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Functional attributes of pea protein isolates prepared using different extraction methods and cultivars

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ABSTRACT

Protein isolates prepared from three pea cultivars by alkali extraction/isoelectric precipitation (AE-IP), salt extraction-dialysis (SE) and micellar precipitation (MP) were assessed for their surface (charge, hydrophobicity) and functional (water/oil holding capacity, solubility, foaming and emulsion capacities/stabilities) properties. Isolate yield was greatest for SE, followed by AE-IP and then MP. Salt extraction and AE-IP (~70%) resulted in higher protein recoveries then MP (~31%). Surface charge was similar for all isolates whereas hydrophobicity was greatest for AE-IP isolates. Overall, differences in functionality between cultivars for a particular extraction method were minimal. Salt-extracted isolates exhibited the highest protein solubility (~89%) and MP isolates the lowest (~46%). Salt-extracted isolates had the highest oil holding capacities (5.3 g/g) and the lowest water holding capacities (0.3–2.6 g/g). Foaming properties were affected by both extraction method and cultivar, however, in general SE isolates tended to have better foaming capacities whereas AE-IP isolates produced more stable foams. Emulsion capacity was greater for SE isolates than AE-IP isolates. All isolates displayed high emulsion stability (~98%). The prepared protein isolates show potential as a plant protein alternative to soy for the food industry, with the exception of possibly meat applications due to the isolates' poor water binding properties relative to soy.

1. Introduction

Field pea (*Pisum sativum* L.) is a commonly grown pulse crop in Canada and represents an important nutritional source for humans and animals as well as being an important export commodity. Its value can be improved significantly through fractionation. Pea can be separated into starch-, fiber- and protein-enriched products for use in the development of novel foods (Tiwari & Singh, 2012). Protein isolates obtained from plant sources represent a growing ingredient market in part due to consumer preferences and their relatively low cost compared to animal-derived proteins. Pea ingredients also are attractive to the food industry because of their low allergenicity, nutritional value and non-GMO status (Barac et al., 2010). While pea does contain anti-nutritional factors that can inhibit digestion and may have other possible deleterious effects pea is still considered a highly nutritious food and is associated with health benefits beyond basic nutrition (Roy, Boye, & Simpson, 2010).

Field pea contains 20–30% protein depending on the variety and environmental factors (Koyoro & Powers, 1987). The main protein classes in pea are albumins and globulins which account for 18–25% and 55–80% of the total protein, respectively, with convicilin, prolamins and glutelins present in minor amounts (Croy, Gatehouse, Tyler, & Boulter, 1980; Guleria, Dua, & Chongtham, 2009; Owusu-Ansah & McCurdy, 1991; Schroeder, 1982; Tsoukala, Papalamprou, Makri, Doxastakis, & Braudo, 2006). The globulins can be further classified based on their sedimentation coefficients into two main types, legumin (11S) and vicilin (7S) (Mertens, Dehon, Bourgeois, Verhaeghe-Cartrysse, & Blecker, 2012). Legumin is a 320–400 kDa protein and consists of six subunit pairs each having an acidic (~40 kDa) and a basic (~20 kDa) subunit linked via a disulfide bond (Barac et al., 2010; Gueguen, Chevalier, Barbot, & Schaeffer, 1988; Mosse & Pernollet, 1983). Vicilin (~150 kDa) has three subunits (~47–50 kDa) with no disulfide bonds present (Casey, 1982; Shewry, Napier, & Tatham, 1995). Vicilin also is glycosylated and has a more hydrophilic surface than legumin which makes it more water soluble. Pea globulins tend to be high in arginine, phenylalanine, leucine and isoleucine, whereas the albumin fraction is higher in tryptophan, lysine and threonine (Swanson, 1990). Pea, like other grain legumes, is deficient in the sulfur-containing amino acids methionine and cysteine but is relatively high in lysine, hence its essential amino acid profile is complementary to that of cereal grains.

Numerous methods for the extraction of protein from pulse flours have been reported. Each extraction method may select for different protein types which in turn influences the final composition and

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functionality of the isolate product. Some common methods to produce protein products (>80% protein) include alkaline extraction-isoelectric precipitation, salt extraction-dialysis and micellar precipitation. Isoelectric precipitation produces isolates composed of mostly globulins, whereas salt extraction generally results in a mixture of both globulins and albumins (Kiosseoglou & Paraskevopoulou, 2011; Liu, Hung, & Bennet, 2008). Micellar precipitation results in a protein isolate having a micelle-type form, likely stabilized by hydrogen bonds (Paredes-Lopez, Ordorica-Falomir, & Olivares-Vazquez, 1991). This isolate may be comprised of both globulins and albumins with the proteins undergoing less denaturation when prepared by this method as compared to isoelectric precipitation (Cordero-de-los-Santos, Osuna-Castro, Borodanenko, & Paredes-Lopez, 2005).

The overall aim of this study was to investigate the physicochemical and functional properties of pea protein isolates derived from cultivars of three market classes of pea using alkali extraction–isoelectric precipitation, salt extraction–dialysis and micellar precipitation. Cultivars used in this study are representative of three market classes grown in Canada and abroad, with high commercial production. Although functionality studies involving pea in the literature are not new, rarely do they compare both extraction method and cultivar-type, along with commercial isolates within the same experimental design. The information obtained from this study may be useful in identifying a pea cultivar and/or extraction method for producing a protein isolate with functionality best suited for a specific end use. The functionality of a variety of commercial protein isolates was also assessed, to put the performance of the prepared isolates in the context of isolates already in the marketplace.

2. Materials and methods

2.1. Materials

Pea cultivars representing three market classes, i.e., CDC Striker (green cotyledon, non-pigmented seed coat), CDC Meadow (yellow cotyledon, non-pigmented seed coat) and CDC Dakota (yellow cotyledon, dun seed coat), were grown in replicate plots from the same field location in Saskatchewan in 2012. Commercial products, including whey protein isolate (Davisco Foods International, Inc., Le Sueure, MN, USA, BiPRO JE Lot #061-7-440), egg protein isolate (Ballas Egg Products Corp., Zanesville, OH, USA, Dried Egg Whites Type H-40), wheat protein isolate (ADM Milling, Keokuk, IA, USA, Pro Lite 100 Lot #026706), soy protein isolate (Cargill Health & Food Technologies, Wayzata, MN, USA, Prolisse Lot #020806PM-01), and pea protein isolate (Nutri-Pea Limited, Portage le Prairie, MB, Propulse) were kindly donated for this project. Protein determination of the commercial isolate products was performed by micro-Kjeldahl analysis (%N × 6.25—egg, wheat, soy and pea; %N 6.38—whey) according to AOAC Official Method 920.87 (AOAC, 2003). Protein levels were found to be 78.0%, 80.6%, 82.5%, 79.9% and 80.0% (w.b.) for the protein isolates from egg, whey, wheat, soy and pea, respectively. All chemicals used were of reagent grade and purchased from Sigma-Aldrich (Oakville, ON, Canada) or VWR (Mississauga, ON, Canada). Milli-Q™ (Millipore Corporation, MA, USA) water was used for all protein extractions and functionality experiments.

