

ANTIOXIDANT ACTIVITIES OF LUPIN SEEDS

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ABSTRACT

Lupin seeds have been used as a source of protein for animal feeding and human consumption. Recently, human consumption of lupins has been increased due to their nutritional values and some functional properties. As lupins are used as an ingredient in foods, the health benefits of lupins are being investigated by researchers.

It was known that antioxidant phytochemicals in foods have many health benefits including prevention of various diseases associated with oxidative stress such as cancer, cardiovascular disease, neuro-degeneration and diabetes. However, there is little knowledge of antioxidant activity in lupins. The purpose of this study is to investigate the antioxidant activity of lupin seeds from 11 different species including four species of lupin grown in Australia: *Lupinus angustifolius*, *L. luteus*, *L. albus* and *L. mutabilis*.

The antioxidant activity of lupin seeds was evaluated as Trolox equivalent antioxidant capacity using *in vitro* DPPH radical scavenging method. The antioxidant activity largely varied with species and varieties. Among them, *L. luteus* cv. Pootalong, *L. micrevthus* and *L. angustifolius* cv. Kalya showed stronger antioxidant activity than the others. The total polyphenolics from lupin seeds were also measured. The relationship of antioxidant activity with total polyphenolics or other phytochemicals is discussed.

KEYWORDS

Lupins, antioxidants, polyphenols, *Lupinus angustifolius*, *L. luteus*, *L. albus* and *L. mutabilis*

INTRODUCTION

Lupins are native to the Mediterranean region, Eastern Africa and north and south America. Crop lupins can generally survive in poor winter environments such as deep sandy, infertile soil and poorer climates that the warm season soybean is not adapted to (Smart *et al.* 1988, Cerletti *et al.* 1983). Lupins have been established in Western Australia successfully since the 1970's and three crop species of lupins- sweet narrow-leaved lupine (*L. angustifolius*), white lupine (*L. albus*) and yellow lupine (*L. luteus*) are

currently cultivated. *Lupinus mutabilis* is currently under development in Western Australia due to its high protein and oil contents (Clements *et al.* 2008, this conference).

Lupins are mostly utilised by stockfeed manufacturers in compound feed rations. There is increasing utilisation in aquaculture (Glencross *et al.* 2003, 2005) and for human food where they are valued for both their nutritional and functional properties. The use of lupin grains as a raw material for industrial processing has been the subject of research in Australia (Pettersson, 1996) and Europe (Bagger *et al.* 1998). Due to the high protein content of lupins, high fibre, they are used to produce dietary fibre product and a specific protein component with high whipping properties (Pettersson, 1996). Southern Europe, Middle East and South American has used high alkaloid bitter lupins as food products, and Western Australia uses low alkaloid lupins for a wide range of food products (Grain Pool of Western Australia, 1991/92). Therefore, many researchers have paid more attention towards the possibility of using lupins as a human food (Pettersson *et al.* 1996) and their potential health benefits. 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 (Hall *et al.* 2005), that they lower blood glucose and insulin levels (Hall *et al.* 2005), and aid bowel health as a faecal bulking agent. However, little is known about their photochemistry (Wang 2003) and antioxidant activity.

There is strong evidence that reactive oxygen species including free radicals can lead to lipid peroxidation and oxidative stress which damage biological structures such as proteins, lipids and DNA (Gülçin *et al.* 2003). They result in body ageing and chronic diseases such as heart disease, stroke, certain cancers, neurodegenerative diseases and lung disorders (Yildirim *et al.* 2001). In general, the human body has its own natural antioxidant system to stand against free radicals using certain enzymes. It is believed that an intake of antioxidants reinforces the defense of human antioxidant system. Red wine, fruit, vegetables and spices are well known for their natural antioxidants. It has been found that high intake of fruit and vegetable has been associated with lower incidences of chronic diseases such as cancer and heart diseases (Bravo, 1998,

Eberhardt *et al.* 2000, Kuo, 1996). Some natural antioxidants are already exploited commercially either as antioxidant additives or as nutritional supplements (Koleva *et al.* 2002).

