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# Alkaline extraction of starch from Australian lentil cultivars Matilda and Digger optimised for starch yield and starch and protein quality

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## Abstract

The Australian lentils, Lens culinaris: Matilda (Green Lentil) and Digger (Red Lentil) contain 44-45% starch (HPLC method) and 30-33% protein (LECO Analyser). This paper describes, first, the identification of optimum starch extraction conditions where yield is high and starch and protein damage is acceptable and, second, the properties of the starch and protein recovered. Starch was extracted from the flour of each variety with water at four temperatures (ambient 22, 30, 35 and 40 °C) and at five pH conditions (distilled water and pH adjusted with NaOH to 8, 8.5, 9.0 and 9.5). Extraction at pH 9.5, for all temperatures, achieved the maximum starch yield (85–95%) for both varieties. Digger gave a lower starch yield than Matilda when extracted in distilled water at all four temperatures. The yields of Digger starch were not significantly different at the four alkaline pHs. Matilda flour showed gradual increase in % starch yield with increase in extraction temperatures and pH (significant to 95% confidence level). Protein vield achieved, from both Digger and Matilda flour, was relatively low: 43-60% of the analysed protein content for Digger and 48-63% for Matilda. No significant difference was observed for extracted Digger protein at the various extraction pHs and temperatures. Matilda protein yields were significantly different between the various extraction conditions. The % starch damage was high for both varieties when extracted at higher temperature and pH. The DSC  $\Delta H$  value increased with increasing pH and temperature. Extraction at higher pH resulted in a smoother and more symmetrical peak, denoting the absence of protein adhering to the starch surface. Even though low pH and low temperature caused less starch damage, these conditions were undesirable because they resulted in lower starch and protein yields. Taking all factors into account, 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. © 2006 Elsevier Ltd. All rights reserved.

Keywords: Extraction; Lentils; Protein; Starch and yield

#### 1. Introduction

Legumes are an important source of food for many people in various parts of the world. They are an excellent source of carbohydrate and provide an inexpensive source of protein (Jood, Bishnoi, & Sharma, 1998). Lentils, botanically classified as *Lens culinaris* (Adsule, Kadam, & Leung, 1989), are an important crop in many developing countries, contributing 2.4% of the world total production for grain legumes. Like most legumes, lentil seeds contain about 67% carbohydrates and 24–30% protein. They are a good source of essential amino acids, such as lysine and arginine, lacking in some cereal-based diets (Longnecker, Kelly, & Huang, 2002). Lentils provide an excellent source of dietary fibre and complex carbohydrate (Adsule et al., 1989; Sotomayor et al., 1999). They have been the basis of diet for many people living in the Middle East and Asia (Borowska, Fornal, Fornal, Rutkowski, & Kaczynska, 1992; Gozalez & Perez, 2002). Although this legume is relatively

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new to Australia, the production and consumption per capita of lentils has increased throughout the past few vears. New legume starches will add variety to the current market. The starch and protein fractions of lentils both show promise as novel ingredients. This research has been initiated by needs of the food industry to search for new protein and starch sources. The project was initiated by the desire of various food industries in Australia to supply current markets and develop new ones. The dairy industry needs to find cheaper functional proteins to replace expensive protein fractions. The cereal industry needs novel proteins for high-protein snacks and breakfast cereals and new starches with new nutritional characteristics: particularly soluble fibre and low glycaemic index (G.I.) starches to meet their current market trends and consumer demands. Similarly, the expanding aqua feed companies are actively seeking for new protein sources for extruded fish-feed to replace their expensive fishmeal, depending on the diet and species types.

Lentils are divided into two main types, based on difference between the seed size and cotyledon colour. Green lentils (brown, yellow, Chilean, Continental or Macrosperma lentils) have a green to brown seed coat with yellow cotyledons. Red lentils (Microsperma or Persian lentils) have a pale grey to dark seed coat with red cotyledons. The green, Matilda, Laird and Invincible, and the red, Aldinga, Callisto, Cobber, Digger and Kye, are varieties commonly grown in Australia.