2.2. Preparation of pea protein isolates

Whole peas were dehulled using a Satake Grain Testing Mill (Satake Engineering Co., Ltd., Japan) and cleaned of loose hull by aspiration (Ames Powercount Co., Brookings, SD, USA). Dehulled peas were then milled by a Cyclone Sample Mill (UDY Corp., Fort Collins, CO, USA) fitted with a 1 mm screen. Prior to protein extraction, all flours were defatted using hexane. In brief, pea flour was mixed with hexane (1:3, w/v) for 40 min using a magnetic stir plate at 500 rpm followed by decanting of the hexane. This process was then repeated two additional times. After the final defatting, the mixture was filtered through Whatman #1 filter paper (Whatman International Ltd., Maidstone, United Kingdom) then air dried for ~18 h in a fumehood. The defatted flour was stored at 4 °C.

2.2.1. Alkali extraction–isoelectric precipitation

The alkali extraction–isoelectric precipitated (AE-IP) pea protein isolate was produced using a modified method of Boye et al. (2010). Briefly, 50 g of defatted pea flour was dispersed in water (1:15, w/v), adjusted to pH 9.50 with 1.0 M NaOH, and stirred at 500 rpm for 1 h at room temperature (21–23 °C). The mixture was centrifuged at 4500 ×*g* (Sorvall RC-6 Plus centrifuge, Thermo Scientific, Asheville, NC, USA) for 20 min at 4 °C. The supernatant was collected and adjusted to pH 4.50 using 1.0 N HCl, then centrifuged again at 4500 ×*g* for 20 min at 4 °C. The pellet was collected and stored at −30 °C. Freeze drying was performed using a Labconco FreeZone 6 freeze drier (Labconco Corp., Kansas City, MO, USA).

2.2.2. Salt extraction-dialysis

The salt extraction–dialysis (SE) pea protein isolate was produced according to the method of Liu, Low, and Nickerson (2009). In brief, 50 g of defatted pea flour was mixed with 0.1 M sodium phosphate buffer (pH 8.00) containing 6.4% KCl at a 1:10 (w/v) ratio. The mixture was stirred at 500 rpm for 24 h at room temperature before centrifuging at 4500 ×*g* for 20 min at 4 °C. The supernatant was collected and dialyzed (6–8 kDa cutoff; Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) against Milli-Q™ water at 4 °C. The water was refreshed three times daily for 72 h (until the conductivity reached ~20 μs/cm). After dialysis, the extract was stored at −30 °C until freeze-dried.

2.2.3. Micellar precipitation

Micellar precipitation (MP) was performed according to the method of Lampart-Szczapa (1996) with slight modifications. To summarize, 50 g of defatted pea flour was suspended in a 1.0 N NaCl solution at a 1:10 (w/v) ratio and stirred (500 rpm) for 2 h at room temperature. After centrifugation at 4000 ×*g* for 20 min at 4 °C, the supernatant was collected and diluted tenfold with cold deionized water (4 °C), and then left for 18 h at 4 °C. The solution was then centrifuged again at 4000 ×*g* for 20 min at 4 °C. The pellet was collected and stored at −30 °C until freeze-dried.

2.3. Analysis of protein isolates

The protein and moisture contents of the nine isolates were determined according to AOAC Official Methods 920.87 (%N × 6.25) and 925.10, respectively (AOAC, 2003). POS Bio-Sciences Corp. (Saskatoon, SK, Canada) determined the amino acid composition of each of the nine protein isolates according to AOAC Official Methods 985.2 and 988.15 (AOAC, 2003; Landry & Delhaye, 1993; White, Hart, & Fry, 1986). In brief, 15.00 ml of 6 N HCl was added to 20 mg of pea protein isolate in individual 20 mm × 150 mm screw cap Pyrex tubes. After flushing with N_2, the tubes were capped and left in an oven for 20 h at 110 °C ± 0.5 °C. The individual amino acids of the acid digested isolates were quantified using the pico-tag amino acid analysis system (Waters Corporation, Milford, MA, USA) via high pressure liquid chromatography.

2.4. Surface characteristics

2.4.1. Surface hydrophobicity

Surface hydrophobicity was determined according to a modification of the method of Kato and Nakai (1980) using the fluorescent probe 8-anilino-1-naphthalenesulfonic acid (ANS). Protein isolates were dispersed (0.025% w/w) in 10 mM sodium phosphate buffer (pH 7.0) by stirring overnight at 4 °C, after which dilutions of 0.005%, 0.010%, 0.015% and 0.020% protein were made in 10 mM sodium phosphate

buffer (pH 7.0). For each protein concentration (0.005–0.025%, 1.6-ml samples), 20 μl of 8 mM ANS solution (in 10 mM sodium phosphate buffer, pH 7.0) was added; samples then were vortexed for 10 s and kept in the dark for 5 min. Fluorescence intensity was measured using a FluoroMax-4 spectrophotometer (Horiba Jobin Yvon Inc., Edison, NJ, USA) with excitation and emission wavelengths of 390 and 470 nm, respectively, and slit widths of 1 nm. Fluorescence intensity values for the ANS blank and protein blanks were subtracted from the fluorescence intensity of the protein solutions containing ANS. The initial slope (S_0) of the plot of the fluorescence intensity against protein concentration was calculated by linear regression analysis and used as an index of the protein surface hydrophobicity (S_0-ANS).

