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Chemical and functional quality of protein isolated from alkaline extraction of Australian lentil cultivars: Matilda and Digger

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Abstract

Starch and protein were extracted from the flour of two Australian lentils, *Lens culinaris*; Matilda (Green Lentil) and Digger (Red Lentil), with water at four temperatures (ambient 22 °C, 30 °C, 35 °C and 40 °C) and under five pH conditions (distilled water and pH adjusted with NaOH to 8, 8.5, 9.0 and 9.5). Upon evaluation of all extraction conditions, pH 9.0 at 30 °C was chosen as an optimum extraction condition for Matilda while pH 8.5 at 35 °C was chosen for Digger.

These extracts were studied by DSC and reversed-phase HPLC. The DSC ΔH value of extracted lentil starch from both Digger and Matilda showed an increasing trend with increases in pH and temperature. Extraction at higher pH resulted in a smoother and more symmetrical peak, denoting the absence of adhered protein on the starch surface. In the study of the functional properties of extracted protein by DSC, the ΔH value of the extracted protein decreased with increasing pH. This trend is more significantly demonstrated in Digger than in Matilda protein. Temperature had less effect than did pH on the ΔH value of protein. Chromatograms from reversed-phase HPLC showed a loss of hydrophilic proteins during extraction. Protein peaks appearing 10–32 min after injection of lentil flour samples were missing from alkaline-extracted protein. The water-holding capability of both Digger and Matilda proteins (adjusted to pH 7.0) increased slightly with increasing pH. Foaming capacity of both proteins decreased with higher extraction pH, while foam stability increased with higher extraction pH. Matilda proteins showed greater foam-forming capacity than did Digger.
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Keywords: DSC; Extraction; Lentils; Protein and reversed-phase HPLC

1. Introduction

Lentils, which are botanically classified as *Lens culinaris* (Adsule, Kadam, & Leung, 1989), are an important crop in many developing countries. They have been the basis of diet for many people living in the Middle East and Asia. Although the lentil is relatively new to Australia, the production and consumption of lentil *per capita* has increased throughout the past few years. Like most legumes, lentil seeds are composed of about two-thirds carbohydrates and 24–30% protein. In addition, lentils are also a good source of certain amino acids, such as lysine and arginine (Longnecker, Kelly, & Huang, 2002), which is important for use in balancing the deficiency of these essential amino acids in cereal-based diets. Both the starch and protein fractions of lentils offer a new source of novel ingredients. New sources of cheaper protein provide new alternatives for the dairy industry, where cheaper protein is required to replace existing proteins. Novel proteins are also highly sought to enable cereal industries and aqua-feed companies to produce new products to meet consumer demand for lower cost and higher nutritional value.

Legume protein isolates are commonly extracted using wet processes. Alkali solution solubilises the protein. The insoluble materials are then physically separated by centrifugation. Acid is then added to the supernatant to precipitate (iso-electrically) solubilised proteins. The final isolated

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protein is then dried using either a spray, drum- or freeze-drying method (Sumner, Nielsen, & Youngs, 1981). Protein isolation and dispersibility has also been investigated in legume flours. Nitrogen of most legumes is least soluble when extracting at pH 4 and its solubility increases after pH 6.0. Almost 80% nitrogen dispersibility is achieved when using an extraction solvent at pH 8.0 and above (Fan & Sosulski, 1974). Protein associated with starch granules affects the extraction of starch. These proteins adhere to the surface of the starch and are relatively difficult to remove. The concentration of residual protein in the extracted starch will determine the grade of starch produced, as each extracting condition will result in a different amount of residual protein on the starch granules (Baldwin, 2001). Functional properties of protein components are also an important aspect when extracting for proteins. Some of the important properties of protein include: emulsion capability (EAI index), emulsification; solubility, water holding capacity (WHC), viscosity; gelation, and foaming properties (Diaz, Pereira, & Cobos, 2004; Hill, 1996; Morr et al., 1985; Patel & Kilara, 1990; Pearce & Kinsella, 1978; Pinnavaia & Pizzirani, 1998; Regenstein & Regenstein, 1984).

This paper describes the effect of alkaline extraction on the protein qualities from two Australian cultivars Matilda and Digger, with the aim of producing extracted protein with minimal changes to its functional properties. The extraction conditions optimising the yield and quality of starch and protein fractions were pH 9.0 at 30 °C for Matilda and pH 8.5 at 35 °C for Digger (Lee, Htoon, & Paterson, 2006).

2. Materials and methods

Two Australian lentils, Matilda (Green lentil) and Digger (Red Lentil) were obtained from The Lentil Company (TLC), Horsham (Victoria). Each sample was ground into fine flour at The University of New South Wales using a Fitz hammer mill (screen aperture 0.79 mm). The flours were packed in plastic bags, sealed and stored in an airtight box prior to analysis.

2.1. Alkaline extraction of lentil flour

Starch and protein were extracted from flour using a modified in-house extraction method of Food Science Australia, CSIRO. The method was described by Lee et al. (2006). Sodium metabisulphite (0.01%) was added to the extraction solution to control microbial contamination and growth. Protein and starch yields were calculated from the amount of mass recovered compared with results obtained from total protein and starch determination.

2.2. Starch damage

Starch damage was determined by the AACC method (76–31), using a starch damage assay kit (Megazyme International, Ireland).

2.3. Differential scanning calorimetry (DSC)

A Perkin–Elmer Pyris-1 DSC (Norwalk, CT, USA) with internal coolant (Intracooler IP) and nitrogen purge gas was used. The enthalpy and melting point of indium were used for the calibration of temperature and heat capacity. The required mass of sample was weighed to 4 decimal places into a stainless steel pan fitted with a rubber O-ring. The dispersant (4% NaCl solution used for protein) was added to attain 70% moisture. The pan was shaken lightly to achieve an evenly distributed sample, and hermetically sealed. The sample was allowed to equilibrate for 1 h before analysis. The sample was scanned from 20 °C to 130 °C at a scan rate of 10 °C/min.

