



Extraction and characterization of protein fractions from Australian canola meals

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ABSTRACT

Canola proteins were sequentially extracted from four Australian canola meals of different species and two industrial meal samples, according to Osborne method (OSB), based on their solubilities in water, 5% NaCl, 0.1 M NaOH and 70% ethanol respectively. These extracts were then compared to the glutelin fraction obtained by direct alkaline extraction method (DIR) in terms of protein yield, recovery, electrophoretic profile, and residual antinutritional components. The OSB method was found to be more effective for protein extraction which resulted in higher cumulative protein yield and recovery of water-soluble protein fractions, although the DIR method produced a glutelin fraction of higher protein content. Phenolic compounds were observed in all the extracted protein fractions although sinapine, the major phenolic compound in canola, was present only in the albumin fractions. The glucosinolate content of all extracts was below the detection limit of the method employed ($<3 \mu\text{mol/g}$). While the electrophoretic profiles of all four OSB fractions were largely different, the DIR fraction was very similar to the glutelins obtained from the OSB method. Isoelectric focusing revealed that many of the proteins in the canola varieties ranged in pI between pH4.6 and 8.3.

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1. Introduction

Canola seed typically contains over 40% oil (Kimber & McGregor, 1995). After the oil has been removed from the seed, the protein-rich meal that is left behind is generally used as a protein source in livestock and aquaculture industries (Canola Council of Canada, 2009). The growing demand for canola oil worldwide implies that more meal will be produced as a result of the increased oil extraction. Therefore, this represents an opportunity to better understand canola proteins (the major constituents in the meal) in order to enhance their utilisation for human food consumption and increase the market value of the meal. This is especially relevant since canola meal has always been recognised as having the potential to be an alternative protein source for human consumption (Uppstrom, 1995) due to their high biological value (Campbell et al., 1981), high protein efficiency ratio compared to soybean (Delisle et al., 1984), and well balanced amino acid composition (Pastuszewska et al., 2000) comparable to the international requirement for adults and young children (FAO/WHO/UNU, 1985). There are also indications that canola protein isolate may possess good technologically functional properties that will enable it to be used as a viable food ingredient (Aluko & McIntosh, 2001; Yoshie-Stark et al., 2008). The incorporation of canola meal in human food is, however, restricted by the presence of antinutritional factors

such as glucosinolates, phytic acid, and phenolics, including sinapine and tannins (Wu & Muir, 2008; Yoshie-Stark et al., 2008). Although it has been shown that at very low concentrations, these components may indeed provide beneficial health effects such as reduced cancer risk (Song & Thornalley, 2007) and strong antioxidative properties (Amarowicz et al., 2000). The most recent reviews on the subject have, however, concluded that the presence of these components in protein extracts remains a major drawback to their utilisation for food manufacture (Aider & Barbana, 2011; Tan et al., 2011).

Canola protein isolates are normally prepared by direct alkaline extraction (DIR) in an environment of high pH followed by acid precipitation, presumably due to the high nitrogen yield obtained in the isolate (Pedroche et al., 2004). These proteins have been characterised mainly by protein nitrogen, as well as molecular mass with gel electrophoresis (Aluko & McIntosh, 2001; Aluko et al., 2005), while there is little information about the level of antinutritional factors in the isolates. Furthermore, proteins prepared by this procedure have poor solubility (Yoshie-Stark et al., 2008), probably due to the extraction process being carried out in a harsh alkaline environment, which may cause irreversible denaturation of proteins. These largely insoluble isolates invariably behave poorly as food ingredients, leading to several attempts to improve them by chemical or enzymatic modifications (Pedroche et al., 2004; Khattab & Arntfield, 2009). It is likely, however, that the Osborne (OSB) method which involves the sequential extraction of proteins using water, salt solution, alkaline and alcoholic solutions respectively (Osborne & Mendel, 1914), could produce canola proteins with better characteristics for incorporation in human food manufacture.

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The industrial toasted meal is the by-product of oil (solvent) extraction process that involves heating the meal at very high temperatures (up to 110 °C), which could affect the quality of proteins isolated from it (Salunkhe et al., 1992). As the canola meal currently remains an industrial by-product of oil processing, and given the economic requirement of desolventising for the economic viability of the process, it is important to study the characteristics of protein isolates from both industrial and non-industrial meals. Furthermore, previous studies have shown possible effects of the environment and genotype on protein content of the meal (Si et al., 2003), as well as the level of antinutritional factors such as glucosinolates (Mailer & Wratten, 1985). To our knowledge, such investigations on profile and characteristics of canola proteins, especially those extracted as individual Osborne fractions are yet to be carried out. As the second largest exporter of canola in the world, it is also important that proteins prepared from Australian canola meal varieties be studied in greater details. In this study, therefore, we compared the common alkaline extraction (DIR) method to the Osborne (OSB) method, for the extraction of protein from meal derived from Australian canola species. The influence of industrial processing of the meal on protein fractions, major antinutritional components and electrophoretic profiles has also been reported.

2. Materials and methods

2.1. Sources of materials and chemicals

B. napus (Monola 76TT) and *B. juncea* (Gladiator 1 mustard) oilseeds were from the Australian National Variety Trials, supplied by Industry & Investment, NSW, Australia. Locally grown *B. oleracea* (Capitata) and *B. rapa* (Chinesis) oilseeds were purchased from Sam's warehouse (Wagga Wagga, NSW, Australia). Industrial pre-toast and toasted *B. napus* meals were supplied by Cargill Oilseeds (Footscray, VIC, Australia). Reagents and chemicals were supplied by Sigma-Aldrich (Castle Hill, NSW, Australia), unless otherwise stated.

2.2. Preparation of canola meals

Whole oilseed samples were ground into powder using a coffee grinder. Petroleum spirit at a meal to solvent ratio of 1:4 was then used to defat the ground seeds. The mixture was filtered through Whatman No. 1 paper, and the wet meal was allowed to dry in a fume hood at room temperature overnight. Industrial meals were used as supplied.