2.4.2. Surface charge

The surface charge of each isolate, at pH 7.0, was determined by measuring the electrophoretic mobility (U_E) of the protein using a Zetasizer Nano (Malvern Instruments, Westborough, MA, USA). Protein isolates were dispersed (0.05% w/w) in 10 mM sodium phosphate buffer (pH 7.0) by stirring overnight at 4 °C. After adjustment to pH 7.0 (with 0.05 M HCl or NaOH) the solution (~1 ml) was inserted into a folded capillary cell and the electrophoretic mobility was measured. The electrophoretic mobility is the measure of the velocity of a particle within an electric field, which can be related to the zeta potential (ζ) using the Henry equation (Eq. (1)), where η is the dispersion viscosity, ε is the permittivity, and $f(\kappa\alpha)$ is a function related to the ratio of the particle radius (α) and the Debye length (κ). Using the Smoluchowski approximation $f(\kappa\alpha)$ equaled 1.5.

$$U_E = \frac{2\varepsilon \cdot \zeta \cdot f(\kappa\alpha)}{3\eta} \quad (1)$$

2.5. Functional properties

The functional attributes of all pea protein isolates prepared using different extraction techniques and cultivars were investigated. The functionality of commercial protein isolate products from whey, wheat, egg, soy and pea were also tested under the same conditions for comparative purposes.

2.5.1. Water and oil holding capacity

Water/oil holding capacity was determined by suspending 0.5 g of protein in 5.0 g of water/oil in a 50 ml screw cap centrifuge tube. Samples were vortexed for 10 s every 5 min for a total of 30 min and then centrifuged (VWR clinical centrifuge 200, VWR International, Mississauga, ON, Canada) at 1000 ×*g* for 15 min. The supernatant was carefully decanted and the remaining pellet was weighed. Water/oil holding capacity was calculated by dividing the weight gained by the isolate by the original sample weight (×100%).

2.5.2. Protein solubility

Protein solubility (%) was determined by dispersing 0.2 g protein (based on weight protein content within the dried powder) in 19 ml of 0.1 N NaCl solution, adjusting the pH to 7.00 using either 0.5 N HCl or NaOH, and stirring (500 rpm) for 1 h at room temperature. Total solution volume then was brought to 20.0 g with 0.1 N NaCl. Mixtures were left to stand for 10 min to foster precipitation. The solution was then centrifuged (VWR clinical centrifuge 200, VWR International, Mississauga, ON, Canada) at 4180 ×*g* for 10 min at room temperature. The protein content of the supernatant was determined using a micro-Kjeldahl digestion and distillation unit. Percent solubility was calculated by dividing the protein content of the supernatant by the total protein in the initial sample (×100%).

2.5.3. Foaming capacity and stability

Foaming properties for each protein isolate were determined according to Liu, Elmer, Low, and Nickerson (2010) with slight modifications. A 1.00% (w/w) protein solution (based on weight protein content within the dried powder) was prepared with 10 mM sodium phosphate buffer (pH 7.00) and stirred overnight at 4 °C. Fifteen ml (V_{li}) of the protein solution was transferred into a narrow 400 ml glass beaker (inner diameter = 69 mm; height = 127 mm; as measured by a digital caliper) and foamed using an Omni Macro homogenizer (Omni International, Marietta, GA, USA) with a 20 mm saw tooth generating probe for a total of 5 min at speed 4 (~7200 rpm). Immediately following homogenization, the foam was transferred to a 100 ml graduated cylinder (inner diameter = 26 mm; height = 25 cm). Foam volume was recorded at time zero and after 30 min. Foaming capacity (FC) and foaming stability (FS) were determined using Eqs. (2) and (3), respectively, where V_{fi} is the volume of foam immediately after homogenization and V_{ft} is the volume of foam remaining after time (time = 30 min).

$$\%FC = \frac{V_{fi}}{V_{li}} \times 100\% \quad (2)$$

$$\%FS = \frac{V_{ft}}{V_{fi}} \times 100\% \quad (3)$$

2.5.4. Emulsion capacity

Emulsion capacity (EC) was tested by creating a series of emulsions using an Omni Macro homogenizer by adding various amounts (by weight) of canola oil to 2.00 g of 1.00% protein solution (based on weight protein content within the dried powder) in 50 ml screw cap centrifuge tubes. Each sample was homogenized for 5 min at speed 4 (~7200 rpm) with the saw tooth generating probe positioned at the oil–water interface. Immediately following homogenization, sample conductivity was measured using an Orion 3-star conductivity meter with a 4-electrode conductivity cell (Thermo Scientific, Waltham, MA, USA). The emulsion capacity was determined to be the point in which an oil-in-water emulsion changed to a water-in-oil emulsion (inversion point) as indicated by a large drop in conductivity. The EC was defined as the average grams of oil emulsified before and after the inversion point per gram of protein. At the inversion point, the oil-in-water emulsion undergoes a phase inversion to form a water-in-oil emulsion.

2.5.5. Emulsion stability

Emulsion stability (ES) was determined according to Stone and Nickerson (2012) using a 1.00% protein solution (based on weight protein content within the dried powder). A 50:50 (5.00 ml protein solution (V_B) and 5.00 ml canola oil) oil-in-water emulsion was made in a 50 ml screw cap centrifuge tube by homogenizing with an Omni Macro homogenizer at speed 4 (~7200 rpm) for 5 min with the saw tooth generating probe positioned at the oil water interface. Immediately following homogenization, the emulsion was transferred to a 10 ml graduated cylinder and allowed to separate for 30 min. Emulsion stability was determined using Eq. (4) where V_A is the volume of the separated aqueous layer after 30 min of drainage.

$$\%ES = \frac{V_B - V_A}{V_B} \times 100\% \quad (4)$$

2.6. Statistical analysis

Protein extractions were performed in triplicate and the resulting isolates were pooled for each cultivar and extraction method. All other experiments were performed in duplicate and reported as the mean ± 1 standard deviation. A two-way analysis of variance

(ANOVA) with a Scheffe post-hoc test was used to detect statistical differences in isolate yield, protein yield and protein content, surface characteristics and functional properties as a function of pea cultivar and method of extraction. A simple Pearson correlation analysis was used to determine correlations between surface characteristics and solubility, and functional properties. All statistical analyses were performed with Systat Version 10.0 software (SPSS Inc., 2000, Chicago, IL, USA).