Starch extraction in the food industry is done by several methods, with different methods showing characteristic extraction efficiencies and functional properties. Ideal extraction conditions cause little or no structural changes to the extracted components. In the case of starch, no damage to its crystalline phase nor depolymerisation is desired (Han & Hamaker, 2002). Fractionation is a physical method used to isolate starches from protein. It consists of a dry milling step, followed by air classification to separate the starch. This method is efficient and convenient and eliminates the need for handling of the large volumes of slurry which occur in wet milling processes. Starch isolated from dry milling, has poorer functional properties than has starch from wet milling (Tian, Kyle, & Small, 1999). Wetmilling is an alternative extraction method, commonly used for extraction of starch from flour (Zheng, Sosulski, & Tyler, 1998). Other methods use alkali to solubilise protein, enabling the extraction of pure starch from flours. Extraction solvents include different alkaline agents, such as detergents and sodium hydroxide. The number of extraction stages for starch extraction varies (Lim, Lee, Shin, & Lim, 1999; Matsunaga, Takahashi, & Kainuma, 2003). In the United States, steeping solution containing 5% sulphur dioxide is used for the extraction of starch from maize. This alkaline extraction technique gives high yield and purity (Han & Hamaker, 2002) but alkali-extracted starch has lower pasting temperature and higher pasting viscosity than has commercially wet-milled starch, perhaps because removal of lipids by sodium hydroxide favours swelling

of the starch granules during extraction. In addition, residual sodium also contributes to an increase in pasting viscosity (Han & Hamaker, 2002).

This study determines the proximate composition of starch and protein in two Australian lentils, Matilda and Digger, evaluates the starch and protein yield and purity from the alkaline extraction of flour, and describes the effect of alkaline extraction on the starch and protein qualities.

# 2. Materials and methods

## 2.1. Materials

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 size 0.79 mm). The flours were packed in plastic bags, sealed and stored in an air-tight box prior to analysis.

#### 2.2. Moisture

Moisture was determined AACC Method 44-19 with modification. The samples were dried at  $130 \,^{\circ}$ C for 24 h instead of the prescribed  $135 \,^{\circ}$ C.

# 2.3. Protein

Estimation of crude protein  $(N \times 6.25)$  was done using an automated LECO Nitrogen Analyser (LECO FD-428, LECO Corporation, St Joseph, MI, USA).

#### 2.4. Fat

Estimation of fat content was by acid hydrolysis: AOAC Official Method 922.06.

# 2.5. Sugars and starch

The sugar and starch contents were analysed using a method modified from Wills, Balmer, and Greenfield (1980). Total soluble sugars were extracted by washing the weighed flour sample three times with boiling 85% ethanol. The ethanol solution containing the sugars was concentrated to about 3 ml on a rotary vacuum evaporator at 45 °C. The concentrate was made up to 10.0 ml with distilled water and passed through an ultrafilter membrane (0.45  $\mu$ m, 47 mm diameter), where about 2.0 ml of the solution was collected.

The sugar-free residue was dried, and 300–400 mg was accurately weighed and added to 10.0 ml distilled water. The mixture was heated in a capped tube to boiling in a water bath for about 4 h to gelatinise the starch. Amyloglucosidase (0.4 ml) was then added, together with 0.3 ml of acetate buffer (pH 4.5), to hydrolyse starch to monosaccharides. The mixture was left overnight at 37 °C. Quantitative

determination of the total soluble sugars and starch was then carried out by HPLC (Hurst, Martin, & Zoumas, 1979; Wills et al., 1980) and the total starch content, as reported in this paper, was used for determination of starch extraction yield.

# 2.6. Alkaline extraction of starch and protein

Starch and protein were extracted from flour using the modified in-house extraction method of Food Science Australia, CSIRO. Four temperatures (ambient 22, 30, 35 and 40 °C) and five different pHs (distilled water and pH 8.0, 8.5, 9.0 and 9.5) were used to determine the effect of alkaline extraction on starch and protein yields. In the initial extraction, the flour sample was extracted with water, adjusted to the required pH with 0.1 M NaOH. The flour-to-water ratio used during washing was 1:3 and, during extraction was, 1:10. Sodium metabisulphite (0.01% w/v) was added to the solution during the first washing step to control bacterial action and growth during extraction. Upon washing, the solution was centrifuged at 3000 rpm for 30 min. The supernatant was collected and combined for the recovery of protein. The top lipid layer was scraped from the surface of each residue and discarded. After twice washing with water at the chosen pH to remove unbound protein, the residue was mixed with water at the chosen pH and extracted for 2 h at the test temperature. During the 2 h of extraction, the pH of the solution was measured at 20-min intervals to check for pH drift. The pH of the solution was corrected using 0.1 M NaOH.

