A practical and economical approach to efficient isolation of lupin protein

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Abstract

The increasing demand for low cost and non-genetically modified vegetable proteins has pushed the food industry to explore alternate sources of protein. Lupin has been found to have similar protein content and amino acid profile to that of soy. In particular, the Australian Sweet Lupin (*Lupinus angustifolius*), a low alkaloid variety of lupin, is high in protein and fibre and low in fat, making it an ideal food ingredient for health and well being. Concentrated form of lupin protein with protein content of 90% was prepared by alkaline extraction at pH 9.0 followed by acidic precipitation at eight different pH levels (4.0, 4.2, 4.4, 4.5, 4.6, 4.8, 5.0 and 5.5). The range of pH employed covered the isoelectric points of major legume proteins. The results revealed that there was no significant difference in protein content and yield of lupin protein isolates precipitated at pH 4.4, 4.5, 4.6, 4.8 and 5.0. The finding indicated that instead of using pH 4.5 for lupin protein precipitation, a higher pH such as 5.0 can be used that would result in decreased acid usage, thus providing a more economical approach to protein isolate production.

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Introduction

Food manufacturers are constantly searching for natural, high quality and low cost food ingredient. In this contest, there is an increasing demand for vegetable protein as a low cost substitution to relatively expensive animal protein. Legumes, such as soybean, pea and chickpea, are commonly used as vegetable protein sources. However among legumes lupin, being the most robust crop that can survive easily under poor growing conditions (Nelson & Delane 1991) and contains high protein content, needs more attention as a source of vegetable protein.

Studies carried out to compare the nutritional quality of Australian Sweet Lupin (*L. angustifolius*) with soy suggested that lupin has similar protein quality to that of soy (Jayasena & others 2004; Kiosseoglou & others 1999). Lupin protein contains essential amino acids that can complement those of wheat to provide a balanced amino acid profile. Moreover, the low alkaloid content of Australian Sweet Lupin (ASL) of less than 15mg/100g is safe for human consumption (Chew & others 2003; Todorov & others 1996). Despite its exceptional nutritional composition, ASL is not extensively used in human food. In order to extend its application to the food industry, the manufacturers have to be convinced that lupin protein can be easily extracted by an economical technique. The lack of information on efficiently isolating lupin protein is one of the main restrictions that limit its incorporation in the food industry (Lqari & others 2002).

Solvent extraction is one of the most widely applied methods in protein extraction due to its efficiency and ease of operation. It has been used in the soy, cottonseed, and fish protein isolating industries for many years (Dennison 1999; Lewis 1996a; Sanchez-Vioque & others 1999; Steytler 1996). Alkaline extraction and acidic (isoelectric) precipitation is one of the commonly applied methods (Chew & others 2003). The basis of such method lies on the application of different solubility and precipitation profiles of proteins (Lewis 1996a). During the process of alkaline extraction and acidic precipitation of protein, the raw material is usually subjected to an alkaline pH level between pH 8 and 12 where the protein is found to be the most soluble (Lewis 1996a). The pH of the soluble protein fraction is then adjusted between pH 4 and 5, where the isoelectric points of most of the vegetable proteins lie (Lewis 1996b; Linden & Lorient 1999; Onweluzo & others 1995; Oshodi & Ekperigin 1989; Padilla & others 1996). Alteration in pH of the protein isolation process can be manipulated to increase yield and it can be easily adopted by the food industry without heavy investment on new machinery. In addition, this method has no significant impact on the amino acid profile of protein (Chew & others 2003).

Research work conducted in Australia showed that ASL, when isolated at pH 4.5, demonstrated good functional properties (Chew & others 2003) and is comparable to that of soy protein isolate (Jayasena & others 2004). However, there was a need to improve the production process to make it more economical. In the present study a range of pH points has been applied to precipitate the lupin protein in order to find any effect on the yield and purity of the isolate in comparison with soy protein isolates. The information would be useful in optimising the process that may help reduce the cost of production of lupin protein isolate.

Materials and Methods

Sample Preparation

ASL flour was supplied by Weston Milling Company, Western Australia, Australia. The flour samples were defatted using hexane as a solvent to a final fat content of less than 0.5%. The reference soy flour was prepared by the same procedure.

Protein Isolation

The defatted flour was subjected to alkaline extraction and acid precipitation at room temperature (20±2°C). The method details are presented in Fig. 1.