3. Results and discussion

3.1. Characterization of the protein isolates

The isolate and protein yields from CDC Striker, CDC Dakota and CDC Meadow pea flours by AE-IP, SE and MP are reported in Table 1. An analysis of variance found that only type of extraction was a significant factor in determining isolate yield ($p < 0.001$), which was greatest for SE methods at 17.4–19.2%, followed by AE-IP and MP methods at 15.3–16.0% and 6.4–7.6%, respectively (Table 1). The SE and AE-IP methods of protein extraction yielded on average 68.2–74.8% and 62.6–76.7%, respectively, which was significantly ($p < 0.001$) higher than the MP method which had a much lower average yield of 30.7–31.1%. Cultivar and the interaction between method and cultivar were not significant ($p > 0.05$) factors in determining protein yield. Abdel-Aal, Shehata, El-Mahdy, and Youssef (1986) found higher protein recovery for micellar precipitation over AE-IP for chickpea and faba bean but the opposite trend for fenugreek. Soetrisno and Holmes (1992) reported a salt-precipitated (calcium and magnesium salts) yellow pea isolate had yields of 67.7% and 62.4%, respectively, which were lower than an AE-IP (73.8%) isolate. Alkali extraction followed by isoelectric precipitation was a more efficient protein extraction method than micellization for safflower (Paredes-Lopez & Ordorica-Falomir, 1986), bambara groundnut and soybean (Adebowale, Schwarzenbolz, & Henle, 2011) and amaranth (Cordero-de-los-Santos et al., 2005). The low protein recovery for micellization in the present study may in part be due to a lack of method optimization. For example, Paredes-Lopez and Ordorica-Falomir (1986) found percent protein extracted from safflower to increase from 44.2% to >65% when the NaCl concentration was increased from 0.2 M to 1.2 M. Arntfield, Ismond, and Murray (1985) attributed the low protein yield of MP from faba bean (42.5%) to lack of protein solubilization in the initial salt extraction step.

The protein contents of the nine isolates are presented in Table 1. A two-way analysis of variance showed that method ($p < 0.001$) and cultivar ($p < 0.05$) were significant factors for protein content; however, their interaction was not ($p > 0.05$). The protein content of the isolates ranged from 71.5% (SE-CDC Meadow) to ~87.8% (MP-CDC Striker). Overall, salt extracted isolates (71.5–79.3%) had significantly ($p < 0.001$) lower protein contents than AE-IP (83.3–86.9%) or MP isolates (81.9–87.8%) which were not significantly ($p > 0.05$) different from each other (Table 1). Differences in protein content based on cultivar have been reported previously by Barac et al. (2010) who found the protein content of the six pea varieties tested to be 22.3–31.8% and the isolates to be ~84–89% protein. Abdel-Aal et al. (1986) reported slightly higher protein contents for faba bean and chickpea isolates produced by a micellar precipitation method over isolates produced by isoelectric precipitation. Can Karaca, Low, and Nickerson (2011) reported that AE-IP (~85.6%) results in higher protein content than SE (~78.4%) for legume protein isolates (i.e. chickpea, lentil, faba bean, soybean and pea). Similar protein contents have been reported by others for laboratory-produced pea protein isolates (Barac et al., 2010; Boye et al., 2010; Can Karaca et al., 2011; Fuhrmeister & Meuser, 2003; Tian, Kyle, & Small, 1999).

The amino acid contents of the protein isolates are reported in Table 2. There were slight differences in the amino acid profiles of the different cultivars and for extraction methods. As expected, the sulfur-containing amino acids, methionine and cysteine, and tryptophan were present at low levels in all nine isolates (Mosse & Pernollet, 1983; Soetrisno & Holmes, 1992). Glutamic acid and aspartic acid were in the greatest abundance followed by arginine and leucine. Soetrisno and Holmes (1992) found only small differences between the amino acid profiles of acid-precipitated and two salt-precipitated pea protein isolates with all samples having a hydrophilic: hydrophobic ratio close to 60:40. Chickpea protein isolates produced by either IP or MP had similar amino acid compositions (Paredes-Lopez et al., 1991). The amino acid profiles in this study are similar to what has been previously reported in literature (Tomoskozi, Lasztity, Haraszi, & Baticz, 2001).

3.2. Surface characteristics

The surface charge for all protein isolates was similar regardless of the method of extraction or cultivar ($p > 0.05$) (Table 3), leading to a net negative zeta potential of approximately −21 mV at pH 7.0. Under these pH conditions, electrostatic repulsive forces between neighboring proteins in solution would be expected to be low, leading to a possible increase in protein-protein interactions and aggregation. Similar surface charge was found for SE and AE-IP pea protein isolates at pH 7.0 (Can Karaca et al., 2011). Analysis of variance for surface hydrophobicity data indicated that both cultivar ($p < 0.05$) and method of extraction ($p < 0.001$) had significant effects. Micellar precipitation and SE gave similar surface hydrophobicities at 14.4–17.0 and 14.5–18.1 a.u. (arbitrary units), respectively, whereas AE-IP resulted in isolates with higher surface hydrophobicities (~22.3–25.9 a.u.). Alkali extraction–isoelectric precipitation resulted in higher surface hydrophobicities at pH 7.0 than SE for chickpea, lentil and pea protein isolates (Can Karaca et al., 2011). The pea globulin fraction had higher surface hydrophobicity than the albumin fraction (Cserhalmi, Czukor, & Gajzago-Schuster, 1998). Can Karaca et al. (2011) hypothesized that AE-IP gave higher surface hydrophobicity because this method of extraction precipitates mainly globulin proteins, whereas SE precipitates both globulins and albumins. Mwasaru, Muhammad, Bakar, and Che Man (1999) found that for AE-IP of legume proteins, the pH of the

Table 1
Isolate yield (%), protein yield (%), protein content (%) and moisture (%) of alkali extracted-isoelectric precipitated, salt extracted and micellar precipitated pea protein isolates from different cultivars. Data represent the mean ± 1 standard deviation (n = 3).

Isolate	Cultivar	Isolate yield (%, based on wt of raw material)	Protein yield (%, based on protein)	Protein content (%, w.b.)	Moisture (%)
AE-IP	CDC Striker	16.0 ± 1.4	62.6 ± 6.0	83.3 ± 1.5	9.49 ± 0.16
	CDC Meadow	15.3 ± 0.3	76.7 ± 1.8	84.7 ± 2.9	9.06 ± 0.00
	CDC Dakota	15.7 ± 0.5	67.9 ± 3.9	86.9 ± 2.1	9.46 ± 0.17
SE	CDC Striker	19.2 ± 1.9	68.2 ± 5.6	76.1 ± 2.5	8.51 ± 0.55
	CDC Meadow	17.4 ± 2.4	74.8 ± 11.4	71.5 ± 0.5	8.12 ± 0.91
	CDC Dakota	18.5 ± 1.2	72.6 ± 1.2	79.3 ± 4.6	8.36 ± 0.08
MP	CDC Striker	7.6 ± 0.8	31.1 ± 2.1	87.8 ± 3.8	8.17 ± 0.44
	CDC Meadow	6.4 ± 0.5	30.9 ± 1.7	81.9 ± 3.5	7.67 ± 0.35
	CDC Dakota	7.2 ± 1.2	30.7 ± 5.7	85.9 ± 3.5	8.80 ± 0.06

Abbreviations: AE-IP, alkali extraction–isoelectric precipitation; SE, salt extraction; MP, micellar precipitation.