Antioxidant activity of plants or fruits work in several mechanisms including free radical scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelating and acting as a substrate for radicals such as superoxide and hydroxide.

There are several methods to measure total antioxidant activity of a compound or plant extract based on hydrogen atom transfer (HAT) reactions and electron transfer (ET) (Huang *et al.* 2005). 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DHHP) is one of a few stable and commercially available organic nitrogen radicals and has a UV-vis absorption maximum at 515 nm (Miliauskas *et al.* 2003). Upon reduction, the solution colour fades. The reaction progress is conveniently monitored by a spectrophotometer. The aim of this study was to study the antioxidant activity of 11 lupin species seeds including *L. albus*, *L. angustifolius*, *L. atlanticus*, *L. cosentinii*, *L. digitatus*, *L. hispanicus*, *L. luteus*, *L. micranthus*, *L. mutabilis*, *L. palaestinus* and *L. pilosus* using the DPPH radical scavenging method. The total phenolics contents from lupin seeds were also measured. The relationship of antioxidant activity with total phenolic contents or other phytochemicals is discussed.

MATERIALS AND METHODS

Materials. Eleven species lupin seeds including *L. albus*, *L. angustifolius*, *L. atlanticus*, *L. cosentinii*, *L. digitatus*, *L. hispanicus*, *L. luteus*, *L. micranthus*, *L. mutabilis*, *L. palaestinus*, *L. pilosus* were obtained via CLIMA, the University of Western Australia. All seeds were milled into a fine powder.

Extraction. Lupin seeds were grounded into fine powder using café grinder. One gram of seed powder was extracted with 20 mL of 80% methanol at room temperature overnight. The sample was centrifuged to separate two layers. The top clear solution was filtered for antioxidant activity test, total phenolic measurement and HPLC analysis.

DPPH radical scavenging method for measuring antioxidant activity. Radical scavenging activity of plants extracts against stable DPPH[•] (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically. When DPPH[•] reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from purple to light yellow) were measured at 515 nm on a UV/visible light spectrophotometer. Radical scavenging activity of extracts was measured by methods as described below.

The DPPH radical solution was freshly prepared daily in methanol to a solution of 0.0768 mg/mL, stored in a flask covered with aluminium foil and kept in the

dark at 4°C between measurements. Trolox was dissolved in ethanol to make solution at 0.63 mg/mL.

Then it was diluted to five different concentrations with methanol as a reference standard. Two mL of lupin seed extract (or trolox solution) was added to 3 mL of DPPH radical solution and 5 mL of methanol. Each sample was incubated for 15 minutes at dark before measurement. The decrease in absorbance at 517 nm for each sample was measured. Methanol was used to zero the spectrometer. A blank sample containing the same amount of methanol DPPH radical solution was prepared and measured. UV analysis on DPPH radical detection was performed in the Chemistry Centre Western Australia using an UV mini 1240 UV-VIS spectrometer supplied from Shimadzu Scientific. The detector was set to measure the wavelength of 517 nm which is the λ_{max} of DPPH radicals. The scavenging activity of DPPH of the sample was calculated based on the following formula:

$$(1 - \text{ABS sample} / \text{ABS methanol solution}) \times 100\%$$

The free radical scavenging activity of lupin seeds was expressed on a received weight basis as mg of Trolox equivalent/100 g, which was calculated using a regression with a calibration curve with five different concentrations of Trolox. Linearity range of the calibration curve was 0.01 mg to 0.08 mg/ml (inhibition from 20 to 80%) ($r^2 = 0.998$).

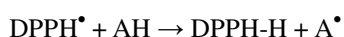
Total phenolics contents. Total phenolics contents were determined by the spectrophotometric method. Two hundred fifty μ l of extract solution was diluted with 14 mL of double distilled water (dd H₂O). A reagent blank using dd H₂O instead of sample was prepared. Two mL of Folin Ciocalteu's phenol reagent was added to the mixture and mixed. After 5 min, 2 mL of 1 M Na₂CO₃ aq solution was added with mixing. The solution was allowed to stand for 90 min and the absorbance was measured at 750 nm using an UV mini 1240 UV-VIS spectrometer supplied from Shimadzu Scientific versus the prepared blank. Total phenolics of lupin seeds were expressed on a received weight basis on mg of gallic acid equiv (GAE)/100 g, which were calculated using a regression with a calibration curve with five different concentrations of gallic acid ($r^2 = 0.998$) ($P < 0.05$).

