer and enhance yields. It forms cluster roots in response to phosphorus starvation (Cheng et al., 2011). The bitter seeds contain the quinolizidine alkaloids lupanine and

sparteine. The presence of these alkaloids limits the use of lupine seed as food and feed. Human consumption of lupines has increased in recent years (Kohajdová et al., 2011). Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxynitrite. Reactive oxygen species generated in tissues and cells can damage DNA, proteins, carbohydrates and lipids (Gill and Tuteja, 2010). Lupin is an economically and agriculturally valuable plant (Sujak et al., 2006; Gulewicz et al., 2008). Lupin grains are particularly rich in dietary fibre, as they store beta-galactan rather than oil or starch. Lupin fibre and lupin protein, mostly from blue lupin, are being developed as functional food additives in Australia. In spite of these benefits and in the context of agricultural intensification, the cultivation of legume crops has gone through a long decline in Europe, leading to a dependence on protein imports (Stoddard et al., 2009). Lupin grains are rich sources of complex carbohydrates, protein, vitamins and minerals. A majority of antioxidants naturally present in foods occur in phenolic structures and especially in flavonoid structures. In addition to fiber, legume grains also contain many substances to improve health such as vitamins, minerals, and other substances, including phenolic compounds (Afshin et al., 2014). Phenolic compounds are resistant to oxidation and protect cell damage to prevent the risk of degenerative diseases thanks to antioxidative, anti-inflammatory, antiallergic and anticarcinogenic activities. (Nderitu et al., 2014; Xu et al., 2009). Cholesterol-free legumes in combination with their low sodium content form a good food stuff not only for people living in developing countries but also for those living in industrialized nations (Sebastia et al., 2001). As a protein source, they are obtained cheaply compared to animal protein sources. Moreover; they are fairly good sources of phytochemicals with antioxidant capacity such as polyphenols, mainly tannins and flavonoids (Zielinska et al. 2008; Martinez-Villalunga et al. 2009). White lupine seeds are generally classified as sweet or bitter depending on the alkaloid content, which ranges from 0.01 to 4% (Bhardwaj and Hamama, 2012). It is an interesting grain legume due to its low alkaloid lines and higher protein content (34 – 45 %), similar to that of soybean (Annicchiarico, 2008; Laudadio and Tufarelli, 2011). Lupine is commonly consumed as a snack in the Middle East and is coming into use as a high-protein soy substitute in the other parts of the world (Kurzbaum et al., 2008). Moreover, white lupine has a higher level of essential amino acids and important dietary minerals (iron and potassium) compared with other rainfed legumes such as pea, faba bean and narrow leafed lupine (*L. Angustifolius* L.), which are useful as innovative ingredients of functional or healthy food products (Annicchiarico et al., 2014; Chiofalo et al., 2012). *Lupinus Mutabilis* is currently under development in Western Australia due to its high protein and oil contents (Clements et al., 2008). Lupin-derived protein ingredients have to provide both adequate nutritional and useful technological functionality to the foods in which they are incorporated in order to meet the needs of consumers and the food industry (Zraly et al., 2008). Lupin is a good source of nutrients, not only proteins but lipids, dietary

fibre, minerals and vitamins (Martínez-Villaluenga et al., 2006). However, although lupin seed is one of the legumes with the lowest levels of non-nutritional compounds (trypsin inhibitors, phytic acid, saponins and lectins) Martínez-Villaluenga et al. (2006), it contains large amounts of α -galactosides (7 – 15%) that are associated with negative physiological functions when consumed in high amounts, as recently reviewed by Martínez-Villaluenga et al. (2008). In contrast to other leguminous plants such as peas, soy beans, *Lupinus Albus* contain extremely low amounts of trypsin inhibitors, lectins, isoflavones, saponins and cyanogens (Joray et al., 2007, Zralý et al., 2007). Lupin have traditionally been used for animal feed but are gaining recognition as a health food for humans, due to their unique dietary composition. Total phenolics and antioxidant activities of grains and germinated grains have been widely investigated. Germination produced high phenolic content and consequently increased the antioxidant activities of lupine seeds. Legumes have been shown to help manage both cholesterol and blood glucose (Bazzano et al., 2011; Sevenpiper et al., 2009). Increased intakes are linked to the prevention of heart disease, diabetes and some cancers (Bazzano et al., 2001; Hwang et al., 2009). It is found that phytochemicals from legumes may be responsible effects (Campost-Vega et al., 2010). The interest in natural antioxidants has increased considerably in recent years because of their beneficial effects of prevention and risk reduction in several diseases. Due to low glycemic index of lupin seeds, it was found that lupin kernel fibres have appetite suppression (Archer et al., 2004) and cholesterol lowering properties, that they lower blood glucose and insulin levels (Hall et al., 2005). The profiles and quantities of polyphenols and tannins in legumes are affected by processing due to their highly reactive tances, which are considered to be natural antioxidants (Rybarczyk and Amarowicz, 2007; Dueñas et al., 2009) nature, which may affect their anti-oxidant activity and the nutritional value of foods (Dlamini et al., 2009). The quantitative determination of phenolic compound content as well as their antioxidant capacity may provide valuable information in considering health-promoting properties of lupin seeds. Lupin does not contain gluten, thus it is sometimes used as a functional ingredient in gluten free foods (Scarafoni et al., 2009). There is potential risk of lupine allergy appear (Zielinska et al., 2008). But some dietary fiber constituents such as inulin are also believed to improve minerals absorption, for example to enhance uptake of calcium by altering the pH value in the colon (Chawla and Patil, 2010). A new research has shown that the choice of carbohydrate consumed, not just the amount, also has an impact on blood glucose levels (McGonigal and Kapustin, 2008). Moreover, there are data suggesting that consuming a high fibre diet (50 g fibre.day⁻¹) reduces glycemia in subjects with type 1 diabetes and glycemia, hyperinsulinemia, and lipemia in subjects with type 2 diabetes (American Diabetes Association, 2008). The phenolic content and composition of *L. Angustifolius*, despite its weak antioxidant capacity, may have positive implications for reducing the risk of cardiovascular disease due to its protective effects on blood vessel health (Oomah et al., 2006). Moreover, lupin seeds also contain constituents that alter satiety and other features of human

health with the prospect of pharmaceutical potential (Lee et al., 2006; Duranti et al., 2008). In general, the human body has its own natural antioxidant system to stand against free radicals using certain enzymes. Antioxidants can be found naturally in foods (Kedare and Singh, 2011). A majority of antioxidants naturally present in foods occur in phenolic structures and especially in flavonoid structures. Sweet lupins, such as those grown in Western Australia, have low levels of alkaloids (Australia New Zealand food authority, 2001). In comparison with the bitter one, sweet *Lupinus Albus* has lesser amount of anti-nutritional factors, particularly quinolizidine alkaloids (Zralý et al., 2007). These sweet varieties have been obtained through breeding programmes. These varieties have advantages of having low alkaloid content but they are also less resistant to disease and herbivore attack (Sanchez et al., 2004).