Table 2
Amino acid composition of pea protein isolates, from different cultivars, produced by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) and micellar precipitation (MP) (n = 2, standard deviation < 5%).

Amino acid (%)	CDC Striker			CDC Meadow			CDC Dakota		
	AE-IP	SE	MP	AE-IP	SE	MP	AE-IP	SE	MP
Aspartic acid (+ Asparagine)	10.2	9.5	10.1	10.8	9.4	10.6	10.4	11.0	10.7
Glutamic acid (+ Glutamine)	15.0	13.3	15.6	15.6	12.7	15.6	15.8	15.4	16.5
Serine	5.2	4.8	4.8	5.5	4.8	5.1	5.3	5.4	5.1
Glycine	3.4	3.3	3.0	3.5	3.3	3.1	3.4	3.7	3.1
Histidine	2.0	1.9	1.9	2.1	1.9	2.0	2.1	2.1	2.1
Arginine	7.4	6.4	7.9	7.5	6.2	8.1	7.5	7.2	8.9
Threonine	3.0	3.2	2.7	3.2	3.0	2.9	3.0	3.5	2.8
Alanine	3.3	3.3	3.1	2.3	3.3	3.1	3.3	3.6	3.2
Proline	3.7	3.7	3.7	3.7	3.6	3.5	3.9	3.9	3.7
Tyrosine	3.2	2.9	2.8	3.3	2.8	2.8	3.1	3.4	2.9
Valine	4.0	3.6	3.7	4.0	3.3	3.5	3.9	3.9	3.6
Methionine	0.7	0.6	0.5	0. 8	0.6	0.5	0.7	0.6	0.5
Cystine	0.7	0.8	0.6	0.8	0.7	0.6	0.8	0.8	0.6
Isoleucine	3.8	3.4	3.8	3.8	3.2	3.5	3.9	3.6	3.5
Leucine	7.2	6.1	7.4	6.9	5.6	6.7	6.8	6.4	7.0
Phenylalanine	4.6	4.1	4.6	4.4	3.70	4.1	4.4	4.3	4.2
Lysine	5.8	5.6	5.7	5.8	5.2	5.5	5.5	5.7	5.4
Tryptophan	0.7	0.6	0.5	0.7	0.6	0.5	0.7	0.7	0.6
Total AA	83.8	77.3	82.4	85.7	74.0	81.7	84.4	85.3	84.6

Abbreviations: AE-IP, alkali extraction–isoelectric precipitation; SE, salt extraction; MP, micellar precipitation.

alkaline solubilization step influenced the surface hydrophobicity of the protein isolate and was dependant on legume type. For pigeon pea, the authors reported that MP protein isolates had lower surface hydrophobicity than AE-IP prepared at pH 9.5 and 10.5, but had higher surface hydrophobicity than the same isolates extracted at pH 8.5, 11.5 or 12.5. For cowpea, the MP isolate only had lower surface hydrophobicity than the AE-IP isolate solubilized at pH 8.5. From these results, as well as the amino acid profiles, the authors (Mwasaru et al., 1999) concluded that the conditions of extraction not only affected the type of proteins being extracted but also the conformation of the proteins, mainly through electrostatic interactions, and this led to differences in the exposed hydrophobic residues. In terms of pea cultivar, CDC Dakota had significantly lower surface hydrophobicities (~17.1 a.u.) than did CDC Meadow and CDC Striker, which had similar results (~19–20 a.u.). A difference in surface hydrophobicity based on pea variety has been previously reported by Cserhalmi et al. (1998) who found that the mixed globulin fractions from five different pea varieties had surface hydrophobicities ranging from 21.81 to 43.11 a.u.

3.3. Protein functionality

3.3.1. Protein solubility

Solubility results (pH 7.0) for the nine isolates are presented in Table 4. Analysis of variance revealed that method of extraction ($p < 0.001$) and the interaction between method and cultivar ($p < 0.05$) were significant factors for solubility. All extraction methods produced isolates with significantly different solubilities with SE producing isolates with the highest solubility (85.7–91.1%) followed by AE-IP (62.7–64.4%) and MP (42.8–49.0%). Different cultivar trends were observed within each method. For example, all AE-IP isolates gave similar values (62.7–64.4%), whereas for SE, CDC Striker (91.11%) had higher solubility than CDC Meadow (85.70%) and CDC Dakota (85.8%). In contrast for MP, CDC Striker (42.78%) had lower solubility than CDC Meadow (48.9%) and CDC Dakota (46.0%). Can Karaca et al. (2011) reported that extraction method significantly affected pea protein isolate solubility with AE-IP (61.4%) resulting in higher solubility than SE (38.1%). The authors attributed this to the difference in surface characteristics of the proteins due to extraction method, as solubility is related to the folding of the protein and exposure of hydrophilic vs. hydrophobic groups, the latter resulting in protein-protein interactions and insolubility. Sumner, Nielson, and Youngs (1981) also found higher solubility for an AE-IP (87%) pea protein isolate than for an SE (64%) isolate. However, Can Karaca et al. (2011) reported higher surface hydrophobicity for AE-IP pea protein than for SE pea protein. In this study, the lower solubility of AE-IP and MP pea protein isolates may be due to protein-protein hydrophobic interactions occurring, preventing the proteins from interacting with the solvent to promote hydration and solubility. Adebowale et al. (2011) found higher solubility for MP than for AE-IP for bambara groundnut and soybean protein isolates due to the conformational changes in the protein induced by micellar precipitation. Higher solubility suggests the proteins extracted are in a more native state (Fuhrmeister & Meuser, 2003). Barac et al. (2010) reported differences in solubility at pH 7 based on pea genotype with two of the three experimental lines tested giving both the highest (L1 = ~85%) and lowest (L2 = 70%) solubility.

In terms of the commercial protein isolates, egg (98.9%) and whey (97.0%) were almost completely soluble, whereas the commercial soy, pea and wheat protein isolates had much lower solubility at 14.9%, 5.0% and 0.7%, respectively (Table 5). The very low solubility of the wheat protein isolate is not surprising as wheat proteins are more alcohol soluble. From the present study, all prepared pea protein isolates showed better solubility than the commercial pea protein isolate, decreasing in order from SE (~88%) to AE-IP (~64%) then to MP-isolates (~46%) (Table 4). Protein isolates produced at the laboratory scale typically have higher solubility than those produced at the pilot or production plant scale as they are not subjected to as harsh of drying conditions (e.g., spray drying).