HPLC instrument. Chromatographic separation of 80% methanol extract was performed using a Water 2695 Separation Module. The HPLC was connected to Water 2996 Photodiode Array Detector. An UV wave length between 210 nm to 550 nm was used for detection of wide range of polyphenolics.

HPLC Column. The column (Apollo C₁₈ 5, 250 mm L x 4.6 mm id, Alltech) was used for separation of polyphenolics, which were identified on the basis of the same retention time and same spectral characteristics of standards. Mobile phases A (acetonitril), B (0.5% phosphoric acid) were applied in separation.

RESULTS AND DISCUSSION

Antioxidant activity. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method is rapid, sensitive, simple, cheap and independent of sample polarity (Koleva *et al.* 2002). It is therefore very convenient for quick screening of many samples for radical scavenging activity. Free radicals are involved in the propagation of lipid (LH) oxidation and many radical species with different reactivity are formed. Relatively stable radicals such as DPPH· are often preferred in assessing radical scavenging activity (Koleva *et al.* 2002). 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:



The remaining DPPH· is measured at 517 nm after a certain time and is inversely corresponding to the radical scavenging activity of the antioxidant. Trolox is an antioxidant with known antioxidant property. It is readily used as a reference compound to compare the antioxidant activity of an unknown antioxidant. The DPPH radical scavenging activity of lupin seed extracts are summarized in Table 1. The free radical scavenging activity of methanol extracts in lupin seed against DPPH greatly varied with species. *L. luteus* cv. Pootalong gave the highest antioxidant activity than other lupin seeds with 0.635 mg trolox eq /g of lupin seed, followed by *L. micranthus* with 0.513 mg trolox eq/g of seed. The white lupin *L. albus* seems to have less antioxidant activity than other nine species of lupin seeds, with 0.153 mg to 0.195 mg trolox eq/g of seed, which is only one third of activity of *L. luteus* cv. Pootalong. Within species, antioxidant activity of lupin seed extracts significant varied with the variety. For *L. angustifolius*, cv. Kalya has a higher antioxidant activity (0.438 mg trolox Eq/g of seed) than other two cv. Belara, 99 WH10 (0.210 mg Trolox Eq/g of seed) and Wongan Hills Telerack (0.163 mg trolox Eq/g of seed). The antioxidant activity of *L. luteus* cv. Pootalong (0.635 mg trolox Eq/g of seed) is much higher than cv Wodjil (0.217mg trolox Eq/g of seed) too. Fig. 1 is the scavenging activity of four lupin seeds against DPPH at three different concentration.

Total polyphenolics of lupin seed extracts. Polyphenolics have shown health benefits due to their antioxidant activity. These compounds can remove free radicals, chelating metal catalysts, activate antioxidant enzymes and inhibit oxidases (Heim *et al.* 2002). The total phenolics in lupin seed (mg/100 g) in methanol extracts, determined from regression equation of calibration curve ($R^2 = 0.99$), expressed as mg GAE (mg galic acid eq)/100 g of seed, as summarized in Table 1. The total phenolic content greatly varied with lupin species, ranging from 374.4 to 2660.4 mg GAE/100 g. The highest total phenolic content was found in *L. mutabilis* P28725. The *L. albus* cv. Etho 66,

L. micranthus, *L. hispanicus*, *L. hispanicus* also produced higher phenolic content with 1661, 1541, 1208, 1208 mg GAE/g respectively. Two varieties of *L. luteus* contained the lowest the phenolic contents with about 374 mg GAE/100 g. The total phenolic contents in species *L. albus* have significantly variation from 1661 mg GAE/100 g in *L. albus* cv. Etho 66 to 444 mg GAE/100 g in cv Andromeda. Similar to *L. albus*, the phenolic contents in *L. mutabilis* P28725 (2660 mg GAE/100 g) is about three times higher than another line (799 mg GAE/100 g). However, the phenolic contents in *L. angustifolius* (from 540 to 580 mg GAE/100 g) and *L. luteus* (365 mg GAE/100 g) did not show significant variation between varieties.

