

Protein quality and physico-functionality of Australian sweet lupin (*Lupinus angustifolius* cv. Gungurru) protein concentrates prepared by isoelectric precipitation or ultrafiltration

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Received 1 October 2002; received in revised form 28 February 2003; accepted 28 February 2003

Abstract

The protein quality and physico-functional properties of Australian sweet lupin protein concentrates, prepared by isoelectric precipitation or ultrafiltration, were assessed. The ultrafiltration process resulted in a higher yield of protein than did the isoelectric precipitation process. The lupin kernel and the two lupin protein concentrates had similar essential amino acid compositions that were inferior to ideal human requirements. True digestibilities of the isoelectrically precipitated (ISO) and the ultrafiltered (UF) lupin protein concentrates were similar but significantly higher ($P < 0.05$) than that of casein. Net protein utilisations (NPU) of the ISO and UF protein concentrates were similar but significantly lower ($P < 0.05$) than that of casein. The UF protein concentrate had higher protein solubility than did the ISO protein concentrate ($P < 0.05$). Low foaming capacity, low viscosity, but high emulsification capacity (particularly at low pH) were observed for lupin protein concentrates.

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Keywords: Lupin; Protein; Ultrafiltration; Protein quality; Functional properties; Isoelectric precipitation

1. Introduction

Australian sweet lupin (*Lupinus angustifolius*) is the largest legume crop grown in Australia (Agriculture Fisheries and Forestry—Australia, 2001). This crop is under-utilised as human food, the majority of the annual Australian harvest of approximately 2 million tonnes being used domestically or overseas for livestock feed (Pettersen, 1998).

Australian sweet lupin is considered suitable for human consumption since it has low levels of bitter-tasting and potentially toxic alkaloids (Pettersen, 1998). In addition, lectins and protease inhibitors, that can

reduce protein digestibility, are found at lower levels in lupin than in many other legumes (Pettersen, Sipsas, & McIntosh, 1997). *L. angustifolius* kernels contain approximately 40% protein (Pettersen et al., 1997), a level that is approaching that of soy. As part of the trend towards the increased use of plant-derived ingredients in formulation of foods such as dairy and meat analogues, lupin has attracted interest worldwide as a potential high protein food ingredient suitable for human consumption (Fudiyansyah, Pettersen, Bell, & Fairbrother, 1995; Johnson & Gray, 1993; Pettersen & Crosbie, 1990).

Although lupin kernel has high levels of protein, rat studies have shown that the protein quality of Australian sweet lupin is inferior to that of egg protein and casein (Fudiyansyah et al., 1995; Ruiz & Hove, 1976; Yen, Grant, Fuller & Pusztai, 1990). This low protein quality can in part be explained by the low proportions of essential sulphur amino acids, methionine and cystine, in the kernel protein of the *Lupinus* species (Ballester et al., 1980; Cerletti, 1983; Schoeneberger, Gross, Cremer, & Elmadfa, 1982; Sgarbieri & Galeazzi,

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1978). Nevertheless, a recent study in human subjects highlighted the high bioavailability of protein from a low-alkaloid *Lupinus albus* variety (Mariotti, Pueyo, Tome, & Mahe, 2002). Lupin-derived protein ingredients need to provide both adequate nutritional and useful technological functionality to the foods in which they are incorporated in order for them to meet the needs of consumers and the food industry. Consumer acceptance of foods can be governed by the physical characteristics of foods (Morr, 1979), which are influenced by the physico-functional properties of their ingredients, in particular, emulsification, foaming, viscosity and solubility characteristics. Solubility in an aqueous environment is considered a particularly influential property of protein food ingredients because high solubility is required if other desirable functional properties are to be achieved (Wolf, 1970). On this basis, it is important to identify and optimise the physico-functional properties of novel protein ingredients, such as those prepared using Australian sweet lupin, in order to maximise the opportunity for food industry applications (Martinez, 1979; Wagner, Sorgentini & Anon, 1992).

Isoelectric precipitation has been the most commonly used method of protein isolation in the commercial production of legume protein food ingredients (Lusas & Riaz, 1995). Unfortunately, this procedure does not fully recover all proteins (Millan, Alaiz, Hernandez-Pinzon, Sanchez, & Bautista, 1994; Oomah & Bushuk, 1983; Ruiz et al., 1976). Of particular concern is the lack of recovery of proteins high in the nutritionally essential sulphur-amino acids (Sgarbieri et al., 1978), which are generally deficient in many vegetable protein sources. Incomplete recovery of proteins by isoelectric precipitation has been identified as the major factor leading to the protein quality of isoelectrically-precipitated lupin protein isolate being lower than that of the lupin flour from which it was extracted (Sgarbieri et al., 1978). Acid precipitation and neutralisation, which are used to prepare isoelectrically precipitated soy protein isolate, can also result in a loss of solubility (KeShun, 1997); this in turn can impact negatively on other important physico-functional properties. Ultrafiltration has been identified as an alternative process to isoelectric precipitation for the manufacture of purified protein ingredients from legumes, resulting in improved protein recovery and improved physico-functional properties (Berot, Gueguen, & Berthaud, 1987; Deeslie & Cheryan, 1988; KeShun, 1997). Limited information is available on the relative merits of isoelectric precipitation and ultrafiltration in the preparation of protein food ingredients from Australian sweet lupin. The aim of this study, therefore, was to identify and compare the protein quality and physico-functional properties of protein concentrates prepared from Australian sweet lupin using isoelectric precipitation or ultrafiltration.