2.4. Agilent bioanalyzer 2100

Protein separation was performed on the Agilent 2100 bioanalyzer using the modified method of Uthayakumaran, Batey, and Wrigley (2005). All reagents were prepared in accordance to the Protein 200 LapChip kit. The Protein 200 ladder and upper marker were used. Results were analysed using the 2100 Expert software provided by Agilent Technologies, USA.

2.5. Reversed-phase HPLC

As a rapid identification method, a reversed-phase Beckman Gold HPLC system, with a Vydac C18 column and a column oven set at 70 °C, was used to separate proteins according to their hydrophobicity (Pollard, Smith, MacRitchie, Bekes, & Wrigley, 1997). Polar proteins elute from the C18 column first, followed by hydrophobic proteins. The system was attached to an automatic sample injector, an in-line filter to remove particulates from the sample or solvent and a UV detector monitoring at 214 nm. The eluting solvents were: 0.05% trifluoroacetic acid in water (Solvent A) and 0.05% trifluoroacetic acid in HPLC grade acetonitrile (Solvent B). The gradient profile used for the RP-HPLC is shown in Table 1.

Protein isolate was extracted by adding 5 mg of flow to 1.0 ml of extraction buffer (0.1 M Tris–HCl, 10% NaCl, pH 7.5). The solution was then subjected to sonification at a speed of 5–6 for 15 s, using a sonification probe, incubated at 65 °C for 1 h, and centrifuged at 14,000 rpm for 15 min. The supernatant was extracted using a 1.0 ml syringe, fil-

Table 1
RP-HPLC gradient used for lentil protein detection

Time (min)	Flow (ml/min)	%A	%B
00:00	1.00	100	0
60:00	1.00	40	60
61:00	1.00	30	70
64:00	1.00	30	70
65:00	1.00	100	0
80:00	1.00	100	0
81:00	0.05	100	0

tered through a 0.45 μm filter and analysed using the RP HPLC.

2.6. Protein functionality

2.6.1. General

Protein isolates were investigated for foaming ability (Patel, Stripp, & Fry, 1988), water-holding capacity (Regenstein & Regenstein, 1984) and emulsification (Yasumatsu et al., 1972).

2.6.2. Foaming ability

The procedure described by Patel et al. (1988) was carried out at room temperature (22–25 °C) with some modifications. 50.0 ml of 1% (w/v) protein dispersion in distilled water, adjusted to pH 7.0, were placed in an ultrasonic bath for 5 min to solubilise the protein. The mixture was transferred to the bowl of a food mixer and whipped for 5 min at the maximum speed (i.e. speed 5) using a two-spindle Kambrook (Cat. No. KSM 230; Power: 230 V; 50 Hz; 230 W) beater. The whipping attachment was then removed and total sample was quickly transferred from the bowl to a 250 ml measuring cylinder. The total volume of foam (FV), including drained liquid, was measured. The foam was then allowed to stand for 30 min at room temperature and the final foam volume and the drained liquid volume were measured (Patel et al., 1988). The foam expansion (FE) is calculated as:

$$\text{FE}\ (\%) = [(\text{FV})/50] \times 100$$

where FV = initial volume of foam.

The foam stability (FS) is calculated as:

$$\text{FS}\ (\%) = [\text{Volume of foam drained after 30 min}/\text{FV}] \times 100$$

2.6.3. Water-holding capacity (WHC)

Dry protein (160.0 mg) was weighed into a weighed 10 ml centrifuge tube. Distilled water (5.0 ml) was added. The solution was adjusted to pH 7.0 and mixed using a magnetic stirrer, heated at 60 °C for 30 min, and cooled in a water bath at ambient temperature for 30 min. The sample at 25 °C was centrifuged at 5000 rpm for 10 min and the supernatant decanted. The centrifuge tube was weighed and the amount of water held per gram of protein sample was determined (Regenstein & Regenstein, 1984; Pinnavaia & Pizzirani, 1998). The water-holding capacity is calculated as:

$$\text{WHC}\ (\%) = [\text{Weight difference (g)}/\text{weight of sample (g)}] \times 100$$

2.6.4. Emulsifying properties

Emulsifying activity and stability were determined by the method of Yasumatsu et al. (1972). Ten millilitre portions of protein solutions (15 mg/ml adjusted to pH 7.0), were dispersed in an ultrasonic bath for 5 min. The dispersed protein solutions were then homogenised with 10 ml of virgin olive oil at a speed of 5 (on a scale varying from 1 to 10) of a homogeniser for 1 min. The emulsions were centrifuged at 1100 *g* for 5 min (Yasumatsu et al., 1972; Bora, 2002). The heights of the emulsified layer and that of the total contents in the tube were measured. The emulsifying activity (EA) was calculated as:

$$\text{EA}\ (\%) = \frac{\text{Height of emulsified layer in tube}}{\text{Height of the total contents in the tube}} \times 100$$

The emulsions were then heated for 30 min at 80 °C and then centrifuged again to determine the emulsion stability. Emulsion stability (ES) was calculated as:

$$\text{ES}\ (\%) = \frac{\text{Height of emulsified layer after heating}}{\text{Height of the emulsified layer before heating}} \times 100$$

3. Results and discussion

3.1. Extraction conditions

Lentil starches from two Australian cultivars, Matilda and Digger, were isolated from flour using an alkaline extraction method. Two factors, pH and temperature, were evaluated to investigate their effects on the efficiency of starch extraction. The % starch yield and protein yield obtained with different extraction pH conditions and temperatures, for both varieties, were as summarised in Tables 2–5. Starch recoveries were generally higher than that achieved for protein. The yields for both starch and protein fractions obtained, were increased with increase in extraction pH and temperatures.

