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Functional Properties, Lipoxygenase Activity, and Health Aspects of *Lupinus albus* Protein Isolates

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To utilize lupin seeds for food and pharmaceutical applications, lupin seeds were pretreated to remove oil using hexane or carbon dioxide. Two types of lupin protein isolate were prepared. Both types of protein isolate showed good foaming activity, comparable to egg white. Protein isolate extracted under acid conditions showed higher foaming activity than protein isolate extracted at neutral pH. The lipoxygenase activity was much reduced in both of the protein isolates. The protein isolate extracted at neutral pH showed a stronger angiotensin converting enzyme inhibition than the protein isolate extracted under acidic pH. In contrast, the protein isolate extracted under acid conditions had a greater sodium cholate binding capacity, comparable to that of cholestyramine. Lupin samples showed less DPPH radical scavenging activity than deoiled soybean. The deoiling method did not affect the functional properties, lipoxygenase activity, angiotensin converting enzyme inhibition, sodium cholate binding, and radical scavenging activity.

KEYWORDS: ***Lupinus albus*; protein isolate; functional properties; lipoxygenase; ACE inhibition; cholic acid binding; DPPH scavenging activity**

INTRODUCTION

Sweet varieties of lupin (*Lupinus albus*) grow well in Mediterranean climates, in slightly acidic soils. Analysis of the nutritional value of sweet lupins has shown that the bioavailabilities of the constituents are comparable to those of soybeans. In general, plant proteins are being increasingly used as food ingredients because they improve key functional properties, for example, stabilization of texture and optimized consumer costs. By nature, the storage lupin proteins include different fractions of (α,β,γ) conglutin (*1*). Native lupin proteins have good solubility under appropriately chosen conditions. The patented method of lupin seed processing (*2*) ensures there is retention of the native protein properties and makes two fractionated protein isolates available. The best pH at which to recover lupin proteins using the described process (*2*) is between pH 7 and 8. Both fractions are highly soluble protein isolates with outstanding emulsification, salt tolerance, and foaming properties. The isolated protein fractions provide the necessary basis for investigating health-related properties.

As we have reported (*3*), lupin protein isolate is useful as a food ingredient for changing the emulsifying capacity and improving the texture of food. We have also reported the different lipoxygenase activities of two types of lupins (*4*). In addition, the isolation of the proteins may give rise to different health-related properties, such as angiotensin I converting enzyme (ACE) inhibition, bile acid binding, and radical scavenging activity.

It is well-known that lipoxygenase (LOX) is responsible for the development of off-flavors in soybeans (*5*), corn (*6*), etc. In general, fractionation is used to nullify or reduce the LOX activity. We have already reported the lipoxygenase activity of deoiled lupin (*4*), and it is also necessary to have information about how the lipoxygenase activity is affected by the isolation of the protein.

ACE plays an important physiological role in regulating blood pressure (*7*). ACE converts an inactive form of decapeptide, angiotensin I, to a potent vasoconstrictor, octapeptide angiotensin II, and deactivates bradykinin, which has a depressor action. Because active ACE causes higher blood pressure in this way, it is recommended to inhibit ACE activity for hypertension. Many ACE inhibitory fractions have been isolated from various protein hydrolysates such as cheese whey (*8*), casein (*9*−*11*), and soybean (*12*). However, no studies are available concerning ACE inhibition in lupin and lupin protein isolate. The present study investigates ACE inhibition in lupin, lupin protein isolate, and their hydrolysates.

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Bile acids are synthesized in the liver from cholesterol (*13*). It is well documented that the sequestering of bile acid anions in the gastrointestinal tract by polymeric solvents is effective in reducing serum cholesterol levels, hence decreasing the risk of cardiovascular diseases (*14*). By binding bile acids, food fractions prevent their reabsorption and stimulate liver cholesterol conversion to additional bile acids. Many papers have mentioned bile acid binding by dietary fibers (*15*, *16*); however, there have been only few reports of bile acid binding by proteins (*17*). Unlike dietary fiber, proteins should be digested in the gastrointestinal tract. We have reported (*18*) bile acid binding by lupin protein isolates, but we have not compared the bile acid binding capacity depending on the deoiling method.

Phenolic compounds, proteins, and peptides have been shown to have antioxidative activities against reactive oxygen species (*19*, *20*). The scavenging of reactive oxygen species is important to prevent diseases such as cancer and coronary heart disease (*21*). One of the objectives in the preparation of protein isolates from lupin is reducing toxic alkaloids; however, this processing may reduce the radical scavenging activity. As a first approach to determine the radical scavenging activity of lupin protein isolates, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scaveinging activity of water extracts of lupin protein isolates was evaluated.

This paper describes an improved procedure for obtaining high-functionality protein isolates from lupin seeds and assesses the functional properties of the products, namely, protein solubility, emulsifying capacity, foaming activity, LOX activity, ACE inhibition, bile acid binding, and radical scavenging activity.

MATERIALS AND METHODS

Materials. White lupin seeds (*L. albus*) were obtained from a Chilean grower (v. Baer). Kernels and hulls were separated in a dry mill, and the kernels were flaked using a roll mill. The lupin flakes were deoiled using hexane or carbon dioxide, and these flakes were used to prepare the protein isolate. The flakes were milled using a Retsch ZM-100 mill (Düsseldorf, Germany) to a powder (<0.1 mm).