MATERIAL AND METHODOLOGY

Material: Lupin Samples were collected from the market, but information such as the variety, were not mentioned, four species of lupin seeds (*L. Albus* (white), *L. Angustifolius* (blue), *L. Luteus* (yellow), *L. Mutabilis* (pearl)) were purchased.

Chemicals and reagents: Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Sodium carbonate (Na_2CO_3), Methanol(CH_3OH).

Extraction: For 12 hours extraction, dry material (5 g) was used and continuously extracted by a Twisselmann extractor with methanol (80%, v/v).

Determination of total polyphenols

Total polyphenols were determined by the method of Lachman et al. (2003) and expressed as mg of gallic acid equivalent per 100 g dry mater. Gallic acid is usually used as a standard unit for phenolics content determination because a wide spectrum of phenolic compounds. The total polyphenol content was estimated using Folin-Ciocalteu assay, Folin-Ciocalteu reagent was added to the extract solutions. After 4 min, 5 mL of Na_2CO_3 (20%) was added, the volume was adjusted to 50 mL by adding distilled water and the mixture was stored at room temperature for 2 h, the absorbance values was measured at 765 nm of wavelength against methanol blank on the spectrophotometer Shimadzu 710 (Japan). The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

Determination of antioxidant activity

Antioxidant activity was measured by the Brand et al. (1995) method-using a compound DPPH[•] (2,2-diphenyl-1-picrylhydrazyl). 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) was pipetted to cuvette (3.9 cm³) then the value of absorbance, which corresponded to the initial concentration of DPPH[•] solution in time A_0 was written. Then 0.1 cm of the lupin extracted was added. Solution in the cuvette was mixed and then was immediately started to measure the dependence $A = f(t)$. The absorbance after 10 minutes was measured at 515 nm in the spectrophotometer Shimadzu UV/VIS-1240 was mixed and measured. The percentage of inhibition

reflects how antioxidant compound are able to remove DPPH[•] radical at the given time.

$$\text{Inhibition (\%)} = (A_0 - A_t / A_0) \times 100$$

Statistical analysis

Results were statistically evaluated by the Analysis of Variance. All the assays were carried out in triplicates and results are expressed as mean \pm SD. The data were subjected to the F-test in the one-way analysis of variance (ANOVA) If the p-value of the F-test is less than 0.05, there is a statistically significant difference between the means at the 95% confidence level; the Multiple Range Tests will tell which means are significantly different from which others. The method currently being used to discriminate among the means of Fisher's least significant difference (LSD) procedure. Analysis was conducted using SAS software 9.4.

RESULTS AND DISCUSSION

On the base of reached results there were estimated changes in the total polyphenols content and also changes in total antioxidant capacity values in dependence on varieties of lupin seeds.

Evaluation of total polyphenol content and values of antioxidant capacity in lupin Species

Phenols are compounds that have the ability to destroy radicals because they contain hydroxyl groups. These important plant components give up hydrogen atoms from their hydroxyl groups to radicals and form stable phenoxyl radicals; hence, they play an important role in antioxidant activity. Therefore, determination of the quantity of phenolic compounds is very important in order to determine the antioxidant capacity of plant extracts (Das and Pereira, 1990). Following the total polyphenol content parameter in lupin species (*L. Albus* L.: white lupin, *L. Angustifolius* L.: blue or narrow-leaved lupin, *L. Luteus* L.: yellow lupin and *L. Mutabilis* L.: pearl or Tarrwi lupin). (Table 1 and Table 2; Figure 1 and Figure 2). According to the obtained results, the polyphenols content (TPC) in the tested was significantly different, and varied with the species, it was detected, that polyphenols content in samples ranges from 367.36 \pm 35.95 mg GAE.100g⁻¹ (in species *L. Mutabilis*) to 696.212 \pm 24.44 mg GAE.100g⁻¹ (in species *L. Angustifolius*). Statistically significant highest value of total polyphenols was recorded in species (*L. Angustifolius*). Statistically significant the lowest content of total polyphenols was recorded in species (*L. Mutabilis*). According to the average contents of total polyphenols in dry matter of lupin there is the following line in present work: *L. Angustifolius* (blue) lupin (696.212 mg GAE.100g⁻¹) > *L. Albus* (white) lupin (614.13 mg GAE.100g⁻¹) > *L. Luteus* (yellow) lupin (467.78 mg GAE.100g⁻¹) > *L. Mutabilis* (pearl) lupin (367.36 mg GAE.100g⁻¹). As shown in (Table 2; Figure 2.), there were statistically significance differences in total polyphenol (TPC) content within lupin species. (Wang and Clements, 2008) were found the highest value of total phenolic content in variety *L. Mutabilis* P28725 (2660.4 mg GAE.100g⁻¹) and the two varieties of

Table 1 Total phenolic content and antioxidant capacity (mean and standard deviation values). In chosen species of lupin.

Species	Colors	TAC %	TPC (mg GAE.100g ⁻¹)
<i>L. Albus</i>	white	43.4363 ±1.05 a	614.13 ±50.40 a
<i>L. Angustifolius</i>	blue	38.2685 ±0.74 b	696.212 ±24.44 b
<i>L. Luteus</i>	yellow	22.2919 ±55.30c	467.78 ±60.26 c
<i>L. Mutabilis</i>	pearl	20.7950 ±0.24 d	367.36 ±35.95 d

Note: Data expressed as means of six replications ±standard deviation. Values in the same column with different letters present significant differences $p < 0.05$ using F-test for independent samples.