3.3.2. Water holding capacity

Water holding capacity (WHC) (i.e., *the amount of water that can be absorbed by 1 g of protein)* values for all nine isolates are given in Table 4. It was determined by analysis of variance that isolation method ($p < 0.001$), cultivar ($p < 0.001$) and their interaction ($p < 0.001$) had significant effects. Overall, MP isolates (3.2–3.6 g/g) gave the highest WHC followed by AE-IP (2.4–2.6 g/g) and SE (0.34–2.6 g/g). However, the effect of cultivar was different for each extraction method. For

Table 3
Surface properties of isoelectric precipitated, salt extracted and micellar precipitated pea protein isolates from different cultivars. Data represent the mean ± 1 standard deviation (n = 2).

Extraction method	Cultivar	Surface hydrophobicity (a.u.)	Zeta potential (mV) at pH 7.0
Alkali extraction–isoelectric precipitation	CDC Striker	25.9 ± 0.1	−20.8 ± 1.4
	CDC Meadow	22.6 ± 1.8	−21.6 ± 0.8
	CDC Dakota	22.3 ± 0.6	−20.5 ± 0.1
Salt extraction	CDC Striker	18.1 ± 1.2	−20.5 ± 0.3
	CDC Meadow	17. 9 ± 0.4	−21.1 ± 1.6
	CDC Dakota	14.5 ± 0.7	−20.9 ± 0.3
Micellar precipitation	CDC Striker	16.4 ± 0.9	−21.2 ± 1.4
	CDC Meadow	17.0 ± 0.2	−20.8 ± 1.1
	CDC Dakota	14.4 ± 1.2	−21.6 ± 1.2

Table 4
Functional properties of pea protein isolates produced by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) and micellar precipitation (MP) from different cultivars. Data represent the mean ± one standard deviation (n = 2).

Isolate	Cultivar	WHC (g/g)	OHC (g/g)	Sol (%)	FC (%)	FS (%)	EC (%)	ES (%)
AE-IP	CDC Striker	2.4 ± 0.1	3.5 ± 0.2	64.1 ± 1.2	183.3 ± 0.0	68.0 ± 1.0	187.5 ± 8.8	96.7 ± 0.4
	CDC Meadow	2.5 ± 0.2	3.7 ± 0.4	62.7 ± 0.7	163.3 ± 4.7	69.6 ± 1.2	193.7 ± 0.0	97.0 ± 1.4
	CDC Dakota	2.6 ± 0.0	3.8 ± 0.2	64.4 ± 0.5	155.0 ± 2.4	68.2 ± 0.1	187.5 ± 0.0	99.9 ± 0.0
SE	CDC Striker	0.3 ± 0.0	5.4 ± 0.1	91.1 ± 2.2	258.3 ± 11.8	48.9 ± 2.0	193.7 ± 0.0	97.6 ± 1.7
	CDC Meadow	2.6 ± 0.3	5.2 ± 0.0	85.7 ± 2.5	163.3 ± 4.7	69.6 ± 1.2	193.7 ± 0.0	97.0 ± 1.4
	CDC Dakota	1.5 ± 0.2	5.2 ± 0.1	85.8 ± 0.7	263.3 ± 4.7	56.3 ± 1.7	243.7 ± 0.0	99.6 ± 0.0
MP	CDC Striker	3.5 ± 0.1	3.6 ± 0.2	42.8 ± 0.1	133.3 ± 0.0	77.8 ± 3.2	N/A	99.7 ± 0.4
	CDC Meadow	3.2 ± 0.0	3.6 ± 0.1	48.9 ± 1.0	161.6 ± 2.3	62.7 ± 0.9	N/A	99.5 ± 0.1
	CDC Dakota	3.6 ± 0.2	3.6 ± 0.1	46.0 ± 1.6	193.3 ± 4.7	52.8 ± 2.7	N/A	99.5 ± 0.1

Abbreviations: AE-IP, alkali extraction–isoelectric precipitation; SE, salt extraction; MP, micellar precipitation; WHC, water holding capacity; OHC, oil holding capacity; Sol, solubility; FC, foaming capacity; FS, foaming stability; EC, emulsion capacity; ES emulsion stability.
Note: N/A (none available): No data were obtained for the MP isolates as they did not exhibit the characteristic emulsifying behaviour needed for the EC test.

instance, all isolates produced by AE-IP had similar WHC (2.4–2.6 g/g), whereas for SE, CDC Meadow (2.60 g/g) had the highest WHC followed by CDC Dakota (1.5 g/g) and CDC Striker (0.3 g/g). The trend was different for MP with CDC Meadow (3.2 g/g) having a lower WHC than CDC Striker and CDC Dakota which had similar values (~3.5 g/g). Sumner et al. (1981) also found water absorption to be affected by extraction method with SE giving a higher WHC (2.0 g/g) than IP (1.1 g/g) for a pea protein isolate; however, the values reported were within the range of what was found in the present study. Protein extraction by micellization resulted in higher WHC than did IP for bambara groundnut and soybean isolates (Adebowale et al., 2011) and chickpea protein isolates (Paredes-Lopez et al., 1991). These authors attributed the higher WHC to greater hydrogen bonding with water by side chains and polar groups exposed on the protein by the micelle method, whereas the isoelectric technique results in proteins with a structure that limits the ability of the proteins to interact with and absorb water. The WHC in the present study are in the range of what has been reported for a laboratory-produced pea protein isolate by Sumner et al. (1981) (AE-IP 1.1 g/g), Tomoskozi et al. (2001) (AE-IP 1.2 g/g), Fuhrmeister & Meuser, 2003 (ultrafiltration 0 g/g, heat-acid precipitation ~2.0 g/g, IP 2.7 g/g) and Boye et al. (2010) (ultrafiltration ~3.8 g/g, AE-IP 4.4 g/g). WHC was negatively correlated with solubility ($r = -0.857$; $p < 0.01$).

In the case of the commercial isolates, WHC could not be detected for whey or egg proteins, since the proteins were almost completely dissolved during the test. Soy in contrast, had poor solubility (<20%) at pH 7.0, and was able to absorb a significant amount of water, having a capacity value of 12.4 g/g. In contrast, the commercial pea protein (also having poor solubility) showed significantly lower amounts of absorbed water (3.1 g/g) (Table 5). Differences in the protein's effectiveness to absorb water relate back to the protein's structure. In general, proteins with higher amounts of hydrophilic groups near the surface abide more water. Testing was unsuccessful for the wheat protein isolate, where proteins formed particulate structures that remained suspended but not dissolved. From the present study, the MP-isolates were slightly better than the commercial pea protein isolate (~3.4 vs. 3.1 g/g), however the SE- (~1.5 g/g) and AE-IP- (2.5 g/g) isolates were poorer (Table 4). All of the prepared pea protein isolates held much less water than the commercial soy product (12.4 g/g) (Table 4).

