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In vitro digestibility, protein composition and techno-functional properties of Saskatchewan grown yellow field peas (*Pisum sativum* L.) as affected by processing

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ABSTRACT

Saskatchewan grown yellow field pea was subjected to different processing conditions including dehulling, micronization, roasting, conventional/microwave cooking, germination, and combined germination and conventional cooking/roasting. Their nutritional and antinutritional compositions, functional properties, microstructure, thermal properties, *in vitro* protein and starch digestibility, and protein composition were studied. Processed field peas including conventional cooked yellow peas (CCYP), microwave cooked yellow peas (MCYP), germinated-conventional cooked yellow peas (GCCYP), and germinated-roasted yellow peas (GRYP) exhibited the significantly higher *in vitro* protein digestibility (IVPD), which was in accordance with their significantly lower trypsin inhibitor activity and tannin content. The SDS-PAGE and size exclusion HPLC profiles of untreated pea proteins and their hydrolysates also confirmed the IVPD result that these four treatments facilitated the hydrolysis of pea proteins to a greater extent. The CCYP, MCYP, GCCYP, and GRYP also exhibited significantly higher starch digestibility which was supported by their lower onset (T_o), peak (T_p), and conclusion (T_c) temperatures obtained from DSC thermogram, their lower pasting properties and starch damage results, as well as their distinguished amorphous flakes' configuration observed on the scanning electron microscopic image. LC/ESI-MS/MS analysis following in-gel digests of SDS-PAGE separated proteins allowed detailed compositional characterization of pea proteins. The present study would provide fundamental information to help to better understand the functionality of field peas as ingredients, and particularly in regards to agri-food industry to improve the process efficiency of field peas with enhanced nutritional and techno-functional qualities.

1. Introduction

Among pulses, field pea constitutes a significant sector of agricultural grain production, as approximately 25 million hectares are grown annually worldwide, where Saskatchewan produced 70% of Canada's approximately 3 million tonnes of field peas in 2012 (Malcolmson et al., 2014). The current market classes of field peas include yellow, green, marrowfat, and dun (Malcolmson et al., 2014). (*Pisum sativum* L.) Field Peas are gradually gaining recognition for their potential as ingredients that can boost the nutritional profile of foods and their beneficial effects that are responsible for preventing several non-communicable disease, including type II diabetes and cardiovascular disease and prevention of the onset of various cancers (Campos-Vega, Loarca-Piña, & Oomah, 2008; Singh & Basu, 2012), and they are recognized globally as a valuable source of protein, dietary fibre, vitamins and minerals (Vankosky, Cárcamo, McKenzie, & Dosdall, 2011). However, field peas are under-exploited mainly due to their low amount of sulfur-containing amino acid, low protein digestibility compared with animal proteins, and the presence of several antinutrititional components such as trypsin inhibitor, lectins, and phytic acids. Food processing including dehulling, soaking, germination, conventional cooking/boiling, microwave cooking and roasting not only improves the flavor and palatability of field peas and peas containing- food products, but also increases the bioavailability of nutrients to various extents by inactivating

Abbreviation: RYPF, raw yellow pea flour; SLYP, split yellow pea; ROYP, roasted yellow pea; MNYP, micronized yellow pea; CCYP, conventional cooked yellow pea; MCYP, microwave cooked yellow pea; GYP, germinated yellow pea; GCCYP, germinated conventional cooked yellow pea; GRYP, germinated roasted yellow pea; ROYPF, roasted yellow pea flour; DSC, differential scanning calorimetry; SEM, scanning electron microscopy; IVPD, *in vitro* protein digestibility; TIA, trypsin inhibitor activity; SEC-HPLC, size exclusion-high performance liquid chromatography; TS, total starch; RS, resistant starch; DS, digestible starch; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel; LC/ESI-MS/MS, liquid chromatography followed by electrospray ionization and tandem mass spectrometry.

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antinutritional components (Khattab & Arntfield, 2009b; Khattab, Arntfield, & Nyachoti, 2009a; Mubarak, 2005; Trugo, Donangelo, Trugo, Knudsen, & Bach, 2000). According to Trugo et al. (2000), germination produced limited increase in protein utilization, whereas the application of heating could be used as a complementary treatment to improve protein utilization and a further decrease in antinutritional compounds. An improvement of protein digestibility of pulses including peas after different processing treatments have been reported previously following partial or complete removal of polyphenols, tannins, phatic acid and trypsin inhibitor (Zia-ur-Rehman & Salariya, 2005; Khattab et al., 2009a; Mubarak, 2005; Siddhuraju & Becker, 2009; Trugo et al., 2000). Meanwhile, the seeds also undergo important physicochemical changes involving gelatinization and swelling of starch, denaturation of protein, solubilisation of some of the polysaccharides, and softening of structure during processing, which could result in a palatable texture with altered functional properties (Aguilera, Esteban, & Benitez, 2009; Ma et al., 2011; Ning, Daun, & Malcolmson, 2003). Applications of peas are in soup mixes, purees and processed products.

The pea starches are reported to have lower digestibility compared with native cereals but are more digestible than native potato and high amylose starches (Liljeberg, 2002). Among the factors that affect the rate and extent of starch digestion, food processing has a major importance. Various processing techniques were able to improve the *in vitro* digestibility of pea starch to varying extents according to previous studies (Zia-ur-Rehman & Salariya, 2005; Bravo, Perumal Siddhuraju, & Sauracalixto, 1998; Eyaru, Shrestha, & Arcot, 2009; Ma, Boye, Azarnia, & Simpson, 2015). The classification of pea proteins is based upon their solubility according to Osborne fractionation method, *i.e.*, albumins are soluble in water, globulins are soluble in dilute salt solutions, and prolamins are soluble in alcohol/water solution. The major storage proteins of peas are globulins which are further classified as legumin (11S), vicilin and convicilin (7S) according to their sedimentation coefficients (Rubio et al., 2014). These proteins are characterized by their high lysine content which is normally deficient in many other plant origins such as cereals (Martínez-Villaluenga, Gulewicz, Frias, Gulewicz, & Vidal-Valverde, 2008). Moreover, both albumin and globulins have been claimed to induce several health beneficial effects (including anticarcinogenic, antihypertensive, hypoglycemic, and hypocholesterolemic properties) upon dietary consumption (Duranti, 2006). Vicilin and convicilin have also been reported as potential major allergens from pea seeds, proteolytic fragments from vicilin are relevant IgE binding pea components (Sanchez-Monge et al., 2004). An electrophoretic method has been used widely for the extraction and separation of peptides following in-gel digests of SDS-PAGE separated proteins. The analysis can be done by capillary LC-ESI-tandem mass spectrometry which is then searched directly against a protein database for identification of the protein from which the peptide originated (Timperman & Aebersold, 2000). Though studies have been done on the investigation of electrophoretic profile of field pea proteins from different locations and genotypes (Alonso, Orúe, Zabalza, Grant, & Marzo, 2000b; Martínez-Villaluenga et al., 2008; Miroljub et al., 2010), the identification and characterization of their major fractions with the application of LC/ESI-MS/MS is still lacking. Therefore this study was aiming to systematically evaluate the impact of different processing treatments (*i.e.*, dry heating, hydrothermal cooking, and combined germination and cooking) on the nutritional composition, antinutritional factors, functional properties, thermal properties, scanning electron microstructure, *in vitro* protein and starch digestibility, as well as the SDS-PAGE and SEC-HPLC profiles of Saskatchewan grown field pea. The protein fractions of field pea were also assessed and characterized by LC/ESI-MS/MS analysis following in-gel digests of SDS-PAGE separated proteins. The results in this study are expected to provide fundamental information to help to better understand the functionalities of field peas as ingredients, and particularly in regards to industrial processes, as well as to modify and obtain value-added field peas through processing with improved physicochemical, functional and nutritional characteristics.

2. Material and method

2.1. Materials

The yellow field peas (*Pisum sativum* L., CDC Centennial Cultivars) were supplied by Canadian International Grains Institute (Winnipeg, MB, Canada) which were grown in randomized complete block design trials in two locations (Meath Park and Wilkie, Saskatoon, SK, Canada) with two replications per location over 2 crop years (2010 and 2011). Trypsin (EC 3.4.21.4), α-chymotrypsin (EC 3.4.21.1), peptidase (EC 3.4.17.1), pancreatic α-amylase (EC 3.2.1.1) amyloglucosidase (EC 3.2.1.3), pepsin (EC 3.4.23.1) were purchased from Sigma-Aldrich (Oakville, ON). Low-molecular mass calibration kits were from Amersham Pharmacia Biotech (Uppsala, Sweden). Precast (10–20%) gradient polyacrylamide Tris-HCl gels and Coomassie brilliant blue R-250 were from Bio-Rad Laboratories (Hercules, CA). Other chemicals and solvents were all of analytical grade and purchased from Sigma-Aldrich (Oakville, ON).