2.3. Protein extraction using the Osborne (OSB) method

Canola protein fractions were prepared using the classical procedure of Osborne and Mendel (1914) with modifications. Meal samples were extracted with 10 volumes of de-ionised water for 1 h on an end-over-end shaker. The extracts were centrifuged at 3000 g for 10 min to obtain the water soluble albumin fractions (supernatant). This process was repeated twice on the supernatant after which the albumin fractions were pooled and filtered under vacuum through Whatman No.1 paper. The residues were then similarly extracted with 5% NaCl, 0.1 M NaOH and 70% ethanol sequentially to obtain globulin, glutelin and prolamin fractions respectively. The meal residues were rinsed with five volumes of de-ionised water and collected for the determination of total protein recovery. Salt in the globulin extract was removed by dialysis at 4 °C using cellulose membrane (molecular weight cut off of 12.4 kDa) against 20 volumes of de-ionised water, for 72 h with water changes every 24 h. For glutelin fractions, pH of the extracts was adjusted to 4.0 with 1 M HCl and centrifuged at 3000 g for 10 min to obtain the precipitates which were then re-suspended in five volumes of de-ionised water and adjusted to pH 7.0 with 1 M NaOH. The supernatant (glutelin residues) was dialysed as above to remove the salt generated as a result of the initial pH adjustment; this

was kept and analysed for the determination of mass balance of the entire process. Ethanol in the prolamin fractions was evaporated from the extracts using Rotavapor R-210 (Buchi Labortechnik, Flawil, Switzerland) at 40 °C. All extracted protein fractions and residues were freeze-dried and stored in the freezer (–20 °C) until further analysis.

2.4. Protein extraction using the direct alkaline (DIR) method

Defatted meal was extracted three times, directly by 0.1 M NaOH solution at a meal to solvent ratio of 1:10 as described for preparation of the glutelin fractions by the OSB method above.

2.5. Chemical analyses

Proximate analysis of the meals was determined by standard Association of Official Analytical Chemists methods (AOAC, 1996). Total phenolic content was determined by Folin–Ciocalteu's reagent method according to Asami et al. (2003) with the absorbance readings measured using FLUOstar Omega Microplate Reader (BMG Labtech, Offenburg, Germany) at 750 nm. The total phenolic content was standardised against sinapic acid and expressed as sinapic acid equivalents (SAE) per gramme sample. Sinapine content was determined by high performance liquid chromatography (HPLC) according to the procedure described by Mailer et al. (2008). Glucosinolate content was determined as glucose from the hydrolysis of the glucosinolates according to the official Australian Oilseeds Federation method 4-1.22 and reported as $\mu\text{mol/g}$ oil-free meal at 10% moisture (Mailer & Pratley, 1990).

2.6. Gel electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) was carried out using a PhastGel Gradient 8–25 on a PhastSystem Separation, Control and Development unit according to manufacturer's protocol (GE Healthcare Bio-Science AB, Uppsala, Sweden). For non-reducing conditions, Tris–HCl buffer solution (pH 8) containing 10% SDS and 0.01% bromophenol blue was used. For reducing conditions, Tris–HCl buffer solution (pH 8) containing 5% 2-mercaptoethanol (ME), 10% SDS and 0.01% bromophenol blue was mixed with samples and boiled for 15 min, then cooled to room temperature. All samples were centrifuged at 16,000 g for 10 min. An aliquot (4 μl) of the supernatant was loaded onto the gel and Precision Plus Protein™ Standards Dual Colour with molecular weight range of 10–250 kDa (Bio-Rad, Hercules, California, USA) was used as the molecular weight standard. The gels were stained with Coomassie brilliant blue, and the bands were scanned and analysed using Gel-Quant™ image analysis software (AMPL Software Pty Ltd, Turramurra, NSW, Australia).

Isoelectric focusing (IEF) was carried out using PhastGel IEF 3–9 on the same PhastSystem but using different separation and development procedures as instructed by the manufacturer (GE Healthcare Bio-Science AB, Uppsala, Sweden). Tris–HCl buffer solution (pH 8) containing only 0.01% bromophenol blue was used in sample preparation. For IEF under reducing conditions, Tris–HCl buffer solution (pH 8) containing 5% ME, 6 M urea and 0.01% bromophenol blue was mixed with samples and boiled for 15 min, then cooled to room temperature. All samples were centrifuged at 16,000 g for 10 min and 2 μl of the supernatant was loaded onto the gel. Broad pI (pH 3–10) IEF Calibration Kit, (GE Healthcare, Bio-Science AB, Uppsala, Sweden) was used as pI standard and the gels were silver-stained by PlusOne™ Silver Staining Kit (GE Healthcare Bio-Science AB, Uppsala, Sweden), scanned and analysed using the Gel-Quant™ software as described above.

2.7. Statistical analysis

All extractions and analyses were carried out in triplicate and the means with standard deviation were reported. Data collected were subjected to analysis of variance (ANOVA), and Fisher's least significant difference (LSD) test was used to determine the means that were significantly different ($P \leq 0.05$) using SPSS™ statistical software version 17 (SPSS Inc, Chicago, IL, USA).

3. Results

3.1. Proximate analysis and meal composition

Proximate analysis of various meal samples is shown in Table 1. Protein, the main component in the meals, ranged from 34.50 to 40.00% depending on the meal types. Protein content of industrial pre-toast meal (40.00%) was similar to that of *B. juncea* (38.38%), *B. oleraceae* (36.38%) and industrial toasted (36.00%) meals, but significantly higher than protein content of *B. rapa* (34.56%) and *B. napus* (34.50%) meals. Moisture content of industrial toasted meal (12.09%) was significantly higher than that of non-industrial *Brassica* samples (9.71–11.27%) while industrial pre-toast meal had the lowest moisture content (8.43%). Ash content of industrial pre-toast (6.33%), toasted (6.35%), and *B. rapa* (6.34%) meals was similar but significantly higher than that in other meal samples (4.19–5.99%). Crude fibre and lipid contents of all the *Brassica* meals prepared in the lab (16.31–20.11%, 9.14–19.34%) were significantly higher in comparison to those of the industrial meals (12.05–13.22%, and 2.46–5.30% respectively). Carbohydrate content had the widest range of all components at 8.35–29.56%.