3.3.3. Oil holding capacity

The oil holding capacity (OHC) (i.e., *the amount of oil that can be absorbed by 1 g of protein*) values for all nine isolates are given in Table 4. Only method of extraction was a significant ($p < 0.05$) factor for OHC. Salt extraction resulted in significantly ($p < 0.05$) higher OHC (5.2–5.4 g/g) than either AE-IP (3.5–3.8 g/g) or MP (3.6–3.7 g/g) which exhibited similar ($p > 0.05$) results. All cultivars within each extraction method gave similar OHC. Salt extraction has previously been found to give higher OHC than IP for protein isolates from pea; however, values in the current study were higher than what the authors reported for all extraction methods (1.22 and 2.30 g/g) (Sumner et al., 1981). Adebowale et al. (2011) found IP produced isolates with higher OHC than MP for bambara groundnut, whereas Abdel-Aal et al. (1986) and Paredes-Lopez et al. (1991) found that MP resulted in higher OHC than IP for chickpea protein isolates. Oil holding capacities in this study were higher than what was previously reported for a pea protein isolate by Boye et al. (2010) (~1.2 g/g), Fuhrmeister and Meuser (2003) (0.87 g/g) and Fernandez-Quintela, Macarulla, Del Barrio, and Martinez (1997) (1.2 g/g). This may be due to different surface properties of the proteins or simply methodology differences in measuring OHC.

In comparison, for the commercial isolate products wheat proteins retained the largest amount of oil (~2.8 g/g), followed by egg and soy (~1.9 g/g), whey (~1.4 g/g) and then pea (~1.0) (Table 5). For all of our prepared pea protein isolates, OHC were much higher than the commercial isolates especially for the SE isolates (~5.3 g/g), followed by AE-IP (~3.6 g/g) and MP (~3.6 g/g) (Table 4).

3.3.4. Foaming properties

Foaming capacity (FC) is the ability of a protein, under certain conditions (i.e. concentration, pH, temperature) to form a foam, whereas FS indicates how well the protein can retain the foam volume over a measured period of time. The effect of extraction method and cultivar on FC

Table 5
Functional properties of commercial protein isolates from various sources performed at pH 7.0 and a 1.00% (w/w) protein concentration. Data represent the mean ± 1 standard deviation (n = 3).

Commercial isolate	WHC (g/g)	OHC (g/g)	Sol (%)	FC (%)	FS (%)	EC (%)	ES (%)
Egg protein	CD[1]	2.0 ± 0.0	98.9 ± 0.9	115.6 ± 16.8	72.7 ± 5.3	197.9 ± 7.2	94.7 ± 2.3
Whey protein	CD[2]	1.4 ± 0.1	97.0 ± 0.9	276.7 ± 5.8	75.5 ± 2.6	210.4 ± 14.4	100.0 ± 0.0
Wheat protein	NM[2]	2.8 ± 0.0	0.7 ± 0.0	182.2 ± 10.2	49.2 ± 8.4	106.2 ± 0.0	24.7 ± 3.1
Soy protein	12.4 ± 0.3	1.8 ± 0.1	14.9 ± 0.8	171.1 ± 16.8	67.7 ± 3.0	172.9 ± 7.2	100.0 ± 0.0
Pea protein	3.1 ± 0.1	1.0 ± 0.0	5.0 ± 0.1	81.1 ± 17.1	27.1 ± 7.4	177.1 ± 7.2	80.7 ± 3.1

[1] CD = completely dissolved.
[2] NM = not measurable, remained suspended in water as particulates (not dissolved).

is summarized in Table 4. Analysis of variance indicated that method ($p < 0.001$), cultivar ($p < 0.001$) and their interaction ($p < 0.001$) were all significant. CDC Meadow (161.6–163.4%) had similar FC for all three extraction methods, whereas SE resulted in much higher FC for CDC Dakota (263.3%) and CDC Striker (258.4%) than the other two extraction methods, indicating these isolates were better able to unfold, adsorb to the air–water interface and greatly decrease the interfacial tension. For MP, CDC Dakota (193.4%) had the highest FC followed by CDC Meadow (161.4%) and CDC Striker (133.3%). A different trend was found for AE-IP where the order was CDC Striker (183.3%) > CDC Meadow (163.4%) > CDC Dakota (155.0%). Sumner et al. (1981) reported foaming properties for a pea protein isolate to be dependent on extraction method (salt vs. IP) as well as method of drying (spray, freeze and drum drying) with spray-dried SE and IP isolates having the highest foaming properties at 433% and 412%, respectively. Paredes-Lopez et al. (1991) found extraction method to have a significant effect on FC for chickpea protein isolates with AE-IP (47.5%) resulting in higher FC than MP (43.3%), although the difference was not large. Barac et al. (2010) reported differences in FC based on pea genotype with Maja (325%) having the highest FC and experimental line L3 having the lowest (175%). Foaming capacities found in this study were higher than what was reported for two commercial pea protein isolates (104% and 96%) (Soral-Smietana, Swigon, Amarowicz, & Sijtsma, 1998). Solubility was positively correlated with FC ($r = 0.678$; $p < 0.01$) indicating that as more protein can migrate to the air–water interface, more foam will be formed. Foaming capacity of the commercial products was found to be greatest for whey protein isolate (~277%), followed by wheat (~182.2%), soy (171.1%), egg (115.6%) and pea (81.1%) (Table 5). Relative to the present study, foam capacity of the prepared pea protein isolates by AE-IP and MP showed similar foam forming properties as wheat and soy. However those prepared by SE, especially CDC-Striker (~258.3%) and CDC Dakota (263.3%) were closer to that of whey protein isolates. For all prepared pea protein isolates, FC was much better relative to the commercial pea product.