Although high extraction pH conditions and temperatures gave higher starch and protein yields, the strong alkaline conditions and temperatures also resulted in a higher % starch damage value achieved in the extracted starches.

Extraction at high pH (pH 9.5) resulted in >1.0% starch damage (Fig. 1). This was not desirable as it causes structural change to the starch granules, resulting in altered rheological and functional properties. Thus, starches extracted using high extraction pH and temperatures are not desired as they cause significant damage to the physical structure of

Table 2
Statistical summary for the effect of pH conditions on % starch yield for Digger flour

	22 °C	30 °C	35 °C	40 °C
Distilled water	73.1 ± 0.4^{a}	76.0 ± 3.0^{a}	74.9 ± 3.4^{a}	80.9 ± 4.7^{a}
pH 8.0	86.3 ± 3.7^{bcde}	88.1 ± 0.9^{bcde}	89.8 ± 1.0^{bcde}	91.4 ± 0.6^{bcde}
pH 8.5	87.4 ± 2.3^{bcde}	88.8 ± 0.6^{bcde}	90.3 ± 1.1^{bcde}	92.7 ± 1.2^{bcde}
pH 9.0	88.6 ± 0.5^{bcde}	88.3 ± 0.9^{bcde}	90.3 ± 2.0^{bcde}	93.3 ± 3.4^{bcde}
pH 9.5	90.5 ± 2.0^{bcde}	89.9 ± 1.6^{bcde}	92.4 ± 3.0^{bcde}	94.8 ± 3.5^{bcde}

Values are means of duplicate analyses ± SD.
[a]Distilled water; [b]pH 8.0; [c]pH 8.5; [d]pH 9.0; [e]pH 9.5.
Means within a column followed by different superscripts are significantly different ($P < 0.05$) at the compared pH conditions.

Table 3
Statistical summary for the effect of pH conditions on % starch yield for Matilda flour

	22 °C	30 °C	35 °C	40 °C
Distilled water	76.2 ± 0.4^{abd}	79.0 ± 0.8^{abc}	80.6 ± 0.4^{ab}	84.9 ± 1.0^{ab}
pH 8.0	76.9 ± 0.9^{abd}	77.6 ± 0.2^{ab}	80.0 ± 0.2^{ab}	85.9 ± 0.9^{ab}
pH 8.5	79.8 ± 0.6^{cde}	80.6 ± 1.0^{ace}	85.6 ± 0.2^{c}	89.2 ± 0.2^{c}
pH 9.0	79.3 ± 2.4^{abcde}	85.2 ± 1.1^{de}	87.6 ± 0.1^{d}	92.0 ± 0.2^{de}
pH 9.5	85.1 ± 2.6^{de}	85.9 ± 2.7^{cde}	90.6 ± 0.4^{e}	95.5 ± 1.8^{de}

Values are means of duplicate analyses ± SD.
[a]Distilled water; [b]pH 8.0; [c]pH 8.5; [d]pH 9.0; [e]pH 9.5.
Means within a column followed by different superscripts are significantly different ($P < 0.05$) at the compared pH conditions.

Table 4
Statistical summary for the effect of pH conditions on % protein yield for Digger

	22 °C	30 °C	35 °C	40 °C
Distilled water	43.8 ± 0.8^{a}	44.8 ± 3.4^{a}	49.3 ± 1.1^{ab}	48.8 ± 0.2^{a}
pH 8.0	54.3 ± 4.1^{bcde}	56.3 ± 2.1^{bcde}	57.9 ± 4.2^{abcde}	60.2 ± 1.8^{bcde}
pH 8.5	54.3 ± 2.1^{bcde}	57.6 ± 3.5^{bcde}	59.3 ± 1.4^{bcde}	60.4 ± 1.3^{bcde}
pH 9.0	56.7 ± 1.3^{bcde}	58.0 ± 1.2^{bcde}	59.5 ± 1.0^{bcde}	61.0 ± 0.8^{bcde}
pH 9.5	59.1 ± 2.5^{bcde}	59.6 ± 2.3^{bcde}	60.3 ± 3.6^{bcde}	62.0 ± 2.0^{bcde}

Values are means of duplicate analyses ± SD.
[a]Distilled water; [b]pH 8.0; [c]pH 8.5; [d]pH 9.0; [e]pH 9.5.
Means within a column followed by different superscripts are significantly different ($P < 0.05$) at the compared pH conditions.

Table 5
Statistical summary for the effect of pH conditions on % protein yield for Matilda

	22 °C	30 °C	35 °C	40 °C
Distilled water	48.5 ± 0.7^{a}	49.1 ± 0.4^{a}	50.9 ± 0.1^{a}	54.2 ± 1.4^{a}
pH 8.0	51.9 ± 0.4^{bcd}	53.5 ± 0.0^{bc}	60.3 ± 0.9^{bcde}	60.3 ± 1.0^{bcd}
pH 8.5	52.4 ± 1.6^{bcd}	52.7 ± 0.7^{bc}	59.0 ± 0.2^{bc}	60.4 ± 0.1^{bc}
pH 9.0	55.8 ± 2.6^{bcde}	56.6 ± 1.5^{de}	60.8 ± 0.2^{bde}	62.4 ± 0.0^{bd}
pH 9.5	60.3 ± 0.5^{de}	59.9 ± 1.9^{de}	60.7 ± 0.4^{bde}	63.4 ± 0.3^{e}

Values are means of duplicate analyses ± SD.
[a]Distilled water; [b]pH 8.0; [c]pH 8.5; [d]pH 9.0; [e]pH 9.5.
Means within a column followed by different superscripts are significantly different ($P < 0.05$) at the compared pH conditions.

the starch granules, resulting in deformed (i.e. irregular shape and swelling) or damaged starch (cracks and bursting of granules).