The lupin proteins were extracted from the deoiled flakes under mild conditions using a two-stage process (*2*). In the first stage, the deoiled flakes were mashed in cold water under acid conditions (pH 4.5). The clarified acid extract was concentrated using a cross-flow membrane filtration unit equipped with 1.7 m^2 Carbosep three-channel tubes (cutoff = 15 kDa). The resulting product was protein isolate F. In the second stage, the main storage protein fraction was extracted at neutral pH, and protein isolate E was enriched using acid precipitation.

Linoleic acid, pepsin, pancreatin, ACE from rabbit lung, a bile acid analysis kit, and DPPH were purchased from Sigma Chemical Co. (St Louis, MO). All other reagents used for the experiments were of analytical grade.

Proximate Analysis. The chemical compositions (dry matter, nitrogen content, ash content, and oil content) of the deoiled lupin flakes and the protein samples were analyzed in accordance with section 35 LMBG (*22*).

Functional Properties. The functional properties of the deoiled lupin flakes and protein isolates were determined using standardized methods.

The protein solubility was determined according to the method of Morr et al. (*23*), and the NSI value was determined following the official AOCS method (*24*) or AACC method (*25*). The protein solubility curve was obtained by mixing protein samples (1 g) in 50 mL of 0.1 M sodium chloride solution at a given pH for 60 min at ambient temperature. The nondissolved fraction was separated by centrifugation at 20000*g* for 15 min. The protein remaining in the solution was determined by nitrogen analysis and then multiplying the recorded nitrogen value by 6.25. The protein solubility is the dissolved protein fraction relative to the protein content of the starting sample, expressed as a percentage.

To determine the (o/w) emulsifying capacity, a 1 L laboratory reactor (IKA-Werke GmbH & Co. KG, Staufen, Germany) with a stirrer and an emulsifying system (Ultra-Turrax, IKA) were used. The protein solution (1% w/w) was agitated in the reactor, which was held at a constant temperature by a cooling or heating system. The oil was automatically added by a titration system (Metrohm GmbH & Co. KG, Herisau, Switzerland). The conductivity was continuously measured and used as a parameter for the determination of the inversion point of the emulsion. The amount of oil that was added up to the inversion point was used to calculate the emulsifying capacity (milliliters of oil per gram of protein).

Foams were generated using a whipping machine (Hobart N 50, Hobart GmbH, Offenburg, Germany). The foaming activities of 5% protein solutions were obtained by relating the foam volume after 8 min of whipping relative to the volume of the prepared protein solution. The foaming activity was calculated using the following equation:

$$\text{foaming activity (\%)} = (\text{foam volume after whipping/volume of protein solution}) \times 100$$

Determination of Lipoxygenase Activity. The extraction of lipoxygenase and the LOX activity assay were carried out following the methods of Ridolfi et al. (*26*), Clemente et al. (*27*), and Axelroad et al. (*28*), with some modification. We prepared crude extract and ammonium sulfate precipitated fraction at 75% saturation as mentioned in our former paper (*4*). The LOX activity was determined spectrophotometrically as mentioned in that paper (*4*) with linoleic acid as a substrate at pH 8.0. The protein concentration was determined by Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA) based on the Bradford dye binding procedure (*29*), using bovine serum albumin as a standard.

Protein Hydrolysis for Determination of ACE Inhibition, Bile Acid Binding, and Radical Scavenging Properties. Protein hydrolysis was performed following the method of Pihlanto-Leppälä et al. (*30*) and Mullally et al. (*31*), with some modification as mentioned in our paper (*18*). The hydrolysates were freeze-dried and stored at 4 °C prior to analysis. The degree of hydrolysis was determined following the method of Nielsen et al. (*32*).

Determination of ACE Inhibition. The ACE inhibition activity was measured spectrophotometrically using hippuryl-L-histidyl-leucine as a substrate, according to the method of Cushman and Cheung (*33*) and Hernández-Ledesma et al. (*34*), with minor modification. Fifteen microliters of a 10 mg/mL (freeze-dried powder) suspension of the hydrolysate was tested for its ability to inhibit ACE activity. In each assay, 15 μL of the sample solution was incubated at 37 °C for 80 min with 110 μL of 13.64 mM hippuryl-His-Leu in 0.2 M potassium phosphate buffer containing 300 mM NaCl at pH 8.3 and 25 μL of ACE solution (150 mU/mL in 50% glycerol). The final concentrations of the hippuryl-His-Leu and ACE were 10 mM and 25 mU/mL, respectively. The reaction was stopped by adding 110 μL of 1 N HCl. Hippuric acid was extracted with 1.0 mL of ethyl acetate. A 750 μL aliquot of the extract was then dried, and the residue was dissolved in 1.0 mL of deionized water. The hippuric acid concentration was determined at 228 nm. The reaction blank was prepared in the same way as indicated above, but the order in which the reagents were added was changed. The addition of HCl was performed before the enzyme was added. Captopril, which is reported to show strong inhibition, was also used in this study.

Determination of Bile Acid Binding. The method used to determine the in vitro sodium cholate binding was a modification of the procedure described by Camire et al. (*35*) and Kahlon and Chow (*36*). As mentioned in our paper (*18*), binding reaction was performed, and then sodium cholate was analyzed spectrophotometrically at 530 nm using the Sigma bile acids analysis kit 450.