2.2. Processing treatments

2.2.1. Dehulling

Whole yellow field pea seeds were coarsely ground at the first stage of milling with a Jacobson 120-B lab scale hammer mill (Minneapolis, MN), using a screen size of 3.2 mm. The material was then transferred into the Buhler ML-202 lab roller mill. When the peas passed through the first break roll to the third break roll, the hulls were removed from the peas and were extracted out of the mill into a collection bin. The remaining material then passed through the remaining three reduction rolls to produce the SLYP (split yellow pea flour).

2.2.2. Grinding

The same method of producing the split yellow flour was used to produce the whole raw yellow field pea flour, with the exception of incorporating the hull fraction back into the split flour. The hulls collected from the roller milling process were pin milled using a Hosokawa Alpine 100 UPZ lab scale pin mill (Summit, NJ) at a speed of 22,000 ± 20 rpm. Both the split roller milled flour (not containing the hull) and the pin milled hull fraction were then blended for 5 min using a P-K Cross-Flow blender (Harsco Industrial Patterson-Kelley, East Stroudsburg, PA) to produce RYPF (raw yellow pea flour).

2.2.3. Roasting

Whole yellow peas were heated in a Picard Electrical Revolving Tray Oven (Quebec, Canada). 2.5 kg of seed were spread out evenly onto 10 trays with the thickness of 20 ± 2 mm and then inserted into the oven and heated for 10 min at 180 ± 2 °C. The seeds were removed from the oven and stirred, then placed back into the oven for another 10 min. The roasted peas were then cooled for 45 min at room temperature and were then stored in pails. The obtained samples are referred to as ROYP (roasted yellow pea).

2.2.4. Micronization

Whole pea seeds were treated with a laboratory scale infrared heating system (Micronizing Company, UK). The seeds were fed onto a vibratory conveyor, and were frequently turned due to the vibration as they passed through the infrared burner. The temperature used to complete the trial was at 110–115 °C. The 25 kg of peas were tempered to 16.5% moisture. The target moisture for the peas was approximately 16%. The obtained samples are referred to as MNYP (micronized yellow pea).

2.2.5. Conventional cooking

The yellow pea seeds were firstly rinsed and were added to boiling water at a seed to water ratio of 1.5:1 (*w*/*v*). The seeds were kept heating slowly for 30 min until majority of the seeds were soft when felt between fingers. The conventional cooked yellow pea seeds were

then drained and freeze dried before grinding. The obtained samples are referred to as CCYP (conventional cooked yellow pea).

2.2.6. Microwave cooking

500 g of pea seeds were put in a glass pot with tap water (1:2 *w*/*v*), and were then cooked in a microwave oven (Kenmore, 1200 W, SEARS, Canada) (on high) for 25 min. Seeds were then drained and freeze dried before grinding. The obtained samples are referred to as MCYP (microwave cooked yellow pea).

2.2.7. Germination-conventional cooking

The field pea seeds were rinsed and soaked in distilled water (1/3 *w*/*v*) for 12 h. The water was then drained off, and the seeds were transferred to a sterile petri dish lined with wet filter paper and were germinated for 72 h at 30 °C at dark. The seeds were sprinkled with distilled water every 12 h in order to maintain an adequate hydration level. The germinated yellow pea seeds were then subjected to conventional cooking following the same procedure for preparing CCYP as described above and then freeze dried before grinding. The prepared samples are referred to as GCCYP (germinated conventional cooked yellow pea).

2.2.8. Germination-roasting

The germinated yellow pea seeds were prepared following the same procedure as described above and then were roasted in an electric double oven (model OD302, Fisher & Paykel Appliances Ltd., Huntington Beach, CA, USA) for 20 min at 100 °C. The obtained samples are referred to as GRYP (germinated roasted yellow pea).

2.2.9. Roasting flours

The raw yellow pea flours (RYPF) as obtained earlier were spread in an aluminum dish and were roasted for 1 min in an electric double oven (model OD302, Fisher & Paykel Appliances Ltd., Huntington Beach, CA, USA) at 200 ± 2 °C. The roasted flours were then stored at 4 °C in polyethylene bags until analyzed. The obtained samples are referred to as ROYPF (roasted yellow pea flour).

2.3. Handling of processed seeds

The same milling method of producing raw yellow pea flour (RYPF) as described above was used to produce the whole roasted/micronized yellow pea flour (ROYP, MNYP). The rest of the processed yellow pea seeds including CCYP, MCYP, GCCYP, and GRYP were ground in a domestic coffee grinder (Black & Decker SmartGrind, Model CBG 100S) for 1 min and then sieved through a 300 μm screen and kept at 4 °C in polyethylene bags until analyzed. All processing treatments as described above in previous section were completed in triplicates for each method.

2.4. Proximate analysis

Protein content was determined with a LECO apparatus (LECO FP-428, LECO Corp., St. Joseph, MI, USA) using the AOAC (1995) Dumas combustion method and a nitrogen conversion factor of 6.25. Moisture was determined according to AACC (1983) official method by drying the samples overnight at 100 °C in a Fisher Isotemp Vacuum Oven (Fisher Scientific Co., Montreal, QC, Canada). Ash content was determined according to AACC (2003) official method. Total fibre content was determined according to AOAC 985.29 (AACC, 1990).

2.5. Amino acid composition

The amino acid content was determined according to the procedures described by the Commission Directive 98/64/EC, and post column analysis of amino acid was based on Pickering cat. No. 0101-0004, version 2.0, July 2003 (Commission Directive, 2003). Sulfur-containing amino acids including cysteine and methionine were determined after performic acid oxidation. The results were reported as grams/100 g of the original sample.

2.6. Antinutritional compounds

The tannin content was determined by the method of Vanillin assay as described by Deshpande and Cheryan (1985). The trypsin inhibitor activity (TIA) was determined colorimetrically using a spectrophotometer at 410 nm according to the procedure as described by Ma et al. (2011). The dilution factor was selected on 1 mL aliquots of each solution producing trypsin inhibition between 40% and 60%.

2.7. In vitro protein digestibility

The *in vitro* protein digestibility (IVPD) was measured according to the pH drop method proposed by Hsu, Vavak, Satterlee, and Miller (1977). This measurement was done on samples including RYPF, SLYP, ROYP, MNYP, CCYP, MCYP, GCCYP, GRYP and ROYPF. Briefly, a total of 62.5 mg protein/mL was suspended in 10 mL of water at pH 8, while stirring in a 37 °C water bath. The multienzyme solution containing 1.6 mg trypsin (14,190 BAEE units/mg protein), 3.1 mg chymotrypsin (60 units/mg powder), and 0.52 mg peptidase (40 units/g powder) per milliliter was added to the sample suspension at a ratio of 1: 10 (*v*/v) which was being stirred at 37 °C. The pH of the sample suspension was recorded after 10 min, and the *in vitro* protein digestibility was calculated according to the following equation: protein digestibility (%) = 210.464–18.103 × pH. The reactions were terminated by heating the samples in boiling water for 10 min followed by centrifugation at 12, 000*g*, 4 °C for 20 min to remove the precipitate. The supernatants were collected and freeze dried for further SDS-PAGE and SEC-HPLC studies. The pH-drop method is based on the principle that hydrolysis results in the release of carboxyl ($-COO^-$) and amino ($-NH3^+$) groups. At neutral and alkali pH, the free amino groups deionize and protons ($H+$) are liberated. The free $H+$ released into the surrounding reaction medium cause a decrease in pH and the drop in pH is recorded at 10 min (Carbonaro, Maselli, & Nucara, 2012).

2.8. In vitro starch digestibility and total starch test

The raw and processed field pea seeds were analyzed for *in vitro* starch digestibility following the method described by Englyst, Kingman, and Cummings (1992). The analysis was done under controlled enzymatic hydrolysis followed by colorimetric measurement of the glucose release using glucose oxidase-peroxidase (GOPOD) kit (K-GLOX, Megazyme Bray, Co. Wicklow, Ireland). Resistant starch was determined using a kit assay (K-RSTAR, Megazyme Bray, Co. Wicklow, Ireland) with slight modification. Samples were initially incubated with 10 mL HCl–KCl buffer (pH 1.5) and 200 μL of pepsin solution (100 mg/mL HCl-KCl buffer) at 40 °C for 1 h with constant shaking (200 strokes/min) to remove proteins. Subsequently, samples were incubated with 4.0 mL of pancreatic α-amylase solution containing dilute amyloglucosidase (300 U/mL) for 16 h at 37 °C with constant shaking (200 strokes/min). The digestible starch (DS) became solubilised and hydrolyzed to glucose by the combined action of the two enzymes. Samples were then washed with 99% (*v*/v) and 50% (*v*/v) ethanol by vigorous stirring on a vortex mixer and centrifugation at 2060*g* for 10 min. The separated pellet from supernatant was further digested with 2 M KOH with vigorous stirring for 20 min in an ice-water bath over a magnetic stirrer. Digested pellet and supernatant were separately incubated at 50 °C for 30 min (with 100 μL of AMG; 3300 U/mL) and 20 min (with 10 μL of AMG; 300 U/mL) respectively. The glucose released was measured using GOPOD kit. Absorbance was measured at 510 nm wavelength against the reagent blank. The glucose content of the supernatant and digested pellet were used in calculation of digestible starch (DS) and resistant starch (RS) respectively by applying the factor of 0.9. Total starch (TS) was then derived as the sum of DS and RS.