As shown in Table 2, all meal samples had significantly different total phenolic contents, ranging from 27.93 SAE/g in industrial toasted meal to 56.48 SAE/g in *B. oleraceae* meal. Phenolic content of pre-toast meal was 50.77 SAE/g, approximately twice the amount of the phenolic content in toasted meal. Although no particular trend of sinapine content was observed when comparing industrial to non-industrial meals, it was in the range of 7.51–12.88 g/kg depending on the meal types. Industrial toasted meal had a significantly reduced sinapine content (7.51 g/kg) in comparison to pre-toast meal (10.47 g/kg), similar to the trend observed for the total phenolic content. Glucosinolate content of *B. oleracea* (123.39 $\mu\text{mol/g}$) meal was significantly higher than the glucosinolate content of *B. juncea* (36.38 $\mu\text{mol/g}$) and *B. rapa* (36.44 $\mu\text{mol/g}$) meals, and were both significantly higher than the glucosinolate content of *B. napus* (9.09 $\mu\text{mol/g}$) and industrial pre-toast (10.47 $\mu\text{mol/g}$) meals; the glucosinolate content of industrial toasted meal was below the detection limit (<3 $\mu\text{mol/g}$). Overall, *B. oleraceae* meal had the highest antinutritional factor content in comparison to all the other meals.

3.2. Extraction yield and protein recovery

Protein yield of albumin fractions obtained from non-industrial *Brassica* meal samples (12.16–15.36%) was significantly higher than that of all the other Osborne fractions (Fig. 1). Furthermore, albumin yield of industrial pre-toast meal (12.15%) was significantly higher

Table 2

Antinutritional composition of canola meals.

Sample	Total phenolics content, SAE/g	Sinapine content, g/kg	Glucosinolate content, $\mu\text{mol/g}$ sample
<i>B. juncea</i>	42.77 $\pm$ 0.44 ^a	7.52 $\pm$ 0.08 ^a	36.38 $\pm$ 3.96 ^a
<i>B. oleraceae</i>	56.48 $\pm$ 0.96 ^b	12.88 $\pm$ 0.67 ^b	123.39 $\pm$ 13.44 ^b
<i>B. rapa</i>	34.59 $\pm$ 0.44 ^c	8.72 $\pm$ 0.17 ^a	36.44 $\pm$ 3.97 ^a
<i>B. napus</i>	52.80 $\pm$ 1.05 ^d	11.24 $\pm$ 0.06 ^c	9.09 $\pm$ 0.99 ^c
Ind. pre-toast	50.77 $\pm$ 1.85 ^e	10.47 $\pm$ 1.07 ^c	10.47 $\pm$ 1.14 ^c
Ind. toasted	27.93 $\pm$ 0.22 ^f	7.51 $\pm$ 0.13 ^a	<3

Data are means of triplicates $\pm$ standard deviation. Within each column, means with different superscripts are significantly different ($P \leq 0.05$).

than that of industrial toasted meal (4.96%). Conversely, protein yield of globulin, glutelin and prolamin fractions extracted from industrial pre-toast meal was significantly higher than that extracted from non-industrial samples (except *B. juncea*). Prolamins were, however, the minor fraction extracted from all samples and were $\leq 2\%$ of the total extracted proteins. Toasting of industrial meal resulted in a significantly decreased yield in albumin, globulin and glutelin fractions, but increased the yield of the prolamin fractions.

Protein yield of the DIR fraction (glutelins) obtained from industrial pre-toast sample (18.89%) was significantly higher in comparison to the non-industrial *Brassica* meal samples (14.21–16.69%), except for *B. oleraceae* which had the highest protein yield of glutelin fractions (19.20%). The desolventising process significantly reduced the yield of DIR glutelins to a minimum of 9.71%. The protein yield of glutelins obtained by the DIR method (9.61–19.20%) was consistently and significantly higher than that of OSB fractions (2.96–7.65%).

As shown in Fig. 1, proteins remaining in the total residues (meal and glutelin residues) obtained from the DIR method were higher than those produced from the OSB method. Irrespective of the extraction method used, industrial toasted meal had the highest amount of proteins in total residues (17.78%, OSB; 17.66%, DIR) left behind after protein extraction in comparison to other meal samples (3.78–5.14%, OSB; 9.16–13.28%, DIR). Furthermore, as shown in Table 3, the recovery of desired protein fractions by OSB method for all the meal samples (39.18–67.81%) was significantly higher than the recovery by DIR method (26.70–52.77%) while industrial toasted meal had the lowest recovery of desired protein fractions disregarding of extraction method.

3.3. Composition of protein fractions

3.3.1. Protein content

Protein content of albumin fractions extracted from non-industrial *Brassica* meal (39.88–45.06%) and industrial pre-toast meal (38.00%) was significantly higher than the protein content of albumins extracted from industrial toasted meal (20.00%). Significantly higher protein content was observed in the globulin fractions extracted from industrial pre-toast meal (68.00%) in comparison to protein content of the globulin fractions extracted from other meal samples (37.44–46.88%). Furthermore, protein content of glutelin fractions extracted by the OSB method from both industrial pre-toast and toasted meals (73.69%, 77.31%) was significantly higher than that extracted from

Table 1

Proximate analysis of canola meals.

Sample	Proximate components, %					
	Moisture	Protein (N $\times$ 6.25)	Crude fibre	Lipid	Ash	Carbohydrates (by difference)
<i>B. juncea</i>	9.71 $\pm$ 0.08 ^a	38.38 $\pm$ 2.17 ^{ab}	20.03 $\pm$ 0.68 ^a	19.34 $\pm$ 0.18 ^a	4.19 $\pm$ 0.02 ^a	8.35
<i>B. oleraceae</i>	11.24 $\pm$ 0.12 ^b	36.38 $\pm$ 2.06 ^{ab}	17.59 $\pm$ 0.60 ^b	12.14 $\pm$ 0.11 ^b	5.99 $\pm$ 0.01 ^b	16.66
<i>B. rapa</i>	11.27 $\pm$ 0.03 ^b	34.56 $\pm$ 1.96 ^{bc}	16.31 $\pm$ 0.55 ^b	9.14 $\pm$ 0.08 ^c	6.34 $\pm$ 0.02 ^c	22.38
<i>B. napus</i>	10.24 $\pm$ 0.02 ^c	34.50 $\pm$ 1.95 ^{bc}	20.11 $\pm$ 0.68 ^a	14.26 $\pm$ 0.13 ^d	5.34 $\pm$ 0.37 ^d	15.55
Ind. pre-toast	8.43 $\pm$ 0.06 ^d	40.00 $\pm$ 2.2 ^a	13.22 $\pm$ 0.45 ^c	2.46 $\pm$ 0.02 ^e	6.33 $\pm$ 0.00 ^c	29.56
Ind. toasted	12.09 $\pm$ 0.09 ^e	36.00 $\pm$ 2.04 ^{ab}	12.05 $\pm$ 0.41 ^c	5.30 $\pm$ 0.05 ^f	6.35 $\pm$ 0.03 ^c	28.21

Data are means of triplicates $\pm$ standard deviation. Within each column, means with different superscripts are significantly different ($P \leq 0.05$).