After 2 h, the solution was passed through a nylon screen (Mesh 325, 44  $\mu$ m). Smaller starch particles passed through the screen with the solution. Any flour sample retained on the screen was extracted again for 2 h using water (adjusted to the chosen pH) at the test temperature. The pH during this extraction step was measured every 20-min and adjusted to the correct pH. After 2 h, the solution was again passed through the screen to separate out the extracted starch. The starch suspensions from both extraction steps were combined and allowed to stand for 90 min. The supernatant was decanted and combined with the supernatant collected from the initial washing step.

The extracted starch was twice washed again with distilled water (1:3) and allowed to settle for 90 min prior to decanting. All supernatant collected during the two sedimentation steps was combined with the initial washing for protein recovery. After the third sedimentation, the extracted starch was washed again with distilled water (1:3) and isolated onto a filter paper by means of suction filtration through a 44  $\mu$ m screen. Isolated starch collected on the filter paper was air-dried (thin air drying) at room temperature for 48 h.

Protein was recovered from the combined supernatant fractions. The isolation and characterisation of the protein fraction is the subject of a second paper. The pH of the supernatant was adjusted to 4.5 using 0.2 M HCl and left

overnight at 4 °C. Lentil protein is the least soluble at about pH 4–5 (Fan & Sosulski, 1974; Shehata, El-Din, & Abd-El-Mottaleb, 1978). The precipitated protein was concentrated by centrifugation (3000 rpm for 30 min) and the protein curd was freeze-dried (Tian et al., 1999). Protein and starch yields were calculated from the amount of mass recovered compared with results obtained from Sections 2.2 and 2.4.

#### 2.7. Scanning electron microscopy

Lentil flours and extracted starches were dispersed on double-stick adhesive tapes mounted on SEM aluminium stubs, coated with a thin layer of gold in a vacuum evaporator (EMITEX K 550X), and examined with a FEI-QUANTA 200 Environmental Scanning Electron Microscope (ESEM), using a large field detector in low vacuum mode.

#### 2.8. Starch damage

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

#### 2.9. 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 four decimal places into a stainless steel pan fitted with a rubber O-ring. The dispersant (distilled water used for starch) 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.

# 3. Results and discussion

# 3.1. Chemical composition

The compositions of Digger and Matilda are given in Table 1.

There was no significant difference of starch or fat contents between Matilda and Digger. Matilda had a significantly higher protein content than Digger (P < 0.05). The protein contents of the two Australian lentils were higher and the starch content was lower than published values for other cultivars (Jood et al., 1998).

# 3.2. Isolation of lentil starch by alkaline extraction

# 3.2.1. General

Lentil starch was isolated from lentil flour using alkaline extraction. Two factors, pH and temperature, were

	Moisture (%) <sup>a</sup>	Protein (%) <sup>b</sup>	Fat (%) <sup>b</sup>	Starch (%) <sup>c</sup>		
Lentil						
Matilda	$10.7\pm0.1$	$32.6\pm0.3$	$2.8\pm0.1$	$45.0\pm5.5$		
Digger	$11.8\pm0.2$	$30.3\pm0.2$	$2.9\pm0.1$	$44.8\pm2.2$		
't-test' $(P < 0.05)$	-13.1	15.5	NS	NS		

 Table 1

 Protein and starch content of lentil cultivars, Matilda and Digger (dry matter basis)

NS, not-significant.

<sup>a</sup> Values are mean  $\pm$  SD of eight independent determinations.

 $^{\rm b}$  Values are mean  $\pm$  SD of five independent determinations.

<sup>c</sup> Values are mean  $\pm$  SD of six independent determinations.

evaluated to investigate their effects on the efficiency of starch extraction. A high sample to water ratio (1:10) was used during the extraction process to increase the efficiency and to reduce the lipid and protein in the extracted starch (Ramirez, 1996). A large volume of distilled water was used after extraction to wash the starch and to reduce the pH, in order to avoid damaging the starch or changing its rheological properties. The starch yield was determined from the ratio of recovered starch to the total starch obtained by HPLC (Section 2.4).

# 3.2.2. Effect of pH

The extraction at pH 9.5, for all temperatures, produced the maximum starch yields (85–95%) for both varieties. In distilled water extraction, at all four temperatures, the starch yield from Digger was lower than that of Matilda. In alkaline extraction, the yield achieved for Digger was slightly higher than that achieved for Matilda at all four temperatures and four pHs, except for pH 9.5 at 40 °C.