2.9. Differential scanning calorimetry

The gelatinization characteristics were determined by differential scanning calorimeter (Q200 Series™ DSC; TA Instruments, New Castle, DE, USA) which was calibrated with indium as standard. Each sample was prepared in 0.01 M phosphate buffer (pH 7.0) with a concentration of 30% (*w*/*v*). Samples (approximately 20 mg) were equilibrated at 4 °C for 12 h and weighed in hermetic alodined pans and tightly sealed with a hermetic alodined lid. The pans were heated under helium from 20 °C to 120 °C at a rate of 5 °C/min. A sealed pan with 20 μL phosphate buffer was used as a reference. The onset (T_o), peak (T_p), and conclusion (T_c) temperatures and enthalpy of the gelatinization endotherm (ΔH) were determined from the thermogram.

2.10. Pasting properties, starch damage and bulk density

The pasting properties were measured using a Rapid Visco Analyzer (RVA; Perten Scientific, Springfield, IL). The pasting variables were determined using the manufacturer supplied software. Experimental condition in RVA was used where samples were equilibrated at 50 °C for 1 min, heated at 3 °C/min to 95 °C, held at 95 °C for 2 min, cooled at 5 °C/min to 50 °C, and held at 50 °C for 1 min. Peak viscosity, final viscosity, breakdown and setback were determined from the viscogram. The starch damage was determined by SDMatic method as described by Medcalf and Gilles (1965). Bulk density was determined according to the following procedure: the tube was filled with samples to 5 mL by constant tapping until no further change in volume. The tube was weighed and bulk density was then calculated from the difference in weight, and expressed as grams of flour per mL.

2.11. Scanning electron microscopy

Scanning electron microscopic observation was conducted according to the method described by Ma et al. (2011). A thin layer of each sample flours was deposited on a double-sided adhesive carbon tape mounted on an aluminum specimen holder, and any unattached particles were removed. The specimen holder was sputter-coated with approximately 10 nm gold using a sputter coater (model 108, Kurt J. Lesker Co., Clairton, PA, USA) and then transferred to a scanning electron microscope (model S-3000N, Hitachi, Tokyo, Japan). Samples were examined at a voltage of 5 kV.

2.12. Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoretic (SDS-PAGE) analysis of raw and all processed peas, as well as the hydrolysates obtained during the IVPD measurement were performed on precast 10–20% gradient polyacrylamide Tris–HCl gel using the Bio-Rad Criterion Cell (Bio-Rad Laboratories, Inc., Mississauga, ON, Canada). Prior to SDS-PAGE analysis, desired amount of samples was loaded by considering the same amount of proteins in dry samples. Equal volumes of Laemmli buffer and sample solutions (undigested and digested) were each mixed and heated at boiling water bath for 5 min, cooled and centrifuged at 10, 000*g* for 10 min. Subsequently, 20 μL of supernatant was loaded in each well along with Amersham low molecular mass marker. For studies under reducing conditions, 5% (*v*/v) β-mercaptoethanol (2-ME) was added to the solutions, and the samples were heated at 100 °C for 5 min prior to the electrophoresis run. Electrophoresis was carried out at 200 V, and the running buffer was 0.1MTris–HCl, and 0.1% (*w*/*v*) SDS. The gel was stained with Bio-Rad Coomassie Blue R-250, followed by destaining in 10% (*v*/v) acetic acid, and 40% (*v*/v) methanol. The low-molecular mass (MM) calibration kit (MM 14.1 to 97 kDa) from Amersham Pharmacia Biotech (GE Healthcare Biosciences, Uppsala, Sweden) was used as molecular markers.

2.13. Protein in-gel tryptic digestion

Twenty four bands of interests from the SDS-PAGE electrophoretic studies were selected for further analysis by liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS). The target bands were cut with a spot/band picker (Gelcompany, San Francisco, CA) and placed in 96-well plates and then washed with water. Tryptic digestion was performed on a MassPrep liquid handling robot (Waters, Milford, USA) according to Shevchenko, Wilm, Vorm, and Mann (1996) with slight modifications suggested by Havliš, Thomas, Šebela, and Shevchenko (2003). Briefly, proteins were reduced with 10 mM DTT and alkylated with 55 mM iodoacetamide. Trypsin digestion was performed using 126 nM of modified porcine trypsin (Sequencing grade, Promega, Madison, WI) at 58 °C for 1 h. Digestion products were extracted using 1% formic acid, 2% acetonitrile followed by 1% formic acid, 50% acetonitrile. The recovered extracts were pooled, vacuum centrifuge dried and then resuspended into 7 μL of 0.1% formic acid. 2 μL of each sample was collected for further LC/ESI-MS/MS analysis.

2.14. Liquid chromatography/electrospray ionization tandem mass spectrometry analysis

The peptide samples obtained from the previous step were separated by reversed-phase nanoscale capillary liquid chromatography and were analyzed by electrospray mass spectrometry. The experiments were performed with a Agilent 1200 nano pump connected to a 5600 mass spectrometer (AB Sciex, Framingham, MA,USA) equipped with a nanoelectrospray ion source. Each tryptic digest sample was injected onto a self-packed PicoFrit column (New Objective, Woburn, MA) packed with Jupiter (Phenomenex) 5 μ, 300 A, C18 (with 10 cm × 0.075 mm internal diameter) as was eluted with one of two buffer systems, 0.1% formic acid in acetonitrile (solution B) or 0.1% formic acid in water (solution A). The elution of peptides started from 2% solution B and increased to 50% in 30 min in a linear gradient manner, at 200 μL/min (obtained by flow-splitting). Mass spectra were acquired using a data dependent acquisition mode by Analyst software version 1.6. Each full scan mass spectrum (400 to 2000 *m*/*z*) was followed by collision-induced dissociation of the seven most intense ions. The dynamic exclusion (30 s of exclusion duration) function was enabled, and the relative collisional fragmentation energy was set to 35%.

All MS/MS samples were analyzed using Mascot software (Matrix Science, London, UK; version 2.3.02). Mascot was set up to search the Uniref100-Fusariumoxy database (release 12–05) assuming the digestion enzyme trypsin. Mascot was searched with a fragment ion mass tolerance of 0.10 Da and a parent ion tolerance of 0.10 Da. Iodoacetamide derivative of cysteine was specified as a fixed modification and oxidation of methionine was specified as a variable modification.

Scaffold (version 3.6.1, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at >95.0% probability as specified by the Peptide Prophet algorithm contained at least 2 identified peptides (Andrew, Nesvizhskii, Eugene, & Ruedi, 2002; Nesvizhskii, Keller, Kolker, & Aebersold, 2003). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

2.15. Size-exclusion high performance liquid chromatography

Size exclusion HLPC (SEC-HPLC) analysis was performed according to the method as described by Xin, Boye, Simpson, and Prasher (2013). Raw and processed field peas and their hydrolysates were dissolved in 0.1 M phosphate buffer (pH 6.8) at a concentration of 20 mg protein per mL, and centrifuged at 500*g* for 3 min. A biosep-SEC S3000 column (300 × 7.8 mm) from phenomenex connected to an Agilent

1200 series HPLC system (Agilent Technologies, USA) was used. 50 μL of sample was loaded on the column, and eluted with the same buffer at a flow rate of 1.0 mL/min, the elution was monitored at 280 nm. The molecular masses of the samples were estimated using different standards containing a mixture of thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylase (200 kDa), albumin bovine serum (66 kDa), carbonic anhydrase (29 kDa), and uridine (0.244 kDa).

2.16. Statistical analysis

All analyses were conducted at least in triplicate and the data were expressed as mean ± SD. Error bars indicate the standard deviation. The statistical significance of difference was evaluated by one-way analysis of variance (ANOVA) using the PRISM software, version 3.02 (GraphPad Software, Inc., San Diego, CA, USA). Significant differences between means were determined by Tukey's multiple comparison test at 5% significance level.