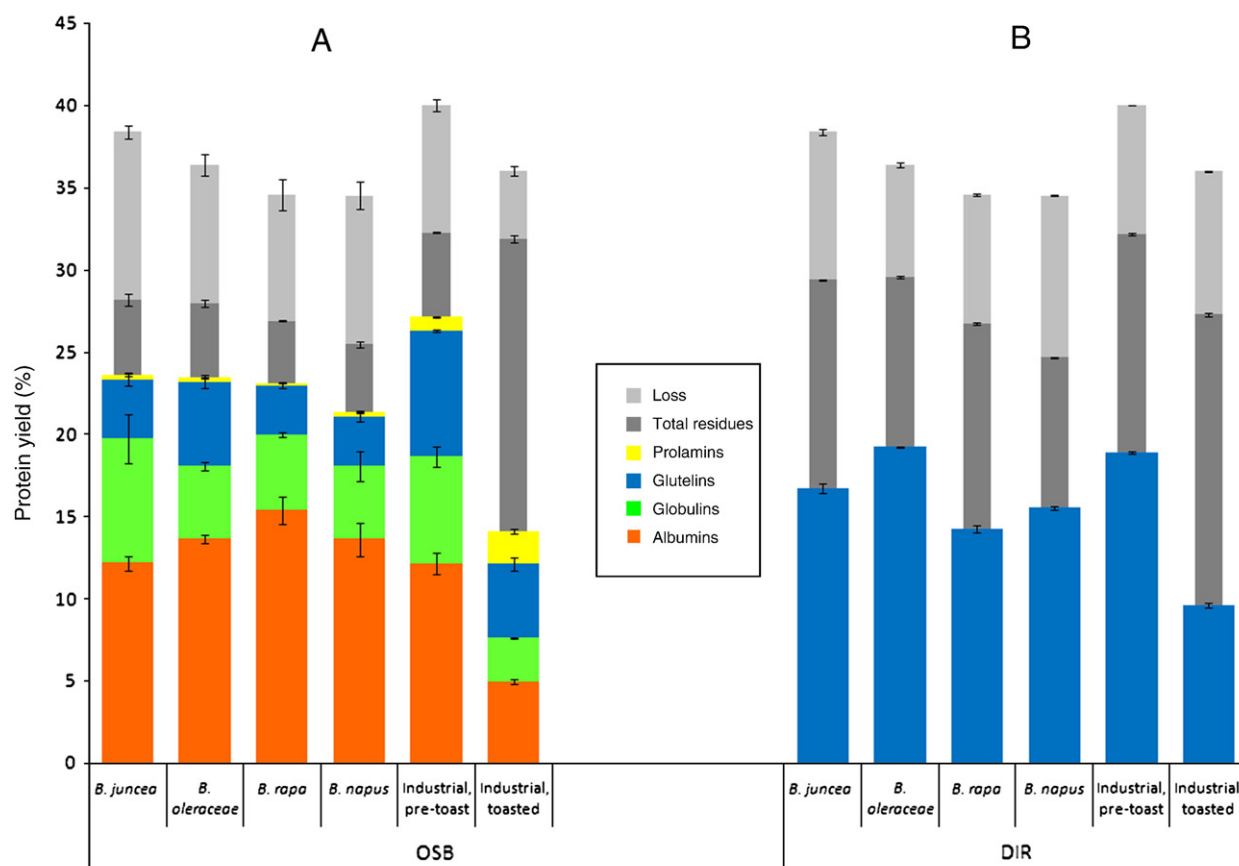


Fig. 1. Protein yield (%) of various samples prepared using (A) Osborne (OSB) and (B) direct alkaline (DIR) extraction method.

non-industrial *Brassica* meals (45.25–47.81%) and the same trend was observed for prolamin fractions. Protein content of DIR fraction (glutelin) obtained from industrial pre-toast meal (83.50%) was not significantly different to that of toasted meal (75.94%), but significantly higher than those extracted from the non-industrial *Brassica* (57.94–69.13%) meals.

3.3.2. Antinutritional content

The composition of several antinutritional constituents of the various extracted protein fractions is shown in Table 4. Compared to

the other fractions, albumin fractions obtained using the OSB method contained the highest amount of total phenolics (79.25–142.35 SAE/g), with the fraction obtained from *B. oleraceae* having the highest phenolic contents (142.35 SAE/g). All extracted globulin fractions had significantly different total phenolics content, ranging from 21.01 to 42.20 SAE/g. Similar trends were observed for the OSB glutelin (9.33–59.59 SAE/g) and prolamin (19.93–62.31 SAE/g) fractions. It is noteworthy that the *B. oleraceae* variety had the highest total phenolics content in all OSB fractions except the globulin fractions. Phenolic contents of DIR fractions extracted from industrial pre-toast and toasted meal (51.28 SAE/g, 51.40 SAE/g) were significantly higher than in the non-industrial *Brassica* extracts.

Sinapine was detected only in the albumin fractions and were significantly different depending on meal samples, with albumin fractions of *B. oleraceae* having the highest (22.99 g/kg), while albumin fractions of *B. napus* had the lowest sinapine content (9.78 g/kg). Sinapine was not detected in DIR fractions. Glucosinolate content of protein fractions extracted by both OSB and DIR methods was below the detection limit (<3 $\mu\text{mol/g}$).