The higher the extraction pH, the higher was the % starch recovered. The effect of pH was more marked in Matilda than in Digger. Matilda flour showed a gradual increase in % starch yield with increase in pH (95% confidence level) (Fig. 1A) at all temperatures. Digger starch yield was more sensitive to pH; it increased from 73–80% (extracted using distilled water for all temperatures) to 86-91% (extracted at pH 8.0 for all temperatures). The differences were significant at the 95% confidence level. However, the yields of Digger starch were not significantly different when compared within the four alkaline pHs

across all four temperatures. At 95% confidence level, the recoveries from the four high pHs were not significantly different at any temperature (Fig. 1B).

## 3.2.3. Effect of temperature

Except for a slight increase at 40 °C, the yield of starch from Digger was unaffected by temperature (P < 0.05). The yield of starch from Matilda increased with increasing extraction temperature (P < 0.05) (Fig. 2).

## 3.3. Isolation of lentil protein

Starch granule-associated protein is a major component that affects the extraction of starch. Proteins adhere to the surface of the starch and are relatively difficult to remove (Baldwin, 2001). In this case, the use of alkali (NaOH) was investigated to determine the efficiency of protein extraction at various extraction pH values. Nitrogen of most legumes is least soluble when extracting at pH 4 and its solubility increases markedly above pH 6.0, where near to 80% nitrogen dispersibility is achieved when using an extraction solvent at pH 8.0 and above (Fan & Sosulski, 1974).

During extraction of starches, alkaline extraction solubilised flour protein. Proteins were extracted from the lentil flours by four consecutive extraction steps, which included the two initial washing and the two extraction steps. The recovery of proteins from the flour was low in both varieties (consistent with the report of Fan & Sosulski (1974)). The protein yield was determined from the ratio of recov-



Fig. 1. Starch yield vs extraction pH for: (A) green lentil (Matilda); (B) red lentil (Digger) at various extraction temperatures. All analysed in duplicate.



Fig. 2. Starch yield vs extraction temperatures for: (A) green lentil (Matilda); (B) red lentil (Digger) at various pHs. All analysed in duplicate.

ered protein mass to the total protein by using a LECO nitrogen analyser (Section 2.2). The protein yields achieved for Digger were 43–60% and for Matilda 48–63% (Tables 2 and 3). The % protein yield achieved by Matilda was higher than that achieved by Digger.

At all temperatures, alkaline conditions extracted significantly more protein from Digger than did distiled water. The yields of Digger proteins, however, were not significantly different when compared within the four alkaline pHs across all four temperatures (P < 0.05) (Table 2).

The yield in Matilda protein extraction differed from Digger. Across all four extraction temperatures, there was no significant difference for % protein yield at pH 8.0 and 8.5. However, most of the other % protein yields achieved were significantly different at different extraction pHs at the same extraction temperature. Thus, extraction pH condition affects the protein extraction efficiency of Matilda more than that of Digger (Table 3).

The same trend was observed, in both Digger and Matilda, when evaluating the effect of temperature on protein extraction efficiency. Matilda was more sensitive to the effect of temperature than was Digger. At each pH, Digger was not sensitive to temperature (Table 4); Matilda showed significant differences which were smaller at high pH (Table 5).

# 3.4. Residual protein

Residual protein attached to starch granules was negligible. No starch sample registered positive protein with the "LECO" protein analyser which has a sensitivity of 0.001%.

### 3.5. Scanning electron microscopy

A SEM was used to observe lentil cotyledon cells (Fig. 3) and extracted starch granules in Digger and Matilda to determine the cleanliness of the starch granules after alkaline extraction. The starch granules shown were from the two extreme extraction conditions used: distilled water at 22 °C (Fig. 4) and pH 9.5 at 40 °C (Fig. 5). The starch granules achieved from the extraction process appeared to be relatively clean but residual materials, possibly protein, were still found in starch extracted using distilled water. No residual materials were observed on the starch granules from the starch extracted at high pH.

#### 3.6. Total % starch damage

In order to ensure good functional starch properties, starch damage should be avoided. A total starch damage value of less than 1% was selected as acceptable. This criterion was met for Digger by all extraction treatments except at 40 °C. Matilda starch was more susceptible to damage. The criterion was met for pH  $\leq$  9.0 and temperature  $\leq$  30 °C. Extraction with distilled water at 35 and 40 °C also gave low starch damage (Fig. 6).