3. Results and discussion

3.1. Proximate analysis

The proximate composition of raw yellow field pea and those subjected to different treatments is summarized in Table 1. There was a general trend that the processing treatments tended to significantly ($P < 0.05$) reduce the protein content of yellow pea seeds. Previous researcher (Mubarak, 2005) also reported that boiling, autoclaving and microwave cooking significantly decreased the crude protein content due to their diffusion into the cooking water. The SLYP also showed decreased protein content ($P < 0.05$) which might be due to the loss of protein during dehulling process. The observation was in accordance with Giami (1993), who reported a decrease of crude protein content by 5.4% after dehulling. The decreased protein content after dry heating treatments was likely to be related to the formation of several nitrogen-containing volatile flavor compounds during roasting and micronization, leading to a significant reduction in total non-protein nitrogen content, and consequently resulting in a significant decrease in total crude protein content (*i.e.*, total nitrogen content × 6.25). Samadi and Yu (2011) also found that dry heating has led to a significant decrease in nonprotein nitrogen fraction of soybeans. Our previous studies supported the proposed assumption (Ma et al., 2015) that roasting could lead to a significant increase in total amount of nitrogenous flavor compounds (including alkylated pyrazines and pyrrole) by identifying and quantifying the volatile flavor composition of different raw and processed legumes including yellow pea seeds using headspace solid-phase microextraction coupled with GC-MS, which, to some extents, could explain the significantly reduced protein content in dry heated samples (including ROYP, MNYP and GRYP, ROYPF) compared with raw yellow field peas in this study. The moisture contents were significantly ($P < 0.05$) reduced after various heat treatments which were caused by loss of water as results of heating. The fibre content was impacted to different extents depending on the specific processing conditions. Samples including CCYP, MCYP, GCCYP, GRYP and ROYPF all showed significantly higher fibre content compared with RYPF. The results were in the same pattern with those reported by Barampama and Simard (1995) who found that soaking and cooking significantly increased the crude fibre content by 6.9% compared with raw beans, possibly due to the protein-fibre complex formed after possible chemical modification induced by soaking and cooking process.

3.2. Amino acid composition

The amino acid composition of raw and treated field pea seeds is shown in Table 2. Asp and Glu were the major amino acids for raw and all treated samples while Cys and Met were found to be the most limiting amino acids in all samples compared with FAO/WHO (1973) reference pattern. Legumes are reported to be rich in Lys but deficient in Trp and the sulfur-containing amino acids Cys and Met (Gupta, 1983). It was observed that total Lys content was significantly increased ($P < 0.05$) after all treatments applied. The observation agreed with those results reported earlier by Khattab et al. (2009a), and Aryee and Boye (2016) who found that lysine content in different legume seeds was significantly increased as a result of different processing treatments. The sulfur rich amino acid Cys was significantly increased after dehulling and micronization, while Met was increased ($P < 0.05$) after dehulling and conventional cooking. Additionally, essential amino acids including His, Ile, Leu, Phe, Thr, and Val contents were all slightly increased after selected treatments. The total essential amino acids were significantly increased after dehulling, micronization, conventional cooking, microwave cooking, germination-conventional cooking, and germination-roasting. The observation agreed with those reported previously by Khattab et al. (2009a) who found that soaking, boiling, microwave cooking and autoclaving generally increased the total essential amino acids in cowpea, kidney bean, and peas. The results were also in accordance with those reported by Saleh and El-Adawy (2006) who found that boiling and microwave cooking of chickpea caused a light increase in total essential amino acids. According to Aryee and Boye (2016), the cooked lentils contained 6 to 25% more of all the essential amino acids (with the exception of valine which were reduced by 2%) than raw lentil flours. The reason of the increased essential amino acid (EAA) including lysine contents, although unexpected and not clear, could be related to the globular structural alternation that occurred during processing, leading to increased accessibility and the release of increased numbers of amino acids that were buried in the interior core of untreated proteins. However, future studies are still needed to further confirm the proposed assumption.

Table 1
Proximate composition of raw and yellow field peas subjected to various processing treatments.

Samples	Protein (db, %)	Ash (%)	Moisture (%)	Fibre (%)	Total tannin content (mg Ecat/g)[a]
RYPF	26.00 ± 0.51^{a}	2.47 ± 0.04^{bc}	8.71 ± 0.11^{a}	14.30 ± 0.00^{a}	2.37 ± 0.01^{a}
SLYP	21.85 ± 0.20^{d}	2.59 ± 0.14^{ab}	7.67 ± 0.90^{b}	9.11 ± 0.05^{b}	1.56 ± 0.11^{c}
ROYP	20.53 ± 0.10^{f}	2.69 ± 0.21^{ab}	7.09 ± 0.05^{bc}	13.4 ± 0.02^{c}	1.45 ± 0.08^{d}
MNYP	20.67 ± 0.06^{ef}	2.74 ± 0.16^{a}	7.31 ± 0.01^{b}	14.15 ± 0.06^{d}	1.60 ± 0.04^{bc}
CCYP	25.11 ± 0.39^{b}	2.12 ± 0.04^{d}	3.43 ± 0.26^{f}	19.55 ± 0.07^{e}	1.28 ± 0.05^{d}
MCYP	24.37 ± 0.07^{c}	2.09 ± 0.02^{d}	2.58 ± 0.76^{g}	19.85 ± 0.1^{e}	1.21 ± 0.08^{f}
GCCYP	20.26 ± 0.09^{f}	1.91 ± 0.14^{d}	4.55 ± 0.59^{e}	20.05 ± 0.02^{e}	1.07 ± 0.04^{g}
GRYP	21.12 ± 0.03^{e}	2.69 ± 0.16^{ab}	6.23 ± 0.89^{cd}	18.55 ± 0.01^{f}	1.31 ± 0.04^{e}
ROYPF	25.29 ± 0.57^{b}	2.61 ± 0.12^{ab}	0.88 ± 0.02^{h}	15.15 ± 0.07^{g}	1.67 ± 0.02^{b}

RYPF (raw yellow pea flour); SLYP (split yellow pea); ROYP (roasted yellow pea); MNYP (micronized yellow pea); CCYP (conventional cooked yellow pea); MCYP (microwave cooked yellow pea); GCCYP (germinated conventional cooked yellow pea); GRYP (germinated roasted yellow pea); ROYPF (roasted yellow pea flour). *For a given parameter, mean values bearing different lower case letters within the same column are significantly different ($P < 0.05$) based on Tukey's multiple comparison test.

[a] Tannin was expressed as mg catechin equivalents on a dry basis.

Table 2
Amino acid content of of raw and yellow field peas subjected to various processing treatments (g/100 g).