3.4. Polypeptide profiles

3.4.1. Meals

Polypeptide profiles under non-reducing and reducing electrophoretic conditions respectively, for *B. napus* meal (lanes 2 and 5), industrial pre-toast meal (lanes 3 and 6), and toasted meal (lanes 4 and 7) are shown in Fig. 2. Under non-reducing conditions, polypeptides of molecular weight (MW) 16, 18, 26, 30, 45, and 53 kDa were the major polypeptides observed in all three profiles. Upon the addition of 2-mercaptoethanol (ME), the major polypeptides of meal proteins (16 and 45 kDa) were broken down, and a group of bands of MW 6, 10,

Table 3

Protein recovery (%) from canola meal samples using Osborne (OSB) and direct alkaline extraction (DIR) methods.

Extraction method	Sample	Desired fractions ¹	Total ²
OSB method	<i>B. juncea</i>	61.44 ± 1.40 ^a	73.36 ± 1.04 ^a
	<i>B. oleraceae</i>	64.38 ± 1.14 ^{ac}	76.83 ± 1.76 ^{bc}
	<i>B. rapa</i>	66.85 ± 2.85 ^{bcd}	77.79 ± 2.81 ^{be}
	<i>B. napus</i>	61.79 ± 2.68 ^{ac}	73.77 ± 2.42 ^{ac}
	Industrial, pre-toast	67.81 ± 0.93 ^d	80.65 ± 0.90 ^{de}
	Industrial, toasted	39.18 ± 1.37 ^e	88.56 ± 0.76 ^f
DIR method	<i>B. juncea</i>	43.50 ± 0.52 ^u	76.54 ± 0.42 ^u
	<i>B. oleraceae</i>	52.77 ± 0.08 ^v	81.22 ± 0.33 ^v
	<i>B. rapa</i>	41.09 ± 0.45 ^w	77.27 ± 0.19 ^w
	<i>B. napus</i>	44.94 ± 0.19 ^x	71.49 ± 0.11 ^x
	Industrial, pre-toast	47.23 ± 0.16 ^y	80.43 ± 0.02 ^y
	Industrial, toasted	26.70 ± 0.26 ^z	75.76 ± 0.10 ^z

Data are means of triplicates ± standard deviation. Within the column of each extraction method, means with different superscripts are significantly different ($P \leq 0.05$).

¹Desired fractions refer to the sum of % protein in the albumin, globulin, glutelin and prolamin fractions for the OSB method or the glutelin fractions only for the DIR method.

²Total refers to the sum of % protein in the desired fractions and all residues.

Table 4

Composition of protein fractions from canola meal prepared using Osborne (OSB) and direct (DIR) extraction method*.

Fractions	Sample	Protein content (N × 6.25), %	Total phenolics content, SAE [#] /g	Sinapine content, g/kg
OSB				
Albumins	<i>B. juncea</i>	43.31 ± 2.45 ^{ab}	125.06 ± 1.44 ^a	15.80 ± 0.42 ^a
	<i>B. oleraceae</i>	41.50 ± 2.35 ^{ab}	142.35 ± 0.39 ^b	22.99 ± 0.83 ^b
	<i>B. rapa</i>	45.06 ± 2.55 ^b	79.25 ± 0.22 ^c	12.74 ± 0.05 ^c
	<i>B. napus</i>	39.88 ± 2.26 ^{ab}	125.57 ± 0.11 ^a	9.78 ± 0.11 ^d
	Ind. pre-toast	38.00 ± 2.15 ^a	120.19 ± 1.16 ^d	19.61 ± 0.01 ^e
	Ind. toasted	20.00 ± 1.13 ^c	130.58 ± 1.63 ^c	18.21 ± 0.29 ^f
Globulins	<i>B. juncea</i>	44.44 ± 2.51 ^a	25.70 ± 0.34 ^a	–
	<i>B. oleraceae</i>	42.56 ± 2.41 ^{ac}	26.09 ± 0.96 ^a	–
	<i>B. rapa</i>	37.44 ± 2.12 ^c	22.97 ± 0.73 ^b	–
	<i>B. napus</i>	37.94 ± 2.15 ^{ac}	21.01 ± 0.27 ^c	–
	Ind. pre-toast	68.00 ± 3.85 ^b	42.20 ± 0.19 ^d	–
	Ind. toasted	46.88 ± 2.65 ^{ad}	32.88 ± 0.69 ^e	–
Glutelins	<i>B. juncea</i>	45.94 ± 2.60 ^a	9.33 ± 0.58 ^a	–
	<i>B. oleraceae</i>	47.81 ± 2.70 ^a	59.59 ± 0.16 ^b	–
	<i>B. rapa</i>	45.25 ± 2.56 ^a	13.14 ± 0.67 ^c	–
	<i>B. napus</i>	46.25 ± 2.62 ^a	17.64 ± 0.24 ^d	–
	Ind. pre-toast	73.69 ± 4.17 ^b	25.38 ± 0.45 ^e	–
	Ind. toasted	77.31 ± 4.37 ^b	39.47 ± 0.22 ^f	–
Prolamins	<i>B. juncea</i>	11.25 ± 0.64 ^a	19.93 ± 0.86 ^a	–
	<i>B. oleraceae</i>	11.13 ± 0.63 ^a	50.58 ± 0.29 ^b	–
	<i>B. rapa</i>	9.13 ± 0.52 ^a	32.43 ± 0.69 ^c	–
	<i>B. napus</i>	9.63 ± 0.54 ^a	34.27 ± 1.55 ^d	–
	Ind. pre-toast	23.75 ± 1.34 ^b	45.63 ± 0.55 ^e	–
	Ind. toasted	36.88 ± 2.09 ^c	62.31 ± 1.14 ^f	–
DIR				
Glutelins	<i>B. juncea</i>	63.44 ± 3.85 ^a	27.36 ± 0.29 ^a	–
	<i>B. oleraceae</i>	69.13 ± 3.91 ^{ac}	40.11 ± 1.15 ^c	–
	<i>B. rapa</i>	60.50 ± 3.42 ^a	26.66 ± 0.11 ^a	–
	<i>B. napus</i>	57.94 ± 3.28 ^{ad}	39.73 ± 0.96 ^c	–
	Ind. pre-toast	83.50 ± 4.72 ^{be}	51.28 ± 0.50 ^b	–
	Ind. toasted	75.94 ± 4.30 ^{ce}	51.40 ± 0.58 ^b	–

*Glucosinolate content of all the extracted protein fractions were below the detection limit (<3 μmol/g).

