



Isolation and structural properties of the major protein fraction in Australian wattle seed (*Acacia victoriae* Bentham)

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ABSTRACT

The major protein fraction of wattle (*Acacia victoriae* Bentham) seed was isolated by anion-exchange and gel permeation chromatography. The protein was then characterised by its amino acid composition, gel electrophoresis, fluorimetry and circular dichroism (CD) in order to elucidate its nature and structural properties. The major amino acids were found to be glutamate (14.4%), aspartate (11.1%) and lysine (9.13%) while the contents of sulphur-containing amino acids (cysteine and methionine) and tryptophan were very low. The native protein, with an isoelectric point of 6.85, was comprised of two subunits of molecular masses 62 and 125 kDa, the bigger unit being joined by at least one disulphide bond. Far-UV-CD spectra showed that the protein consisted mainly of equal amounts of β -sheets and random structures (39% each), about 19% β -turns and relatively little α -helix (3.6%). These structures were also found to be very stable to changes in pH (3–9), temperature and ionic strength. Based on the fluorescence emission and near-UV-CD data, however, the tertiary structure was more sensitive to pH, temperature and ionic strength.

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1. Introduction

Acacia victoriae Bentham, also known as prickly wattle, is the most common of about a thousand *Acacia* species found in Australia (Maslin & McDonald, 2004). The seed, known to be rich in proteins, complex carbohydrates, dietary fibre and fat, has been used for thousands of years for food, by the Aboriginal people, in different parts of Australia and there is significant interest in the plant due to its potential nutritional and technological food-functional properties which have been recognised (Forbes-Smith & Paton, 2002; Seigler, 2002). Consequently, apart from the traditional wild harvest, it is also being increasingly sourced as a cultivated plant (Ahmed & Johnson, 2000).

Currently, the roasted, ground wattle seed is mainly used as a flavorant in manufactured foods, such as condiments, dairy products and baked goods, and also as a coffee analogue (Hegarty & Hegarty, 2001; Maslin & McDonald, 2004; Maslin, Thomson, McDonald, & Hamilton-Brown, 1998). However, due to the high protein and carbohydrate contents of its non-roasted dry form, research has commenced on the utilisation of its extracts as food manufacturing ingredients. For example, the protein profile and the emulsifying properties of its water extract have been documented (Agboola, Ee, Mallon, & Zhao, 2007), as was the nature of

its protease inhibitors, especially its heat resistance (Ee, Zhao, Rehman, & Agboola, 2008). The influence of heat processing on the emulsifying and other food-functional properties of the extract, including foam formation and gelation characteristics, has also been studied (Ee, Rehman, Agboola, & Zhao, 2009). Due to the fact that the studied extracts contained both proteins and soluble carbohydrates, it was not possible to isolate the relative contribution of the protein to the measured functional properties. The proteins, therefore, needed to be isolated, purified and characterised, in order to obtain more relevant information about their properties.

Plant proteins remain a very important source of food and feedstock, for human and animal consumption, respectively. The study of their nature and structural characteristics has traditionally served to widen their utilisation accordingly (deMan, 1999). Analyses, such as amino acid composition, secondary and tertiary structures, hydrophobicity, molecular weight and isoelectric profiles, are keys to understanding the nature of these proteins and thus their suitability for various functional purposes (Damodaran, 1996).

It has already been reported that several proteins were present in the water extracts of wattle seed (Agboola et al., 2007). However, it is more common to extract plant proteins (being studied for their biochemical properties) with slightly alkaline buffers (Liu, 1997). Furthermore, previous results were limited to the elucidation of their electrophoretic profiles under reducing conditions. In this study, therefore, the major protein fraction from

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isolates of wattle seed has been further purified and studied in more detail, especially its spectral properties under different environmental conditions, as well as the measurement of its amino acid composition, structure and electrophoretic profiles under reducing and non-reducing conditions. The results are expected to assist in the evaluation of wattle seed protein isolate as a functional food ingredient.

2. Materials and methods

2.1. Material

Whole wattle seeds were supplied by Outback Bushfoods, Alice Springs, Australia. Reagents and chemicals were supplied by Sigma Chemicals (St. Louis, MO, USA) and Fisher Scientific (Oakville, ON, Canada).

2.2. Extraction of crude wattle seed protein isolate

Whole wattle seeds were ground using a ZM 100 ultra centrifugal mill (Retsch GmbH, Germany) to pass through a 0.11 mm mesh and extracted with 50 mM tris-HCl buffer (pH 8.1) in a ratio of 1:10 (flour to buffer), by stirring for 1 h at room temperature (25 °C). The slurry obtained from the extraction was then centrifuged at 3000g for 10 min, after which the supernatant was fractionated by using increasing concentrations of ammonium sulphate in 25% increments, as described by Bollag, Rozycki, and Edelstein (1996). The precipitates were then dialysed (molecular weight cut-off 10,000 Da) against 50 mM tris-HCl buffer (pH 8.1) at 4 °C, for at least 48 h with three changes of buffer. Finally, the extract was dried on a Christ-Alpha 1-4 freeze dryer (Biotech International, Germany) and stored at -20 °C until used.