Correlation between free radical scavenging activity and total phenolic contents. The free radical scavenging activity is not well correlated with total phenolic contents ($R = 0.204$) (Fig. 2) although it showed a trend that the higher phenolic contents have a stronger DPPH scavenging activity. It was correspondence to Oomah *et al.* (2006)s observation that is antioxidant activity of lupin genotypes not related to phenolic contents of seeds. It was found that DPPH scavenging activity was not correlated with total phenolic contents in some plants (Miliauskas *et al.* 2003). The phenolic compounds may contribute directly to antioxidant activity. It is suggested that the antioxidant activities can be ascribed to the different mechanisms exerted by different phenolic compounds, and to the synergistic effects of different compounds. It is known that only phenolic compounds of a certain structure and particularly hydroxyl position in the molecular determine antioxidant activity; in general these properties depend on the ability to donate hydrogen or electron to a free radical. The methanolic extracts of lupin seed were analysed by high performance liquid chromatography to see the phenolic profiles (Wang unpublished). The chemical profiles significantly varied with lupin species and varieties. For *L. albus*, the chemical profiles in cv ETHO 66 (Fig. 3a) are significantly different from those of cv ANDROMEDA (Fig. 3b). Their chemical structures are being identified. Similarly, two lines of *L. mutabilis* P28725 have different chemical profiles.

Methanol extracts in lupin seeds exhibited weak DPPH scavenging activity compared to other legumes seeds such as field peas (1.333 mg trolox eq/g) and black bean (0.982 mg trolox eq/g) (Wang unpublished). Lampart-Szczapa *et al.* (2003) have reported that lupin seed contained high levels of tocopherol with 0.264% for *L. luteus* var Juno and 0.186% for *L. albus* var. Wat. It is suggested that lupin seeds may have higher antioxidant activity in lipid-soluble substances (Oomah *et al.* 2006). Amarowicz *et al.* (2004) have reported total antioxidant activity of some legumes seeds with the order: adzuki bean > red bean > faba bean > green lentil > red lentil > broad bean > pea.

CONCLUSION

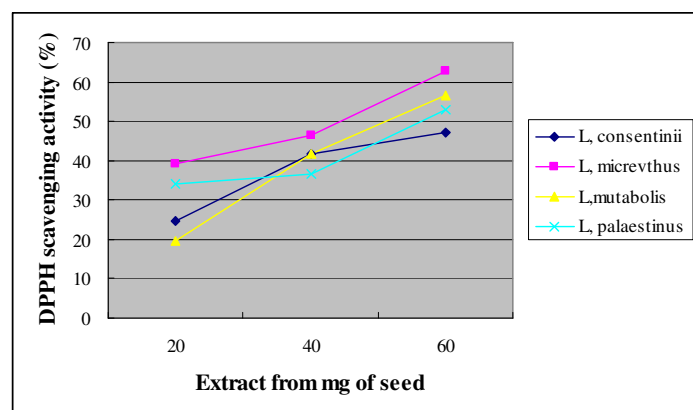
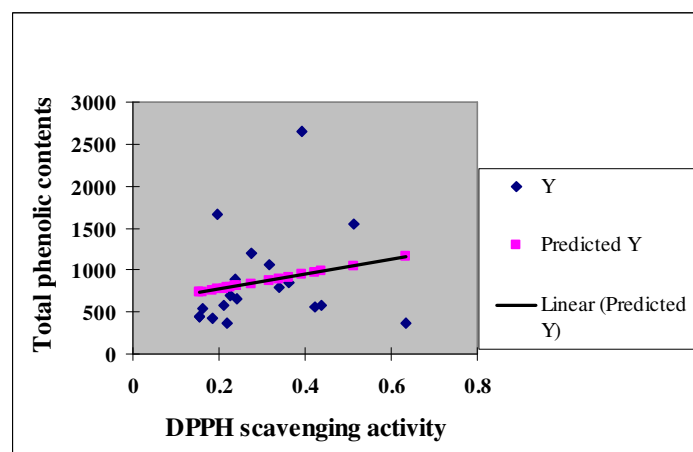
The antioxidant activity of eleven lupin species seed in methanol extract was evaluated by DPPH free radical scavenging activity. Correlation between antioxidant properties and total phenolic contents was not found. In further work, it will be worthwhile determining the antioxidant activity in lipid-soluble extract of lupin seeds.