[#]Sinapic acid equivalent.

–Not detected.

Within the column of each fraction, means with different superscripts are significantly different ($P \leq 0.05$).

and 19–32 kDa appeared. Polypeptide of MW 18 kDa remained as one of the most prominent polypeptide bands under reducing condition.

3.4.2. Extracted protein fractions

Polypeptide profiles of albumin fraction obtained from *B. napus* and industrial samples under non-reducing (lanes 2–4) and reducing conditions (lanes 5–7) were very similar to those from their respective meals. Polypeptides of MW 16, 18, 26, 30, 45, and 53 kDa were the major bands observed under non-reducing conditions. An extra polypeptide band of MW 14 kDa was observed only in the polypeptide profiles of *B. napus* (lane 2) and industrial pre-toast (lane 3) samples.

Polypeptide profiles of globulins extracted from *B. napus* and industrial meals show major bands of MW 16, 18, 28, 30, 45, and 60 kDa and a minor polypeptide of MW 6 kDa. Under reducing conditions, the most prominent polypeptide band of MW 16 kDa was broken down to 6 and 10 kDa. The polypeptide bands of MW 45 and 60 kDa of globulin fractions obtained from *B. napus* and industrial toasted meal (lanes 2 and 4 respectively) also disappeared, but a group of protein bands of MW of 18–37 kDa appeared. Polypeptide profiles of all the globulin fractions under reducing conditions were very similar to those of the albumin fractions but the bands had less intensity compared to the polypeptide profiles of albumins.

The glutelin fractions obtained using the OSB method had only one prominent polypeptide band of MW 16 kDa (lanes 2 and 3). There was no prominent band observed in the glutelin fractions obtained from the industrial toasted meal. Polypeptide profiles of glutelins under reducing conditions were similar to its non-reducing polypeptide

profile except for the presence of extra polypeptides of high MW (100–140 kDa) under reducing conditions as shown in Fig. 2.

Polypeptide profiles of DIR (glutelin) fractions were generally similar to the polypeptide profiles of OSB glutelin fractions, except that there were more bands in the DIR fractions under both non-reducing and reducing conditions, including a high MW band of 110 kDa not previously observed in any of the meals. Under reducing conditions, polypeptide profiles of DIR fractions showed bands of MW 6 and 10 kDa which were not shown in reduced polypeptide profile of glutelins obtained from the OSB method. There was no prominent polypeptide band in polypeptide profiles of prolamin fractions under either non-reducing or reducing conditions (results not shown).

3.5. IEF

IEF profile of *B. napus* meal in native buffer shows a wide but diffuse range of isoelectric point (pI) values at pHs ranging from 6.1 to 7.2 and from pH 5.4 to 5.8 (figure not shown). IEF profiles of albumin and globulin fractions were in the range of pH 6.0–6.9 and 6.0–7.4 respectively. The glutelin (OSB and DIR), and prolamin fractions, were non-responsive at the same isoelectric focusing environment. Under reducing conditions, the IEF profile of the *B. napus* meal was similar to that of the albumin fraction. Prominent bands showing pIs of 5.4, 5.6, 5.8, 6.2, 6.8, 7.7, 7.9, and 8.3 were observed in both IEF profiles, except that major fractions with pIs close to neutral pH were present in higher concentrations in the albumins. Furthermore, IEF profiles of the albumins show a larger group of high acid pIs within the pH range 4.6–5.2 in comparison to that of the meal. IEF profile of globulins under reducing conditions was slightly different, showing a range of pIs at 5.6, 5.9, and 7.7, although not as prominent as the equivalent counterparts in the meal and albumin fractions. Similar to results obtained in native buffer, no IEF bands were observed for the glutelin and prolamin fractions under reducing conditions.

4. Discussion

4.1. Proximate analysis and meal composition

In this study, the proximate composition of the meal samples was in agreement with the data reported by Seberry et al. (2009) whereby the average protein content for the 2009 Australian canola harvest was 40.1% in oil free meal. Moisture and ash content of all meal samples were also in agreement with values reported previously (Pedroche et al., 2004; Khattab & Arntfield, 2009), indicating that there is limited variation in the composition of canola meal samples. Significantly higher lipid contents of the non-industrial *Brassica* meals in our study suggest that the defatting process carried out in the lab was less effective in comparison to the industrial oil extraction protocol which involved a more rigorous process with high temperature treatment.

The significant differences in total phenolic and sinapine contents between different meal samples were in agreement with a previous study on antinutritional components in Australian canola meal (Mailer et al., 2008). The reduced sinapine content in industrial toasted meal in comparison to the pre-toast meal is possibly a consequence of the formation of oligomeric sinapine derivatives from monomeric sinapine in high temperature conditions during the desolventising process as suggested by Larsen et al. (1983). Although the glucosinolate content of *B. napus* and industrial pre-toast meal was in agreement with the average glucosinolate content of canola in Australia's National Variety Trial 2009/2010 (Seberry et al., 2009), those of the other *Brassica* meals which exceeded the Canola Council limit, indicates that *B. oleraceae*, *B. juncea*, and *B. rapa* samples investigated in this study were not of canola grade. Glucosinolates were not detected in the industrial toasted meal possibly due to the heat treatment during the desolventising process. Jensen et al. (1995) demonstrated that high temperature destroyed glucosinolates and improved meal flavour and palatability.