DPPH Radical Scavenger Study of Water Extract. Following the sample treatment of Duh et al. (*37*), the sample powder was suspended with water at the concentration of 10 mg/mL and kept at 99 °C by thermomixer for 10 min. After cooling, samples were centrifuged, and crude water soluble extract taken as a supernatant was used for DPPH radical scavenger study. Extracted protein was analyzed according to the Bio-Rad protein assay, using bovine serum albumin as a standard.

Table 1. Chemical Composition and Functional Properties of Lupin Protein Isolates

	hexane deoiled		carbon dioxide deoiled	
sample	protein isolate E	protein isolate F	protein isolate E	protein isolate F
chemical composition				
dry matter (%)	96.17 ± 0.34[a]	93.53 ± 0.77[a]	95.83 ± 0.07	93.43 ± 0.67
protein (N × 6.25) (%)	100.6 ± 0.30[a]	91.44 ± 0.09[a]	99.41 ± 0.01	87.83 ± 0.94
oil (%)	1.16 ± 0.16[a]	0.24 ± 0.02[a]	2.35 ± 0.27	0.63 ± 0.02
alkaloids (%)	<0.002	<0.002	<0.002	<0.002
functional properties				
nitrogen resolubility at pH 7 (%)	64.1 ± 0.30	71.0 ± 0.45	59.8 ± 0.25	68.3 ± 0.30
emulsification capacity (EC) (mL/g)	450 ± 10	370 ± 5.0	445 ± 25	505 ± 7.5
foaming activity (%)	1102 ± 16	2083 ± 63	1134 ± 25	1718 ± 15
foaming stability (%)	73 ± 2.0	95 ± 3.0	52 ± 2.0	98 ± 2.0

[a] Data are from Yoshie-Stark and Wäsche (*24*).

After passing through the solid extraction column (Waters C_{18}, Waters GmbH, Eschborn, Germany), total soluble phenol in the test extracts was determined with Folin−Ciocalteu reagent according to the method of Sigleton et al. (*38*) using gallic acid as a standard.

The scavenging activity of sample extracts against DPPH radical was measured according to the method of Matthäus (*39*) with some modification. An aliquot (50 mL) of sample extract was mixed with 700 mL of 100 mM sodium phosphate buffer, pH 7.0, and then 750 mL of 100 mM DPPH in methanol was added. The mixture was shaken vigorously and allowed to stand for 30 min in the dark. The absorbance at 517 nm was measured. A blank test was prepared by adding water instead of extract. From a calibration curve obtained with different concentrations of extract, the median effective dose (ED_{50}) was calculated. The ED_{50} was that concentration of an antioxidant which was required to quench 50% of the initial DPPH radicals under the experimental conditions given.

Statistical Analysis. Results are presented as mean values ± standard error of the mean (SEM) ($n = 3-5$). ANOVA was used to calculate significant differences.

RESULTS AND DISCUSSION

Chemical Composition. Protein isolate E and protein isolate F were prepared using a two-stage protein isolation process. Protein isolate E represents the fractions that are separated by alkaline extraction and isoelectric precipitation. Protein isolate E comprises the high molecular weight proteins (α-, β-conglutin). Protein isolate F, which is recovered by acid extraction and ultrafiltration, is enriched in the γ-conglutin fraction. **Table 1** shows the dry matter, protein, oil, and alkaloid contents of the lupin protein isolates that were used in this work and the corresponding raw material. Both samples of deoiled lupin flakes contained 57% protein as dry matter. Immediately after harvest, the lupin seeds contained approximately 40% protein and 12% oil (*3*). After deoiling, the residual oil content was extremely low, with the oil content of both samples of deoiled lupin flakes being <2%. After processing, the protein contents of protein isolate E from the hexane- and CO_2-deoiled samples were, respectively, 100.6 and 99.4% (N × 6.25). The oil contents were, respectively, 1.16 and 2.35%. Both of the protein isolate F samples contained approximately 90% protein and <1% oil. This shows that the protein isolation process was efficient in yielding a higher protein concentration. The oil content was, however, not reduced by this processing.

Functional Properties. Figure 1 shows the solubility of protein isolates E and F from the two deoiling processes as a function of pH. All of the samples showed high protein resolubility up to 72%. Protein isolates deoiled with hexane showed slightly higher solubility than the samples deoiled using carbon dioxide. In both cases the maximum solubility was reached at pH ∼7 (60−70%). The protein solubility of protein isolate F was less affected by pH (50−70%), whereas protein isolate E had a minimum solubility at pH ∼5 (12−15%). We have already reported the effect of pH on the solubility of proteins from deoiled flakes (*3*). Hexane deoiled flakes had minimum solubility between pH 4 and 5. The solubility profile for protein isolate E was similar to that of the deoiled flakes. López et al. (*40*) reported the solubility of commercial soy protein and commercial sesame protein. Commercial soy protein showed a minimum solubility of 52% at pH 4.0 and a maximum solubility of 62% at pH 10.0. Commercial sesame protein showed a minimum solubility of 8% at pH 6.0 and a maximum solubility of 75% at pH 2.0. In addition, it was known that egg white ovalbumin has an isoelectric point at pH 4.5 (minimum solubility). Our results indicate that lupin protein has a solubility similar to that of egg white and soybean protein. This means that lupin protein may be useful as a replacement for egg white in food production. In contrast, it is not possible for sesame protein to replace egg white. Achouri et al. (*41*) described the solubility of soy protein isolate at different pH values. They reported a minimum protein solubility of 60% at pH 5 and a maximum protein solubility of 95% at pH 8. Molina Ortiz and Wagner (*42*) also reported a minimum solubility for soy protein isolate at pH 4.5. Our protein isolate F was extracted under acidic pH conditions; therefore, the solubility problem at pH ∼4−5 was dissolved with a solubility of 55−65%.