2. Experimental

2.1. Preparation of protein concentrates

Based on previously published methods (Manrique, 1977; Ruiz et al., 1976), split, dehulled Australian sweet lupin kernels (*Lupinus angustifolius* cv. Gungurru), supplied by Speciality Ingredients (Aust.) Ltd (Fremantle, 6160, Western Australia), were soaked in distilled water at 1:3 w/v ratio for 3 h at room temperature. Further water was then added to give an original dry kernel: added water ratio of 1:10 w/v, prior to homogenisation for 1 min at maximum speed in a Waring blender (Model 31BL44). The pH of the slurry was adjusted to 8–9 using 0.5 mol/l NaOH_(aq). The slurry was then stirred for 30 min, and, following this, separated at 2060 g for 30 min at 4 °C using a Beckman Model GS-6R centrifuge (Beckman Instruments Inc, Palo Alto, CA, 94304, USA). The alkaline protein extract supernatant was removed by decantation and the extraction procedure was repeated on the residue pellet at an original dry kernel:added water ratio of 1:5 w/v. The resulting supernatants from the two alkaline extractions were combined for use in protein concentrate preparation.

Isoelectrically-precipitated (ISO) protein concentrate was obtained by acidifying the alkaline protein extract to pH 4.5 at 4 °C using HCl_(aq) (see Fig. 1) (Ruiz et al., 1976). The precipitated protein was separated at 2060 g at 4 °C for 30 min using a Beckman Model GS-6R centrifuge.

Ultrafiltered (UF) protein concentrate was prepared from the alkaline protein extract using an Amicon Model DC10L ultrafiltration unit (Amicon Corp., Beverly, MA, 01915, USA), fitted with a spiral-wound Type S10Y10 membrane cartridge (nominal molecular weight cut-off of 10,000 Daltons, membrane area 0.1 m²). The alkaline protein extract was pre-heated to 40 °C and concentrated five-fold by ultrafiltration. An additional four volumes of deionised water (40 °C) was added to the resulting retentate, followed by five-fold re-concentration (diafiltration). The diafiltration step was then repeated.

The ISO and UF protein concentrates were neutralised to pH7.0±0.1 with HCl_(aq) or NaOH_(aq), prior to being spray-dried to a free-flowing powder, using a Niro Model HD06805K spray-dryer (NiroNiro A/S, Soeborg, 2860, Denmark) with inlet and outlet air temperatures of 170 and 74 °C, respectively.

Protein contents of the lupin kernels, alkaline protein extract, and final dry powdered ISO and UF protein concentrates were measured by Kjeldahl nitrogen distillation, based on Association of Official Analytical Chemist (AOAC) standard procedures (AOAC, 1995), using a semi-micro Kjeldahl autoanalyser (Gerhardt GmbH & Co., Bonn, 53119, Germany) and a nitrogen conversion factor of 5.5 (Mosse, 1990). All reagents

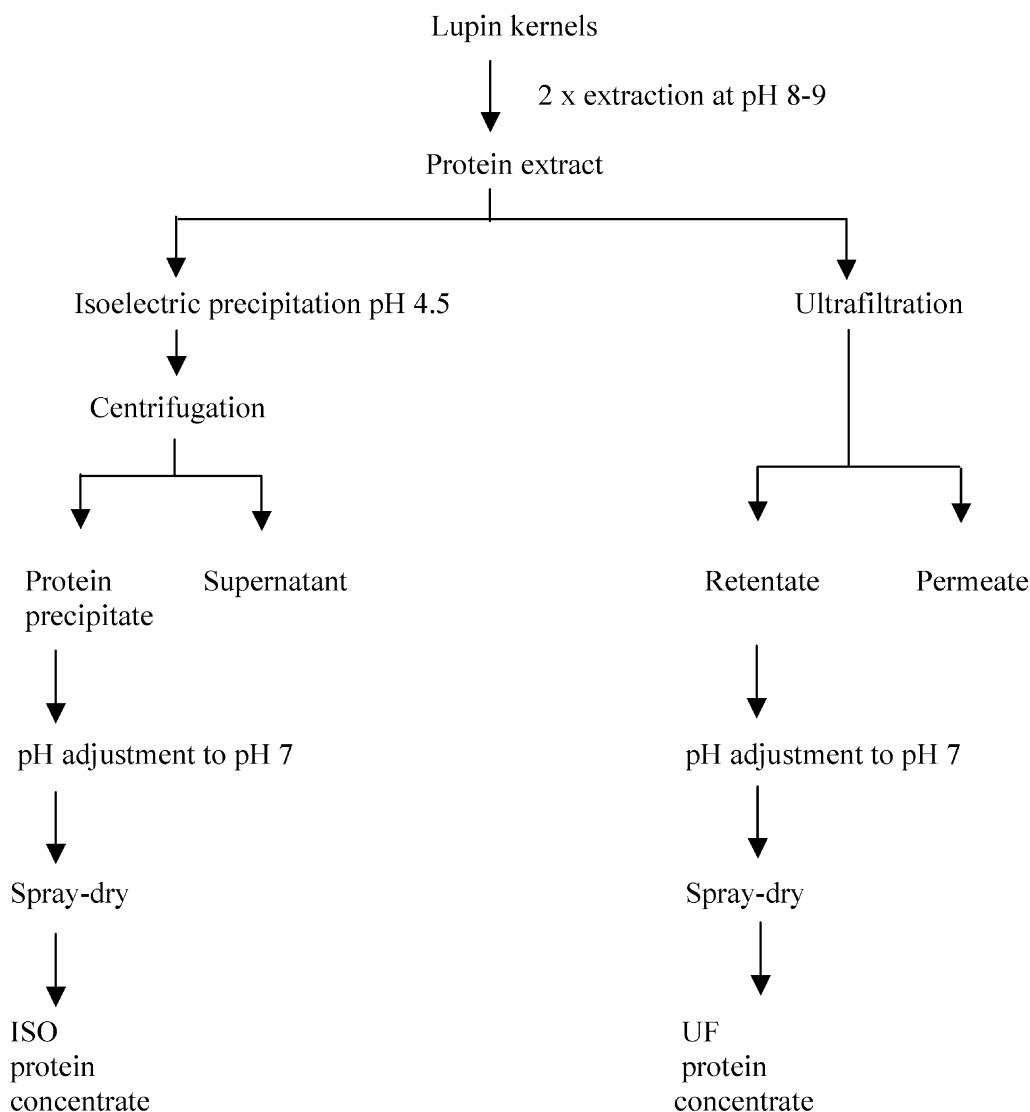


Fig. 1. Overview of process for protein concentrate preparation.

used for protein concentrate preparation and analysis were of Analytical Grade (BDH Laboratory Supplies, Poole, BH15 ITD, England).