2.3. Fast protein liquid chromatography (FPLC)

Fast protein liquid chromatography was carried out on a using a Hiload 26/10 Q-Sepharose high performance anion-exchange column equilibrated with 50 mM phosphate buffer (pH 7) and connected to an AKTA FPLC system (Amersham Biosciences, Montreal, Canada). All samples were filtered using a 0.2 µm Millipore membrane (Millipore Corporation, Millford, MA, USA) prior to loading. An aliquot (10 mg/ml) of the filtered crude isolate solution was loaded, after which a linear gradient of 0–100% (0.5 M) NaCl over five column volumes was used to elute the bound proteins at a flow rate of 5 ml/min. For the second stage of purification, pooled peaks from anion-exchange chromatography were desalted by dialysis and freeze dried (as above). An aliquot (2 mg/ml) of dried sample was then loaded onto a Hiload 16/60 Superdex 75 preparative grade size exclusion column equilibrated with 50 mM phosphate buffer (pH 7), similarly connected to the AKTA FPLC system. For both stages of purification, the eluted proteins were monitored at 214 nm and 5 ml fractions were collected.

2.4. Native and sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE)

Native and SDS-PAGE were carried out on crude protein isolate and purified protein fractions using the PhastSystem Separation and Development units according to the manufacturer's instructions (Pharmacia LKB, Montreal, Canada). For native PAGE, 5 mg/ml of each sample in 50 mM tris/HCl buffer (pH 9) containing 0.01% bromophenol blue (dye) was prepared, out of which an aliquot (2 µl) was loaded onto an 8–25% gradient gel. For SDS-PAGE, sample concentration was 6.6 mg/ml and the buffer's pH was lowered to 8.0 while also containing 10% (w/v) SDS. SDS-PAGE, under

reducing conditions, was similar except for the addition of 5% (v/v) β-mercaptoethanol. All SDS-PAGE samples under reducing and non-reducing conditions were boiled for at least 5 min, cooled to room temperature and centrifuged (16,000g) for 10 min and an aliquot (2 µl) of the supernatant was loaded onto the 8–25% gradient gel. A similar aliquot of molecular weight standards, ranging from 10 to 225 kDa, was also loaded onto the same gradient gel.

2.5. Isoelectric focussing

Freeze-dried protein fraction and crude isolate were characterised by performing isoelectric focussing on the Phastsystem apparatus, as described by manufacturers on a PhastGel IEF 3-9. The standards, run on the same gel, were ampholytes in the Pharmacia broad range pI calibration kit containing proteins with various isoelectric points, ranging from 3 to 10. The protein bands were developed using the silver-staining method, according to the manufacturer's instructions (Pharmacia LKB, Montreal, Canada).

2.6. Circular dichroism (CD)

Far- and near-UV-CD spectra were measured using a JASCO model J-815 spectropolarimeter (JASCO Corporation, Tokyo, Japan) at 25 °C unless otherwise stated. The far-UV-CD spectrum was measured on the 4 mg/ml protein fraction in phosphate buffer (pH 7) at 190–240 nm, using a quartz cell with path length of 0.5 mm. The pH of the buffer was adjusted to between 3 and 9 with either 1 M HCl or 1 M NaOH to determine the pH effect on the structures. The effect of calcium ions (up to 50 mM CaCl₂) and temperature sweeps (25–90 °C) were carried out at pH 7. Deconvolution of far-UV spectra was performed using the CONTIN-LL secondary structure algorithm (Whitmore & Wallace, 2004) accessed via the DICHROWEB website (<http://www.cryst.bbk.ac.uk/cdweb/html/home/html>). The near-UV spectrum was measured at 250–320 nm under the same conditions as above but using a 10 mg/ml protein concentration and a quartz cell path length of 1 mm. Molar ellipticity was calculated from the near-UV data, as described by Schmid (1990), using the molecular mass of 187 kDa for the protein fraction and a mean residue weight of 115.

2.7. Intrinsic fluorescence intensity

The fluorescence measurements were carried out on a JASCO FP 6300 spectrofluorimeter (JASCO Corporation, Tokyo, Japan). The excitation wavelength was fixed either at 280 nm (tyrosine and tryptophan fluorescence) and the emission measured between 290 and 400 nm or fixed at 295 nm (tryptophan fluorescence only) and emission was monitored between 300 and 450 nm. The effect of pH was measured at 25 °C, using 125 µg/ml of protein fraction in 50 mM phosphate buffer adjusted to between pH 3 and 9 while temperature effect was monitored at pH 7 with temperature adjusted to between 25 and 90 °C and maintained by an external water bath. The effect of calcium was monitored similarly at pH 7 (25 °C) with up to 0.5 M CaCl₂ in the buffer.