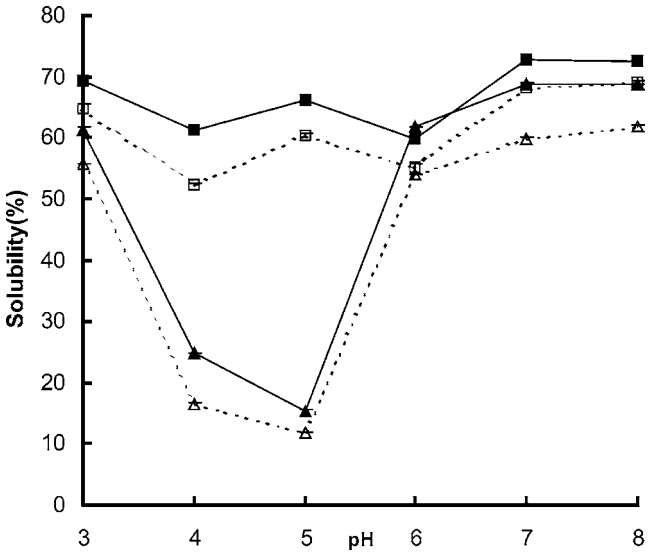

Figure 1. Protein solubility as a function of pH for deoiled lupin flakes and lupin protein isolates: (■) hexane-deoiled protein isolate E; (▲) hexane-deoiled protein isolate F; (□) CO_2-deoiled protein isolate E; (△) CO_2-deoiled protein isolate F.

Table 2. Comparison of Lipoxygenase Activity of Lupin Flakes and Lupin Protein Isolates[a]

sample	protein (mg)	total activity (units)	specific activity (units/mg of protein)	yield (%)
hexane-deoiled lupin flakes				
crude extract	214[b]	65300[b]	305[b]	100[b]
0–75% $(NH_4)_2SO_4$	201[b]	62100[b]	309[b]	95.1[b]
protein isolate E	217	8187	39	
protein isolate F	147	4924	33	
CO_2-deoiled lupin flakes				
crude extract	220	61000	277	100
0–75% $(NH_4)_2SO_4$	217	60000	274	98.4
protein isolate E	200	3880	19	
protein isolate F	121	3750	31	
hexane-deoiled soybean				
crude extract	173[b]	597900[b]	3460[b]	100[b]
0–75% $(NH_4)_2SO_4$	152[b]	531000[b]	3500[b]	89[b]

[a] Data are expressed on the basis of 1 g of lupin or soybean samples. [b] Data are from Yoshie-Stark and Wäsche (*4*).

Nitrogen resolubility values at pH 7 are shown in **Table 1**. Protein isolate E from hexane-deoiled lupin showed a value of 64.1%, and for the CO_2-deoiled lupin this was 59.8%. Protein isolate F showed nitrogen resolubilities of 71.0% for hexane-deoiled protein and 68.3% for CO_2-deoiled lupin. There was no significant difference between the nitrogen resolubility values at pH 7. El-Adawy et al. (*43*) reported values for the nitrogen resolubility of proteins isolates prepared from sweet lupins (*L. albus*) and bitter lupins (*L. termis*). These values were 22.6 and 21.7%, respectively. Lqari et al. (*44*) reported the nitrogen resolubility of protein isolates prepared from blue lupins (*L. angustifolius*). Compared to our protein extraction conditions, this protein was extracted at very high pH (pH 10−12). The nitrogen resolubility was 19.2−33.8% at pH 7. However, the higher the nitrogen resolubility of the native lupin protein, the better their technofunctional properties (foaming, emulsification) for food applications.

Table 1 summarizes the emulsification and foaming properties of lupin protein isolates E and F made from hexane-deoiled lupins and CO_2-deoiled lupins. Both types of protein isolate had a good emulsification capacity, with a value of 450 mL of oil/g of protein on average. There are many papers relating to the emulsification capacity of plant proteins. Sweet lupin protein isolate, bitter lupin protein isolate, soy protein isolate, pea protein isolate, and sesame protein isolate were reported to have emulsification capacities of, respectively, 169 and 164 mL of oil/g of protein (*43*), 107 and 69−76 mL of oil/g of protein (*45*), and 130 mL of oil/g of protein (*46*). In our study, all of the protein isolates showed higher emulsification capacity than these reported values. When we determined the functional properties of whole egg and egg white for comparative purposes, the foaming activity and foaming stability of protein isolate F were found to be nearly the same as for egg white.

Concerning protein isolate F, the product recovered from CO_2-deoiled lupins showed a better value than the product recovered from hexane-deoiled lupins. Both protein isolate types E and F showed very good foaming activity that was comparable to that of egg white reference foams. Protein isolate F from both hexane- and CO_2-deoiled lupins showed 1.5−1.9 times higher foaming activity than protein isolate E.