2.2. Assessment of protein quality

The amino acid compositions of the lupin kernels and the ISO and UF lupin protein concentrates were determined by high-performance liquid chromatography (Rayner, 1985), and compared to the human amino acid requirement for a 1-year-old infant and for an adult (Food and Agriculture Organisation/World Health Organisation, 1991).

Protein digestibility and net protein utilization (using whole body nitrogen analysis) of the ISO and UF lupin protein concentrates were determined in rats. Ethics approval for the rat studies was given by the Animal Experimental Ethics Committee of Deakin University.

Four isonitrogenous and isoenergetic rat diets were formulated according to the American Institute of Nutrition Rodent Diet, AIN-93G (Reeves, Nielsen, & Fahey, 1993), using ISO lupin protein concentrate, UF lupin protein concentrate or casein as the only sources of protein to formulate diets that contained 10% protein, or egg albumin to formulate the low protein control diet that contained 4% protein (see Table 1). Soy oil was used to balance the fat content of the diets to correct for the different levels of fat in the protein sources.

Weanling, male rats (*Rattus norvegicus*, Sprague–Dawley strain) were allocated to either ISO lupin protein concentrate ($n=5$), UF lupin protein concentrate ($n=5$), casein control (CC) ($n=4$) or low protein control (LPC) ($n=5$) dietary groups. The rat weight for each group (mean \pm standard deviation) was 106.6 ± 3.3 g. The rats were housed individually in Nalgene stainless steel mesh-bottom, clear metabolic cages that incorporated

Table 1
Composition of experimental diets (g kg⁻¹)

Ingredient	Diet			
	ISO lupin protein concentrate	UF lupin protein concentrate	Casein	Low protein control
ISO lupin protein concentrate	125.3	0.0	0.0	0.0
UF lupin protein concentrate	0.0	112.4	0.0	0.0
Casein	0.0	0.0	116.1	0.0
Egg albumin	0.0	0.0	0.0	40.0
Soy bean oil	53.4	63.1	58.2	58.2
Cellulose	50.0	50.0	50.0	50.0
Sucrose	100.0	100.0	100.0	100.0
Starch	626.3	629.5	630.7	706.8
Mineral mix (AIN-93G-MX) ^a	35.0	35.0	35.0	35.0
Vitamin mix (AIN-93-VX) ^a	10.0	10.0	10.0	10.0
Total	1000.0	1000.0	1000.0	1000.0

^a Prepared according to Reeves et al. (1993).

faeces and urine separation and collection mechanisms (Nalge Nunc International Corporation, Rochester, NY, 14625, USA). Cages were housed in an air-conditioned room maintained at 20 °C with a 12-h light and dark cycle. A 2-day acclimatisation period on normal stock diet (A.R.M. Cubes, Clark King & Co., Gladesville, 2111, Australia) was followed by a 4-day preliminary period on the experimental diets and then a 5-day balance period also on the experimental diets. During the preliminary and balance periods, each rat received a diet equivalent to 150 mg nitrogen and 10 g dry matter per day; water was provided ad libitum. On each day of the balance period, the unconsumed diet was collected and weighed in order to calculate nitrogen intake. Faeces were also collected daily during the balance period, weighed and stored at -20 °C. At the end of the balance period, rats were asphyxiated with CO₂ and the carcasses stored at -20 °C.

The total faecal production during the balance period of each individual rat was dried at 105 °C overnight in a convection oven and ground with a pestle and mortar to a fine powder in preparation for nitrogen determination. Rat carcasses were freeze-dried to a constant weight, using a DynaVac Engineering Fd3 Freeze Dryer (DynaVac Engineering Pty Ltd, Wantirna South, 3152, Australia), then individually homogenised to a coarse powder using a Waring blender. 1.0 ± 0.1 g faeces and 0.5 ± 0.1 g carcass were analysed in duplicate for nitrogen contents using Kjeldahl distillation as previously described.

The protein quality indices of true digestibility (TD) and net protein utilisation (NPU) using whole-body nitrogen analysis were calculated using the following formulae (Miller & Bender, 1955):

$$TD = \frac{N_{\text{intake}} - (N_{\text{faecal}} - N_{\text{metabolic}})}{N_{\text{intake}}} \times 100$$

where,

N_{intake} = Nitrogen consumed on experimental diet during balance period

N_{faecal} = Total nitrogen excreted in faeces on experimental diet during balance period