2.8. Surface hydrophobicity

Aromatic surface hydrophobicity was determined as described by Haskard and Li-Chan (1998), using 1-anilino-naphthalene-8-sulphonate (ANS) as the probe. The protein concentration ranged from 25 to 200 µg/ml in 50 mM phosphate buffer while the ANS concentration was maintained at 100 µM throughout the experiment. Surface hydrophobicity was determined from the initial slope (S_0) of the linear regression analysis of the plot of relative fluorescence intensity (RFI) against percent protein concentration. The RFI was measured under different environmental conditions,

using the JASCO FP 6300 spectrofluorimeter (JASCO Corporation, Tokyo, Japan). The excitation wavelength was 390 nm while the emission was measured at 470 nm.

3. Results and discussion

3.1. Isolation and characterisation of purified wattle protein fraction

Fig. 1 shows the profiles of both ion-exchange (A) and size exclusion (B) chromatographs of crude wattle proteins precipitated by increasing concentrations of ammonium sulphate. In Fig. 1A, there were two major regions with several peaks in each and classified as groups 1 and 2, respectively. The peaks eluting around 20 min (group 1) were much smaller than those eluting after 40 min (group 2). When the group 1 proteins were analysed using gel electrophoresis, there was very little protein detected, in spite of the high absorbance values of their major peaks. Comparatively, the proteins in group 2 were quite well-defined and apparently accounted for the major fraction in the wattle seed extract (Fig. 1B insert, lanes A–D). Pooling of all the peaks in group 2 of the ion-exchange chromatogram, followed by separation on a size exclusion column, yielded a single peak, which accounted for about 95% (by area) of the total (Fig. 1B). The single peak in Fig. 1B is shown to be a single band in native protein gel electrophoresis (lane E in Fig. 1B insert). It is possible that the proteins in group 1 were highly con-

jugated with significant amounts of carbohydrate moieties, especially given the well-known nature of proteins extracted from other Acacia species (Goodrum, Patel, Leykam, & Kieliszewski, 2000). Furthermore, native protein bands of the group 2 peaks, as shown in Fig. 1a insert, showed significant similarities in the number of major and minor bands to suggest that the reasons why they tended to be eluted as different peaks from those of ion-exchange chromatography are probably related to the degree of glycosylation. This is typical of storage proteins from leguminous plants (Sathe & Venkatachalam, 2007) and cereals (Shewry, 2007).

SDS-PAGE electrophoretograms of wattle isolate and purified fractions under reducing and non-reducing conditions are shown in Fig. 2. The profile of the crude protein isolate under non-reducing conditions confirmed the presence of about a dozen bands ranging from 145 kDa to less than 10 kDa (lane D), and the major band had a molecular mass of 62 kDa. In the absence of mercaptoethanol, the purified protein was resolved into two main bands with molecular masses of 62 and 125 kDa, respectively (lane E). Therefore, it is apparent that there is no disulphide bond between the two main polypeptide chains present in the purified wattle protein. In contrast, the 125 kDa polypeptide contains intra-disulphide bonds as evidenced by the disappearance of this band when mercaptoethanol was added (lane B). The appearance of a single band equivalent to 62 kDa after addition of mercaptoethanol suggests that the 125 kDa band was most likely a dimer with each subunit being about 62 kDa in size, joined presumably by one or more disulphide bond(s). This is in agreement with an earlier study of wattle protein molecular mass under reducing conditions whereby Agboola et al. (2007) reported a similar profile with a major band of 61 kDa molecular mass. The native purified protein fraction, which occurred as a single band (Fig. 1B insert, lane E) would therefore be expected to have a molecular mass of 187 kDa which is slightly less than that of the 7S globulin of soy protein isolate (deMan, 1999). This figure was, therefore, used in calculations involving measurement of all properties of the purified wattle protein fraction reported in this study.