Lipoxygenase Activity. The results of LOX analysis from hexane-deoiled lupin crude extract and hexane-deoiled soybean were referred from our paper (*4*). As shown in **Table 2**, 201, 217, and 152 mg of protein were collected by 75% ammonium sulfate precipitation from 214, 220, and 152 mg of protein in the crude extract from hexane-deoiled lupin flakes, CO_2-deoiled lupin flakes, and hexane-deoiled soybean flakes. The total activity and specific activity of soybean lipoxygenase were ∼10 times that of lupin LOX. The total activity and specific activity of the LOX from hexane-deoiled lupins were ∼10% higher than that from CO_2-deoiled lupin flakes. However, there was no big difference in the LOX from the two types of deoiled lupin flakes. The LOX activity in the protein isolates was ∼6−12% of the LOX activity in deoiled lupin crude extracts. This indicates that protein isolation was effective in removing lipoxygenase. In the case of protein isolates E and F from lupins, both the hexane-deoiled protein isolates and the CO_2-deoiled protein isolates showed much lower LOX activity than the original deoiled lupin flakes. Compared to hexane-deoiled lupin protein isolate E, CO_2-deoiled lupin protein isolate E showed only half the total activity and specific activity. There was no difference between hexane deoiled lupin protein isolate F and CO_2-deoiled lupin protein isolate F.

These results have been converted to the amount of hydroperoxide (micromoles) produced by using the factor $\epsilon = 25000$ M^{-1} cm^{-1} in order to make comparisons with other reports. LOX crude extract from hexane-deoiled lupin produced 2612 μmol of hydroperoxide/min according to the calculation. LOX crude extract from CO_2-deoiled lupin and LOX crude extract from soybean produced 2440 and 24000 μmol of hydroperoxide, respectively. LOX from hexane-deoiled protein isolates (E and F) and LOX from CO_2-deoiled protein isolates (E and F) produced 327, 197, 155, and 150 μmol of hydroperoxide, respectively. Clemente et al. (*27*) reported that broad bean deoiled powder produced ∼38000 μmol of hydroperoxide after calculating the sample amount to adjust for comparison. Jaren-Galan et al. (*47*) reported that olive extract produced 6800 μmol of hydroperoxide. Although broad beans showed greater LOX activity than soybeans, lupin LOX showed <50% of the activity of olive LOX. Durum wheat semolina was reported to have LOX activity to produce 1570 μmol of hydroperoxide (*48*), and a crude extract of lupin LOX showed higher activity. However, after processing into protein isolates E and F, they showed 10−20% of the activity of wheat LOX.

Lipoxygenase inhibitors with phenolic structures have been widely reported. For example, Sadik et al. (*49*) examined the inhibition activity of soybean LOX by several flavonoids. It showed strong inhibition by luteolin, fisetin, and quercetin. Najid et al. (*50*) carried out comparative research on lupin LOX inhibition and soybean LOX inhibition. They mentioned that esculetin was the strongest inhibitor for purified lupin LOX, whereas esculetin was not effective in inhibiting soybean LOX. One of our objectives in separating proteins from lupin seeds is to reduce the LOX activity. Our results showed a dramatic reduction in LOX activity in protein isolates E and F. The reduction of LOX activity was also achieved by LOX inhibitors such as polyphenols. However, the study of Najid et al. (*50*) mentioned different characteristics (i.e., their optimum pH was 6.0, not 8.0) for purified lupin LOX, and therefore further research is needed to find suitable LOX inhibitors for production scale in food applications.

ACE Inhibition. In this study, results were expressed as percent ACE inhibition by 150 μg samples (the final concentration in the reaction tube was 1 mg/mL). From our preliminary experiments, the concentration of captopril was fixed at 1.5 ng (0.01 μg/mL) in this experiment.

As shown in **Figure 2**, ACE was inhibited by deoiled lupin and its hydrolysate to a degree of 7.3−48.9%, by protein isolate

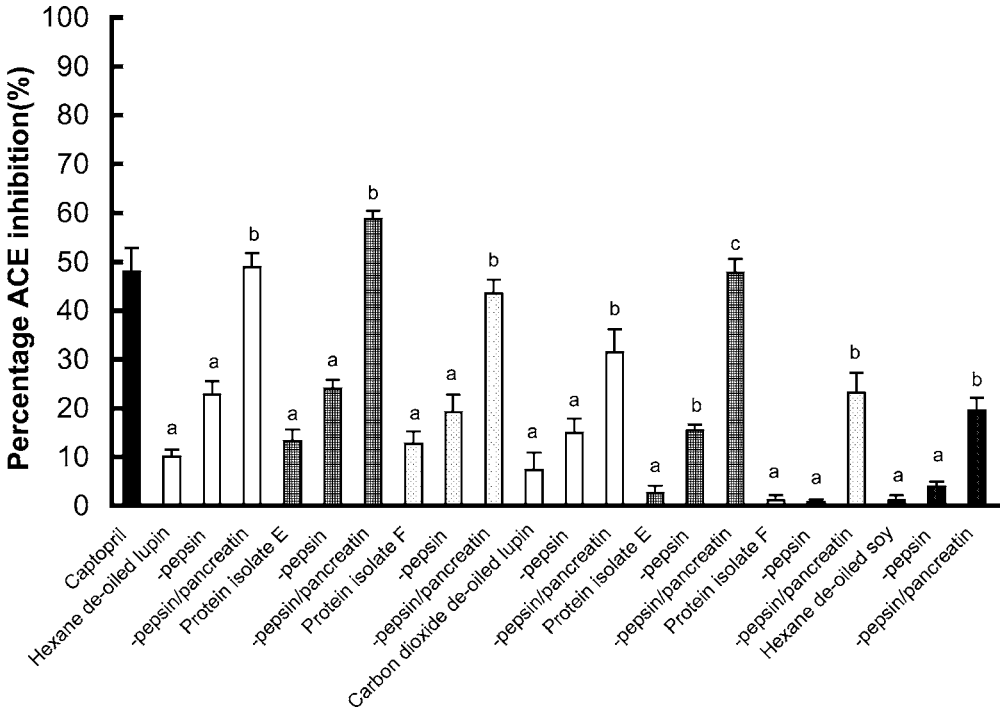

Figure 2. ACE inhibition of deoiled lupin, lupin protein isolates, deoiled soybean, and their hydrolysates. Different letters indicate significant differences among the same samples by hydrolysis ($p < 0.05$).