$N_{\text{metabolic}}$ = Total nitrogen excreted in faeces on low-protein diet during balance period

$$NPU = \frac{TBN_{\text{exp}} - (TBN_{\text{lp}} - N_{\text{lp}})}{N_{\text{exp}}} \times 100$$

where,

TBN_{exp} = Total body nitrogen on experimental diet after balance period

TBN_{lp} = Total body nitrogen on low protein diet after balance period

N_{lp} = Nitrogen intake on low protein diet during balance period

N_{exp} = Nitrogen intake on experimental diet during balance period

2.3. Evaluation of physico-functional properties

Based on previously published methods (Morr et al., 1985), protein solubilities of ISO and UF lupin protein concentrates were determined by stirring 500 mg of each protein concentrate for 1 h in 40 ml 0.1 mol/l NaCl_{aq} at pH 2, 4, 6 or 8, the pH being adjusted and maintained using 0.1 mol/l HCl_(aq) or 0.1 mol/l NaOH_(aq). Each sample was adjusted to 50 ml, volumetrically, with 0.1 mol/l NaCl_(aq), then centrifuged at 6860 g for 30 min at 20 °C in a Jouan CR 411 centrifuge (Jouan, Winchester, VA 22602, USA). The resulting supernatant was filtered through Whatman no.1 filter paper and the nitrogen content determined in triplicate using Kjeldahl distillation as previously described. Protein content was calculated using a nitrogen conversion factor of 5.5 (Mosse, 1990). Protein solubility was calculated as:

Protein solubility (%) =

$$\frac{\text{Supernatant protein conc. (mg/ml)} \times 50}{\text{Sample wt. (mg)} \times \frac{\text{Sample protein content (\%)}}{100}} \times 100$$

The foaming capacity of ISO and UF lupin protein concentrates was assessed in quadruplicate at pH 2, 4, 6 or 8, using previously published methods (Gruener & Ismond, 1997). Samples were dispersed in distilled water (2% w/v) and pH was adjusted using 0.1 mol/l HCl_(aq) or 0.1 mol/l NaOH_(aq). The dispersion was stirred for 1 h at room temperature using a magnetic stirrer, then transferred to a 50 ml graduated measuring cylinder. The dispersed samples were then homogenised for 1 min using a Tekken 50 homogeniser (Ika Works, Rawang

Indah, Rawang-Selangor, 4800, Malaysia) on the 'high' speed setting. The volume of the resulting foam layer was noted 30 s after the end of the homogenisation. Foam capacity was calculated as follows;

Foam capacity (%) =

$$\frac{\text{Volume of foam phase (ml) 30 s post-homogenising} \times 100}{\text{Volume of original solution (ml)}}$$

The emulsion capacity of the ISO and UF lupin protein concentrates was evaluated in quadruplicate at pH 2, 4, 6 or 8 using previously published methods (King, Aguirre, & de Pablo, 1985). A sample (2.00 g) was homogenised for 30 s in 100 ml of distilled water using a Tekken 50 homogeniser, after which pH was adjusted using 0.1 mol/l NaOH_(aq) and 0.1 mol/l HCl_(aq). The dispersion was then transferred into a Waring blender to which canola oil was added at a rate of 40 ml/min using a BioRad Econo pump (Selby Biolab, Clayton, 3168, Australia), whilst blending to produce an emulsion. A resistance meter was used to monitor the electrical resistance of the emulsion. The time from start of oil addition until the separation of the emulsion, as indicated by a rise in electrical resistance to infinity, was recorded. Emulsion capacity was calculated as follows:

Emulsion capacity (ml oil/g protein) =

$$\frac{\text{Time (min) of oil addition} \times \text{rate of oil addition (ml/min)}}{\text{Sample wt. (g)} \times \frac{\text{Sample protein content (\%)}}{100}}$$

The apparent viscosity of ISO and UF lupin protein concentrates was measured in quadruplicate at 10, 20, 30, 50 and 70 °C at each of pH 4, 6, 7 or 8. 1% (w/v) dispersions of the protein concentrates in distilled water were prepared by blending for 30 seconds using a Tekken 50 homogeniser on setting '7'. The pH of suspensions was adjusted using 0.1 mol/l NaOH_(aq) and 0.1 mol/l HCl_(aq). Apparent viscosity measurements were performed using a Brookfield DV-1+ viscometer (Brookfield Engineering, Stoughton, MA, 02072, USA) with a UL adaptor (Spindle 00) and a spindle speed of 50 rpm (shear rate 61.2 s⁻¹).

2.4. Statistical analysis

The data were analysed using SPSS 10.0 for Windows statistical software (SPSS Inc., Chicago, IL 60606, USA). Comparisons of group means were subject to one way ANOVA using the Student Newman Kuels post hoc test. In all analyses, $P < 0.05$ was considered significant.

3. Results and discussion

3.1. Preparation of protein concentrates

Alkaline extraction of the lupin kernels resulted in solubilisation of 87% of the kernel protein, of which 59% was recovered by isoelectric precipitation, a value lower than the 70–85% protein recovery previously reported using this method (Millan et al., 1994; Ruiz et al., 1976; Sgarbieri et al., 1978). In terms of protein recovery, ultrafiltration appeared more effective than isoelectric precipitation, recovering 92% of the total protein solubilised by alkaline extraction of the kernels. The loss of the acid-soluble protein fraction during ISO lupin protein concentrate manufacture but apparent recovery during the ultrafiltration process is of possible nutritional significance, given that it has been reported that the acid-soluble fraction of legume protein contains a higher proportion of nutritionally essential sulphur amino acid-rich proteins than does the acid-precipitable fraction, and many legumes are well known for their overall lack of sulphur amino acids (Cerletti, Duranti, & Restani, 1983; Oomah et al., 1983).

The ISO lupin protein concentrate contained 671 g/kg protein in comparison to the UF protein concentrate, which contained 751 g/kg protein. Qualitative assessment of the colour indicated that the UF lupin protein concentrate was less yellow in colour than the ISO lupin protein concentrate. The paler colour of the UF protein concentrate could be advantageous for its incorporation into food products.