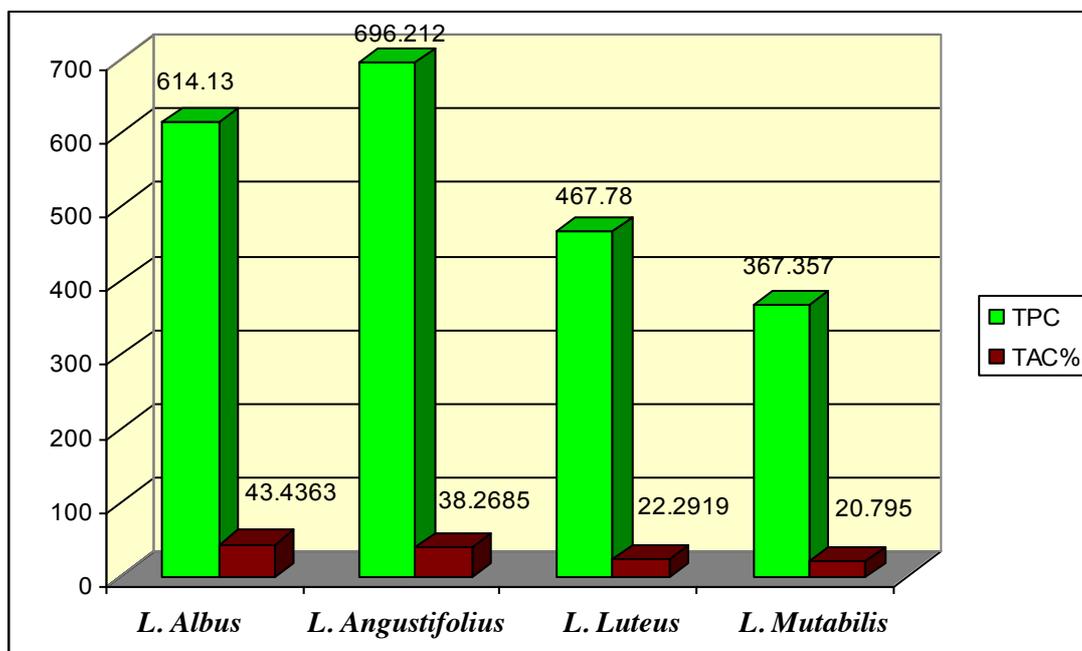


Figure 1 Average content of total polyphenols TPC (mg GAE.100g⁻¹) and Average content of total antioxidant capacity TAC (%) in chosen four species of lupin seeds.

L. Luteus were contained the lowest total phenolic (369.2, 374.4 mg GAE.100g⁻¹). In *L. Albus*. Etho 66, was 1661.2 mg GAE.100g⁻¹, in Andromeda variety was 451.6 mg GAE.100g⁻¹, in unknown variety name was 444.4 mg GAE.100g⁻¹. In comparison to determined values of polyphenols their results were lower value than present result in *L. Albus* with (614.13 mg GAE.100g⁻¹) with exception Etho 66 variety with 1661.2 mg GAE.100g⁻¹. Also the polyphenols content in *L. Angustifolius* in present result with the value (696.212 mg GAE.100g⁻¹) higher than their results with (553.4, 578.4, 578.0, 535.1 mg GAE.100g⁻¹). Our result in *L. Luteus* with (467.78 mg GAE.100g⁻¹) higher than their results with (374.4, 369.2 mg GAE.100g⁻¹), but in *L. Mutabilis* their results with (799.1, 2660.4 mg GAE.100g⁻¹) were higher than present result with (367.36 mg GAE.100g⁻¹). **Siger et al. (2012)**, reported the highest values of total polyphenols in *L. Luteus*, Parys Cultivar with (317.88 ±2.69 mg GAE.100g⁻¹ d.m), followed by *L. Albus*, Boros Cultivar with (271.25 ±3.75 mg GAE.100g⁻¹ d.m) and *L. Angustifolius* Bojar Cultivar with (269.72 ±9.97mg GAE.100g⁻¹ d.m). When we comperesen present result, in *L. Luteus*

unknown Cultivar with (467.78 mg GAE.100g⁻¹ d.m) in *L. Albus* with (614.13 ±50.40 mg GAE.100g⁻¹ d.m), in *L. Angustifolius* with (696.212 ±24.44 mg GAE.100g⁻¹ d.m) their results were lower value of phenolic contents than the present result and present result higher value phenolic contents than the result of **Wang and Clements (2008)**, in both Cultivar of *L. latus* with (374.4 mg CAE.100g⁻¹, 369.2 mg CAE.100g⁻¹). **Ahmed (2014)** determined the content of total polyphenols in lupine flour with [(138.17 ±8.35 µg GAE.g⁻¹ d.w) which equal to 1381.7 ±8.35 mg CAE.100g⁻¹]. Also **Walaa et al. (2015)** determined the content of total polyphenols in lupine flour with [(136 ±8.33 µg GAE.g⁻¹ d.w) which equal to 1360 ±8.33 mg CAE.100g⁻¹]. In comparison to our determined values of total polyphenols their results were higher than our results value of polyphenols content in all species of lupin seeds in present result. **Gamal and Ismail (2013)** determined the content of total polyphenols in Lupinustermis seeds with [(14.23 ±1.38 µg GAE.g⁻¹ d.m) which equal to 142.3 ±8.35 mg CAE.100g⁻¹], their result was lower than present results.

Table 2 Total phenolic content and antioxidant capacity (mean and standard deviation values with the group). In chosen four species of lupin seeds.

species	TAC%			TPC (mg GAE.100g ⁻¹)		
	Mean	Std dev	Group	Mean	Std dev	Group
<i>L. Albus</i>	43.44	1.05	a	614.13	50.40	a
<i>L. Angustifolius</i>	38.27	0.74	b	696.21	24.44	b
<i>L. Luteus</i>	22.29	0.55	c	476.78	60.26	c
<i>L. Mutabilis</i>	20.80	0.24	d	367.36	35.95	d

Note: All the assays were carried out in triplicates and results are expressed as mean ±SD. The data were subjected to one-way analysis of variance (ANOVA) and the differences between various concentrations were determined Fisher LSD test using SAS software.