3.2. Differential scanning calorimetry

The DSC ΔH of extracted lentil starch, from both Digger and Matilda, increased with increases in pH and temperature. Extraction at higher pH resulted in a smoother and more symmetrical peak, denoting the absence of adhered protein on the starch surface.

Comparing the DSC endotherms obtained for starches extracted using distilled water (22 °C) to those starches extracted at pH 9.5 (40 °C), the ΔH values and peak temperatures increased slightly with increased severity of extraction conditions. With increased severity in extraction conditions, the gelatinisation temperature increased from 65.7 °C to 66.4 °C for Digger and from 63.6 °C to 65.6 °C for Matilda, while the corresponding enthalpy values, ΔH, increased from 16.12 ± 0.36 J/g to 17.55 ± 0.28 J/g and from 14.94 ± 0.99 J/g to 16.05 ± 0.54 J/g, respectively.

Full fat soy protein was used as a reference to identify the presence of 7S and 11S globulin proteins. The unfolding of 7S and 11S proteins was detected at about 92 °C and 110 °C, respectively. The 11S protein peak, at around 110 °C, was absent in all 3 samples of extracted Digger proteins (Fig. 2). No peak was detected at about 110 °C for any of the extracted proteins. The absence of 11S protein in all extracted lentil proteins could be related to the loss of water-soluble hydrophilic proteins, which are not collected in the acid precipitation step.

A trial was carried out by boiling a small sample of the collected supernatant after acid precipitation to dryness and a very small amount of residual substances was retained in the beaker after heating. This may be the 11S globulin left in the supernatant. It was not economical to recover this small quantity of protein, as a significant amount of heat energy was required to vaporise the large volume of supernatant achieved throughout the entire extraction process.

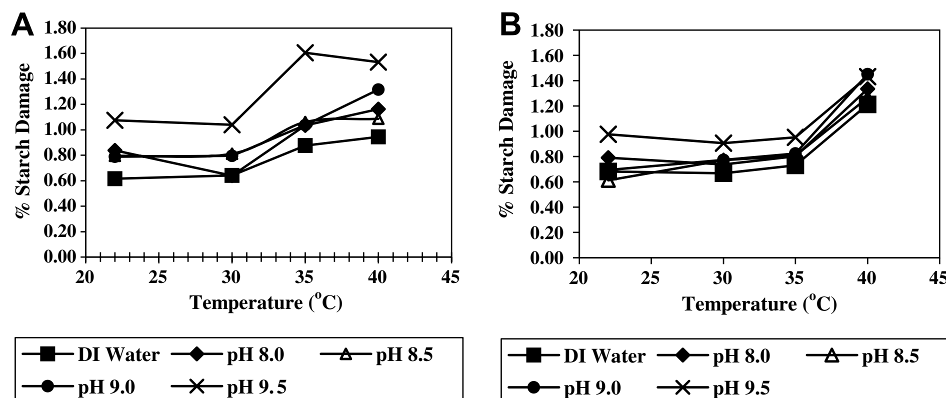

Fig. 1. Starch damage vs extraction temperature for: (A) green lentil (Matilda); and (B) red lentil (Digger) under various pH extraction conditions.

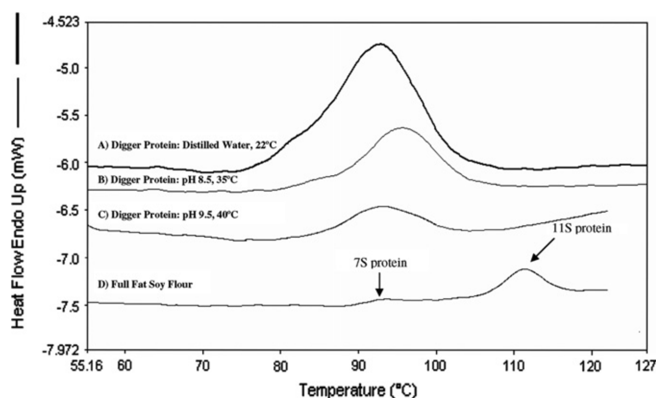

Fig. 2. DSC endotherms of extracted digger protein under three different extraction conditions and full fat soy flour; (A) distilled water, 22 °C; (B) pH 8.5, 35 °C; (C) pH 9.5, 40 °C and (D) full fat soy flour.

Table 6
Effect of extraction pH on the enthalpy, ΔH, (J/g), of Digger protein

	22 °C	30 °C	35 °C	40 °C
Distilled water	10.6 ± 0.2^{ab}	10.6 ± 0.2^{a}	10.3 ± 0.2^{ab}	11.0 ± 0.2^{a}
pH 8.0	10.4 ± 0.6^{ab}	10.1 ± 0.1^{b}	9.85 ± 0.0^{ab}	8.67 ± 0.2^{b}
pH 8.5	8.66 ± 0.4^{c}	8.87 ± 0.1^{c}	8.49 ± 0.1^{c}	6.74 ± 0.1^{cd}
pH 9.0	6.84 ± 0.3^{d}	7.07 ± 0.5^{d}	6.85 ± 0.1^{d}	6.83 ± 0.2^{cd}
pH 9.5	5.04 ± 0.2^{e}	4.84 ± 0.2^{e}	5.90 ± 0.1^{e}	5.72 ± 0.0^{e}

Values are means of duplicate analyses ± SD.
[a]Distilled water; [b]pH 8.0; [c]pH 8.5; [d]pH 9.0; [e]pH 9.5.
Means within a column followed by different superscripts are significantly different ($P < 0.05$) at the compared pH conditions.