Samples	RYPF	SLYP	ROYP	MNYP	CCYP	MCYP	GCCYP	GRYP	ROYP
Aspartic acid	2.26 ± 0.01^{d}	2.43 ± 0.00^{c}	2.31 ± 0.01^{d}	2.45 ± 0.00^{c}	2.54 ± 0.01^{b}	2.41 ± 0.00^{c}	2.85 ± 0.00^{a}	2.04 ± 0.00^{e}	2.42 ± 0.00^{c}
Glutamic acid	3.21 ± 0.01^{f}	3.31 ± 0.01^{e}	3.06 ± 0.01^{g}	4.46 ± 0.01^{a}	3.50 ± 0.00^{c}	3.34 ± 0.01^{de}	3.96 ± 0.01^{b}	3.38 ± 0.01^{de}	3.41 ± 0.01^{d}
Alanine	0.87 ± 0.00^{de}	0.92 ± 0.00^{cd}	0.85 ± 0.00^{e}	0.89 ± 0.01^{de}	0.99 ± 0.00^{b}	0.95 ± 0.00^{bc}	1.12 ± 0.01^{a}	0.63 ± 0.00^{f}	0.88 ± 0.01^{de}
Arginine	1.49 ± 0.00^{d}	1.45 ± 0.00^{d}	1.38 ± 0.01^{e}	1.83 ± 0.00^{a}	1.70 ± 0.00^{b}	1.58 ± 0.01^{c}	1.73 ± 0.00^{b}	1.34 ± 0.01^{e}	1.50 ± 0.00^{d}
Cystine	0.25 ± 0.00^{bc}	0.29 ± 0.00^{b}	0.22^{c} ± 0.01	0.34 ± 0.00^{a}	0.29 ± 0.00^{b}	0.22 ± 0.00^{c}	0.25 ± 0.00^{bc}	0.23 ± 0.00^{c}	0.29 ± 0.01^{b}
Glycine	0.84 ± 0.00^{de}	0.32 ± 0.01^{g}	0.82 ± 0.00^{e}	1.08 ± 0.00^{a}	0.91 ± 0.00^{bc}	0.86±0.01^{cde}	0.95 ± 0.00^{b}	0.82 ± 0.00^{e}	0.89 ± 0.01^{cd}
Histidine	0.46 ± 0.00^{cd}	0.50 ± 0.00^{bc}	0.45 ± 0.00^{cd}	0.63 ± 0.01^{a}	0.53 ± 0.00^{b}	0.50 ± 0.00^{bc}	0.61 ± 0.00^{a}	0.43 ± 0.00^{d}	0.46 ± 0.01^{cd}
Isoleucine	0.67 ± 0.01^{de}	0.67 ± 0.00^{de}	0.65 ± 0.00^{e}	0.96 ± 0.01^{a}	0.76 ± 0.01^{bc}	0.72 ± 0.01^{cd}	0.82 ± 0.01^{b}	0.71 ± 0.00^{cde}	0.66 ± 0.00^{de}
Leucine	1.32 ± 0.00^{e}	1.34 ± 0.01^{e}	1.32 ± 0.01^{e}	1.93 ± 0.00^{a}	1.53 ± 0.00^{c}	1.41 ± 0.01^{d}	1.60 ± 0.00^{b}	1.43 ± 0.00^{d}	1.33 ± 0.00^{e}
Lysine	1.13 ± 0.01^{g}	1.28 ± 0.00^{d}	1.15 ± 0.00^{fg}	1.60 ± 0.00^{a}	1.28 ± 0.00^{d}	1.23 ± 0.01^{de}	1.50 ± 0.00^{b}	1.2 ± 0.01^{ef}	1.36 ± 0.00^{c}
Methionine	0.15±0.00^{bc}	0.18 ± 0.01^{ab}	0.15 ± 0.00^{bc}	0.15 ± 0.00^{bc}	0.21 ± 0.00^{a}	0.16 ± 0.01^{abc}	0.18 ± 0.00^{ab}	0.11 ± 0.01^{cd}	0.15 ± 0.01^{bc}
Phenylalanine	0.86 ± 0.01 ^{f}	0.88 ± 0.00^{ef}	0.90 ± 0.00^{def}	1.29 ± 0.00^{a}	1.01 ± 0.00^{c}	0.95 ± 0.00^{d}	1.11 ± 0.00^{b}	0.93 ± 0.01^{de}	0.87 ± 0.01^{f}
Proline	0.29 ± 0.00^{ef}	0.70 ± 0.00^{c}	0.28 ± 0.00^{f}	0.79 ± 0.00^{b}	0.34 ± 0.00^{e}	0.77 ± 0.01^{b}	0.96 ± 0.01^{a}	0.49 ± 0.00^{d}	0.95 ± 0.00^{a}
Serine	0.99 ± 0.01^{f}	1.05 ± 0.00^{de}	1.00 ± 0.00^{ef}	1.16 ± 0.00^{b}	1.14 ± 0.00^{bc}	1.08 ± 0.01^{d}	1.36 ± 0.00^{a}	0.93 ± 0.00^{g}	1.10 ± 0.00^{cd}
Threonine	0.72 ± 0.01^{c}	0.81 ± 0.00^{b}	0.79 ± 0.01^{b}	0.80 ± 0.00^{b}	0.81 ± 0.00^{b}	0.83 ± 0.00^{b}	0.90 ± 0.00^{a}	0.67 ± 0.00^{c}	0.83 ± 0.01^{b}
Tyrosine	0.45 ± 0.00^{f}	0.39 ± 0.01^{g}	0.50 ± 0.00^{de}	0.75 ± 0.00^{a}	0.65 ± 0.00^{b}	0.56 ± 0.00^{c}	0.54 ± 0.00^{cd}	0.39 ± 0.00^{g}	0.49 ± 0.00^{ef}
Valine	0.79 ± 0.00^{c}	0.77 ± 0.00^{c}	0.76 ± 0.01^{c}	0.88 ± 0.00^{b}	0.96 ± 0.01^{a}	0.79 ± 0.01^{c}	0.99 ± 0.01^{a}	0.60 ± 0.00^{e}	0.70 ± 0.00^{d}

RYPF (raw yellow pea flour); SLYP (split yellow pea); ROYP (roasted yellow pea); MNYP (micronized yellow pea); CCYP (conventional cooked yellow pea); MCYP (microwave cooked yellow pea); GCCYP (germinated conventional cooked yellow pea); GRYP (germinated roasted yellow pea); ROYPF (roasted yellow pea flour). *For a given parameter, mean values bearing different lower case letters within the same row are significantly different ($P < 0.05$) based on Tukey's multiple comparison test.

3.3. Antinutritional compounds

3.3.1. Tannin

A general reduction in tannin content was observed in this study as shown in Table 1, where samples including CCYP, MCYP, GCCYP, and GRYP exhibited the significantly lower values (1.07–1.45 mg Ecat/g). The observation could be due to the fact that tannins are water soluble and consequently leach into liquid medium due the hydrothermal treatments applied (Reddy & Pierson, 1994). Furthermore, the decrease could also be related to the fact that these compounds are heat labile and degrade upon both dry and wet thermal treatments (Rakić et al., 2007). The finding agreed with those reported by Khattab and Arntfield (2009b), Zia-ur-Rehman and Salariya (2005), Udensi, Ekwu, and Isinguzo (2007), and Alonso, Aguirre, and Marzo (2000a). In addition, the predominance of tannin content in seeds coats have been confirmed in the present study by its significantly lower value in dehulled yellow peas (SLYP) compared with the whole seeds (RYPF) (Table 1).

3.3.2. Trypsin inhibitor activity

Differences in trypsin inhibitor activity (TIA) before and after processing are shown in Fig. 1. All treatments has caused a significant decrease ($P < 0.05$) in TIA except for dehulling compared with the raw peas (Fig. 1). The maximum reduction (−83.1% to −87.2%) was found in samples including CCYP, MCYP, GCCYP, and GRYP. The observation was in good agreement with those results found in tannin content in this study as discussed previously, *i.e.*, the four treatments (*i.e.*, CCYP, MCYP, GCCYP, and GRYP) that led to the maximum reduction in TIA happened to be those same treatments that resulted in significantly lower tannin content. The split yellow peas had significantly higher TIA than whole pea seeds which agreed with those reported by Deshpande, Sathe, Salunkhe, and Cornforth (1982) on beans. Heating could generally inactivate trypsin inhibitors as a result of denaturation of these heat-liable proteins (Vidal-Valverde et al., 1994). These results were consistent with the findings of Khattab and Arntfield (2009b) that micronization caused 88.8%–94.4% reduction in TIA, whereas boiling and microwave cooking generally brought a total removal of trypsin inhibitors of legume seeds including cowpea, kidney bean and pea. As reported by Wang, Daun, and Malcolmson (2003), cooking of yellow field pea for 30 min reduced the TIA level by approximately 84.3%. A complete inactivation of trypsin inhibitor upon the combined germination and heating treatments was also reported for different legumes including lupin, soybean and black bean by Trugo et al. (2000).

3.4. In vitro protein digestibility

The low protein digestibility of peas has been one of the main drawbacks that limit the full utilization of these plants. The impact of different treatments on *in vitro* protein digestibility (IVPD) of yellow field pea seeds is shown in Table 3. There was a general trend that heating tended to improve the IVPD of pea seeds. The results were in reasonable accordance with those reported previously (Khattab et al., 2009a; Mubarak, 2005). Among all samples, CCYP, MCYP, GCCYP, and GRYP exhibited the significantly higher protein digestibility with 4.0%–10.1% enhancement compared with raw samples (RYPF). These were supported by the trends observed for TIA and tannin contents as discussed earlier that the samples including CCYP, MCYP, GCCYP and GRYP which showed the significantly lower trypsin inhibitor activity and tannin contents had the significantly higher IVPD values. The results suggested that the protein digestibility was negatively correlated with TIA and tannin contents. The protein digestibility could be greatly affected by the globular structure and conformation of protein, apart from this, the presence of antinutritional factors could also have an impact. Heating

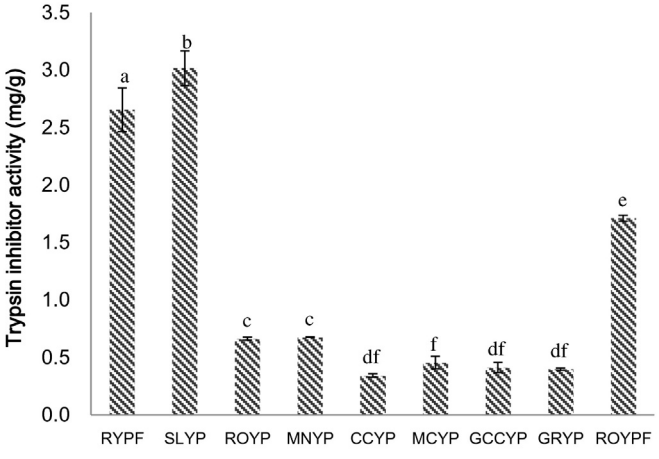

Fig. 1. Trypsin inhibitor activity of raw and processed yellow field peas, where RYPF (raw yellow pea flour); SLYP (split yellow pea); ROYP (roasted yellow pea); MNYP (micronized yellow pea); CCYP (conventional cooked yellow pea); MCYP (microwave cooked yellow pea); GCCYP (germinated conventional cooked yellow pea); GRYP (germinated roasted yellow pea); ROYPF (roasted yellow pea flour); YPCF (yellow pea coarse fibre); YPFF (yellow pea find fibre). *For a given parameter, mean values bearing different lower case letters are significantly different ($P < 0.05$) based on Tukey's multiple comparison test.