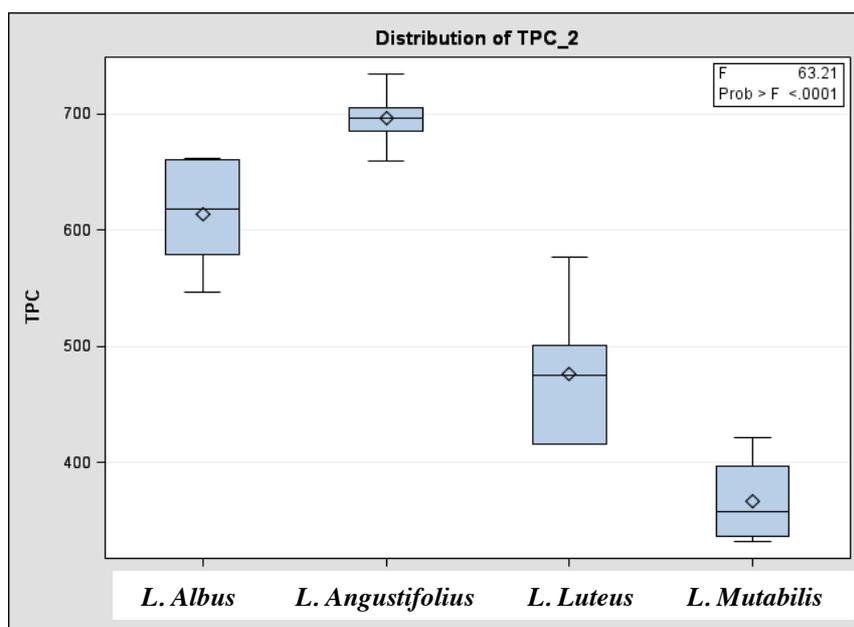
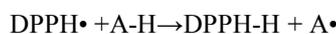


Figure 2 Box-plot of the content of total polyphenols TPC (mg GAE.100g⁻¹) in four species of lupin.

Statistical evaluation of antioxidant capacity(TAC) differences significance within the frame of chosen species

Another indicator that has been evaluated and compared was the changes in total antioxidant capacity values in lupin seeds. This method is based on decreased absorbance of DPPH radical read at 515 nm, due to the action of antioxidants. A 0.1 mL volume of the sample was added to 3.9 mL of DPPH• dissolved in methanolic solution and carefully homogenized. Free radical was freshly prepared before each assay while protected from light. The DPPH method is a preferred method because it is fast, easy and reliable and does not require a special reaction and device. The free radical scavenging activities of extracts depend on the ability of antioxidant compounds to lose hydrogen and the structural conformation of these components (Shimada et al., 1992). The DPPH free radical, which is at its maximum wavelength at 517 nm, can easily receive an electron or hydrogen from antioxidant molecules to become a stable diamagnetic molecule (Soares et al., 1997). Owing to the DPPH radical's ability to bind H, it is considered to have a radical scavenging property. A solution of DPPH radicals prepared in methanol is converted into DPPH-H (diphenylhydrazine) molecules in the presence of an

antioxidant agent, as shown in the following equation. Discoloration occurs due to the decreasing quantity of DPPH radicals in the environment. The discoloration of the DPPH therefore reflects the radical scavenging activity of the analyzed extract (Guo et al., 2007). The method is based on the reduction of alcoholic DPPH• solutions in the presence of a hydrogen donating antioxidant (AH) to the non radical form DPPH-H.



In the present work as shown in (Table 1, 2; Figure 1, 3), it was found that, antioxidant capacity in samples ranges from 20.795% to 43.4363%. Statistically the highest value of antioxidant capacity (43.4363%) was recorded in *L. Albus* species (white color). The lowest value of antioxidant capacity (20.795%) was recorded in *L. Mutabilis* species (pearl color). In the case of *L. Angustifolius* (blue color) was (38.2685%), and (22.2919%) for Luteus species (yellow color). Ahmed (2014) published that the value of antioxidant capacity in lupine flour was (20.26 ±1.22). Gamal and Ismail (2013) determined that antioxidant activity by DPPH (Inhibition %) was (19.21 ±0.345%) in lupin seeds, (*Lupinus termis* L.).

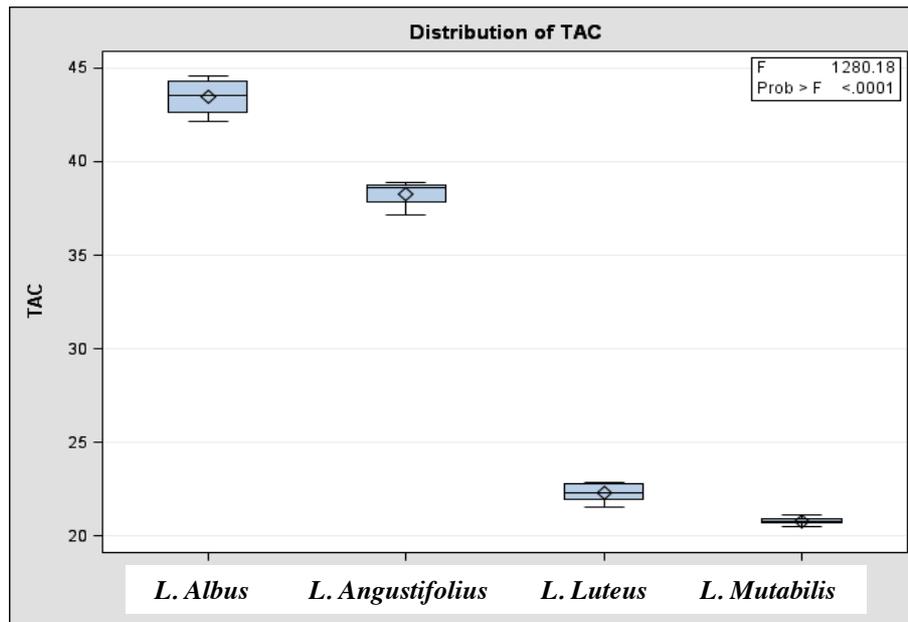


Figure 3 Box-plot of the content of total antioxidant capacity TAC (%) in four species of lupin.

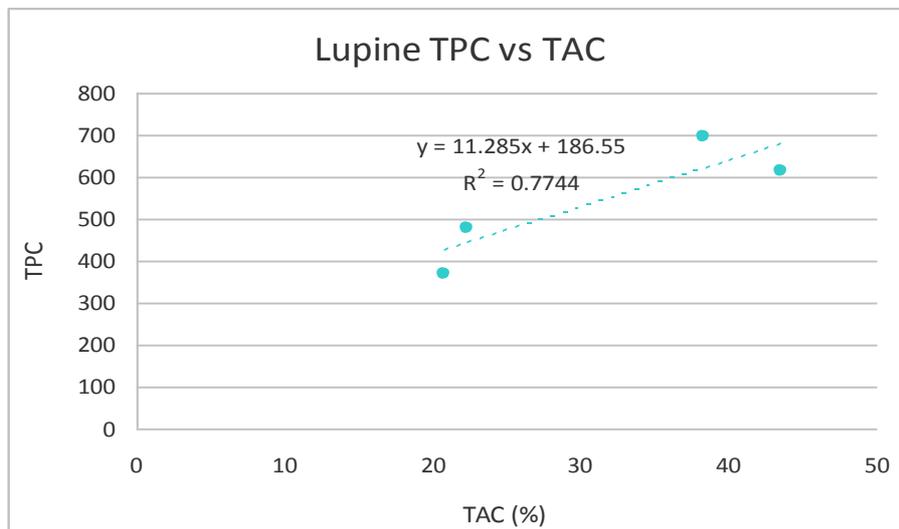


Figure 4 Correlation between TP and TAC of four species of lupin.