Table 7
Effect of extraction pH on the enthalpy, ΔH, (J/g), of Matilda protein

	22 °C	30 °C	35 °C	40 °C
Distilled water	10.9 ± 0.2^{a}	11.0 ± 0.1^{ac}	10.8 ± 0.0^{abc}	11.6 ± 0.2^{ac}
pH 8.0	11.6 ± 0.0^{b}	11.9 ± 0.2^{bc}	11.2 ± 0.7^{abc}	12.6 ± 0.1^{b}
pH 8.5	11.4 ± 0.0^{c}	11.2 ± 0.3^{abc}	11.2 ± 0.6^{abc}	11.2 ± 0.1^{ac}
pH 9.0	10.1 ± 0.1^{d}	9.98 ± 0.1^{d}	9.23 ± 0.6^{de}	10.4 ± 0.1^{d}
pH 9.5	9.25 ± 0.1^{e}	8.92 ± 0.2^{e}	9.29 ± 0.6^{de}	8.32 ± 0.7^{e}

Values are means of duplicate analyses ± SD.
[a]Distilled water; [b]pH 8.0; [c]pH 8.5; [d]pH 9.0; [e]pH 9.5.
Means within a column followed by different superscripts are significantly different ($P < 0.05$) at the compared pH conditions.

Protein quality, as shown by ΔH and functional properties, was affected by pH rather than temperatures. ΔH values decreased significantly with increase in pH ($P < 0.05$) (Tables 6 and 7). This trend was more pronounced in Digger. The decrease in ΔH occurred because the protein structure uncoiled as the pH moved further from the iso-electric point. The ΔH for either lentil did not change significantly between the four different temperatures at the same extraction pH (Tables 8 and 9).

3.3. Agilent 2100 bioanalyzer

A typical elution profile is shown in Fig. 3. The molecular weight for the proteins extracted from both Digger and Matilda generally fell within the range 10–140 kDa. When comparing the gel patterns and elution profiles achieved for Matilda proteins extracted under the same pH conditions (distilled water and pH 9.5) but at different temperatures, no distinct difference in pattern was observed (Fig. 4A and B). Thus, extracting temperatures had no effect on the molecular weight range of the proteins.

Extraction pH did affect the molecular weight of extracted proteins. From the protein gels and elution profiles obtained for Matilda protein extracted at 22 °C, the intensities of the bands at 85 kDa and 90 kDa were more intense from those extracted at pH 9.0 and 9.5 than from those extracted in distilled water, or at pH 8.0 and 8.5 (Figs. 5A and 6A). Matilda proteins extracted at 40 °C, when compared among the various extraction pH conditions also showed the same trend (Figs. 5B and 6B). Therefore protein extracted at higher pH had increased high molecular weight and reduced low molecular weight protein fractions. Thus, although, qualitatively, the molecular weight profile did not change significantly with extraction conditions, the intensities of the bands at certain molecular weights (85 and 90 kDa) changed with increase in extraction pHs.

The same trend was also observed for Digger proteins. Extraction temperatures had little effect on the molecular weight of extracted proteins. Increased extraction pH increased the intensity of high molecular weight proteins and decreased the proportion of low molecular weight proteins.

3.4. Reversed-phase HPLC

The pH and temperature of extraction affected the proportion of hydrophilic to hydrophobic protein (Fig. 7A–C). Hydrophilic protein peaks eluted at about 10.4 min

Table 8
Effect of extraction temperature on the enthalpy, ΔH, (J/g), of Digger protein

	Distilled water	pH 8.0	pH 8.5	pH 9.0	pH 9.5
22 °C	10.6 ± 0.2^{abcd}	10.4 ± 0.6^{abc}	8.66 ± 0.4^{abc}	6.84 ± 0.3^{abcd}	5.04 ± 0.2^{ab}
30 °C	10.6 ± 0.2^{abcd}	10.1 ± 0.1^{ab}	8.87 ± 0.1^{ab}	7.07 ± 0.5^{abcd}	4.84 ± 0.2^{ab}
35 °C	10.3 ± 0.2^{abc}	9.85 ± 0.0^{ac}	8.49 ± 0.1^{ac}	6.85 ± 0.1^{abcd}	5.90 ± 0.1^{cd}
40 °C	11.0 ± 0.2^{abd}	8.67 ± 0.2^{d}	6.74 ± 0.1^{d}	6.83 ± 0.2^{abcd}	5.72 ± 0.0^{cd}

Values are means of duplicate analyses ± SD.
[a]22 °C; [b]30 °C; [c]35 °C; [d]40 °C.
Means within a column followed by different superscripts are significantly different ($P < 0.05$) at the compared pH conditions.

Table 9
Effect of extraction temperature on the enthalpy, ΔH, (J/g), of Matilda protein

	Distilled water	pH 8.0	pH 8.5	pH 9.0	pH 9.5
22 °C	10.9 ± 0.2^{abc}	11.6 ± 0.0^{abc}	11.4 ± 0.0^{abcd}	10.1 ± 0.1^{abc}	9.25 ± 0.1^{abcd}
30 °C	11.0 ± 0.1^{abc}	11.9 ± 0.2^{abc}	11.2 ± 0.3^{abcd}	9.98 ± 0.1^{abc}	8.92 ± 0.2^{abcd}
35 °C	10.8 ± 0.0^{abc}	11.2 ± 0.7^{abc}	11.2 ± 0.6^{abcd}	9.23 ± 0.6^{abcd}	9.29 ± 0.6^{abcd}
40 °C	11.6 ± 0.2^{d}	12.6 ± 0.1^{d}	11.2 ± 0.1^{abcd}	10.4 ± 0.1^{cd}	8.32 ± 0.7^{abcd}

Values are means of duplicate analyses ± SD.
^{a}22 °C; ^{b}30 °C; ^{c}35 °C; ^{d}40 °C.
Means within a column followed by different superscripts are significantly different ($P < 0.05$) at the compared pH conditions.