3.2. Assessment of protein quality

The essential amino acid compositions of the lupin kernel and the ISO and UF protein concentrates are shown in Table 2. These data illustrate that the lupin kernel and the ISO and UF protein concentrates appear

Table 2
Essential amino acid composition and score of *Lupinus angustifolius* fractions

Amino acid	Human amino acid requirements (mg amino acid/g protein) ^a		Essential amino acid content (mg amino acid/g protein)		
	1 year old	Adult	Kernel flour	ISO protein concentrate	UF protein concentrate
Histidine	26	16	25.1	22.7	22.5
Isoleucine	46	13	35.7	40.8	37.5
Leucine	93	19	61.1	71.0	65.4
Lysine	66	16	39.2	38.8	42.2
Threonine	43	9	31.8	32.4	34.3
Tryptophan	17	5	6.8	7.6	6.0
Valine	55	13	34.4	36.9	34.7
Methionine & cysteine	42	17	15.2	16.1	14.5
Tyrosine & phenylalanine	72	19	63.5	75.3	76.7

^a Food and Agriculture Organisation (1991).

generally similar in their essential amino acid profile and fulfil the requirements of a human adult for all essential amino acids except for the sum of cysteine and methionine, which was slightly deficient. With respect to the amino acid requirements of a 1-year-old human infant, all of the essential amino acids (except tyrosine and phenylalanine in the ISO and UF protein concentrates) were deficient. Lupin kernel, and ISO and UF protein concentrates, were found to be generally deficient in essential amino acids compared to published values for the essential amino acid composition of important protein sources for human nutrition, such as casein, beef, egg wheat, soy and wheat (Friedman, 1996; Yen et al., 1990). In the present study, the sum of cysteine and methionine was found to be limiting in all lupin samples. This deficiency in sulphur amino acids has been commonly reported for other legumes, including protein isolates from other lupin species (Donovan, McNiven, McLeod, & Anderson, 1991; El-Adawy, Rahama, El-Bedawy, & Gafar, 2001; Friedman, 1996). Lysine has been reported to occur in high proportions in legume proteins, such as soy, compared to the proportions found in grains, such as wheat (Friedman, 1996; Kinsella, 1979). The findings of the present study, like those previously reported for Australian sweet lupin, as well as other *Lupinus* species (Pettersen et al., 1997), indicate that lupin protein, though generally having a higher lysine concentration than wheat, is deficient in this amino acid compared to many other more commonly consumed legume species. Isoelectric precipitation did not dramatically modify the essential amino acid profile of the lupin protein concentrate compared to the lupin kernel and, in agreement with a previous study on *L. albus* (Alamanou & Doxastakis, 1995), no notable differences in the essential amino acid profiles of ISO and UF lupin protein concentrates were found.

The true digestibility (TD) and net protein utilisation (NPU) of the ISO and UF lupin protein concentrates and casein are presented in Table 3. In contrast to reports that legumes have low protein digestibility compared to animal protein (Mongeau, Sarwar, Peace, & Brassard, 1989), the lupin protein concentrates in the present study showed significantly higher true digestibility than did casein ($P < 0.05$) and higher digestibility

Table 3
The nutritional indices of lupin protein concentrates and casein

	TD (%)	NPU (%)
ISO lupin protein concentrate	98.3 ± 1.3a	45.4 ± 16.8a
UF lupin protein concentrate	98.2 ± 1.7a	45.9 ± 23.9a
Casein	94.8 ± 1.6b	78.1 ± 28.2b

Results are expressed as mean ± standard deviation. $n = 5$ for ISO and UF lupin protein concentrates; $n = 4$ for casein. TD = true digestibility; NPU = net protein utilisation. Means within the same column with different letters are significantly different ($P < 0.05$).

than reported for a range of other legumes (Friedman, 1996). The high digestibility of lupin protein concentrates found in the present study may in part be explained by the fact that antinutritional factors such as lectins and protease inhibitors, which can reduce protein digestion, are found in relatively low levels in *L. angustifolius* compared to many other legumes (Pettersen et al., 1997) and that the protein has been released for the kernel matrix in the preparation of the concentrates. The NPU values of both ISO and UF lupin protein concentrates were significantly lower ($P < 0.05$) than that for casein, although no significant difference in NPU between the ISO and UF lupin protein concentrates was found ($P > 0.05$). These results indicate that the lupin protein concentrates have an NPU that is comparable to that reported for many other legumes such as the lentil, the broad bean and the peanut but lower than that reported for soy (Friedman, 1996).

3.3. Evaluation of physico-functional properties

The effect of pH on the protein solubility of UF and ISO lupin protein concentrates is illustrated in Fig. 2. Both concentrates showed similar trends in solubility, which was maximal at pH 2 and 8 and minimal at pH 4–5. The UF lupin protein concentrate had a higher solubility ($P < 0.05$) at all pH levels tested except pH 8. It appears that the UF process resulted in a protein concentrate that had improved solubility compared to that of the protein concentrate prepared by isoelectric precipitation, consistent with a previously published study investigating *L. albus* protein (Alamanou et al., 1995). For optimum functional applications of vegetable proteins, such as soy, over 90% protein solubility is required (KeShun, 1997). Although the lupin protein concentrates in the present

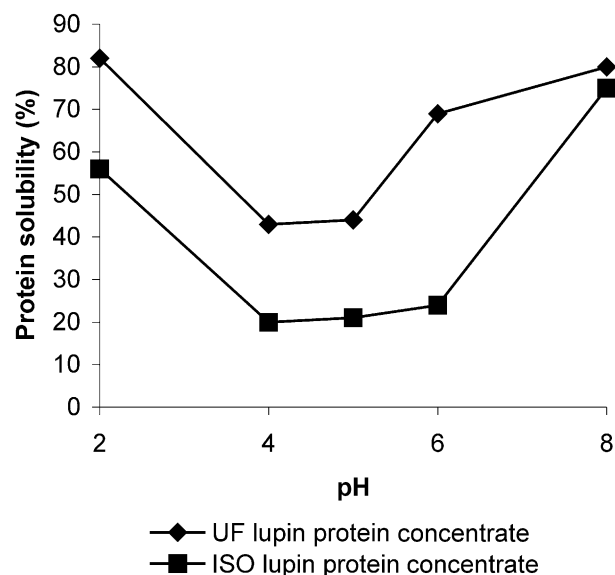


Fig. 2. Protein solubility of lupin protein concentrates (mean, $n = 4$).