Walaa et al. (2015) published that the value of antioxidant capacity in sweet lupine flour was (20.7 ± 1.22%). The results by those authors in comparison to our measured values in *L. Mutabilis* species (pearl color), their results were similar to our result. (Wang and Clements, 2008) determined that antioxidant activity in *L. Luteus* unknown variety name was (0.217 mg Trolox Eq.g⁻¹), and in *L. Angustifolius* unknown variety name was (0.424 mg Trolox Eq.g⁻¹), in two varieties of *L. Albus* were (0.153 mg Trolox Eq.g⁻¹) In comparison to our measured values of antioxidant activity their result was similar to the present value in *L. Luteus* with (22.29%) and present result in *L. Albus* with (43.44%) higher than their results in *L. Albus* but present result in *L. Angustifolius* with (38.27%) lower than their result in *L. Angustifolius* with (0.424 mg Trolox Eq.g⁻¹).

Correlation between the total antioxidant activity values and total phenolics contents

ANOVA linear correlation coefficients were used to assess the relationships between TPC and TAC, Correlation: Our result confirmed a strong statically correlations between total polyphenol content and total antioxidant capacity values. A statistically strongly significant correlation (R = 0.88; p < 0.05) was found (Figure 4). Wang and Clements (2008) found that the free radical scavenging activity is not well correlated with total phenolic contents (R = 0.204) in different species and varieties of lupin seeds. Amarowicz et al. (2005) analyzed the extracts of fababean, broad bean, adzuki bean, red bean, pea, red lentil and green lentil seeds using 80% (v/v) acetone and confirmed a statistically significant correlation between the total antioxidant activity values and total phenolics (p = 0.01). A strong correlation between total polyphenol content and antioxidant activity (R = 0.86; p < 0.05) was observed also by Akond et al. (2011) in common bean and a statistically strongly significant correlation (p-value 2.391E-06; R = 0.802) was found between total

polyphenol content and total antioxidant capacity values by Dalaram et al., (2013) in lentil cultivar. A strong correlation between total polyphenol content and antioxidant activity ($R = 0.783645$; $p < 0.05$) was observed by Dalaram (2015) in different cowpea species and varieties. According these authors this finding suggests that total polyphenolic content is a good predictor of in vitro antioxidant activity.

CONCLUSION

In this study we compared the total phenolic content and antioxidant capacity of (four species lupin seeds). The correlation between total phenol content and antioxidant activity was good, suggesting that phenolic compounds are the most responsible compounds contributing to antioxidant activity of investigated samples despite the *L. Angustifolius* with a higher (TPC) content than *L. Albus* (TPC) content, recorded the lower antioxidant capacity (TAC%). It may be due to the phenolic compound structure and particularly hydroxyl position in the molecule determine antioxidant activity or on the ability to donate hydrogen or electron to a free radical. The positive interrelationship between these two parameters demonstrates that the antioxidant activity depends mainly on polyphenols contents.