F and its hydrolysate to 0.7−43.4%, and by protein isolate E and its hydrolysate to 2.6−58.7%. It was also inhibited by deoiled soybean and its hydrolysate to a degree of 1.1−8.0% and by 0.01 μg/mL captopril to 48.0%.

Both hexane-deoiled lupin and CO_2-deoiled lupin showed stronger ACE inhibition than hexane-deoiled soybean. Deoiled lupin and soybean had a 56−61% protein concentration, and their degree of hydrolysis (DH) was in a range up to 13.2%. With the same approximate protein content and DH values, pepsin hydrolysate from hexane-deoiled lupin showed 22.8% inhibition (DH = 2.88%), whereas pepsin hydrolysate from deoiled soybean showed 3.75% inhibition (DH = 3.34%). After pepsin−pancreatin digestion, the DH of the deoiled lupin reached a maximum of 13.2%; however, the deoiled soybean showed a DH of 5.66%. Even with this difference in DH, the hexane-deoiled lupin had >6 times higher ACE inhibition (48.9%) than the ACE inhibition by deoiled soybean (8.03%). ACE inhibition by deoiled lupin was significantly more effective than soybean. These results demonstrated that lupins possess more effective compounds for inhibiting ACE than soybeans. We have carried out tests with soy at a final concentration of 1 mg/mL with 61% protein. Only 5.7% was hydrolyzed. This means that ~0.035 mg/mL soy hydrolysate (peptide) gave 8.0% ACE inhibition. If the IC_{50} value is calculated from the linear increase of inhibition with increased amount of peptide, soybean hydrolysate has an IC_{50} value at ~0.2−0.3 mg/mL. Wu and Ding (*51*) reported the IC_{50} value of the enzymatic hydrolysate of soy protein at 0.34 mg/mL. We estimate the ACE inhibition by soybean hydrolysate was performed with quite similar activity. Captopril was reported to show a variety of IC_{50} values under various experimental conditions, namely, 0.023 μM = 0.005 μg/mL (*52*), 1.60 nM = 0.00035 μg/mL (*53*), and 0.39−0.42 μM = 0.085−0.092 μg/mL (*54*). As 47% inhibition was observed in our experiment with 0.01 μg/mL = 0.048 μM, we tested four or five more concentration points to give an IC_{50} value. Under our test conditions, the IC_{50} value of captopril was 0.015 μg/mL.

The protein isolates had 88−100% protein content (N × 6.25). The protein isolate E samples from hexane-deoiled lupin and CO_2-deoiled lupin both showed significantly higher ACE inhibition than the protein isolate F samples. The highest inhibition was observed by pepsin−pancreatin digested protein isolate E to a degree of 58.7% with DH 11.5%.

In the same way, we also determined the IC_{50} values for pepsin−pancreatin hydrolyzed samples. Pepsin−pancreatin hydrolyzed deoiled lupin, protein isolate F, and protein isolate E were found to have IC_{50} values of approximately 0.29, 0.21, and 0.33 mg/mL, respectively. Yust et al. (*55*) reported an IC_{50} of 0.18 mg/mL for legumin hydrolysate, and Vermeirssen et al. (*53*) reported an IC_{50} of 0.90 mg/mL for whey digest and 1.36 mg/mL for pea protein digest. Compared to these reports and considering the small degree of hydrolysis, deoiled lupin and protein isolate E might be more powerful ACE inhibitors. However, as Fujita et al. (*56*) reported, further digestion may produce more binding sites for ACE, resulting in a reduction of the IC_{50} value, for example, 0.045 mg/mL for themolysin-digested chicken muscle, 0.083 mg/mL for thermolysin-digested ovalbumin, and 0.043 mg/ml for pepsin-digested ovalbumin.

For potentially replacing egg protein in baked products with lupin protein, it is useful to know the ACE inhibition per serving. For the same sample amount, the fact that the hydrolysates of lupin protein and protein isolate have higher ACE inhibition activities than soybean and its hydrolysate is important. It was also very apparent that after pepsin−pancreatin digestion, the hexane-deoiled lupins and their protein isolates showed significantly higher ACE inhibition than the CO_2-deoiled samples.