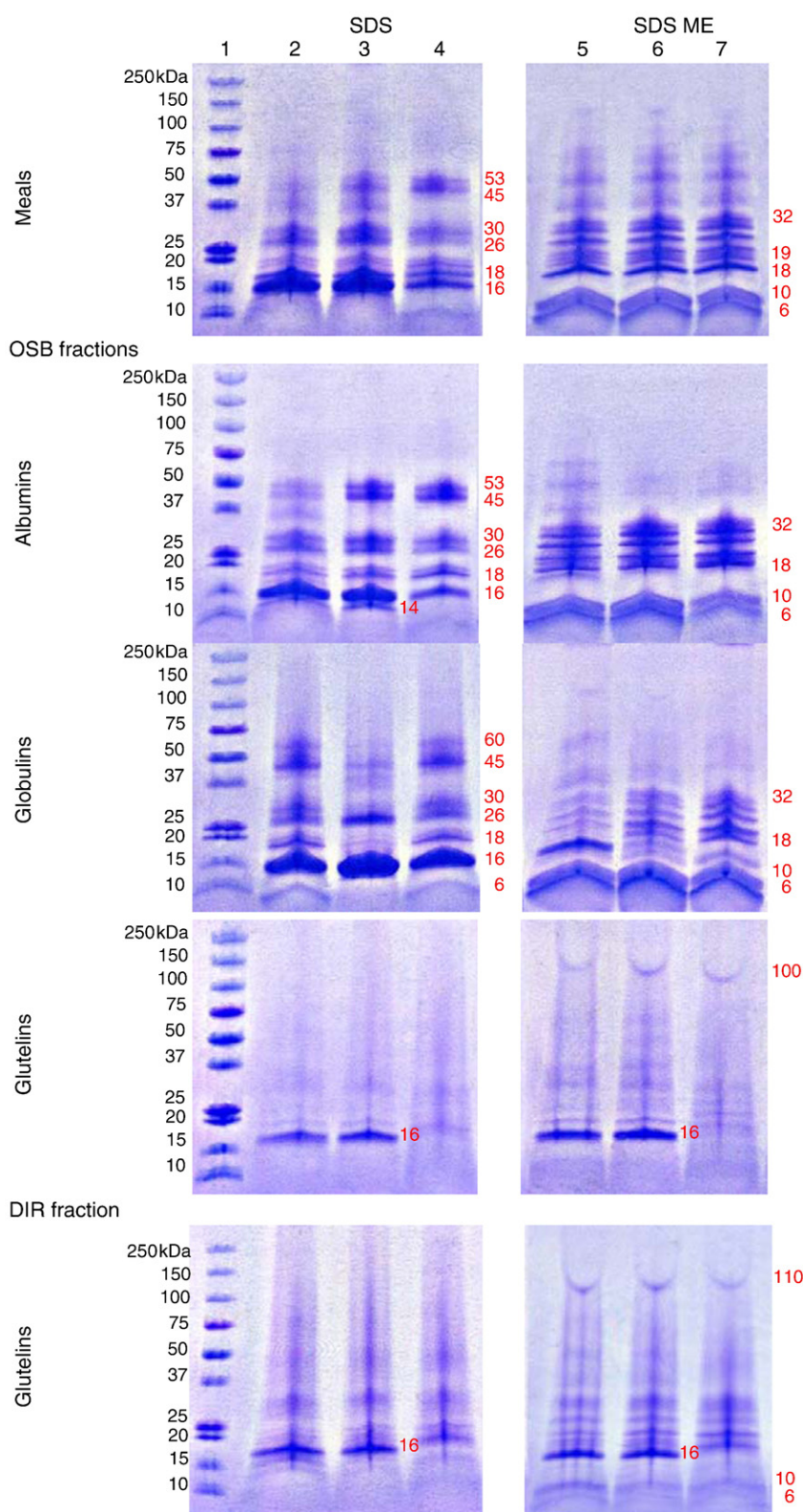


Fig. 2. Polypeptide profiles of canola meals, OSB fractions and DIR fractions, under non-reducing (SDS) and reducing (SDS-ME) conditions. Lane 1, protein molecular weight standard marker; lanes 2 and 5, *B. napus*; lanes 3 and 6, industrial pre-toast; lanes 4 and 7, industrial toasted. Lanes 2–4, SDS profiles; lanes 5–7, SDS-ME profiles. Calculated molecular weights have been inserted on major bands.

4.2. Extraction yield and protein recovery

The desolventising process, which decreased the meal protein content by only 10%, translated into a significant decrease in recovery of desired protein fractions by a margin of at least 40% for both OSB

and DIR method. Results indicated that the industrial toasted meal had a significantly higher amount of (non-extractable) proteins in the residues in comparison to the other meal samples irrespective of protein extraction method, suggesting that the desolventising process carried out at high temperature affected the overall meal

solubility in all extraction solvents, resulting in reduced protein extractability.

Protein recovery of desired OSB fractions and DIR fractions in this study was comparable to the protein recovery values (33–65%) by various extraction methods reported previously (Gillberg & Tornell, 1976; Tzeng et al., 1988; Xu & Diosady, 1994). The significantly lower protein yield of DIR fractions in comparison to OSB fractions could possibly be due to the acid precipitation step. According to Gillberg and Tornell (1976), rapeseed (canola) has a very complex protein composition, with widely different isoelectric points. In this study, however, only the fractions that were insoluble around pH 4.0 were collected. This has caused a considerably large amount of proteins which solubilised at pH 4.0 to be left behind. However, the protein content of this fraction was considerably higher. According to Rohani and Chen (1993) and Gillberg and Tornell (1976), the acid precipitation step of the DIR method, although resulting in low protein yield, is an effective technique in preparing extracts of higher protein content or purity. The overall lower protein content of OSB fractions, especially the albumins and the globulins in comparisons to DIR fractions could also be due to the solubilisation of other non-protein components such as carbohydrates and antinutritional components along with the extracted protein fractions in the different solvents used accordingly (Pedroche et al., 2004).

4.3. Antinutritional content

The major phenolic compounds in rapeseed or canola are sinapate esters with sinapine being the most prominent one (Shahidi & Naczk, 1992). The detection of sinapine only in the albumin fractions could possibly be due to the solubilisation of sinapine in water at the first step of the OSB method, which also explains the absence of sinapine in the subsequent protein fractions. Sinapine content in the albumin fractions was higher than in its respective meals except for the *B. napus* meal sample, suggesting a species-specific reason. The reason why this did not occur in the industrial pre-toast sample (*B. napus*) is, however, not clear and requires further investigation. Higher sinapine content in extracted albumins in relation to the meals may, however, be due to the removal of other water insoluble components as precipitates during the extraction of albumin fractions, thus concentrating the sinapine in the water extracts. Sinapine was not detected in the DIR fraction as a result from the alkaline extraction condition whereby sinapine was hydrolysed to sinapic acid and choline (Austin & Wolff, 1968). The significantly reduced sinapine content in albumins extracted from toasted meal in comparison to pre-toast meal could possibly be due to the significantly lower sinapine content in the heat-processed starting meal.