REFERENCES

- Abdelrahman R. A. 2014. Influence of Chemical Properties of Wheat-Lupine Flour Blends on Cake Quality. *American Journal of Food Science and Technology*, vol. 2, no. 2, p. 67-75. <https://doi.org/10.12691/ajfst-2-2-4>
- Afshin, A., Micha, R., Khatibzadeh, S., Mozaffarian, D. 2014. Consumption of nuts and legumes and risk of incident ischemic heart disease, stroke, and diabetes: A systematic review and meta-analysis. *The American Journal of Clinical Nutrition*, vol. 100, no. 1, p. 278-288. <https://doi.org/10.3945/ajcn.113.076901> PMID:24898241
- Akond, G. M. A. S. M., Khandaker, L., Berthold, J., Gates, L., Peters, K., Delong, H., Hossain, K. 2011. Anthocyanin, total polyphenols and antioxidant activity of common bean. *American Journal of Food Technology*, vol. 6, no. 5, p. 385-394. <https://doi.org/10.3923/ajft.2011.385.394>
- Amarowicz, R., Troszynska, A., Barylko-Pikielna, N., Shahidi, F. 2004. Polyphenolics extracts from legume seeds: correlations between total antioxidant activity, total phenolics content, tannins content and astringency. *Journal of Food Lipids*, vol. 11, no. 4, p. 278-286. <https://doi.org/10.1111/j.1745-4522.2004.01143.x>
- Amarowicz, R., Troszyńska, A., Barylko-Pikielna, N., Shahidi, F. 2005. Polyphenolics extracts from legume seeds: Correlations between total antioxidant activity, total phenolics content, tannins content and astringency. *Journal of Food Lipids*, vol. 11, no. 4, p. 278-286. <https://doi.org/10.1111/j.1745-4522.2004.01143.x>
- American Diabetes Association. 2008. Nutrition recommendations and interventions for diabetes. *Diabetes Care*, vol. 31, no. suppl. 1, p. S61-S78.
- Aniess, W. I. M., Khalil, A. F., Mosa, Z. M. 2015. Phenolic compounds and antioxidants capacity of sweet lupine derivatives-wheat Flour mixtures and the effects on diabetic rats. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, vol. 9, no. 5, p. 61-69.
- Annicchiarico, P. 2008. Adaptation of cool-season grain legume species across climatically contrasting environments of southern Europe. *Agronomy Journal*, vol. 100, p. 1647-1654. <https://doi.org/10.2134/agronj2008.0085>
- Annicchiarico, P., Manunza, P., Arnoldi, A., Boschin, G. 2014. Quality of *Lupinus Albus L.* (white lupin) seed: extent of genotypic and environmental effects. *Journal of Agricultural and Food Chemistry*, vol. 62, no. 28, p. 6539-6545. <https://doi.org/10.1021/jf405615k> PMID:24934884
- Archer, B. J., Johnson, S. K., Devereux, H. M., Baxter, A. L. 2004. Effect of fat replacement by inulin or lupin-kernel fibre on sausage patty acceptability, postmeal perceptions of satiety and food intake in men. *British Journal Nutrition*, vol. 91, no. 4, p. 591-599. <https://doi.org/10.1079/BJN20031088> PMID:15035686
- Asian Productivity Organization, 2003. Processing and Utilization of Legumes [online] s.a. [cit. 2016-10-25] Available at: http://www.apo-tokyo.org/00e-books/AG-12_Legumes/AG-12_Legumes.pdf.
- Australia New Zealand Food Authority, 2001. Lupin alkaloids in food. A toxicological review and risk assessment. *Technical Report Series*, no. 3, p 1-21.
- Azo, W. M., Lane, G. P. P., Davis, W. P., Cannon, N. D. 2012. Bi-cropping white lupins (*Lupinus Albus L.*) with cereals for wholecrop forage in organic farming: The effect of seed rate and harvest dates on crop yield and quality. *Biological Agriculture and Horticulture*, vol. 28, no. 2, p. 86-100. <https://doi.org/10.1080/01448765.2012.671722>
- Bazzano, L. A., He, J., Ogden, L. G., Loria, C., Vupputuri, S., Myers, L., Whelton, P. K. 2001. Legume consumption and risk of coronary heart disease in US men and women. NHANES I epidemiologic follow-up study. *Archives of Internal Medicine*, vol. 161, no. 21, p. 2573-2578. <https://doi.org/10.1001/archinte.161.21.2573> PMID:11718588
- Bazzano, L. A., Thompson, A. M., Tees, M. T., Nguyen, C. H., Winham, D. M. 2011. Non-soy legume consumption lowers cholesterol levels: a meta-analysis of randomized controlled trials. *Nutrition, Metabolism and Cardiovascular Disease*, vol. 21, no. 2, p. 94-103. <https://doi.org/10.1016/j.numecd.2009.08.012> PMID:19939654
- Bhardwaj, H. L., Hamama, A. A. 2012. Cultivar and growing location effects on white lupin immature green seeds. *Journal of Agricultural Science*, vol. 4, no.2, p. 135-138. <https://doi.org/10.5539/jas.v4n2p135>
- Brand-Williams, W., Cuvelier, M. E., Berset, C. 1995. Use of free radical method to evaluate antioxidant activity. *Food Science and Technology*, vol. 28, no. 1, p. 25-30. [https://doi.org/10.1016/s0023-6438\(95\)80008-5](https://doi.org/10.1016/s0023-6438(95)80008-5)
- Campos-Vega, R., Loarca-Piña, G., Oomah, B. D. 2010. Minor components of pulses and their potential impact on human health. *Food Research International*, vol. 43, no. 2, p. 461-482. <https://doi.org/10.1016/j.foodres.2009.09.004>
- Clements, J., Prilyuk, L., Quealy, J., Francis, G. 2008. Interspecific a crossing among the new world lupin species for lupinus Mutabilis crop improvement. In *Lupinus for Health and Wealth*, Fremantle : Westwrm Australia, Proceedings of the 12th International Lupin Conference, pp. 324-327.
- Dalaram, I. S. 2015. Total polyphenol content and antioxidant capacity of cowpea effect of variety and locality, *Potravinárstvo*, vol. 9, no. 1, p. 358-364. <https://dx.doi.org/10.5219/508>