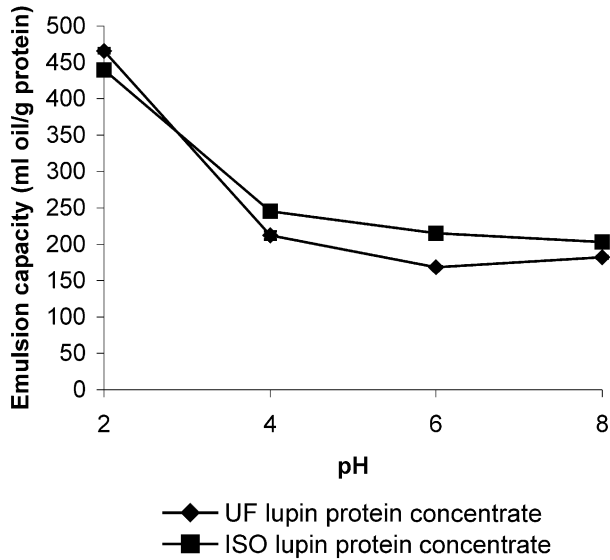


Fig. 3. Emulsion capacity of lupin protein concentrates (mean, $n=4$).

study do not meet this desired value, the generally higher solubility of the UF protein concentrate highlights its greater potential than the ISO lupin protein concentrate for having useful functional properties.

Both the ISO and the UF protein concentrates exhibited very low foaming capacity (data not shown). Lack of foaming ability precludes the use of the lupin protein concentrates investigated in the present study in foaming applications, such as egg white replacement in meringues, ice-creams and whipped desserts. Since chemical modification of vegetable proteins, such as soy, improves foaming properties (Kinsella, 1979) and preliminary studies have indicated that protein fractions with excellent foaming properties can be isolated from Australian sweet lupin (Johnson, unpublished 1994), alternative approaches to the preparation of foaming lupin protein ingredients are worthy of investigation.

The emulsion capacities of ISO and UF lupin protein concentrates were similar and tended to decrease with

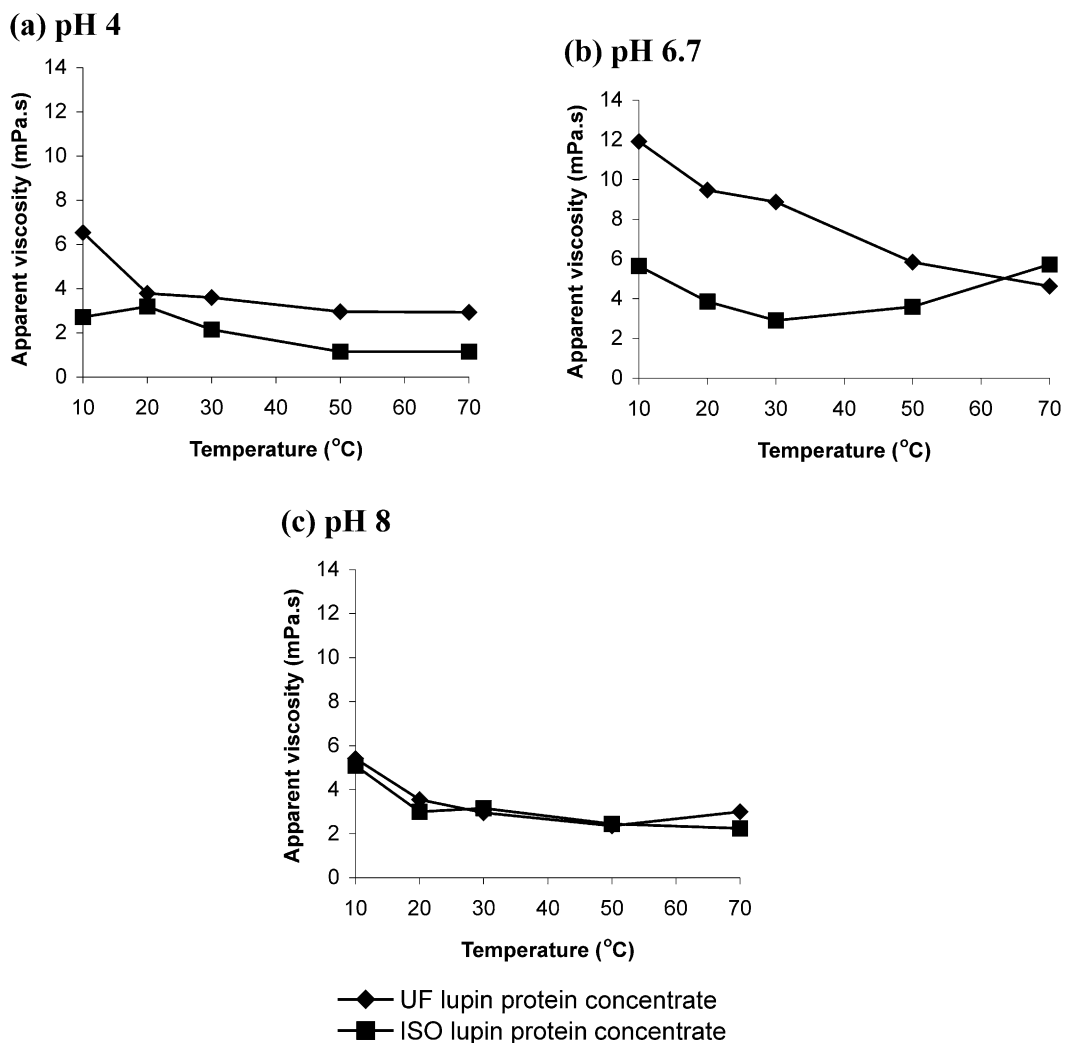


Fig. 4. Apparent viscosity of ISO and UF lupin protein concentrates (mean, $n=4$).