The absence of glucosinolates in all extracted protein fractions prepared by either OSB or DIR methods could be due to the decomposition of glucosinolates in the presence of water as demonstrated by Tripathi et al. (2000). This does not necessarily imply, however, that these fractions are safe for human consumption. For example, in the OSB method, presence of water and myrosinase enzyme from the meals would result in hydrolysis of glucosinolates and the formation of toxic degradation products such as thiocyanates and nitriles (Gillberg & Tornell, 1976) which are biologically active (Tripathi & Mishra, 2007). Therefore, the significant sinapine content and possible accumulation of toxic degradation products of glucosinolates in the albumin fractions need to be further investigated, if this fraction of canola meal protein is to be viable as a human food ingredient. This is especially important if technological functionality of the albumin fractions was proved to be significant in subsequent studies.

4.4. Polypeptide profiles

4.4.1. Meals

The polypeptide profile of *B. napus* meal proteins was in agreement with the study by Aluko and McIntosh (2001), except for the 26 and

45 kDa polypeptide bands which appeared only in the profiles of Australian varieties (lanes 2–4). In this study, no prominent polypeptide band with MW > 53 kDa was observed. In contrast, Aluko and McIntosh (2005) in their study on Canadian canola meal (unknown variety) reported that MW of the polypeptides in *Brassica* meals ranged up to 80 kDa.

In the presence of ME, the breakdown of major polypeptides of MW 16 and 45 kDa proteins (lanes 2–4) suggests that polypeptides in canola meals were stabilised by disulfide bonds. Similarly, a study by Menendez-Arias et al. (1987) showed that the 16 kDa polypeptide isolated from the seeds of the same Brassicaceae family (*Sinapis alba*) dissociated into two polypeptides of MW 5 and 9.5 kDa. ME, however, had no effect on the polypeptide of MW 18 kDa, suggesting that this particular polypeptide could have been stabilised by non-covalent bonding other than conventional disulfide linkages.

4.4.2. Extracted protein fractions

Polypeptide profiles of albumins were very similar to its respective meal counterparts except for the absence of minor high MW fractions possibly due to their decreased water solubility. Polypeptide band of MW 14 kDa, according to Wu and Muir (2008), was the major polypeptide in 2S albumin and was observed in *B. napus* and industrial pre-toast albumin polypeptide profiles. The polypeptide profiles of globulins were similar to the meal and albumin polypeptide profiles. Wu and Muir (2008) isolated 12S globulins which contain major polypeptide bands of MW 29.5, 44, and 50 kDa. All of these polypeptide bands (except the 50 kDa band) in the globulin fraction were observed under non-reducing conditions in this study. However, these three bands were not fraction-specific as they were also found in polypeptide profiles of albumins. This could indicate that the polypeptide categorised as 12S globulins can also be partly isolated with de-ionised water.

Polypeptide profiles of glutelins obtained by using OSB methods under reducing and non-reducing conditions were similar except for the emergence of 110–140 kDa polypeptide bands under reducing conditions. This was presumably from the breakdown of polypeptide of MW more than 250 kDa. This presumption is based on the observation that compared to the electrophoresis under non-reducing conditions, less residues remained at the loading zone under reducing conditions although the same volume and concentration of samples were applied. It is also apparent that NaOH, a strong solvent, was able to extract these high MW components which were not observed in the albumin and globulin fractions where they are likely to have been left behind with the residues. The streaking pattern of bands in both polypeptide profiles could possibly be due to the reduced solubility of denatured proteins resulting from the use of strong alkali to prepare the glutelin fractions.

Polypeptide profiles of DIR fractions, although similar to those of OSB glutelins, had more bands, possibly due to their higher protein content (Table 4). The DIR method apparently was able to extract more protein bodies which would have been extracted as albumins and glutelins in the OSB method. Polypeptides profiles of the DIR fraction under non-reducing and reducing conditions were different to the profiles reported by Aluko et al. (2005) for *Brassica* samples of Canadian varieties, in terms of total number of polypeptide bands and the appearance of prominent polypeptide bands, suggesting a possible variation in polypeptide composition of canola proteins extracted from canola seed varieties grown in different environments.

Polypeptide profiles of prolamins had no prominent bands under either non-reducing or reducing conditions, suggesting that the proteins in the extracted fractions were not very soluble in the reagent buffer, and were possibly denatured by the organic solvent used for their extraction. Furthermore, it is probable that the relatively low protein content of most of the prolamins fractions affected their detection using SDS-PAGE.

4.5. IEF

IEF profiles (not shown) of canola meal, albumins and globulins under reducing conditions had wide pI range of pH 4.6 to 8.3 with major polypeptide fractions having pIs close to neutral pH. This finding is in agreement with the study by Lonnerdal et al. (1977) who concluded that rapeseed protein had a very complex protein composition, with a wide range of protein isoelectric points from pH 4 to 11. This finding also supports the low protein yield of DIR extraction as it involved the acid precipitation step at pH 4, thus explaining why the OSB method extracts canola proteins more comprehensively than the DIR method.

5. Conclusion

OSB method was found to be an effective and potentially better alternative for canola protein extraction as this method provides higher cumulative protein yield with higher recovery of desired protein fractions, even though DIR method produced a protein fraction of higher protein content. Industrial desolventising process was found to negatively affect the meal solubility in water, thus resulting in decreased recovery of the more desirable (soluble) protein fractions, although the level of antinutritional components was lowered. This study confirmed the complexity of canola proteins in terms of electrophoretic profiles which may further inform the extraction process for viable food ingredients from the meal. Additionally, further research regarding the nutritional quality of the individual protein fractions is still needed, as research on food functional properties of the individual OSB and DIR fractions is currently in progress.

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