- Dalaram, S. I., Vollmannova, A., Timoracka, M. 2013. Bioactive compounds in commonly utilized legume cultivars from Iraq. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 2, sp. no. 1, p. 2032-2042.
- Das, N. P., Pereira, T. A., 1990. Effects of flavonoids on thermal autooxidation of palm oil: structure-activity relationship. *Journal of the American Oil Chemists' Society*, vol. 67, no. 4, p. 255-258. <https://doi.org/10.1007/BF02540652>
- Dlamini, N. R., Dykes, L., Rooney, L. W., Waniska, R. D., Taylor, J. R. N. 2009. Condensed tannins in traditional wet-cooked and modern extrusion-cooked sorghum porridges. *Cereal Chemistry*, vol. 86, no. 2, p. 191-196. <https://doi.org/10.1094/CCHEM-86-2-0191>
- Dueñas, M., Hernández, T., Estrella, I., Fernández, D. 2009. Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (*Lupinus Angustifolius* L.). *Food Chemistry*, vol. 117, no 4, p. 599-607. <https://doi.org/10.1016/j.foodchem.2009.04.051>
- Duranti, M., Consonni, A., Magni, Ch., Sessa, F., Scarafoni, A. 2008. The major proteins of lupin seed: Characterisation and molecular properties for use as functional and nutraceutical ingredients. *Trends in Food Science and Technology*, vol. 19, no. 12, p. 624-633. <https://doi.org/10.1016/j.tifs.2008.07.002>
- Ghazal, G. M., Shahhat, I. M. A. 2013. Polyphenols, Flavonoids, Carotenoids and Antioxidant Activity of Lupine (*Lupinus termis* L.) Seeds Affected by Vitamin C, vitamin B3 and Turmeric Rhizomes Extract. *Advances in Environmental Biology*, vol. 7, no. 14, p. 4914-4924.
- Gill, S. S., Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, vol. 48, no. 12, p. 909-930. <https://doi.org/10.1016/j.plaphy.2010.08.016> PMID:20870416
- Gulewicz, P., Martinez-villaluenga, C., Frias, J., Ciesiolka, D., Gulewicz, K., Vidal-Valverde, C. 2008. Effect of germination on the protein fraction composition of different lupin seeds. *Food Chemistry*, vol. 107, no. 2, p. 830-844. <https://doi.org/10.1016/j.foodchem.2007.08.087>
- Guo, X. Y., Wang, J., Wang, N. L., Kitanaka, S., Yao, X. S., 2007. 9,10-Dihydrophenanthrene derivatives from *Pholidota yunnanensis* and scavenging activity on DPPH free radical. *Journal of Asian Natural Products Research*, vol. 9, no. 2, p. 165-174. <https://doi.org/10.1080/10286020500480522> PMID:17454314
- Hall, R. S., Johnson, S. K., Baxter, A. L., Ball, M. J. 2005. Lupin kernel fibre-enriched foods beneficially modify serum lipids in men. *European Journal of Clinical Nutrition*, vol. 59, no. 3, p. 325-33. <https://doi.org/10.1038/sj.ejcn.1602077> PMID:15508014
- Hwang, Y. W., Kim, S. Y., Jee, S. H., Kim, Y. N., Nam, C., M. 2009. Soy food consumption and risk of prostate cancer: a meta-analysis of observational studies. *Nutrition and Cancer*, vol. 61, no. 5, p. 598-606. <https://doi.org/10.1080/01635580902825639> PMID:19838933
- Chawla, R., Patil, G. R. 2010. Comprehensive Reviews in Food Science and Food Safety. *Acta Chimica Slovaca*, vol. 4, no. 1, p. 95-114.
- Cheng, L., Bucciarelli, B., Shen, J., Allen, D., Vance, C. P. 2011. Update on white lupin cluster root acclimation to phosphorus deficiency. *Plant Physiology*, vol. 156, no. 3, p. 1025-1032. <https://doi.org/10.1104/pp.111.175174> PMID:21464472
- Chiofalo, B., Lo Presti, V., Chiofalo, V., Gresta, F. 2012. The productive traits, fatty acid profile and nutritional indices of three lupin (*Lupinus* spp.) species cultivated in a Mediterranean environment for the livestock. *Animal Feed Science and Technology*, vol. 171, p. 230-239. <https://doi.org/10.1016/j.anifeedsci.2011.11.005>
- Jensen, E. S., Peoples, M. B., Boddey, R. M., Gresshoff, P. M., Hauggaard-Nielsen, H., Alves, B. J., Morrison, M. J. 2012. Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries: a review. *Agronomy for Sustainable Development*, vol. 32, no. 2, p. 329-364. <https://doi.org/10.1007/s13593-011-0056-7>
- Joray, M. L., Rayas-Duarte, P., Mohamed, A., Van Santen, E. 2007. Coated Lupin Bean Snacks. *Journal of Food Quality*, vol. 30, no. 2, p. 267-279. <https://doi.org/10.1111/j.1745-4557.2007.00120.x>
- Kedare, S. B., Singh, R. P., 2011. Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, vol. 48, no. 4, p. 412-422. <https://doi.org/10.1007/s13197-011-0251-1> PMID:23572765
- Kirmizi, S., Guleryuz, G. 2007. Monitoring Protein Mobilization During Seed Germination of Broad Bean (*Vicia faba* L.). *Asian Journal of Plant Sciences*, vol. 6, p. 374-378.
- Kohajdová, Z., Karovičová, J., Schmidt, Š. 2011. Lupin composition and possible use in bakery a review. *Czech Journal of Food Science*, vol. 29, no. 3, p. 203-211. <https://doi.org/10.3923/ajps.2007.374.378>
- Kurzbaum, A., Safori, G., Monir, M., Sinsolo, C. 2008. Anticholinergic syndrome in response to lupin seed toxicity. *Israeli Journal of Emergency Medicine*, vol. 8, no. 2, p. 20-22.
- Lachman, J., Proněk, D., Hejtmánková, A., Pivec, V., Faitová, K. 2003. Total polyphenol and main flavonoid antioxidants in different onion (*Allium cepa* L.) varieties. *Scientia Horticulturae*, vol. 30, p. 142-147.
- Laudadio, V., Tufarelli, V. 2011. Dehulled-micronised lupin (*Lupinus Albus* L. cv. *Multitalia*) as the main protein source for broilers: influence on growth performance, carcass traits and meat fatty acid composition. *Journal of the Science of Food and Agriculture*, vol. 91, no. 11, p. 2081-2087. <https://doi.org/10.1002/jsfa.4426> PMID:21520452
- Lee, Y. P., Mori, T. A., Sipsas, S., Barden, A., Puddey, I. B., Burke, V., Hall, R. S., Hodgson, J. M. 2006. Lupin-enriched bread increases satiety and reduces energy intake acutely. *The American Journal of Clinical Nutrition*, vol. 84, no. 5, p. 975-80. PMID:17093146
- Martín-Cabrejas, M., Diaz, M., Aguilera, Y., Benítez, V., Mollá, E., Esteban, R. 2008. Influence of germination on the soluble carbohydrates and dietary fibre fractions in non-conventional legumes. *Food Chemistry*, vol. 107, no. 3, p. 1045-1052. <https://doi.org/10.1016/j.foodchem.2007.09.020>
- Martínez-Villaluenga, C., Bringe, N. A., Berhow, M. A., Gonzalez de Mejia, E. 2008. Beta-conglycinin embeds active peptides that inhibit lipid accumulation in 3T3-L1 adipocytes in vitro. *Journal of Agricultural and Food Chemistry*, vol. 56, no. 22, p. 10533-10543. <https://doi.org/10.1021/jf802216b> PMID:18947234
- Martínez-Villaluenga, C., Dia, V. P., Berhow, M., Bringe, N. A., Gonzalez de Mejia, E. 2009. Protein hydrolysates from beta-conglycinin enriched soybean genotypes inhibit lipid accumulation and inflammation in vitro. *Molecular Nutrition and Food Research*, vol. 53, no. 8, p. 1007-1018. <https://doi.org/10.1002/mnfr.200800473> PMID:19603404