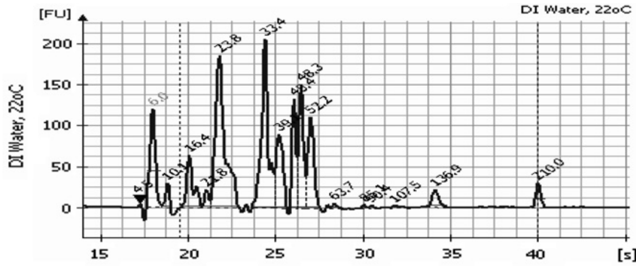

Fig. 3. Electropherogram of Matilda protein extracted using distilled water at 22 °C.

for Digger and 11.1 min for Matilda flour. Chromatograms from reversed-phase HPLC showed the loss of hydrophilic proteins from extracts. Peaks eluted only after 32 min, for both Digger and Matilda proteins, denoting the presence of more hydrophobic proteins in all alkaline extracted conditions as compared to lentil flour. The loss in hydrophilic proteins may have been caused by loss of water-soluble proteins during the extraction process, in particular during acid precipitation. This conclusion is corroborated by the low protein yield achieved and the absence of 11S proteins shown by DSC (Fig. 2).

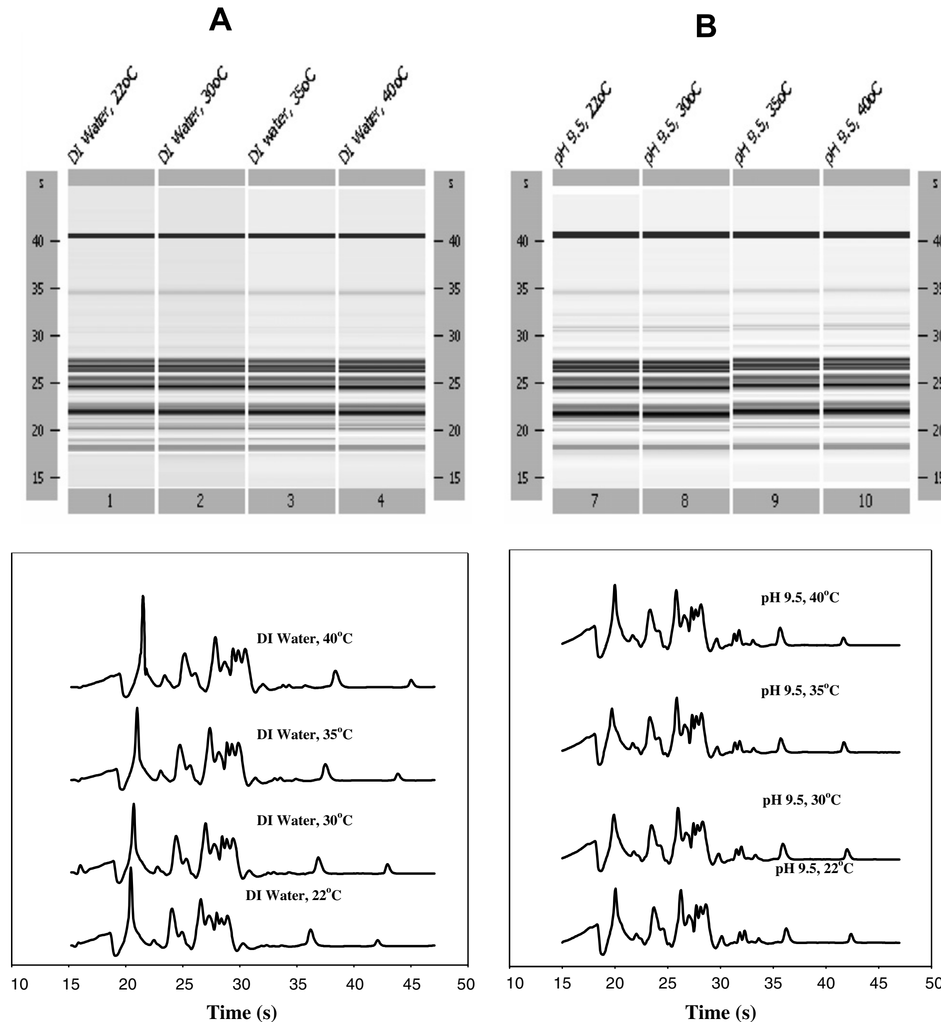

Fig. 4. Gels and electropherograms achieved for Matilda proteins extracted at same pH with varying temperatures; (A) distilled water and (B) pH 9.5.