Although mild extraction pH gave relatively low % starch damage values, starch and protein yields were lower. Low extraction temperature, 22 °C, also gave relatively low

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

	22 °C	30 °C	35 °C	40 °C	
Distilled water	$43.8\pm0.8^{\rm a}$	$44.8\pm3.4^{\rm a}$	$49.3 \pm 1.1^{\rm ab}$	$48.8\pm0.2^{\rm a}$	
pH 8.0	$54.3 \pm 4.1^{bcde}$	$56.3 \pm 2.1^{bcde}$	$57.9 \pm 4.2^{\mathrm{abcde}}$	$60.2 \pm 1.8^{bcde}$	
рН 8.5	$54.3 \pm 2.1^{bcde}$	$57.6 \pm 3.5^{bcde}$	$59.3 \pm 1.4^{\mathrm{bcde}}$	$60.4 \pm 1.3^{bcde}$	
рН 9.0	$56.7 \pm 1.3^{bcde}$	$58.0 \pm 1.2^{bcde}$	$59.5 \pm 1.0^{bcde}$	$61.0 \pm 0.8^{bcde}$	
рН 9.5	$59.1 \pm 2.5^{bcde}$	$59.6 \pm 2.3^{bcde}$	$60.3 \pm 3.6^{bcde}$	$62.0 \pm 2.0^{\mathrm{bcde}}$	

Values are means of duplicate analyses  $\pm$  SD.

Superscripts: <sup>a</sup>distilled water; <sup>b</sup>pH 8.0; <sup>c</sup>pH 8.5; <sup>d</sup>pH 9.0; <sup>e</sup>pH 9.5.

Means within a column followed by different superscripts are significantly different ( $P \le 0.05$ ) at the compared pH conditions.

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	22 °C	30 °C	35 °C	40 °C	
Distilled water	$48.5\pm0.7^{\rm a}$	$49.1\pm0.4^{\rm a}$	$50.9\pm0.1^{ m a}$	$54.2\pm1.4^{\rm a}$	
рН 8.0	$51.9 \pm 0.4^{ m bcd}$	$53.5\pm0.0^{ m bc}$	$60.3 \pm 0.9^{ m bcde}$	$60.3 \pm 1.0^{\mathrm{bcd}}$	
рН 8.5	$52.4 \pm 1.6^{ m bcd}$	$52.7\pm0.7^{ m bc}$	$59.0\pm0.2^{ m bc}$	$60.4\pm0.1^{ m bc}$	
рН 9.0	$55.8 \pm 2.6^{bcde}$	$56.6 \pm 1.5^{de}$	$60.8\pm0.2^{ m bde}$	$62.4\pm0.0^{ m bd}$	
рН 9.5	$60.3 \pm 0.5^{\mathrm{de}}$	$59.9 \pm 1.9^{\mathrm{de}}$	$60.7\pm0.4^{ m bde}$	$63.4\pm0.3^{\rm e}$	

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

Values are means of duplicate analyses  $\pm$  SD.

Superscripts: <sup>a</sup> distilled water; <sup>b</sup>pH 8.0; <sup>c</sup>pH 8.5; <sup>d</sup>pH 9.0; <sup>e</sup>pH 9.5.

Means within a column followed by different superscripts are significantly different (P < 0.05) at the compared pH conditions.

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Statistical summary	for the effect	t of temperature	on % protein	yield for	Digger
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	Distilled water	pH 8.0	pH 8.5	pH 9.0	рН 9.5
22 °C	$43.8\pm0.8^{ab}$	$54.3 \pm 4.1^{abcd}$	$54.3 \pm 2.1^{\mathrm{abc}}$	$56.7 \pm 1.3^{\mathrm{abc}}$	$59.1 \pm 2.5^{abcd}$
30 °C	$44.8 \pm 3.4^{\mathrm{abcd}}$	$56.3 \pm 2.1^{abcd}$	$57.6 \pm 3.5^{\mathrm{abcd}}$	$58.0 \pm 1.2^{ m abcd}$	$59.6 \pm 2.3^{\mathrm{abcd}}$
35 °C	$49.3 \pm 1.1^{bcd}$	$57.9 \pm 4.2^{\mathrm{abcd}}$	$59.3 \pm 1.4^{\mathrm{abcd}}$	$59.5 \pm 1.0^{ m abcd}$	$60.3 \pm 3.6^{\mathrm{abcd}}$
40 °C	$48.8\pm0.2^{ m bcd}$	$60.2 \pm 1.8^{ m abcd}$	$60.4 \pm 1.3^{\mathrm{bcd}}$	$61.0\pm0.8^{ m bcd}$	$62.0\pm2.0^{ m abcd}$

Values are means of duplicate analyses  $\pm$  SD.

Superscripts: <sup>a</sup>22 °C; <sup>b</sup>30 °C; <sup>c</sup>35 °C; <sup>d</sup>40 °C.