Isoelectric point (*pI*) is an intrinsic property of proteins which is defined as the pH whereby the protein has the lowest solubility and information could be crucial in determining the utilisation of the protein, especially in food processing. The *pI* of the major wattle seed protein is determined as 6.85 (IEF gel not shown) and

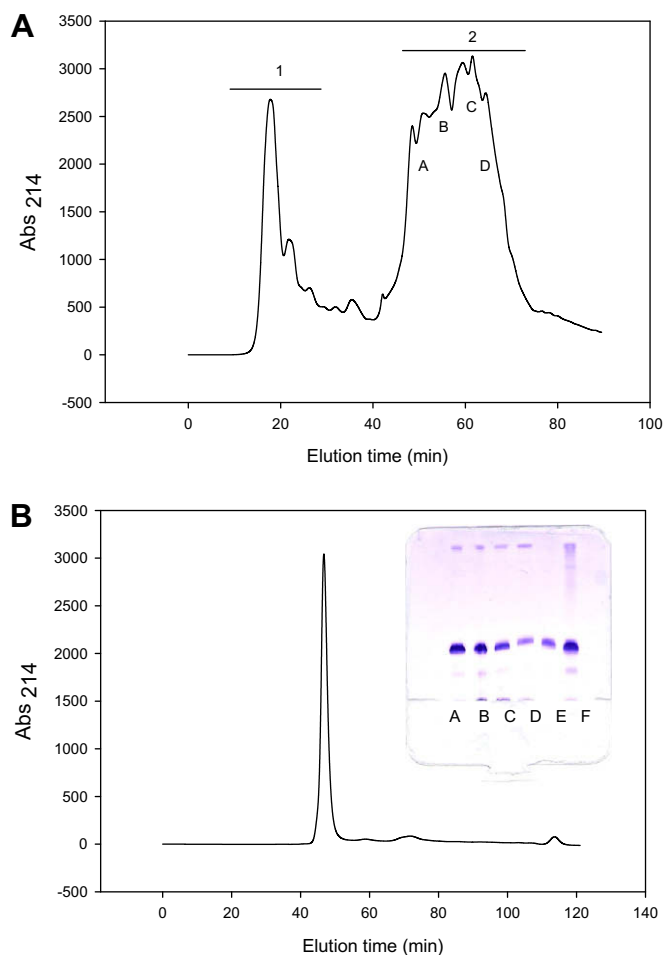


Fig. 1. Chromatographs obtained during the purification of crude wattle protein isolate with (A) anion-exchange and (B) size exclusion (SE) FPLC chromatography. Native gel electrophoretograms of group 2 eluents (lanes A–D), the SE purified protein (lane E) and the crude protein isolate (lane F) are inserted in Fig. 1B.

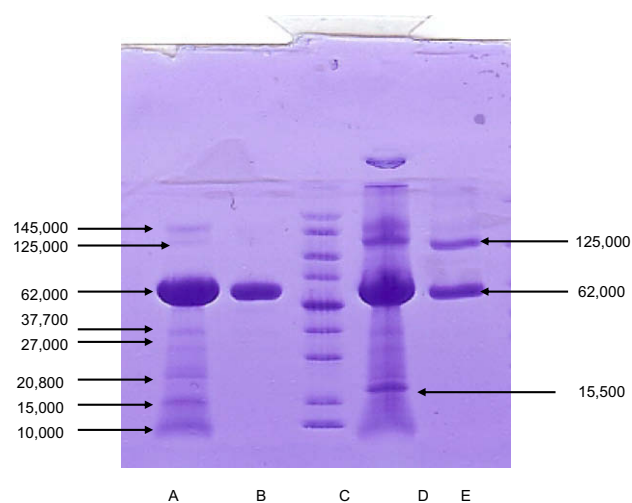


Fig. 2. SDS-PAGE electrophoretograms of crude protein isolate (lanes A and D) and purified major fraction (lanes B and E) under reducing (lanes A and B) and non-reducing conditions (lanes D and E). Protein standards in lane C are (from top to bottom) 225, 150, 100, 75, 50, 35, 25, 15 and 10 kDa).

within the range of most acidic subunits of seed globulins (Chung, Lei, & Li-Chan, 2005; Peterson & Brinegar, 1982).

Table 1 shows the amino acid compositions in the wattle seed flour and the major protein fraction. To our knowledge, this is the first report of amino acid analysis of wattle seed flour and the result satisfies the Food and Agricultural Organisation's requirements (FAO/WHO/UNU, 1985) for essential amino acids. Considering that all of the minor proteins were not included in the analysis of the major fraction, it is understandable why the compositions vary between the flour and the major fraction whereby some amino acids were more concentrated by purification than others. The major amino acids in the purified protein fraction were found to be glutamate (14.4%), aspartate (11.1%) and lysine (9.13%) while the contents of sulphur-containing amino acids, cysteine (0.67%) and methionine (0.99%), as well as tryptophan (0.75%) were very low. The comparatively high amount of amide (glutamic acid–glutamine, aspartic acid–asparagine, arginine) is similar to those of other 7S globular seed proteins and most likely stemmed from a storage type role in the plant (Marcone, 1999).