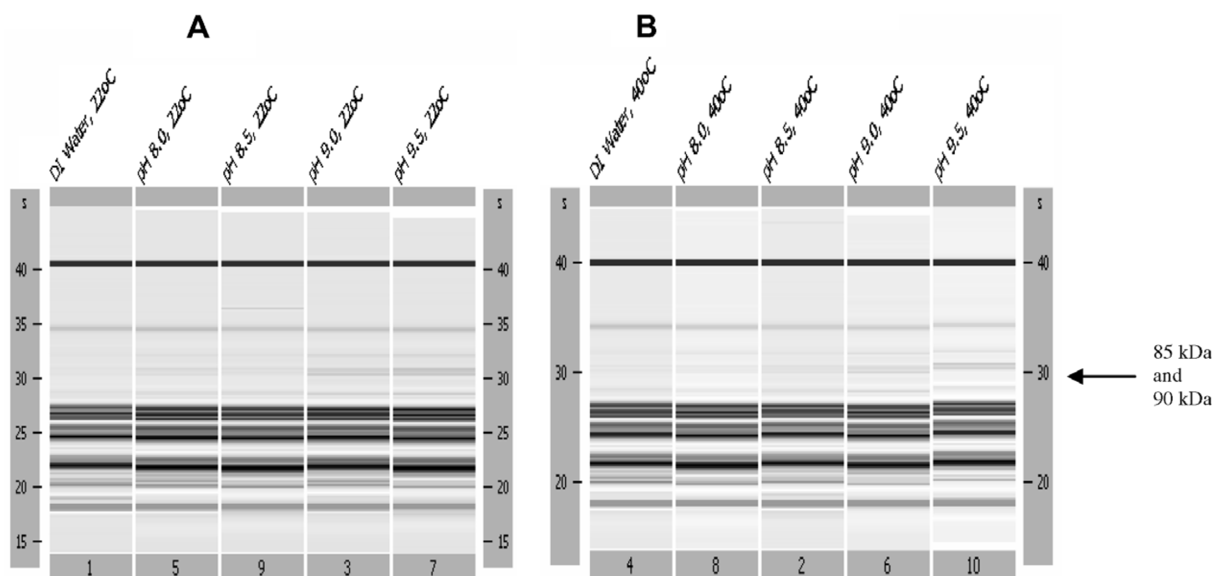

Fig. 5. Gels for Matilda proteins extracted at two temperatures with varying extraction pHs; (A) 22 °C and (B) 40 °C.

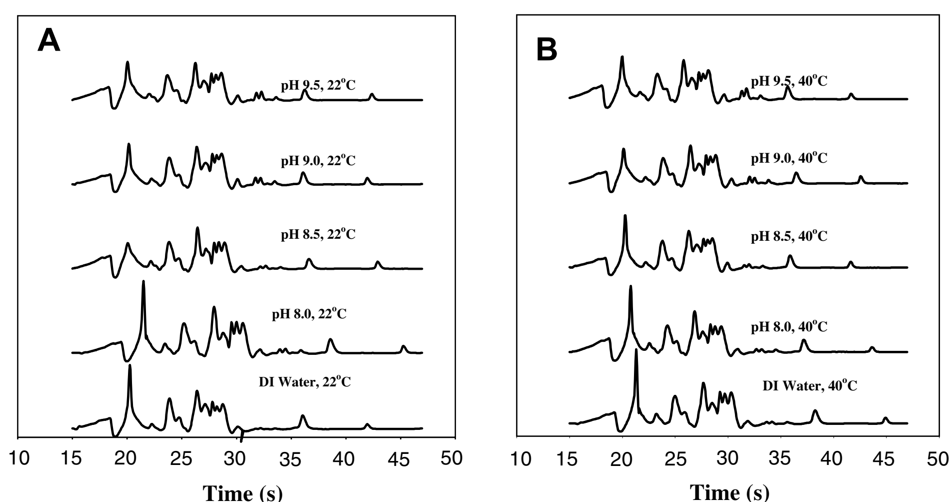

Fig. 6. Electropherograms for Matilda proteins extracted at two temperatures with varying extraction pHs; (A) 22 °C and (B) 40 °C.

In order to evaluate the effect of pH, reversed-phase HPLC chromatograms from the two most extreme extraction conditions (protein extracted at pH 9.5, 40 °C and protein obtained using distilled water, 22 °C) were compared for Digger and Matilda proteins. Fig. 7B and C shows the reversed-phase HPLC chromatogram for Digger proteins extracted under the two extreme conditions. Values and standard deviations were achieved from four replicate analyses. The difference in observed peaks may be due to alteration of the protein during extraction. Thus, protein quality was affected by extraction conditions: particularly extraction pH. The extracted proteins for both Digger and Matilda were more hydrophobic than was the original protein.

Therefore, results from both the Agilent 2100 bioanalyzer and the reversed-phase HPLC showed changes in protein quality with changes in extraction conditions. The decrease in the number of peaks and intensity of certain peaks, as well as the changes in molecular weight with proteins extracted at high extraction pHs, alters the behaviour of the lentil proteins. The strong alkaline extraction may have unfolded the protein structure (uncoiling and re-coiling the lentil protein structure), which resulted in a decrease in enthalpy (ΔH). Out of the 20 peaks found, 10 peaks were significantly different in terms either of absence of peaks or decreased peak intensity ($P < 0.05$). Similarity, in the case of Matilda protein, 11 out of 16 detected peaks were significantly different with increase in process severity.

3.5. Functional properties

Table 10 summarises the effect of three extraction conditions on some of the functionalities of extracted proteins for Digger and Matilda. Both the emulsifying activity