ACE plays a major role in the regulation of blood pressure, especially in raising the blood pressure. There are many patients with hypertension in developing countries, and inhibition of ACE is important for them. Captopril is a powerful ACE inhibitor and is used as a medicine to suppress hypertension. However, for long-term use and for preventing hypertension in the first place, it is recommended to replace medicines with standard foods or food fractions. Enzymatic hydrolysates of food

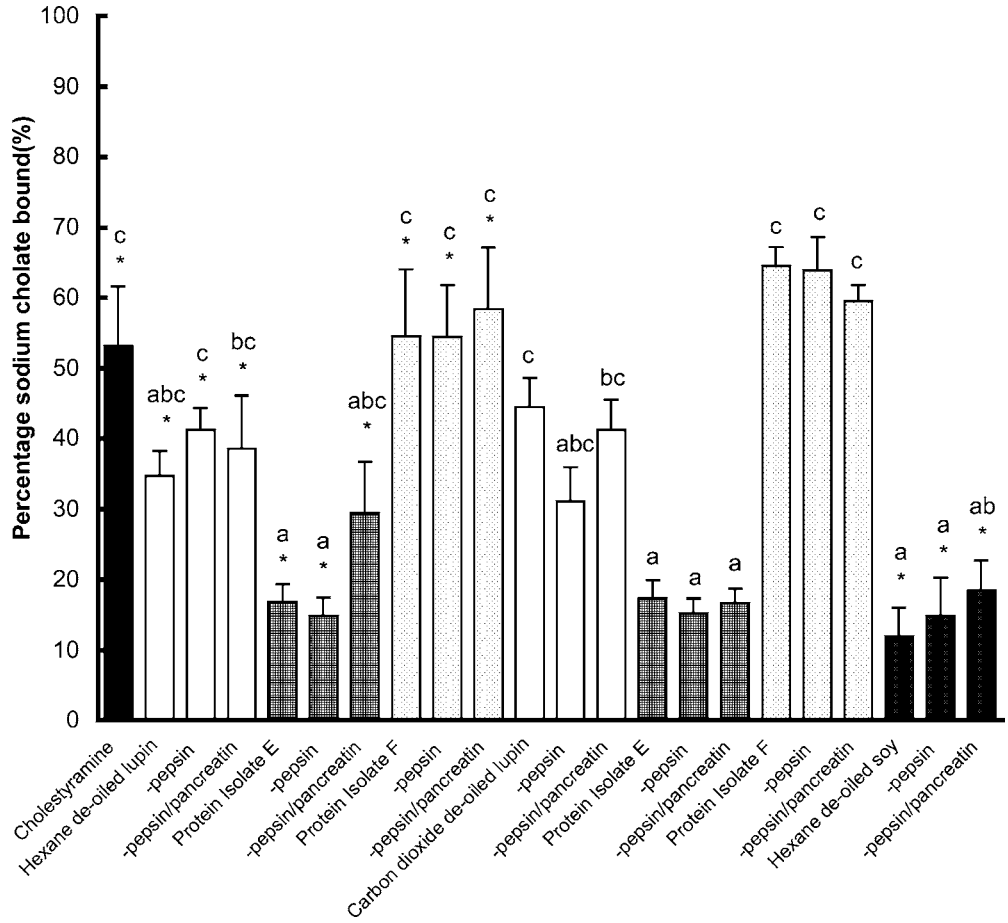

Figure 3. Bile acid binding by deoiled lupin, lupin protein isolates, deoiled soybean, and their hydrolysates. Different letters indicate significant differences ($p < 0.05$). * indicates data from Yoshie-Stark and Wäsche (*24*).

proteins have been reported to have ACE inhibition activity. Many peptides with ACE inhibition from casein, whey, soy, pea, etc. (*31*), have been described.

For further research, hydrolyzed samples from protein isolate E should be separated to give an effective peptide fraction and the precise IC_{50} must be determined for that peptide. From a different standpoint, consideration should be given to a recommendable daily intake to reduce ACE activity based on the food model.

Cholic Acid Binding. The results of sodium cholate binding by hexane-deoiled lupin, hexane-de-oiled lupin protein isolates, soybean, and cholestyramine were referred from our paper (*18*). As shown in **Figure 3**, sodium cholate was bound by deoiled lupin and its hydrolysate to a degree of 31.1−44.4%, by protein isolate F and its hydrolysate to a degree of 54.4−64.4%, and by protein isolate E and its hydrolysate to a degree of 14.8−29.4%. It was also bound by cholestyramine to a degree of 53.1% and by soybean and its hydrolysate to a degree of 11.9−18.5%. The degree of hydrolysis increased as a result of the digestion process; however, the sodium cholate binding capacity was not significantly affected by hydrolysis. There was no significant difference between hexane-deoiled lupin and CO_2-deoiled lupin. Deoiled lupin, lupin protein isolate F, and cholestyramine showed significantly higher sodium cholate binding than deoiled soybean and its hydrolysate. Protein isolate E had significantly lower cholate binding than protein isolate F, except for pepsin−pancreatin digested, hexane-deoiled protein isolate E. Camire et al. (*35*, *57*) reported cholate binding of 75% by cholestyramine, 15−20% by three types of raisin, and 10% by wheat bran. Deoiled lupin and lupin protein isolate F showed higher cholate binding capacities compared to alfalfa, raisins, and wheat bran. Some papers have mentioned that hydrophobic undigested fractions of soy protein have been shown to lower cholesterol and bind bile acids to an even greater extent than soy protein (*58*, *59*). However, in our study, the bile acid binding capacity was not affected by hydrolysis (digestion) up to a DH = 20%.