3.2. Circular dichroism (CD) spectra

Fig. 3 shows effects of pH and calcium ions on the wattle protein secondary structure, as measured by far-UV-CD data. Analysis of the protein secondary structure shows equal amounts of β -sheets and random structures (39% each), about 19% β -turns and relatively little α -helix (3.6%). The effect of pH (Fig. 3A), shows a CD spectrum that is atypical of any of the standard secondary structure patterns (Kelly & Price, 2000). The positive maximum around 210 nm is typical of type I β -turn protein (Johnson, 1990) and this reduces in intensity with increasing pH from 3 to 9. The results, however, show no major changes in the backbone secondary structure of the wattle protein under the influence of pH. The effect of calcium was more dramatic on the CD spectrum with the positive maximum around 210 nm changing to negative peaks which is typical of increasing α -helical structures (Schmid, 1990). The effect of calcium did not, however, translate into significant changes in the fraction of secondary structures. It is possible that the available structure determination algorithm was not particularly suitable for the wattle protein fraction since a much higher protein concentration (10 mg/ml) was utilised in order to obtain a reasonable signal, probably due to a considerable degree of glycosylation.

Table 1

Amino acid composition (g/100 g protein) of whole wattle seed and the major protein fraction in isolate.^a

Amino acid	Whole seed (flour)	Major protein fraction
Aspartic acid ^b	12.1 ± 0.06	11.1 ± 0.06
Threonine	4.04 ± 0.02	4.68 ± 0.03
Serine	7.06 ± 0.04	6.34 ± 0.03
Glutamic acid ^c	15.7 ± 0.07	14.4 ± 0.06
Proline	4.48 ± 0.02	6.28 ± 0.03
Glycine	8.10 ± 0.04	5.14 ± 0.02
Alanine	4.05 ± 0.01	5.39 ± 0.02
Cysteine	1.49 ± 0.01	0.67 ± 0.00
Valine	7.08 ± 0.03	4.73 ± 0.02
Methionine	1.60 ± 0.01	0.99 ± 0.01
Isoleucine	3.21 ± 0.02	3.35 ± 0.01
Leucine	6.87 ± 0.03	9.25 ± 0.04
Tyrosine	2.58 ± 0.02	4.36 ± 0.02
Phenylalanine	3.59 ± 0.02	4.50 ± 0.02
Histidine	3.07 ± 0.02	3.17 ± 0.02
Lysine	7.01 ± 0.03	9.13 ± 0.05
Arginine	5.83 ± 0.03	5.73 ± 0.03
Tryptophan	2.12 ± 0.01	0.75 ± 0.00

^a Values are means of duplicate determinations ± standard deviation.

^b Includes asparagine.

^c Includes glutamine.

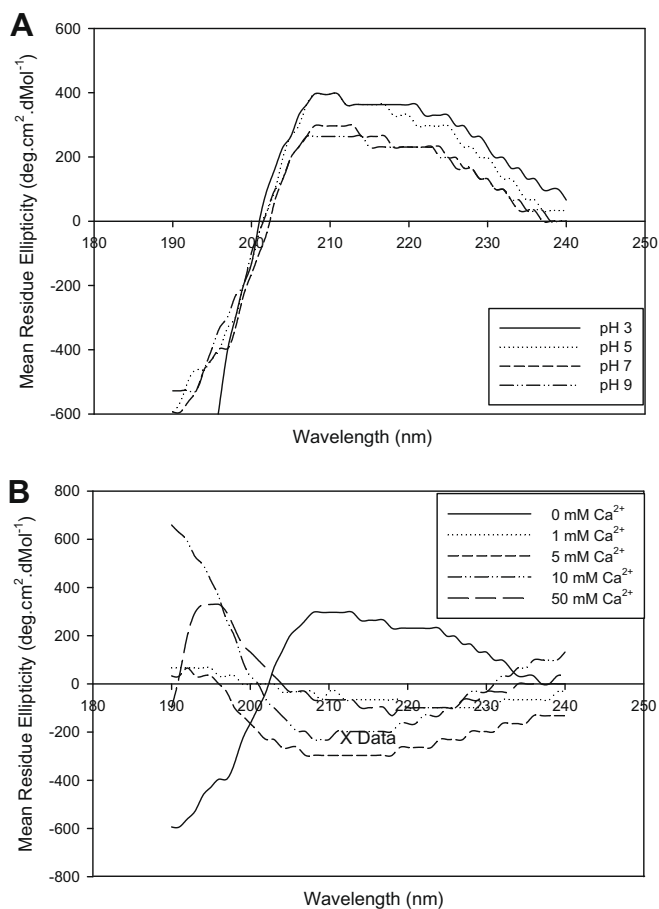


Fig. 3. Effects of pH (A) and calcium ions (B) on the far-UV-CD spectra of purified wattle protein fraction.