Table 3
In vitro protein and starch digestibility of raw and yellow field peas subjected to various processing treatments.

Samples	IVPD (%)	TS (%)	RS (%)	DS (%)
RYPF	83.99 ± 1.15^{ab}	42.81 ± 0.75^{c}	33.99 ± 1.05^{b}	8.82 ± 0.3^{f}
SLYP	85.63 ± 0.38^{adf}	46.52 ± 0.75^{b}	37.74 ± 0.35^{a}	8.79 ± 0.4^{f}
ROYP	86.72 ± 0.10^{df}	41.67 ± 0.64^{cd}	30.59 ± 0.8^{c}	11.08 ± 0.16^{e}
MNYP	85.45 ± 0.38^{ad}	38.8 ± 1.53^{e}	28.38 ± 0.65^{d}	10.41 ± 0.87^{e}
CCYP	92.45 ± 1.17^{c}	48.35 ± 1.12^{a}	30.16 ± 1.58^{cd}	18.2 ± 0.46^{c}
MCYP	89.25 ± 0.64^{e}	41.76 ± 0.22^{cd}	11.35 ± 0.5^{g}	30.42 ± 0.28^{a}
GCCYP	87.38 ± 1.21^{ed}	49.27 ± 0.35^{a}	21.34 ± 1.21^{e}	27.92 ± 1.56^{b}
GRYP	87.86 ± 0.21^{ef}	45.46 ± 0.35^{b}	14.7 ± 0.7^{f}	30.76 ± 0.35^{a}
ROYPF	85.34 ± 1.01^{ad}	41.38 ± 0.16^{d}	28.92 ± 0.1^{cd}	12.46 ± 0.16^{d}

RYPF (raw yellow pea flour); SLYP (split yellow pea); ROYP (roasted yellow pea); MNYP (micronized yellow pea); CCYP (conventional cooked yellow pea); MCYP (microwave cooked yellow pea); GCCYP (germinated conventional cooked yellow pea); GRYP (germinated roasted yellow pea); ROYPF (roasted yellow pea flour); IVPD: *In vitro* protein digestibility (%); TS: total starch; RS: Resistant starch (%); DS: Digestible starch (%).*For a given parameter, mean values bearing different lower case letters within the same column are significantly different ($P < 0.05$) based on Tukey's multiple comparison test.

has a significant influence on improving the IVPD of peas either by inactivating the antinutrients or by denaturing the proteins which exposes the interior parts of protein structure allowing greater access of gastrointestinal enzymes for hydrolysis. Two antinutrients that are closely related to protein digestion are tannins and trypsin inhibitors. Tannins are reported to reduce the protein digestibility of legumes by binding to proteins through hydrogen bonding and hydrophobic reactions and thereby reducing their nutritional quality (Solanki, Kapoor, & Singh, 1999), whereas the adverse impact of low molecular weight trypsin inhibitors is due to the irreversible binding between trypsin inhibitors to the endopeptidase trypsin to form an inactive protein complex, thereby inhibiting the activity of trypsin and thus lead to a decrease in the diet protein digestibility (Chavan & Kadam, 1989). Previous studies (Chau & Pck, 1997; D. Kaur & Kapoor, 1990; Vijayakumari, Siddhuraju, Pugalenthi, & Janardhanan, 1998) also indicated that the decreased levels of certain antinutrients could be partially responsible for the improved protein digestibility of legumes.

3.5. In vitro starch digestibility and Total starch

The total starch (TS), digestible starch (DS) and resistant starch (RS) contents of raw and processed field peas are given in Table 3. Hydrothermal processing generally resulted in a significant increase of total starch in yellow field peas except for MCYP. An increased TS in cooked legumes compared to the respective raw seed was also reported by Siddhuraju and Becker (2009), Bravo et al. (1998), and Periago, Englyst, and Hudson (1996). The increased starch content could be due to the elevated levels of reducing sugars in cooked pea seeds during the measurement of starch by analyzing glucose content obtained after hydrolyzing starch by amylase, pancreatin and amyloglucosidase (Eyaru et al., 2009). The leguminous seed starches are digested more slowly *in vitro* by human digestive juices compared to cereals (Jenkins et al., 1980) which consequently promote slow and moderate postprandial glucose and insulin responses. Digestible starch, calculated as the difference between TS and RS, was found to be significantly enhanced after various thermal treatments. The higher digestible starch content ($P < 0.05$) was found for CCYP, MCYP, GCCYP, and GRYP, which was consistent with the higher *in vitro* protein digestibility values observed. The improved starch digestibility could be attributed to the ruptured starch granules and more opened starch structure obtained after processing which facilitated more randomized configuration for hydrolysis by α-amylase (Eyaru et al., 2009). Furthermore, the partial removal of antinutrients could also contribute to the improved starch digestibility by creating a large space within the matrix and increasing the susceptibility to enzymatic attack (Kataria, Chauhan, & Punia, 1989). In the case of GCCYP and GRYP samples, it was suggested that cooking the germinated legumes could promote metabolic and structural changes with modifications of the nature of interaction between legume starch and protein/fibre, rendering the former more readily digestible (Faki, Venkatarama, & Desikachar, 1984). According to previous studies, the *in vitro* starch digestibility was also increased for two varieties of Indian Tribal pulses after applying various domestic processing treatments including soaking, dehulling, ordinary and pressure cooking, as well as sprouting (Siddhuraju & Becker, 2009).

The raw field peas are one of the richest sources of nutritionally important enzyme resistant starch (~34% RS) which are mostly in the form of RS_2 that are entrapped inside the starch granules and are protected from digestion by their compact conformation or structure. Except that the resistant starch content was significantly increased after dehulling, all thermal treatments applied in this study has led to a decreased RS value as presented in Table 3. The general increase in TS and decrease in RS could be both attributed to gelatinization and dispersion of the starch molecules during thermal processing rendering them more prone to be attacked by starch hydrolyzing enzymes (Eyaru et al., 2009). The observation of the reduced RS level was also reported previously for soaked and/or cooked peas, chickpeas and lentils (Costa, Queiroz-Monici, Reis, & Oliveira, 2006; Periago et al., 1996). It has been suggested that factors including the presence of intake tissue/cell structures enclosing starch granules that hinder the swelling and solublization of starch, the formation of retrograded starch in cooked starch, the presence of high viscous and soluble dietary fibres, high amylose/amylopectin ratio, and the presence of antinutrients may all lead to the apparent reduction of enzyme hydrolysis of starch in cooked legumes (Siddhuraju & Becker, 2009). Nevertheless, it was noticed in this study that ROYP, MNYP, CCYP, GCCYP, and ROYPF still possess a significant fraction of starch remaining undigested by amylases with RS content between 21.34 and 30.59%, unlike potatoes and raw bananas which normally have nearly complete removal of RS after processing and ripening. Future studies are needed to elucidate the mechanism of structural modification in these samples that maintained high levels of RS after processing, *i.e.*, whether due to the formation of retrograded starch (RS_3) or due to the protection of the tissue/cell structure enclosing the starch crystallinity.

3.6. Thermal characteristics

The onset (T_o), peak (T_p), and conclusion (T_c) temperatures, and ΔH (enthalpy) of raw and processed field peas are shown in Table 4. Their thermograms are given in Fig. 2. As can be seen, except for CCYP, MCYP, and GCCYP which were samples obtained by hydrothermal treatments, the rest of the DSC thermograms including those of raw and dry heated peas were characterized by the appearance of two endotherms, firstly a large and higher temperature transition was observed and followed by a small and lower temperature transition (Fig. 2). The first peak at lower temperature is due to starch gelatinization, and the second peak at a higher temperature (80–100 °C) represents the melting of the amylose-lipid complex. The second endotherm had significantly lower gelatinization enthalpies compared with that of the first peak (Table 4), suggesting that more thermal energy is required to gelatinize starch than the organization of amylose-lipid complexes during gelatinization. The transition temperatures of the two endotherms and the gelatinization range (*Tc-To*) of the raw field pea was similar to those reported by Chung, Liu, Hoover, Warkentin, and Vandenberg (2008). The onset and peak gelatinization temperatures were significantly decreased ($P < 0.05$) for samples after the application of thermal treatments in comparison with raw field peas. Particularly it was noticed that samples including CCYP, MCYP, GCCYP, and GRYP exhibited the significantly lower values of T_o, T_p, and T_c (Table 4). The results indicated that the field peas after these treatments were much easily to achieve gelatinization where lesser energy was needed to break the intermolecular bonds in starch granules. In addition, a striking correlation was found among the results of DSC, and *in vitro* protein and starch digestibility analyses, where CCYP, MCYP, GCCYP and GRYP which had the

Table 4
Differential scanning calorimetric characteristics of raw and yellow field peas subjected to various processing treatments.