increasing pH (see Fig. 3). This finding is contradictory to previously reported effects of pH on the emulsion capacity of cowpea, soy and *L. albus* proteins (Aluko & Yada, 1997; King et al., 1985; Kinsella, 1979); these studies demonstrated that emulsion capacity increased as pH moved away, either higher or lower, from the isoelectric point. The emulsion capacities of the ISO and UF protein concentrates were comparable to those reported for protein isolates of *Lupinus termis* and *Lupinus albus* (El-Adawy et al., 2001), which in turn were considered comparable to those of soybean proteins, thus highlighting the potential of lupin protein in products such as meat analogues. The high emulsification capacity of the ISO and UF lupin protein concentrates at low pH suggests they may be particularly suitable as vegetable protein emulsifiers to replace animal derived emulsifiers in foods, particularly those which are highly acidic in nature.

The apparent viscosity of ISO and UF lupin protein concentrates is illustrated in Fig. 4. Both lupin protein concentrates had low viscosities, indicating that they may be useful in protein-based beverages, in which high viscosity might adversely affect their acceptability to consumers. Nevertheless, the UF lupin protein concentrate did have a higher apparent viscosity than the ISO protein concentrate ($P < 0.05$) at pH 4 (10, 30, 50 and 70 °C), pH 6.7 (10 °C) and pH 8 (70 °C); this may be related to the higher protein solubility of the UF protein concentrate. Further studies of important rheological properties of UF and ISO lupin protein concentrates, such as yield stress and intrinsic viscosity (Rao, 1999) would be valuable.

4. Conclusion

Compared with isoelectric precipitation for the preparation of lupin protein concentrate, the ultrafiltration process used in the present study did result in improved protein recovery and increased protein solubility, but it did not result in improved protein quality. Australian sweet lupin does appear to have potential as a source of vegetable protein ingredients for manufactured food. The findings of the present study indicate the need to identify and evaluate alternative approaches for the manufacture of lupin protein concentrates and isolates with improved nutritional quality and a wider range of useful functional properties. This should help to ensure that lupin can provide protein-based food ingredients to satisfy the diverse demands of the food industry and consumers.

Acknowledgements

The authors are grateful to Dr. John Pearce and Mr. Phillip Clark for their technical assistance in ultrafiltration

and spray-drying, Ms. Lu Zhong Xian for her valuable contribution in the rat diet formulation, Ms. Maree McGlynn for her generous assistance during the rat study, Mr. Maurice Kerr for performing the amino acid analysis and Dr. Sing Kai Lo for his helpful guidance in statistical analysis. This work was funded by Deakin University.

References

- Agriculture Fisheries and Forestry—Australia (2001). *Australian food statistics 2001*. Canberra: Agriculture and Fisheries—Australia.
- Alamanou, S., & Doxastakis, G. (1995). Physico-chemical properties of lupin seed proteins (*Lupinus albus*, ssp. Graecus). *Lebensmittel Wissenschaft und Technologie (Food Science and Technology)*, 28(6), 641–643.
- Aluko, R. E., & Yada, R. Y. (1997). Some physicochemical and functional properties of cowpea (*Vigna unguiculata*) isoelectric protein isolate as a function of pH and salt concentration. *International Journal of Food Sciences and Nutrition*, 48(1), 31–39.
- AOAC (1995). *Official methods of analysis*. Washington, DC: Association of Official Analytical Chemist.
- Ballester, D., Yanez, E., Garcia, R., Erazo, S., Lopez, F., Haardt, E., Cornejo, S., Lopez, A., Pokniak, J., & Chichester, C. O. (1980). Chemical composition, nutritive value, and toxicological evaluation of two species of sweet lupine (*Lupinus albus* and *Lupinus luteus*). *Journal of Agricultural and Food Chemistry*, 28(2), 402–405.
- Berot, S., Gueguen, J., & Berthaud, C. (1987). Ultrafiltration of faba-bean (*Vicia faba* L.) protein extracts: process parameters and functional properties of the isolates. *Lebensmittel Wissenschaft und Technologie (Food Science and Technology)*, 20(3), 143–150.
- Cerletti, P. (1983). Lupin seed proteins. In B. J. F. Hudson (Ed.), *Developments in food proteins, vol. 2* (pp. 133–171). Barking: Applied Science Publications.
- Cerletti, P., Duranti, M., & Restani, P. (1983). Properties of lupine proteins relevant to their nutritional performance. *Qualitas Plantarum Plant Foods in Human Nutrition*, 32(2), 145–154.
- Deeslie, W. D., & Cheryan, M. (1988). Functional properties of soy protein hydrolysates from a continuous ultrafiltration reactor. *Journal of Agricultural and Food Chemistry*, 36(1), 26–31.
- Donovan, B. C., McNiven, M. A., McLeod, J. A., & Anderson, D. M. (1991). Protein quality of two cultivars of lupin seeds evaluated in weanling rats. *Animal Feed Science and Technology*, 33, 87–95.
- El-Adawy, T. A., Rahama, E. H., El-Bedawey, A. A., & Gafar, A. F. (2001). Nutritional potential and functional properties of sweet and bitter lupin seed protein isolates. *Food Chemistry*, 74(4), 455–462.
- Friedman, M. (1996). Nutritional value of proteins from different food sources. A review. *Journal of Agricultural and Food Chemistry*, 44(1), 6–29.
- Fudiyansyah, N., Petterson, D. S., Bell, R. R., & Fairbrother, A. H. (1995). A nutritional, chemical and sensory evaluation of lupin (*L. Angustifolius*) tempe. *International Journal of Food Science and Technology*, 30(3), 297–305.
- Gruener, L., & Ismond, M. A. H. (1997). Effects of acetylation and succinylation on the functional properties of the canola 12s globulin. *Food Chemistry*, 60(4), 513–520.
- Johnson, S. K., & Gray, D. M. (1993). Ingredients derived from lupin—strong potential for a range of dietary fibre applications. *International Food Ingredients*, 5, 18–23.
- KeShun, L. (1997). *Soybeans: chemistry, technology and utilisation*. London: Chapman and Hall.
- King, J., Aguirre, C., & de Pablo, S. (1985). Functional properties of lupin protein isolates (*Lupinus albus* cv. Multolupa). *Journal of Food Science*, 50(1), 82–87.