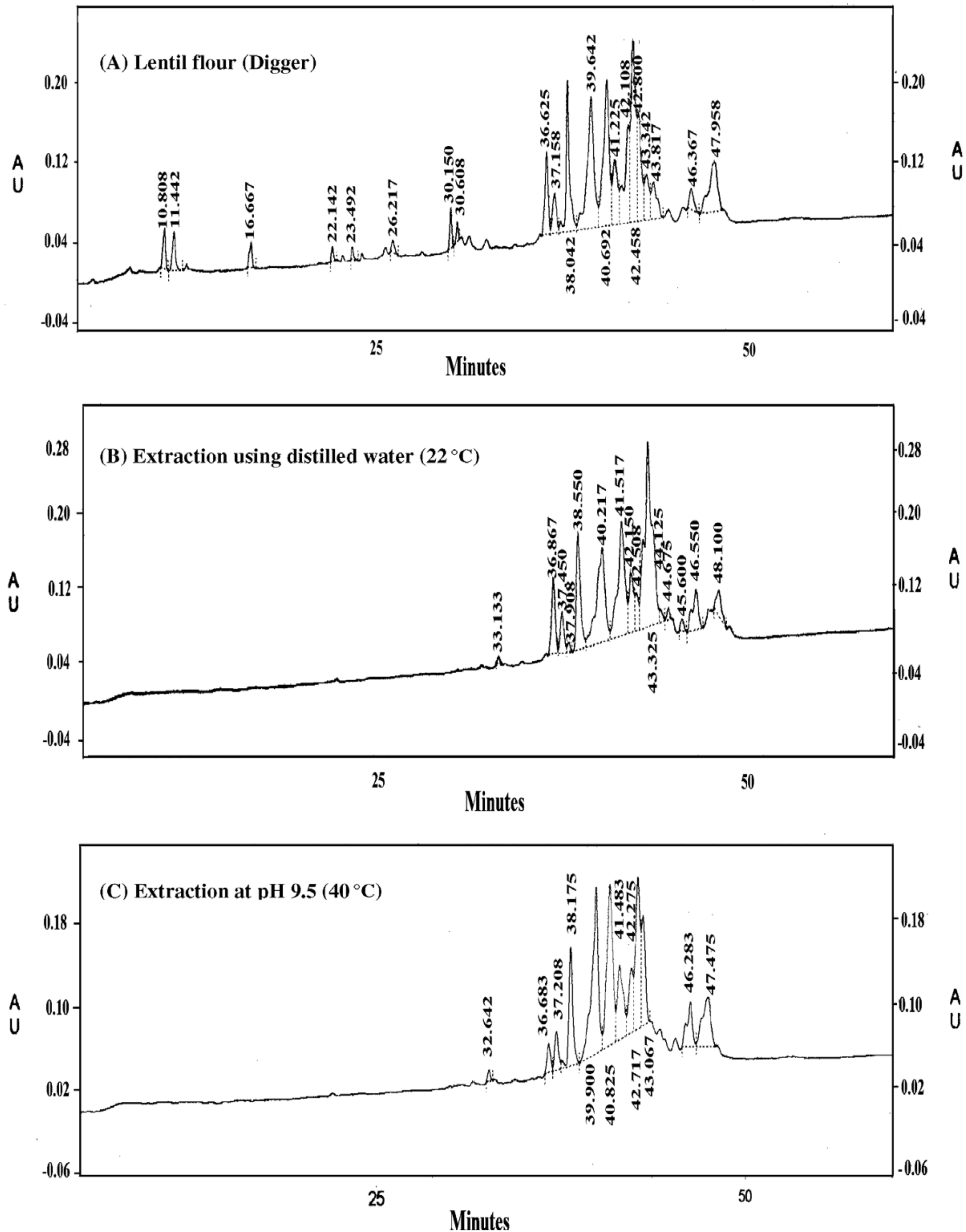

Fig. 7. Chromatograms of reversed-phase HPLC under two extreme extraction conditions for red lentil protein (Digger): (A) lentil flour; (B) protein extracted with distilled water (22 °C) and (C) protein extracted at pH 9.5 (40 °C).

and emulsifying stability decreased with increases in extraction pH and temperature. Digger proteins extracted with distilled water at 22 °C had the highest emulsifying activity (46.7%). This emulsifying property was decreased to about 41.1% when the Digger protein was extracted by the most severe extraction condition (pH 9.5, 40 °C). Although the emulsifying activity did not decrease significantly in either Digger or Matilda, the decrease in emulsion stability was notable. It decreased from about 90–82% for Digger and about 89–79% for Matilda. This decrease in emulsifying properties would possibly be due to changes in peak intensities and peak numbers, as observed by reversed-phase

Table 10
Emulsifying, foaming properties and water holding capacity of extracted Digger and Matilda proteins under three different extraction conditions

Lentil protein extraction condition	Emulsion activity (%)	Emulsion stability (%)	Water-holding capacity (%)	Foam expansion (%)	Foam stability (%)
Red Lentil (Digger)					
Distilled water, 22 °C	46.7 ± 1.0	89.9 ± 0.3	2.7 ± 0.2	43.3 ± 11.0	20.9 ± 6.1
pH 8.5, 35 °C	45.9 ± 0.8	83.2 ± 0.5	2.8 ± 0.4	31.3 ± 4.2	22.8 ± 5.4
pH 9.5, 40 °C	41.1 ± 0.5	82.0 ± 0.9	3.1 ± 0.1	24.0 ± 2.0	31.8 ± 6.7
Green Lentil (Matilda)					
Distilled water, 22 °C	46.3 ± 0.6	89.4 ± 4.3	2.1 ± 0.3	68.0 ± 11.1	13.3 ± 5.1
pH 8.5, 35 °C	44.9 ± 0.6	82.3 ± 2.0	3.3 ± 0.1	54.0 ± 3.5	28.4 ± 2.1
pH 9.5, 40 °C	42.9 ± 0.9	79.1 ± 0.6	3.8 ± 0.1	42.0 ± 7.2	62.4 ± 7.1

Values are triplicate means ± SD.

HPLC for protein extracted under harsher alkaline conditions. With extraction at pH 9.5 and 40 °C, fewer elution peaks were obtained, thus resulting in loss of emulsifying properties, as protein peaks resolved from the HPLC chromatograms were hydrophobic proteins.

The water-holding capability of both Digger and Matilda proteins (adjusted to pH 7.0) increased slightly with increasing extraction pH. This would not be expected from protein depleted in hydrophilicity. The foaming capacity of both proteins decreased and foam stability increased with higher extraction pH. The % foam expansion for Digger proteins, extracted using distilled water (22 °C), decreased from 43.3% to about 24.0% when extracted at pH 9.5 (40 °C) while their foaming stability increased. Matilda protein extracted at pH 9.5 (40 °C) had a higher % foam expansion than had protein extracted using distilled water (22 °C). In all, Matilda proteins showed greater foam-forming capacity than Digger.

4. Conclusions

Dissolving in alkali and precipitating in acid caused loss of most of the hydrophilic proteins, indicated by the absence of 11S globulin. Heat and alkaline conditions denatured the protein by unfolding the protein structure. Severe extraction conditions (high pH and high temperature) produced deterioration in the quality of protein and starch isolates. The results from the DSC, bioanalyzer and HPLC chromatograms show the changes in protein profiles with extraction conditions. Protein functionality changed in both lentil varieties. Therefore, even though high extraction pHs gave high protein yields, they changed the protein quality, which was not desirable.

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