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 temperature on % protein yield for Matilda

	Distilled water	pH 8.0	pH 8.5	pH 9.0	pH 9.5
22 °C	$48.5\pm0.7^{ab}$	$51.9\pm0.4^{\rm a}$	$52.4\pm1.6^{\rm a}$	$55.8\pm2.6^{abc}$	$60.3\pm0.5^{abc}$
30 °C	$49.1\pm0.4^{\rm ab}$	$53.5\pm0.0^{ m b}$	$52.7\pm0.7^{ m ab}$	$56.6 \pm 1.5^{\mathrm{ab}}$	$59.9 \pm 1.9^{\mathrm{abc}}$
35 °C	$50.9\pm0.1^{ m c}$	$60.3\pm0.9^{ m cd}$	$59.0 \pm 0.2^{\circ}$	$60.8\pm0.2^{ m ac}$	$60.7\pm0.4^{ m abc}$
40 °C	$54.2\pm1.4^{\rm d}$	$60.3\pm1.0^{\rm cd}$	$60.4\pm0.1^{ m d}$	$62.4\pm0.0^{\rm d}$	$63.4\pm0.3^{\rm d}$

Values are means of duplicate analyses  $\pm$  SD.

Superscripts: <sup>a</sup>22 °C; <sup>b</sup>30 °C; <sup>c</sup>35 °C; <sup>d</sup>40 °C.

Means within a column followed by different superscripts are significantly different (P < 0.05) at the compared pH conditions.



Fig. 3. Fragment of cotyledon cell in raw lentil flour: (A) Digger; and (B) Matilda. Smooth starch granule surface with spherical protein bodies embedded in matrix. Magnification 2000×.

yield. Therefore, after considering all the results obtained from extraction yield, scanning electron micrographs and % starch damage values, pH 9.0 at 30 °C was chosen as an optimum extraction condition for Matilda and pH 8.5 at 35 °C was chosen for Digger.

#### 3.7. Differential scanning calorimetry

DSC confirmed the absence of protein, shown by SEM of starch granules. The DSC  $\Delta H$  of extracted lentil starch, from both Digger and Matilda, increased with increases in



Fig. 4. Starch extracted using distilled water at 22 °C: (A) Digger; and (B) Matilda. There were some residual materials around starch granules. Magnification 2000×.



Fig. 5. Starch extracted using pH 9.5 at 40 °C: (A) Digger; and (B) Matilda. Contained some deformed and cracked starch granules. Magnification: (A) 1000× and (B) 2000×.



Fig. 6. Starch damage vs extraction temperature for: (A) green lentil (Matilda); and (B) red lentil (Digger) at various pH extraction conditions.

pH and temperature. Extraction at higher pH resulted in a smoother and more symmetrical peak (Fig. 7).

A shouldering peak adhering to the starch peak was observed in Fig. 7(A) and (B). Higher extraction pH, which

helps to solubilise protein adhering to the starch granules, reduced the shouldered peak. A much smoother peak was observed for the Digger starch extracted at pH 9.5 than for Digger starch extracted using distilled water.



Fig. 7. DSC endotherms of extracted Digger starch compared at three different extraction conditions; (A) distilled water, 22 °C; (B) pH 8.5, 35 °C and (C) pH 9.5, 40 °C.

Matilda starch, extracted at pH 9.5, also showed a smoother peak.

As extraction pH and temperatures increased, the  $\Delta H$  values and peak temperatures increased. The gelatinisation temperature increased from 65.7 to 66.4 °C for Digger and from 63.6 to 65.6 °C for Matilda, while the corresponding enthalpy values,  $\Delta H$ , increased from  $16.12 \pm 0.36$  to  $17.55 \pm 0.28$  J/g and from  $14.94 \pm 0.99$  to  $16.05 \pm 0.54$  J/g, respectively.

#### 4. Conclusion

Lentil flour can be extracted to produce clean starch and protein fractions with acceptable yields by alkaline extraction. The extraction procedure was optimised to low % starch damage to avoid alteration of the true rheological properties of the lentil starch. The optimised samples will allow rheological study to provide a basis for their uses in new product areas, such as a functional ingredient in the extrusion process to create new cereal-based products. Alkaline-extracted lentil starch has higher gelatinisation temperature and higher enthalpy values than starch extracted using distilled water, due to the removal of surface proteins. The optimum conditions for alkaline extraction of the starch from both varieties of Australian lentils were pH 9.0 at 30 °C for Matilda and pH 8.5 at 35 °C for Digger.

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