Foaming stability also was affected by extraction method ($p < 0.001$), cultivar ($p < 0.001$) and the interaction between method and cultivar ($p < 0.001$), the results of which can be found in Table 4. All AE-IP cultivars had similar FS (68.0–69.6%), whereas the three cultivars for each of the SE and MP methods varied considerably. CDC Striker produced by MP had the highest FS of the nine isolates at 77.8% whereas the same cultivar produced by SE had the lowest FS (48.95). For the MP isolates, CDC Dakota (52.8%) had the lowest FS followed by CDC Meadow (62.7%). The trend was different for the SE isolates, with CDC Meadow (69.6%) having the highest FS followed by CDC Dakota (56.3%). Paredes-Lopez et al. (1991) found higher FS for an AE-IP (66.6%) chickpea isolate over a MP (59.6%) isolate, with values being similar to what was found in this study. Foam volume stabilities of two commercial isolates (51% and 56%) were similar to the lowest values found in this study (Soral-Smietana et al., 1998). Low FS of a protein isolate indicates a weak interfacial film of the adsorbed proteins. In terms of foam stability, egg, whey and soy (~72%) were comparable; whereas wheat (49.2%) and pea (27.1%) were notably lower (Table 5). Overall the foam stabilities for the lab produced pea protein isolates ranged from ~48.9% to 77.8% depending on the extraction method and cultivar (Table 4).

3.3.5. Emulsifying properties

Results for emulsion capacity (i.e., *the amount of oil that can be emulsified by a protein relative to the total weight of emulsion, after which the emulsion undergoes an inversion from an oil-in-water emulsion to a water-in-oil emulsion*) for all nine isolates are shown in Table 4. No data was obtained for the MP isolates as they did not exhibit the characteristic emulsifying behaviour needed for the EC test. For the remaining six isolates, method, cultivar and their interaction ($p < 0.05$) were significant. CDC Meadow had the same EC for both AE-IP (193.8 g/g) and SE (193.8 g/g), whereas CDC Dakota had the highest EC of the three cultivars and increased from 187.5 g/g to 243.7 g/g for AE-IP and SE, respectively. CDC Striker had similar values (~190.6 g/g) for both methods. Abdel-Aal et al. (1986) found that fababean, chickpea and fenugreek protein isolates produced by MP had higher EC than those produced by AE-IP, which the authors attributed to the higher solubility of the MP isolates. Emulsification properties have previously been found to be similar for SE and AE-IP pea protein isolates (Can Karaca et al., 2011), whereas Sumner et al. (1981) found that SE gave better emulsification properties than AE-IP. Boye et al. (2010) reported that extraction technique, specifically ultrafiltration and AE-IP, did not affect the emulsifying properties of legume protein isolates. A simple Pearson correlation analysis showed that EC was negatively correlated with surface hydrophobicity ($r = -0.732$; $p < 0.01$), a result supported by Can Karaca et al. (2011). In terms of the commercial products tested, EC values for whey and egg proteins were found to be 210.4 g/g and 197.9 g/g, respectively, which was higher than that of soy (172.9 g/g) and pea (~177.1 g/g), and substantially higher than that of wheat (~106 g/g) (Table 5). Overall prepared pea protein isolates from the present study (with the exception of MP-isolates) were found to display similar emulsion forming properties as whey and egg, and were slightly better than the commercial soy and pea (Table 4).

The effects of extraction method and cultivar on ES were examined and results are reported in Table 4. All nine isolates had high ES (>96%) under the conditions tested, indicating the oil droplets were successfully dispersed throughout the aqueous phase due to adequate strength of the viscoelastic film at the interface, as well as sufficient charge repulsion and/or steric hindrance between droplets to prevent coalescence of the droplets. Extraction method ($p < 0.05$) and cultivar ($p < 0.05$) both were significant factors for ES, however all isolates had stabilities above 96%. Alkali extracted-isoelectric precipitated isolates ranged from 96.7% to 100.0% whereas MP isolates all had very similar ES (99.5–99.7%). CDC Dakota (~99.7%) had significantly ($p < 0.05$) higher ES than the other two cultivars (~98.0%), which were not significantly different ($p > 0.05$) from each other. Koyoro and Powers (1987) found the ES of mixed globulins, extracted by IP after solubilization in salt, to be much lower than that of the isolates in this study at 57.6%. Micellar precipitation (94%) has been reported previously to result in higher ES than AE-IP (85%) for chickpea (Paredes-Lopez et al., 1991). Adebowale et al. (2011) also found MP to give higher emulsion stability indices than AE-IP for bambara groundnut and soy protein isolate. Fuhrmeister and Meuser (2003) reported differences in ES for pea protein isolates produced by several different extraction methods, with a commercial, acid-extracted isolate having lower ES than an isolate produced by ultrafiltration. Commercial products tested as part of this study showed that ES was greatest for whey and soy at 100.0%, followed by egg protein isolate (94.7%), pea (80.7%) and then wheat (24.7%) (Table 5). Similar to EC values, wheat proteins because of their poor solubility within an aqueous medium failed to integrate sufficiently with the oil–water interface. Our prepared pea protein isolates had similar ES to that of whey, soy and egg.

4. Conclusion

Overall, this study examined the effect of cultivar and the type of extraction method used to produce a protein isolate, on pea protein functionality. Although both factors were found to influence protein functionality, the effect of cultivar-type was minimal relative to the method of extraction. In general, SE isolates had better OHC, solubility and FC than the other isolates but lower WHC. All nine isolates successfully stabilized the 50:50 water/oil emulsions, whereas only the SE and IP isolates had emulsion capacity properties. Based on these results, SE produced isolates with the best results overall, regardless of cultivar, in terms of both extraction yield and functionality.

Further to our main aforementioned conclusion, comparison of the prepared pea protein isolates to other commercial protein ingredients is important to put the functionality in the context of current products within the marketplace. The pea protein isolates prepared using SE

showed high solubility, and were within 10% of that of whey protein isolate, but performed much better than the other commercial plant protein ingredients tested (wheat, soy and pea). Overall the prepared isolates displayed much higher OHC than the commercial products, but had poorer WHC. The prepared isolates were also found to have similar foaming properties to soy, and were much better than the commercial pea ingredient. In the case of emulsion capacity, the prepared pea protein isolates (with the exception of MP-isolates) displayed similar properties to whey and egg, and were slightly better than soy. And finally, the prepared isolates formed emulsions with similar stability as whey, egg and soy emulsions.

Applications in the food industry of the prepared pea protein isolates could include beverages, sauces, salad dressings, baked products and so on. Based on its reduced WHC, their use in meat applications as binders may be limited. Major differences between the pea protein prepared in the lab and a commercially processed pea protein indicates the need for further research in less harsh production techniques. The pea protein isolates produced in this study had favorable functionality for their use in different types of food products; however isolate flavor and color were not studied and may create significant challenges for product developers.

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