Fig. 4 shows the result of temperature sweeps at 220 nm (A) and 195 nm (B), respectively. Changes at 220 nm are normally used as indicators of α -helix structure while the changes at 195 nm are usually indicative of changes in β -sheet structure. The results show better stability of the α -helical structures at pH 3 and 6 compared to pH 9, especially after heating beyond 70 °C. On the other hand, changes in the β -sheets were not detected at pH 6, probably because the protein was very close to the isoelectric point and maintained a very rigid structural conformation. Slight changes in β -sheet structural conformation were recorded at pH 3 throughout the temperature scan, while significant changes were observed at pH 9, becoming more pronounced after about 56 °C. These results show that the wattle protein fraction has fairly stable secondary structures, even at high temperatures, especially at pH values different from the *pI*. However, the protein structure becomes slightly susceptible to high temperatures at high pH values. This behaviour is similar to those of other stable seed storage proteins, such as phaseolin, whose denaturation requires simultaneous exposure to more than one denaturing agent (Deyer, Nelson, & Murai, 1992; Rocha et al., 2007).

Changes in tertiary structure of the wattle protein fraction, under the influence of pH, temperature and calcium ions, have been estimated using the near-UV-CD spectrum, as well as fluorescence intensity. Fig. 5 shows the effect of pH (A) and temperature (B) on the near-UV spectrum. CD bands in the near-UV region depend on changes to the aromatic amino acids, tyrosine, tryptophan and phenylalanine, which are all present in the wattle protein fraction (Table 1). At the neutral pH of 7, there appeared to be two negative peaks, at 267 nm and 273 nm, and a slightly positive peak at

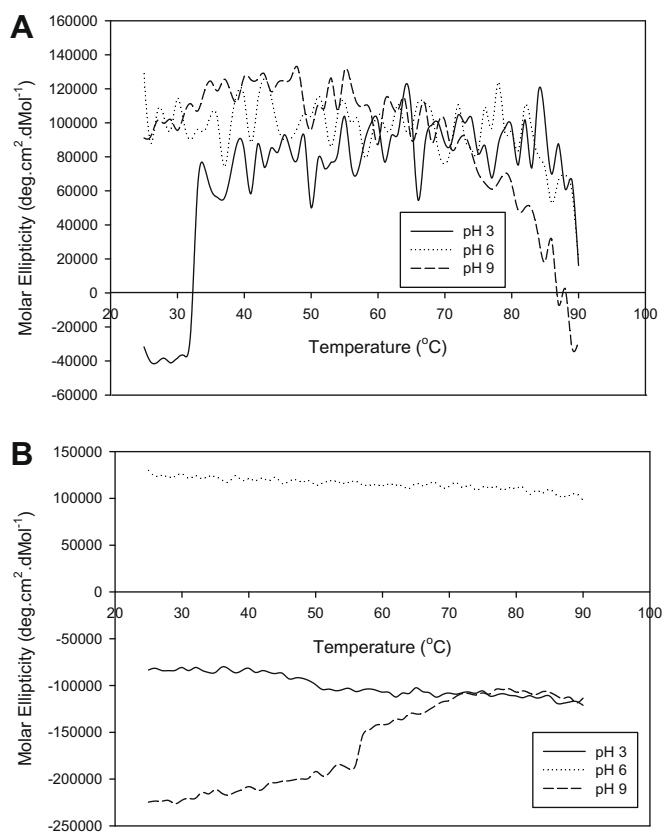


Fig. 4. Effects of temperature on molar ellipticity of wattle protein fraction measured at (A) 220 nm and (B) 195 nm at different pH values.

290 nm (tryptophan). The major negative peak (273 nm) falls between responses for phenylalanine (255–270 nm) and tyrosine (275–280) while the minor peak is in the upper range of phenylalanine response (Kelly & Price, 2000; Strickland, 1974). These results show that the residues were located in three different but stable environments. There was a dramatic change in the spectrum as the pH fell to 6, with all the peaks shifting upwards into the positive region. On the other hand, an increase to pH 8 resulted in increased intensities of the negative peaks while the positive peak disappeared. The results showed that minor changes in pH of the aqueous environment could result in major changes to the tertiary structure of the wattle protein fraction. This protein appears to be different from other globulin storage proteins, such as phaseolin and glycinin, which require more pronounced shifts in pH for similar extents of conformational changes (Deyer et al., 1992; Renkema, Lakemond, deJongh, Gruppen, & vanVliet, 2000).

The influence of temperature (pH 7) on the near-UV-CD spectrum (Fig. 5B) showed an initial increased intensity of the negative 260–280 nm bands (phenylalanine and tyrosine residues) but decrease in intensity of the positive 285–290 nm band (most likely tryptophan) territory without changes in wavelength between 25 and 60 °C. The results indicate temperature-dependent increased interactions of the phenyl alanine and tyrosine residues with each other or with adjacent aromatic residues. In contrast, it seems that the tryptophan residues interacted less with each other or adjacent aromatic residues as the temperature increased from 25 to 60 °C. Between 60 and 70 °C, however, the reverse occurred with decreased intensity of the tyrosine and phenylalanine bands but increased intensity of the tryptophan bands. Therefore, it can be concluded that the purified wattle protein showed more notable changes in tertiary structure than in secondary structure under the influence of temperature.