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                              Defatted flour
                                    |
                                    v
              Mixing with deionised water (1:10 w/v) for 30 min

              1 M NaOH  ---> |
                             v
                   Stirring for 1 h (pH 9.0)
                             |
                             v
                 Centrifugation (3000 rpm, 15 min)
                             |
              +--------------+--------------+
              v                             v
          Supernatant                Residue (dietary fibre fraction)

   1 M HCl ---> |
               v
     pH adjustment and stirring 1
               |
               v
          Precipitation
               |
               v
   Centrifugation (3000 rpm, 15 min)
               |
       +-------+-------+
       v               v
  Whey fraction   Protein Residue
       |               |
       v               v
  Freeze drying     Washing
                       |
                       v
                  Freeze drying
                       |
                       v
                  Protein isolate
```
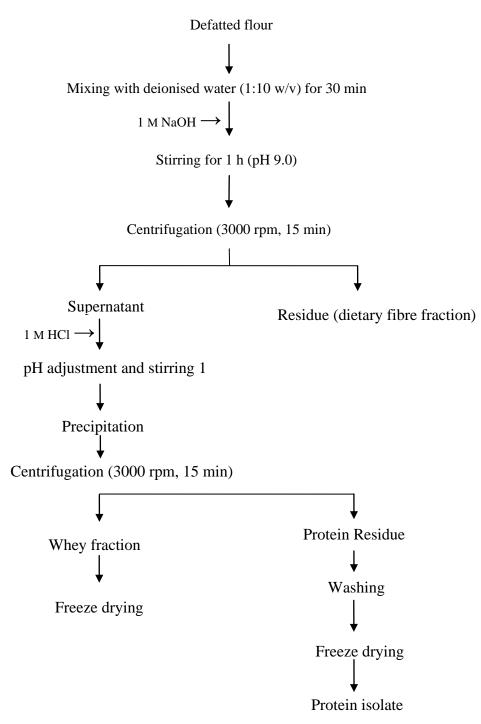
Fig. 1. Flow diagram for the preparation of protein isolates

The pH of the supernatant was adjusted to 4.0, 4.2, 4.4, 4.5, 4.6, 4.8, 5.0 or 5.5. The pH points were selected to find the optimum protein precipitation point. The resulted lupin protein isolates were named as LPI-4.0, LPI-4.2, LPI-4.4, LPI-4.5, LPI-4.6, LPI-4.8, LPI-5.0 and LPI-5.5, respectively. The procedure was carried out in triplicate for each type of protein isolate. The LPI-4.5 served as the control (control-A) as pH 4.5 is the most commonly used for lupin and soy protein precipitation. For a comparison, soy protein isolate was prepared by

treating defatted soy flour in a similar way with protein precipitation at its known isoelectric point of pH 4.5 and was referred to as SPI-4.5 (control-B).

Yield of protein isolates
The yields of protein isolate (PI) samples were calculated based on the weights of PI and defatted flour on the dry basis.

$$\text{Yield of protein isolate (\%)} = \frac{\text{Weight of protein isolate (g)}}{\text{Weight of flour (g)}} \times 100$$

Protein recovery
Protein contents of the defatted flour and protein isolate were used in calculating the protein recovery by using the following formula:

$$\text{Protein Recovery (\%)} = \frac{\text{PI Wt. (g) x PI protein contents (\%)}}{\text{Flour Wt. (g) x Flour protein contents (\%)}} \times 100$$

Chemical analysis
AOAC (2000) methods were used to determine moisture contents (method 925.09), fat contents (method 920.39C) and protein (N x 5.7) contents (method 979.09).

Statistical analysis
Data were statistically analyzed using one-way analysis of variance (ANOVA). The Duncan's post-hoc test was used to compare means at *$P \leq 0.05$*. All data were analyzed by SPSS 17.0 software.

Results and Discussion

Chemical analysis
The moisture, fat and protein contents of defatted lupin flour and defatted soy flour are reported in Table 1. The protein content of defatted lupin flour (36.8%) was lower than that of defatted soy flour (51.9%). However protein content of lupin flour was higher than other common legumes, such as chickpeas (24.7%), mung beans (26.3%), field peas (28.8%) and broad beans (32.5%) (Coffmann & Garcia 1977; Makri & others 2005; Sanchez-Vioque & others 1999; Tian & others 1999). The results are in agreement to those of Wasche & others (2001) who reported protein contents of whole lupin seeds (*L. angustifolius*) as 34.4 % (N x 6.25).

Protein extraction
Alkaline extraction of lupin flour at pH 9.0 resulted in extraction of 85% of the protein content (Table 2). The results are in agreement to the reported values of 80 to 87% at pH 9.0 for lupin flour (Chew & others 2003; Sgarbieri & Galeazzi 1978). Soybean, however, demonstrated a lower level of protein extraction (~70%) under same conditions resulting in higher protein losses in the residue. Soy flour had 31.6% and lupin flour had 15.3% of the protein retained in the residue. The protein loss could be reduced by extracting twice, however it would result in the higher production cost of the protein isolates. Most of the protein (~85%) in the lupin flour could be extracted by using a single extraction. The solubility of lupin protein can be increased to 97% by increasing pH to 11 (Ruiz & Hove 1976) however this will lead to an increase in the production cost.