Samples	T_o (°C) (peak I)	T_o (°C) (peak II)	T_p (°C) (peak I)	T_p (°C) (peak II)	T_c (°C) (peak I)	T_c (°C) (peak II)	ΔH (J/g) (peak I)	ΔH (J/g) (peak II)
RYPF	62.28 ± 1.07^{ab}	81.89 ± 0.40^{b}	72.63 ± 0.11^{b}	86.15 ± 0.19^{b}	81.81 ± 0.49^{bc}	89.91 ± 0.48^{c}	0.68 ± 0.05^{bc}	0.02 ± 0.00^{a}
SLYP	60.32 ± 1.81^{bc}	82.88 ± 0.42^{b}	72.63 ± 0.19^{b}	86.84 ± 0.32^{b}	81.19 ± 0.70^{c}	91.58 ± 0.11^{bc}	0.77 ± 0.13^{b}	0.02 ± 0.00^{a}
ROYP	58.51 ± 1.27^{c}	82.08 ± 0.72^{b}	73.11 ± 0.10^{ab}	86.32 ± 0.85^{b}	90.77 ± 0.40^{a}	90.30 ± 0.51^{bc}	1.48 ± 0.05^{a}	0.02 ± 0.00^{a}
MNYP	63.86 ± 1.00^{a}	88.22 ± 2.50^{a}	73.64 ± 0.02^{a}	91.97 ± 1.57^{a}	82.86 ± 0.79^{b}	97.69 ± 1.96^{a}	0.56 ± 0.03^{d}	0.02 ± 0.00^{a}
CCYP	44.65 ± 1.37^{d}	–	57.63 ± 0.55^{d}	–	70.05 ± 0.55^{g}	–	0.58 ± 0.05^{cd}	–
MCYP	44.34 ± 0.32^{d}	–	57.69 ± 1.34^{d}	–	71.73 ± 1.34^{f}	–	0.58 ± 0.03^{cd}	–
GCCYP	44.38 ± 1.59^{d}	–	56.61 ± 0.24^{e}	–	70.31 ± 0.24^{g}	–	0.72 ± 0.07^{b}	–
GRYP	46.35 ± 1.21^{d}	80.70 ± 1.34^{b}	56.21 ± 0.49^{e}	86.17 ± 0.99^{b}	66.49 ± 1.33^{h}	92.26 ± 1.44^{b}	0.33 ± 0.03^{e}	0.09 ± 0.06^{b}
ROYPF	62.06 ± 0.39^{ab}	82.54 ± 0.07^{b}	72.98 ± 0.27^{ab}	86.83 ± 0.36^{b}	81.93 ± 0.2^{bc}	91.13 ± 0.50^{bc}	0.75 ± 0.07^{b}	0.02 ± 0.00^{a}

RYPF (raw yellow pea flour); SLYP (split yellow pea); ROYP (roasted yellow pea); MNYP (micronized yellow pea); CCYP (conventional cooked yellow pea); MCYP (microwave cooked yellow pea); GCCYP (germinated conventional cooked yellow pea); GRYP (germinated roasted yellow pea); ROYPF (roasted yellow pea flour); T_o (onset temperature); T_p (peak temperature); T_c (conclusion temperature); ΔH (enthalpy of the gelatinization endotherm). * For a given parameter, mean values bearing different lower case letters within the same column are significantly different ($P < 0.05$) based on Tukey's multiple comparison test.

significantly higher *in vitro* protein and starch digestibility exhibited the significantly lower values of T_o, T_p, and T_c. This observation further conformed the assumption that these samples were more vulnerable for the breakage of intermolecular bonding in their structure with pre-exposed protein and starch molecules due to the above four treatments applied.

3.7. Pasting properties, starch damage and bulk density

The pasting properties of raw and processed field peas are summarized in Table 5. All samples exhibited a gradual increase in viscosity with the increase in temperature accompanied by the removal of water from the extruded amylose by the granules as they swell (Ghiasi, Varrianomarston, & Hoseney, 2000). The pasting properties in terms of setback, breakdown, peak and final viscosity were all significantly higher for SLYP and ROYP compared with RYPF. The peak and final viscosity which indicates the ability of starch to form a viscous paste were significantly decreased for CCYP, MCYP, GCCYP and GRYP with significantly lower values observed ($P < 0.05$). The reduction could be due to thermal degradation of starch granules during heat treatment, which could be further confirmed by their significantly lower digestible starch values as observed (Table 3). The results agreed with those reported earlier by Kaur, Sandhu, Ahlawat, and Sharma (2015). The setback (*i.e.*, measurement of syneresis of starch upon cooling of cooked starch paste) and breakdown (*i.e.*, measurement of susceptibility of cooked starch to disintergradation) were not observed for CCYP, MCYP, GCCYP and GRYP, which was possibly due to the already disintegrated starch granules with absence of granule fragments.

Bulk density has been reported to be dependent on the combined effects of interrelated factors including the intensity of attractive interparticle forces, particle size, and number of contact points within the sample (Wani, Sogi, Shivhare, & Gill, 2014). The bulk density values exhibited by raw and processed peas in this study were comparable to those of other common legumes reported by Aguilera et al. (2009), and Jood, Bishnoi, and Sharma (1998). There was minor difference observed for samples before and after processing except for MCYP, GCCYP, GRYP which showed significantly increased bulk densities compared with RYPF (Table 5).

Starch damage refers to a process during which starch granules being physically altered from their native granular form under various processing conditions (Chen & d'Appolonia, 1986). The Starch damage values were significantly increased after all thermal treatments applied in this study, where CCYP, MCYP, GCCYP, and GRYP exhibited the significantly higher values (Table 5). The damaged starch granule implies

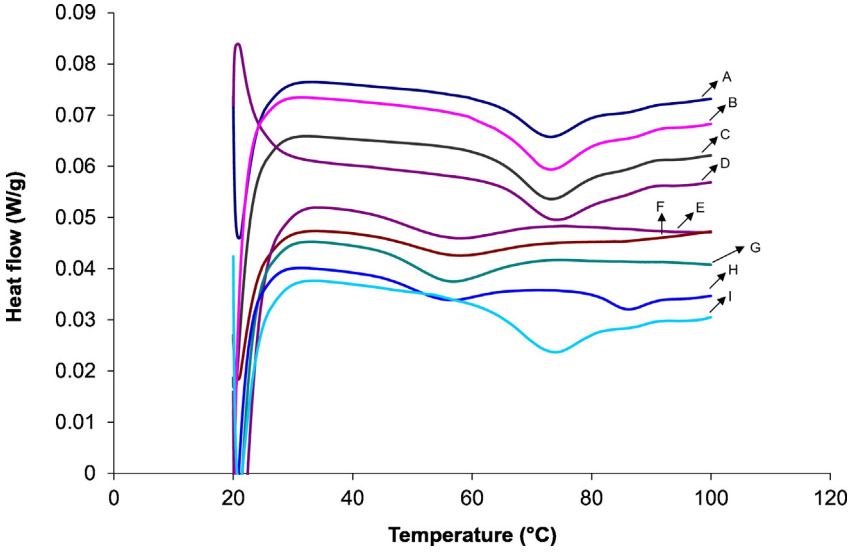

Fig. 2. Differential scanning calorimetry results of raw and processed yield field peas, where A: RYPF (raw yellow pea flour); B: SLYP (split yellow pea); C: ROYP (roasted yellow pea); D: MNYP (micronized yellow pea); E: CCYP (conventional cooked yellow pea); F: MCYP (microwave cooked yellow pea); G: YPFF (yellow pea find fibre); H: YPCF (yellow pea coarse fibre); I: GCCYP (germinated conventional cooked yellow pea); J: GRYP (germinated roasted yellow pea); K: ROYPF (roasted yellow pea flour).

Table 5
Bulk density, starch damage, and pasting properties of raw and yellow field peas subjected to various processing treatments.