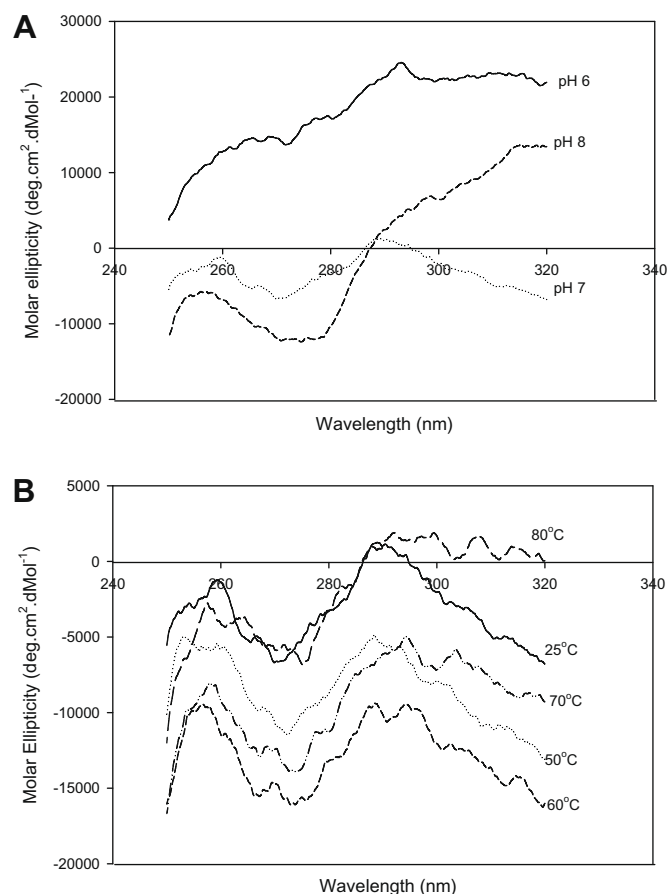


Fig. 5. Effects of pH (A) and temperature (B) on near-UV-CD spectra of purified wattle protein fraction.

3.3. Intrinsic fluorescence spectra and surface hydrophobicity

Fig. 6 shows changes in maximum fluorescence intensity of solutions of the wattle protein fraction (F_{\max}) with changes in pH, calcium ion concentration and temperature. The fluorescence spectrum is defined chiefly by the polarity of the environment of the tryptophan and tyrosine residues, and by their specific interactions (Schmid, 1990). The F_{\max} was above 140 and remained fairly stable between pH 3 and 6 after which it experienced a steep reduction before stabilising at pH 9, suggesting an open structure at lower pH values but a more globular conformation at high pH values. The results also suggest the presence of net charged groups at lower pH values, which will help to maintain an open structure as a result of increased electrostatic repulsions. As pH increased, neutralisation of the electrostatic charges could have encouraged a more closed conformation. On the other hand, the F_{\max} rose very steeply between 0 and 100 mM Ca^{2+} (maximal value) before falling slightly at 500 mM Ca^{2+} , suggesting a calcium-dependent opening of the protein structure but up to a maximum at 500 mM. Thus, the effect of calcium is opposite to that of pH, which is expected, since addition of calcium increases electrostatic repulsions, similar to the situation at low pH levels. The effect of temperature on F_{\max} at pH 7 was a steep reduction until 70 °C after which it rose slightly, suggesting a temperature-dependent reduction in the degree of exposure of the aromatic amino acid residues. In all of these measurements, there was very little change in wavelength of the maximum fluorescence intensity (λ_{\max}) which was around 330 nm, characteristic of fluorescence profile of tryptophan residues in a hydrophobic environment, e.g., the interior of a globular