DPPH Scavenging Activity. Figure 4 shows DPPH scavenging activity as a median effective dose (ED_{50}). Because protein and phenol concentrations were expressed on the basis of hot water extract, ED_{50} was also calculated as the sample concentration of the suspension of hot water extraction. Hexane-deoiled lupin, protein isolates E and F, and their hydrolysates showed ED_{50} to the concentration of 118−238 mg/mL, whereas CO_2-deoiled lupin had ED_{50} to the concentration of 111−272 mg/mL. Hexane-deoiled lupin, CO_2-deoiled lupin, both protein isolates F, and their hydrolysates showed similar ED_{50} values to deoiled soybean and hydrolysates. Soybean samples did not show a stronger effect to inhibit ACE or to bind bile acid than lupin samples; however, they showed slightly higher radical scavenging activity than lupin samples. Deoiled lupin and protein isolate F did not show a dramatic change of ED_{50}; however, protein isolate E from both hexane-deoiled and CO_2-deoiled lupin showed the decrease of ED_{50} by hydrolysis. Phenol concentration of hot water extract was also analyzed; there was an inverse tendency between phenolic content and the EC_{50} of DPPH scavenging activity. Extracted total phenol increased while ED_{50} value decreased following the hydrolysis step with the samples of hexane-deoiled lupin and hexane-deoiled and CO_2-deoiled protein isolate E. However, they did not have clear

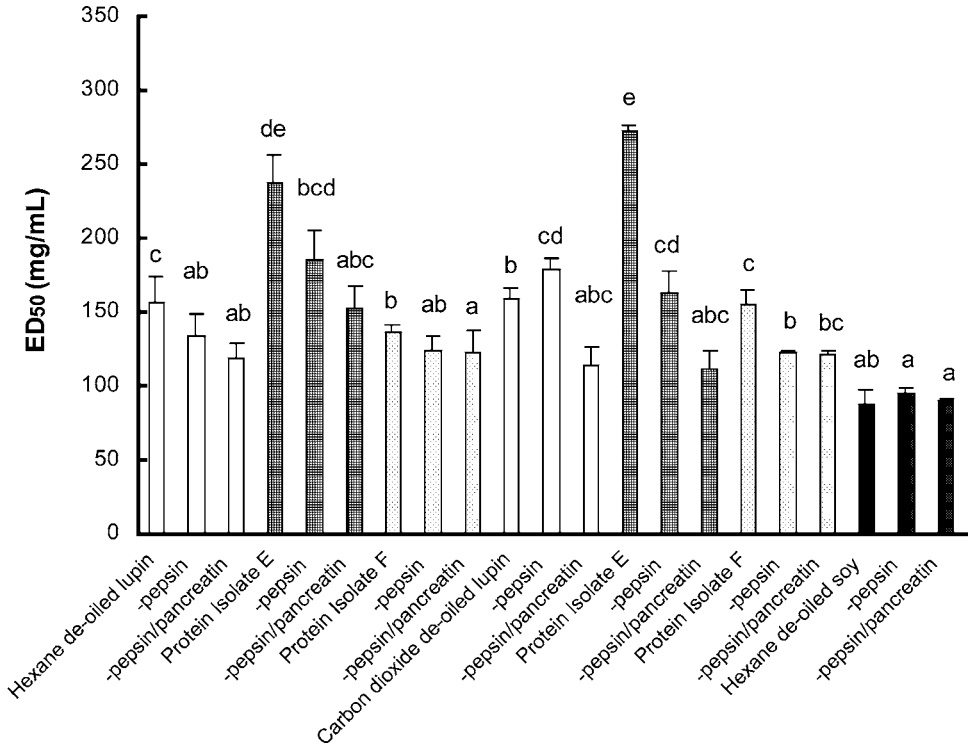

Figure 4. Solubilized protein, solubilized total phenol, and ED_{50} of DPPH radical scavenging study of deoiled lupin, lupin protein isolates, deoiled soybean, and their hydrolysates. Different letters indicate significant differences ($p < 0.05$).

correlation ($R = 0.52$). From lupin samples, extracted phenol concentration increased following the hydrolysis. Highest phenol concentration was detected from hexane-deoiled protein isolate F with pancreatin digestion, at the concentration of 98.2 mg/L; however, that sample did not show the smallest ED_{50}.

Aluko and Monu (*60*) mentioned that higher DPPH scavenging activity was shown from a smaller molecular weight fraction of quinoa protein. The peptide fragments from soybean protein digests (*61*) were also reported to have radical scavenging activity with relationship to hydrophobicity. Smaller molecular weight fractions such as peptides or phenols are supposed to be effective from these references and also from our results; however, further research is required to identify most effective compounds to scavenge radicals. Matthäus (*39*) reported the DPPH radical scavenging activities of several fractionated extracts by solvent from oilseeds plants, and that paper mentioned the extracts of 70% methanol and 70% acetone had the greatest DPPH radical scavenging activity.

As a first approach to radical scavenging activity of lupin samples, we prepared only hot water extract for DPPH radical scavenging study, and further research with some organic solvent extracts is also required.

Conclusions. Lupin protein isolates were found to have interesting functional properties. Their physicochemical properties demonstrated the potential for lupin protein to replace egg protein in food production. Deoiling method did not affect functional properties, LOX activity, or bioactive properties. Isolation of protein from lupin reduced LOX activity dramatically. Lupin protein isolate E showed greater ACE inhibition than protein isolate F. However, with regard to the bile acid binding capacity, protein isolate F showed a higher binding capacity. Lupin protein isolate F showed higher DPPH radical scavenging activity than protein isolate E. Overall, the two protein isolates have different physiological effects. Deoiled lupin and protein isolates showed more effects on ACE inhibition and bile acid binding than deoiled soybean; in contrast, they had weaker radical scavenging activity than deoiled soybean.

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