Solid residue (Dietary fibre fraction)
The residue comprised largely of dietary fibre from the high dietary fibre contents of lupin flour (Guillon & Champ 2002; Lqari & others 2002). Most of the fibre in lupin is non-starch

polysaccharides with a negligible amount (0.4%) of starch (Nelson & Delane 1991), making it a product with low glycaemic index (GI). Lupin has the advantage of having lower oligosaccharides level (4.6%) than soy (5.7%), thus reducing the uneasiness accompanied by indigestion of soy oligosaccharides (Petterson & Crosbie 1990). The soluble and insoluble dietary fibre in these fractions should be investigated to determine its application.

Chemical analysis of the fibre fraction revealed that it contained around 13% protein (Table 2). The high amount of dietary fibre coupled with a considerable amount of protein makes these dietary fibre fractions an attractive ingredient in the food industry for use in different healthy foods. Further investigations on the potential food applications of this fraction could lead to development of novel healthier food ingredients. Its application in the food industry can also resolve the disposal problem, leading to a more sustainable production.

Effect of pH on the protein contents of whey fraction and protein isolates

There was a clear effect of precipitation pH on the protein recovery in the whey fraction and in the protein isolates. The protein recovery and protein contents in the whey fraction were lower at pH around 4.6 (Fig. 2). Protein recovery in the protein isolates was lower at pH 4.0 and 4.2 as compared to higher pH points. However, there was no significant difference ($P \leq 0.05$) in protein recovery and protein contents of the protein isolates prepared at pH range of 4.4-5.0 (Table 3). At this pH range (pH 4.4-5.0), 75-79% of the soluble protein was recovered as protein isolate. The protein recovery was lower than the 83% reported earlier by Ruiz & Hove (1976) but higher than the 59% reported by Chew & others (2003). Protein recovery can be improved by using different precipitation aids such as sodium hexametaphosphate and sodium sulphate (Jayasena & others 2006; Ruiz & Hove 1976).

The pH 4.5 is mostly used in protein isolation (Chew & others 2003; Kiosseoglou & others 1999; Ruiz & Hove 1976) however, our results revealed that a pH ranging from 4.4 to 5.0 has no significant effect ($P \leq 0.05$) on protein recovery in lupin protein isolates. Production of lupin protein isolate at a higher pH level such as pH 5.0 reduces the cost of production as less amount of acid is required at the precipitation step.

Protein contents of the lupin protein isolates were not significantly affected ($P \leq 0.05$) by the selected precipitation pH points (Table 3). All of the lupin protein isolates were high in purity containing around 90% protein (N x 5.7). The protein contents of the isolates were similar to that reported by Kiosseoglou & others (1999) and Lqari & other (2002). In contrast, Chew & others (2003) showed lower purity both by isoelectric precipitation (67%) and by ultrafiltration (75%). The protein contents (N x 5.7) of the lupin protein isolates were higher than the protein isolates prepared from soybean (82.2%), field peas (80.3%), faba bean (86.3%) and chickpea (78%) (Sanchez-Vioque & others 1999; Sosulski & Mc Curdy 1987). The results also indicate that the protein contents of lupin protein isolates prepared at pH 4.4-5.5 were significantly higher than that of soy protein isolate (Table 3). In other words, the purity of lupin protein isolate was better than soy protein isolate.

The high protein contents of lupin protein isolates (~90%) indicate that the extraction pH of 9.0 as well as the range of acidic pH values applied in this study are effective in producing highly concentrated protein isolates. Protein isolates with high protein contents are preferable in the food industry since lesser amount is required to achieve desirable nutritional and functional properties in food products.