- Kinsella, J. E. (1979). Functional properties of soy proteins. *Journal of the American Oil Chemists' Society*, 56(3), 242–258.
- Lusas, E. W., & Riaz, M. N. (1995). Soy protein products: processing and use. *Journal of Nutrition*, 125(3 Suppl.), 573s–580s.
- Mariotti, F., Pueyo, M. E., Tome, D., & Mahe, S. (2002). The bioavailability and postprandial utilisation of sweet lupin (*Lupinus albus*)-flour protein is similar to that of purified soybean protein in human subjects: a study using intrinsically ¹⁵N-labelled proteins. *British Journal of Nutrition*, 87(4), 315–323.
- Martinez, W. H. (1979). Functionality of vegetable proteins other than soy. *Journal of the American Oil Chemists' Society*, 56(3), 280–284.
- Millan, F., Alaiz, M., Hernandez-Pinzon, I., Sanchez, R., & Bautista, J. (1994). Study of lipid components of *Lupinus mutabilis* meal and isolates. *Biosci Biotech Biochem.*, 58(12), 2258–2260.
- Miller, D. S., & Bender, A. E. (1955). The determination of the net protein utilization of proteins by a shortened method. *British Journal of Nutrition*, 9, 382–388.
- Mongeau, R., Sarwar, G., Peace, R. W., & Brassard, R. (1989). Relationship between dietary fibre levels and protein digestibility in selected foods as determined in rats. *Plant Foods for Human Nutrition*, 39(1), 45–51.
- Morr, C. (1979). Technical problems and opportunities in using vegetable proteins in dairy products. *Journal of the American Oil Chemists' Society*, 56(3), 383–385.
- Morr, C. V., German, B., Kinsella, J. E., Regenstein, J. M., Van Buren, J. P., Kilara, A., Lewis, B. A., & Mangino, M. E. (1985). A collaborative study to develop a standardized food protein solubility procedure. *Journal of Food Science*, 50(6), 1715–1718.
- Mosse, J. (1990). Nitrogen to protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its definition and determination. Variation according to species and to seed protein content. *Journal of Agricultural Food Chemistry*, 38(1), 18–24.
- Oomah, B. D., & Bushuk, W. (1983). Characterization of lupin proteins. *Journal of Food Science*, 48(1), 38–41.
- Petterson, D., Sipsas, S., & McIntosh, J. B. (1997). *The chemical composition and nutritive value of Australian pulses*. Canberra: Grains Research and Development Corporation.
- Petterson, D. S. (1998). Composition and food uses of lupins. In J. S. Gladstones, C. A. Atkins, & J. Hamblin (Eds.), *Lupins as crop plants: biology, production and utilization* (pp. 353–384). Wallingford, Oxon: CAB International.
- Petterson, D. S., & Crosbie, G. B. (1990). Potential for lupins as food for humans. *Food Australia*, 42(5), 266–268.
- Rao, M. A. (1999). *Rheology of fluid and semisolid foods*. Gaithersburg: Aspen Publishers.
- Rayner, C. (1985). Protein hydrolysis of animal feeds for amino acid content. *Journal of Agricultural and Food Chemistry*, 33(4), 722–725.
- Reeves, P. G., Nielsen, F. H., & Fahey, G. C. Jr. (1993). AIN-93 purified diets for laboratory rodents: final report of the American institute of nutrition ad hoc writing committee on the reformulation of the AIN-76a rodent diet. *Journal of Nutrition*, 123(11), 1939–1951.
- Ruiz, L. P., & Hove, E. L. (1976). Conditions affecting production of a protein isolate from lupin seed kernels. *Journal of the Science of Food and Agriculture*, 27(7), 667–674.
- Schoeneberger, H., Gross, R., Cremer, H. D., & Elmadfa, I. (1982). Composition and protein quality of *Lupinus mutabilis*. *Journal of Nutrition*, 112(1), 70–76.
- Sgarbieri, V. C., & Galeazzi, M. A. M. (1978). Some physicochemical and nutrition properties of a sweet lupin (*Lupinus albus* var. Multolupa) protein. *Journal of Agricultural and Food Chemistry*, 26(6), 1438–1442.
- Wagner, J. R., Sorgentini, D., & Anon, M. C. (1992). Effect of physical and chemical factors on rheological behaviour of commercial soy protein isolates: protein concentration, water imbibing capacity, salt addition, and thermal treatment. *Journal of Agricultural and Food Chemistry*, 40(10), 1930–1937.
- Wolf, W. J. (1970). Soybean proteins: their functional, chemical and physical properties. *Journal of Agricultural and Food Chemistry*, 18(6), 969–976.
- Yen, P., Grant, G., Fuller, M. F., & Pusztai, A. (1990). Nutritional evaluation of sweet lupinseed (*Lupinus angustifolius*). *Proceedings of the Nutrition Society*, 49(2), 145A.