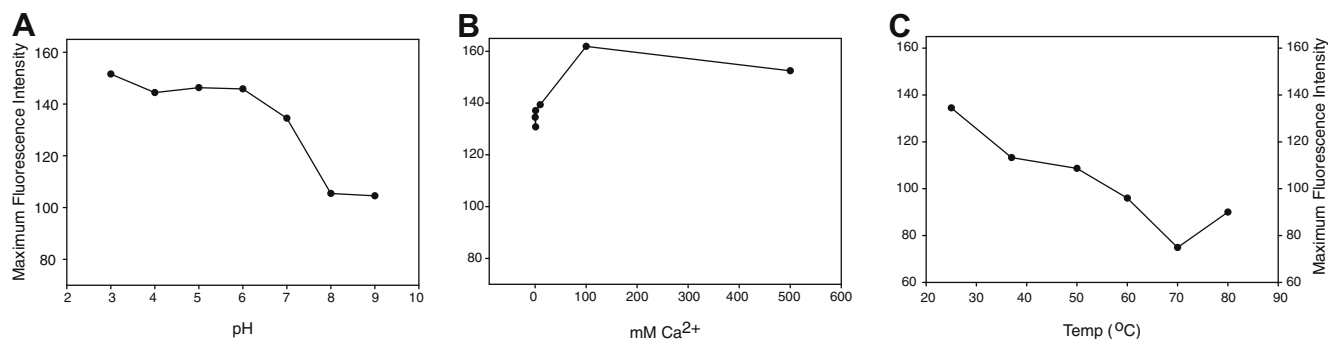


Fig. 6. Maximum fluorescence intensity of purified wattle protein fraction under the influence of (A) pH, (B) calcium ions and (C) temperature. Excitation was carried out at 280 nm and the emission was monitored between 290 and 400 nm.

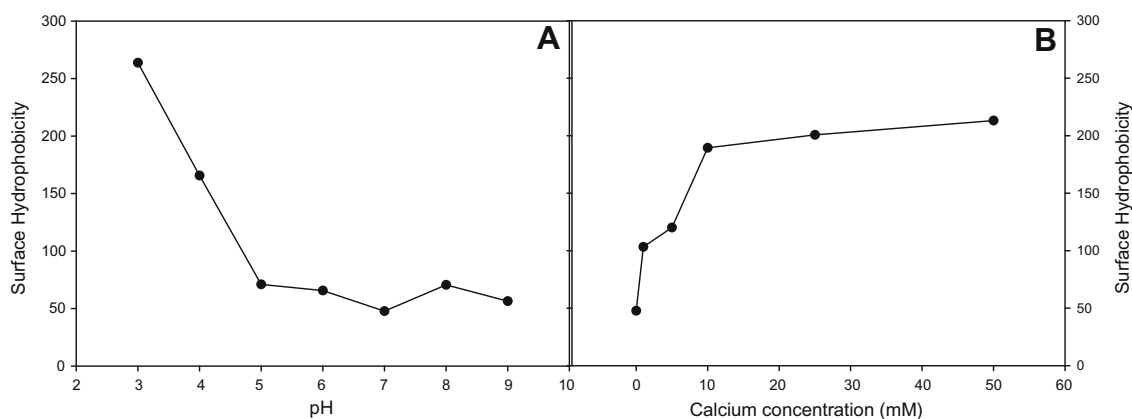


Fig. 7. Effects of (A) pH and (B) calcium concentration on aromatic (ANS) surface hydrophobicity (S_0) of purified wattle protein fraction. Excitation was at 390 nm and emission at 470 nm.

protein. The only significant change was a sudden blue shift in λ_{\max} to 321 nm when the sample was heated from 60 to 70 °C, and movement back up to 330 nm at 80 °C. This would suggest that, apart from this specific temperature effect, the polarity of the environment did not change significantly during these measurements (Schmid, 1990). Overall the results showed that increasing pH and temperature generally led to a more compact protein structure (lower F_{\max}), while the presence of calcium ions resulted in partial unfolding and exposure of hydrophobic residues in the wattle protein fraction.

Surface hydrophobicity (S_0) is a very influential property of proteins in evaluating their functional properties, e.g., interfacial tension and emulsifying activity, many of which are important for their role in food manufacture (Haskard & Li-Chan, 1998). Fig. 7 shows that the S_0 was reduced with increasing pH while it was increased with calcium ion concentration. The results appears to follow the same trend as F_{\max} plots (Fig. 6) whereby the protein became more compact with increasing pH while the addition of calcium ions resulted in exposure of hitherto buried hydrophobic residues. These results are also in agreement with data from other globular proteins from cowpea (Aluko & Yada, 1995; Mwasaru, Muhammad, Bakar, & Che Man, 1999). However, the results of S_0 measurements could also have been due to masking of the protein charge at low pH and increasing ionic strength with calcium ions (Kinsella, Damodaran, & German, 1985). Furthermore, it is understandable that the exposure of hydrophobic residues would decrease as the pH was increased from pH 3 towards the pI (pH 6.85). With respect to functionality, however, the protein should be able to form very stable emulsions, especially in acidic foods, due to high S_0 below neutral pH values.

4. Conclusions

This study demonstrated the nature and properties of the major protein fraction from wattle seed. Significantly, the nutritional quality of the total seed flour was established as being within requirements for the essential amino acids, and the purified protein fraction was shown to be similar in size to the major 7S globulin from leguminous plants, with a very stable secondary structure dominated by β -sheets and random structures. The tertiary structure was, however, more susceptible to environmental changes, e.g., pH, temperature and ionic strength, suggesting that further studies on its utilisation as a functional food ingredient should be performed under these conditions.

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