Effect of pH on the yield of protein isolate

Yield of protein isolates ranged from 22.5% to 27.1% of the starting material (lupin flour) as a function of the pH range applied for protein precipitation (pH4.0-5.5). However statistical analysis demonstrated that there was no significant difference in yields of protein isolates precipitated at pH 4.2 to pH 5.5 (Table 3). The yields of all lupin protein isolate samples were

significantly lower than that of soy protein isolate. It was mainly due to substantially higher protein contents in the soy flour than the lupin flour.

The protein isolate yield could be increased by different techniques such as repeating the extraction process (Kiosseoglou & others 1999), increasing the pH of the extracting solution to 11 or 12 (Lqari & others 2002; Ruiz & Hove 1976), using extraction aids such as Na_2SO_3 (Sanchez-Vioque & others 1999) or using various precipitating aids such as sodium hexametaphosphate and sodium sulphate (Jayasena & others 2006; Ruiz & Hove 1976). However all these practices incur an additional cost and could result in deterioration of the functional properties which may not be feasible technically and economically. In case of using higher pH for extraction or repeating the extraction step a higher volume of acid will be required to lower the pH of the protein suspension during the acid precipitation step. Another major drawback of the application of extreme alkaline pH is protein denaturation. The subsequent inferior quality will limit the applications of such protein in the food industry.

Functional properties

Emulsifying activity and emulsion stability

Conclusions

Lupin protein isolates containing high protein content of above 90% with the potential to replace soy protein isolate as a new, low-cost source of vegetable protein can be prepared by using a simple method. For the production of lupin protein isolates, the commonly applied precipitation point of pH 4.5 may not be required. A protein isolate with similar protein content and yield can be produced at a higher pH point (for example pH 5.0) which will reduce the volume of acid required for the precipitation of protein, thus reducing the production cost of protein isolates. Reduced acid utilization will also help reduce environmental pollution. Although it could not be done in this study, other properties such as colour of the protein isolate may be important and could be considered in the future studies.

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Table 1. Composition of the defatted lupin and soy flour

Contents	Defatted Lupin Flour	Defatted Soy Flour
Moisture (%)	9.7±0.1	10.1±0.0
Protein (% d.b.)	36.8±1.4	51.9±0.4
Fat (% d.b.)	0.2±0.0	1.2±0.2

Table 2. Composition of solid residue after protein extraction at pH 9.0

Contents (d.b.)	Defatted Lupin Flour	Defatted Soy Flour
Solid residue (g/100 of flour)	40.6±5.1	39.7±2.1
Protein content (g/100g)	13.1±0.6	37.2±1.3
Protein recovery (g/100g of total)	15.3±1.6	31.6±1.5

Table 3. Effect of pH on the protein content, protein recovery and yield of the protein isolates

Samples	Protein content (%, d.b.)	Protein recovery (% of the total protein, d.b.)	Protein recovery (% of the soluble protein, d.b.)	Yield (% of flour, d.b.)
LPI 4.0	89.3±1.9^{ab}	50.0±1.9^{c}	63.3±2.4^{f}	22.5±2.0^{c}
LPI 4.2	89.4±1.3^{ab}	55.9±0.4^{b}	68.8±2.1^{e}	24.9±0.4^{bc}
LPI 4.4	92.2±2.7^{a}	60.9±1.7^{a}	74.8±3.3^{bcd}	25.7±1.2^{b}
LPI 4.6	92.2±2.2^{a}	62.3±1.4^{a}	78.8±4.0^{b}	26.5±0.7^{b}
LPI 4.8	94.4±1.1^{a}	61.2±1.3^{a}	78.5±4.6^{b}	25.8±1.1^{b}
LPI 5.0	90.8 ±4.8^{a}	59.4±1.7^{a}	75.8±0.4^{bcd}	26.9±1.5^{b}
LPI 5.5	94.5 ±2.3^{a}	59.6±2.9^{a}	72.4±0.8^{de}	24.6±1.0^{bc}
Control-A (LPI 4.5)	89.7 ±1.3^{ab}	59.7±1.1^{a}	75.5±2.7^{bcd}	27.1±1.2^{b}
Control-B (SPI 4.5)	85.2 ±5.4^{b}	57.7±2.2^{b}	86.0±0.4^{a}	35.5±0.5^{a}

Values (expressed as means ± s.d.; n = 3) with the same letter in a column are not significantly different (p≤ 0.05); d.b.= dry basis.

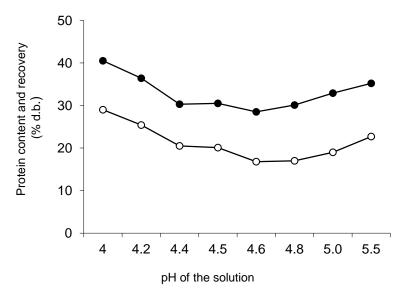Fig. 2. Effect of pH on the protein contents and protein recovery of the whey fraction of lupin protein (protein contents ─●─, protein recovery ─○─).