- Martínez-Villaluenga, C., Frias, J., Vidal-Valverde, C. 2006a. Functional lupin seeds (*Lupinus Albus* L. and *Lupinus Luteus* L.) after extraction of α -galactosides. *Food Chemistry*, vol. 98, no. 2, p. 291-299. <https://doi.org/10.1016/j.foodchem.2005.05.074>
- Martínez-Villaluenga, C., Zielinski, H., Frias, J., Piskula, M. K., Kozłowska, H., Vidal-Valverde, C. 2009. Antioxidant capacity and polyphenolic content of highprotein lupin products. *Food Chemistry*, vol. 112, no. 1, p. 84-88. <https://doi.org/10.1016/j.foodchem.2008.05.040>
- McGonigal, A., Kapustin, J. 2008. Low glycemic index diets: should they be recommended for diabetics? *The Journal for Nurse Practitioners*, vol. 4, no. 9, p. 688-696. <https://doi.org/10.1016/j.nurpra.2008.07.020>
- Mülayim, M., Tamkoç, A., and Babaoglu, M. 2002. Sweet white lupins versus local bitter genotype: agronomic characteristics as affected by different planting densities in the Göller region of Turkey. *European Journal of Agronomy*, vol. 17, no. 3, p. 181-189. [https://doi.org/10.1016/S1161-0301\(02\)00007-2](https://doi.org/10.1016/S1161-0301(02)00007-2)
- Nderitu, A. M., Dykes, L., Awika, J. M., Minnaar, A., Duodu, K. G. 2013. Phenolic composition and inhibitory effect against oxidative DNA damage of cooked cowpeas as affected by simulated in vitro gastrointestinal digestion. *Food Chemistry*, vol. 141, no. 3, p. 1763-1771. <https://doi.org/10.1016/j.foodchem.2013.05.001> PMID:23870889
- Oomah, B. D., Tiger, N. Olson, M., Balasubramanian. P. 2006. Phenolics and antioxidative activities in narrow-leaved lupins (*Lupinus Angustifolius* L.). *Plant Foods for Human Nutrition*, vol. 61, no. 2, p. 91-97. <https://doi.org/10.1007/s11130-006-0021-9> PMID:16804740
- Reinhard, H., Rupp, H., Sager, F., Streule, M., Zoller, O. 2006. Quinolizidine alkaloids and phomopsins in lupin seeds and lupin containing food. *Journal of Chromatography A*, vol. 1112, no. 1-2, p. 353-360. <https://doi.org/10.1016/j.chroma.2005.11.079> PMID:16359686
- Rybarczyk, A., Amarowicz, R. 2007. Silica gel column chromatography of phenolic compounds in sweet lupin seeds extract. *Bromatologia i Chemia Toksykologiczna*, vol. 4, p. 375-379.
- Sanchez, M. C., Altares, P., Pedrosa, M. M., Burbano, C., Cuadrado, C., Goyoaga, C., Muzquiz, M., Jimenez-Martinez, C., Davila-Ortiz, G. 2004. Alkaloid Variation During Germination in Different Lupin Species. *Journal of Food Chemistry*, vol. 90, no. 3, p. 347-355. <https://doi.org/10.1016/j.foodchem.2004.04.008>
- Scarafoni, A., Ronchi, A., Duranti, M. 2009. A realtime PCR method for the detection and quantification of lupin flour in wheat flour-based matrices. *Food Chemistry*, vol. 115, no. 3, p. 1088-1093. <https://doi.org/10.1016/j.foodchem.2008.12.087>
- Sebastia, V., Barbera, R., Farre, R and Lagarda, M. J. 2001. Effects of Legume Processing on Calcium, Iron and Zinc Contents and Dialysabilities. *Journal of Sciences of Food and Agriculture*, vol. 81, no. 12, p.1180-1185. <https://doi.org/10.1002/jsfa.927>
- Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T., 1992. Antioxidative properties of xanthone on the auto oxidation of soybean in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*, vol. 40, p. 945-948. <https://doi.org/10.1021/jf00018a005>
- Sievenpiper, J., Kendall, C. W., Esfahani, A., Wong, J. M., Croleton, A. J., Jiang, H. Y., Bazinet, R. P., Vidgen, E., Jenkins, D. J. 2009. Effect of non-oil-seed pulses on glycaemic control: a systematic review and meta-analysis of randomised controlled experimental trials in people with and without diabetes. *Diabetologia*, vol. 52, no. 8, p. 1479-1495. <https://doi.org/10.1007/s00125-009-1395-7> PMID:19526214
- Siger, A., Czubinski, J., Kachlicki, P., Dwiecki, K., Lampart-Szczapa, E., Nogala-Kalucka, M. 2012. Antioxidant activity and phenolic content in three lupin species. *Journal of Food Composition and Analysis*, vol. 25, no. 2, p.190-197. <https://doi.org/10.1016/j.jfca.2011.10.002>
- Soares, J. R., Dins, T. C. P., Cunha, A. P., Almeida, L. M., 1997. Antioxidant activity of some extracts of *Thymus zygis*. *Free Radical Research*, vol. 26, no. 5, p. 469-478. <https://doi.org/10.3109/10715769709084484> PMID:9179593
- Stoddard, F. L., Hovinen, S., Kontturi, M., Lindström, K., Nykänen, N. 2009. Legumes in Finnish agriculture: History, present status and future prospects. *Agricultural and Food Science*, vol. 18, no. 3-4, p. 191-205. <https://doi.org/10.2137/145960609790059578>
- Sujak, A., Kotlarz, A., Strobel, W. 2006. Compositional and nutritional evaluation of several lupin seeds. *Food Chemistry*, vol. 98, no. 4, p. 711-719. <https://doi.org/10.1016/j.foodchem.2005.06.036>
- Uzun, B., Arslan, C., Karhan, M., Tokar, C. 2007. Fat and fatty acids of white lupin (*Lupinus Albus* L.) in comparison to sesame (*Sesamum indicum* L.). *Food Chemistry*, vol. 102, no. 1, p. 45-49. <https://doi.org/10.1016/j.foodchem.2006.03.059>
- Wang, S., Clements, J. 2008. Antioxidant Activities of Lupin Seeds. *Lupinus for Health and Wealth, Fremantle : Westwrn Australia, Proceedings of the 12th International Lupin Conference*, pp. 324-327. ISBN 0-86476- 153-8.
- Xu, J. G., Tian, C. R., Hu, Q. P., Luo, J. Y., Wang, X. D., Tian, X. D. 2009. Dynamic changes in phenolic compounds and antioxidant activity in oats (*Avena nuda* L.) during steeping and germination. *Journal of Agricultural and Food Chemistry*, vol. 57, no. 21, p. 10392-10398. <https://doi.org/10.1021/jf902778j> PMID:19827789
- Zielinska, D., Frias, J., Piskula, M. K., Kozłowska, H., Zielinski, H., Vidal-Valverde, C. 2008. Evaluation of the antioxidant capacity of lupin sprouts germinated in the presence of selenium. *European Food Research and Technology*, vol. 227, p. 1711-1720. <https://doi.org/10.1007/s00217-008-0898-7>
- Zralý, Z., B. Písaříková, B., Trčková, M., Doležal, M., Thiemel, J., Simeonovová, J., Jůzl, M. 2008. Replacement of soya in pig diets with white lupine cv. Butan. *Czech. Journal of Animal Science*, vol. 53, p. 418-430.
- Zralý, Z., B. Písaříková, B., Trčková, M., Herzigl, I., Jůzl, M., Simeonová, J. 2007. The Effect of White Lupine on the Performance, Health, Carcass Characteristics and Meat Quality of Market Pigs. *Veterinarní Medicina*, vol. 52, no. 1, p. 29-41.

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