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Table 1. The antioxidant activity and total phenolic contents of lupin seeds

Species name	Variety name	Antioxidant activity mg Trolox Eq/g	Total phenolics content mg GAE/100 g
<i>L. albus</i> L.	Etho 66	0.195	1661.2
<i>L. albus</i>		0.153	444.4
<i>L. albus</i>	Andromeda	0.153	451.6
<i>L. angostifolius</i>		0.424	553.4
<i>L. angostifolius</i>	Belara, 99 WH10	0.210	578.4
<i>L. angostifolius</i>	Kalya	0.438	578.0
<i>L. angostifolius</i>	Wongan Hills Telerack	0.163	535.1
<i>L. atlantieus</i>		0.240	661.2
<i>L. consentinii</i>		0.227	706.0
<i>L. digitatus</i>		0.362	845.3
<i>L. hispanicus</i>		0.277	1208.1
<i>L. luteus</i>		0.217	374.4
<i>L. luteus</i>	Pootalong	0.635	369.2
<i>L. micrevthus</i>		0.513	1541.0
<i>L. mutabilis</i>		0.339	799.1
<i>L. mutabilis</i>	P28725	0.394	2660.4
<i>L. palaestinus</i>		0.238	898.4
<i>L. pilosus</i>		0.316	1056.1
04D 505-006		0.186	431.2

**Fig. 1.** The DPPH scavenging activity (%) of four lupin seeds at three different concentrations.**Fig. 2.** Relationship between DPPH scavenging activity and total phenolic contents of different species of lupin seeds ($P < 0.05$, $R = 0.204$).

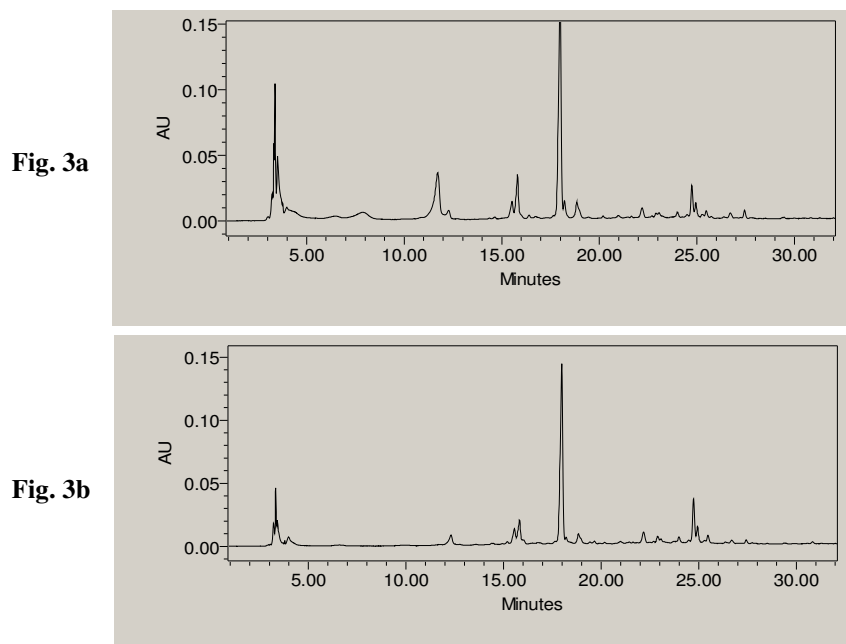


Fig. 3. The HPLC chromatogram of methanol extract of *L. albus* var ETO66 (3a) and var Andromeda (3b).