Samples	Bulk density (g/mL)	Starch damage (Ai%)	Pasting properties			
			Setback (RVU)	Breakdown (RVU)	Peak viscosity (RVU)	Final viscosity (RVU)
RYPF	0.72 ± 0.03^{ef}	95.4 ± 0.04^{f}	88.59 ± 0.83^{c}	12.46 ± 0.18^{b}	162.21 ± 3.36^{d}	238.0 ± 2.83^{e}
SLYP	0.74 ± 0.00^{de}	96.6 ± 0.1^{e}	166.5 ± 2.83^{a}	19.5 ± 6.36^{a}	196.21 ± 1.71^{a}	343.21 ± 1.82^{a}
ROYP	0.76 ± 0.01^{cde}	95.6 ± 0.2^{f}	111.95 ± 2.16^{b}	20.21 ± 1.12^{a}	185.5 ± 2.72^{b}	277.08 ± 3.54^{b}
MNYP	0.75 ± 0.02^{de}	96.8 ± 0.07^{de}	88.71 ± 0.76^{c}	9.67 ± 0.23^{b}	164.0 ± 1.06^{d}	242.84 ± 0.23^{d}
CCYP	0.76 ± 0.02^{cde}	98.5 ± 0.03^{b}	–	–	27.5 ± 0.71^{f}	60.5 ± 0.71^{g}
MCYP	0.81 ± 0.01^{bc}	98.2 ± 0.1^{c}	–	–	21.5 ± 2.12^{g}	50.0 ± 1.41^{h}
GCCYP	0.83 ± 0.01^{b}	98.4 ± 0.02^{bc}	–	–	35.0 ± 1.41^{e}	70.5 ± 2.12^{f}
GRYP	0.94 ± 0.06^{a}	98.8 ± 0.1^{a}	–	–	17.0 ± 1.41^{h}	31.5 ± 2.12^{i}
ROYPF	0.69 ± 0.03^{f}	96.9 ± 0.2^{d}	91.45 ± 0.64^{c}	19.5 ± 0.71^{a}	175.0 ± 0.01^{c}	247.0 ± 1.41^{c}

RYPF (raw yellow pea flour); SLYP (split yellow pea); ROYP (roasted yellow pea); MNYP (micronized yellow pea); CCYP (conventional cooked yellow pea); MCYP (microwave cooked yellow pea); GCCYP (germinated conventional cooked yellow pea); GRYP (germinated roasted yellow pea); ROYPF (roasted yellow pea flour). *For a given parameter, mean values bearing different lower case letters within the same column are significantly different ($P < 0.05$) based on Tukey's multiple comparison test.

their increased susceptibility to be attacked by α-amylase enzymes, which was in accordance with their significantly higher digestible starch content as well as their higher peak and final viscosity observed.

3.8. Scanning electron microscopy

The microstructures of raw and thermally treated field peas are presented in Fig. 3. Starch granules, characterized by smooth surfaces varying in shape from void to spherical with heterogeneous size ranging from 20 to 35 μm in length and 15 to 25 μm in width, were visible for the raw field pea seeds. No major differences were observed among microstructures of RYPF, SLYP, ROYP, MNYP, and ROYPF which all maintained the integral starch granules in their structure. It was interestingly noticed that the integral starch granules were disappeared in the micrographs of CCYP, MCYP, GCCYP, and GRYP, instead pronounced changes with more amorphous flakes were observed in these samples (Fig. 3e–h). This fact could be mainly due to the endocorrosion of starch granules and the alternation of protein structure with the occurrence of gelatinization and cross-linking during processing that led to the formation of amorphous extracellular material in the seeds. The granule swelling was accompanied by the leaching of granular constituents, predominantly amylose, into the external matrix resulting in a continuous matrix composed cross-inked amylose, amylopectin and modified protein molecules. Similar observation were also found in pre-boiled lentil, pea, and chickpeas (Ma et al., 2011), and germinated cowpea seeds (Błaszczak et al., 2007). The microscopic observation

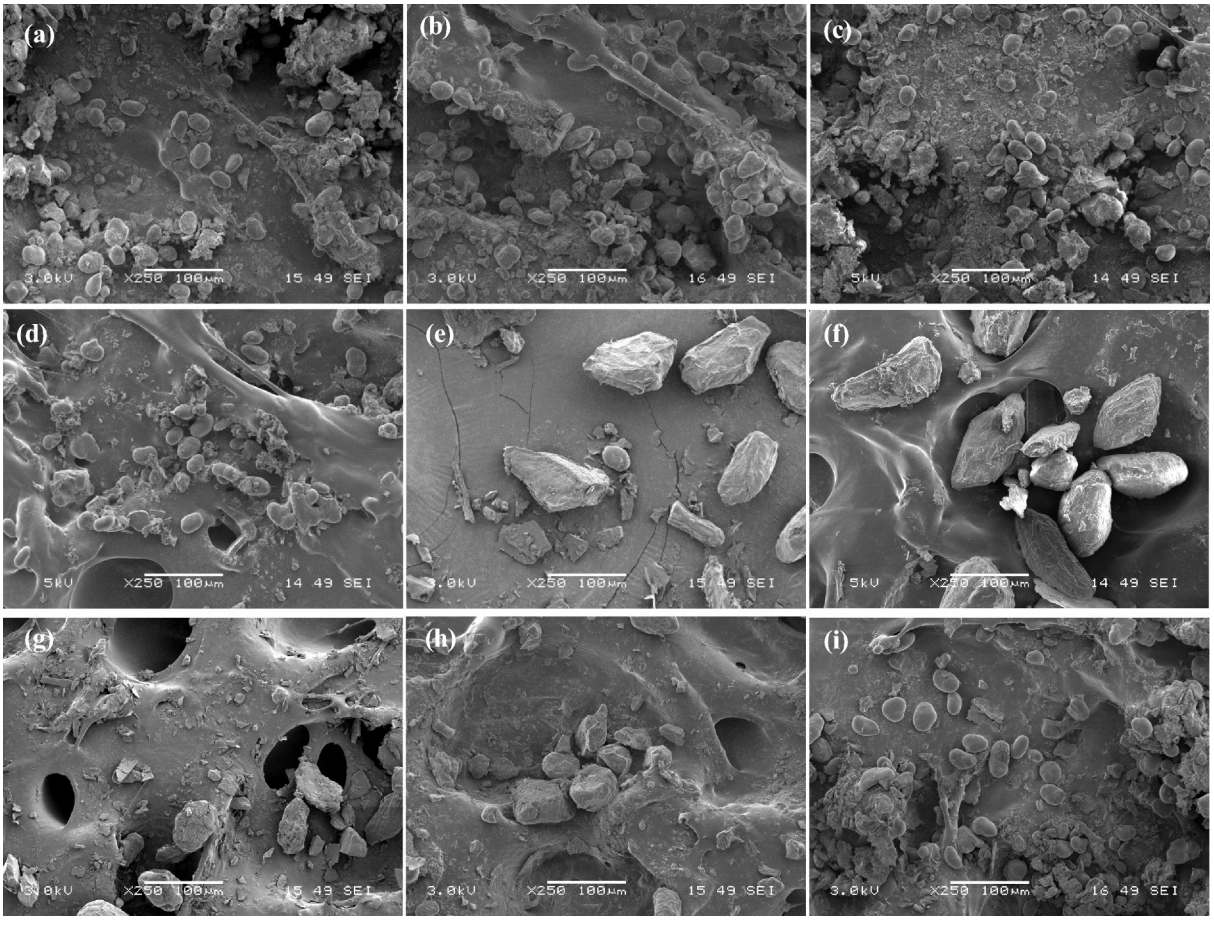

Fig. 3. Scanning electron microscopic images of (a) RYPF; (b) SLYP; (c) ROYP; (d) MNYP; (e) CCYP; (f) MCYP; (g) GCCYP; (h) GRYP; (i) ROYPF; (j) YPCF; (K) YPFF.

was consistent with the changes of *in vitro* protein and starch digestibility, as well as the trends observed for functional properties of CCYP, MCYP, GCCYP, and GRYP. The modification of microstructure, could, in part, explain the increased starch digestibility and the corresponded functional properties of the above four types of processed seeds, *i.e.*, the starch gelatinization and breakdown of the cell walls made starch granules more susceptible to amylolytic enzymes (Marconi, Ruggeri, Cappelloni, Leonardi, & Carnovale, 2000).

3.9. Protein compositional characterization of raw and processed peas based on SDS-PAGE and LC/ESI-MS/MS analyses

Fig. 4 shows the SDS-PAGE profiles of raw field peas and those subjected to various treatments under both non-reducing (Fig. 4a) and reducing (Fig. 4b) conditions. A multitude of bands ranging in size from approximately 16 to 97 kDa was observed for all samples. The bands marked with letters in the SDS-PAGE profile as shown in Fig. 4 and Fig. 5 were identified by LC/ESI-MS/MS using the Mascot search engine by accurate matching of peptide mass. The putative identifications of their major components are listed in Table 6.

Band A and B (as shown in Fig. 4a) with estimated MM (molecular mass) of 98 kDa and 97 kDa which were bared affected by the presence of β-Me, were confirmed to be linoleate 9S-lipoxygenase. The identification was consistent with those reported previously (Barac et al., 2010; Szymanowska, Jakubczyk, Baraniak, & Kur, 2009). Band C, with a MM of 73 kDa, was assigned to be pea alpha-dioxygenase, which, according to Liu et al. (2006), was responsible for converting fatty acids to 2-hydroperoxy products that are important in plant signaling pathways. Band D with a MM of 72 kDa was assigned to be convicilin, an important storage protein of peas which was confirmed in earlier studies by Croy,

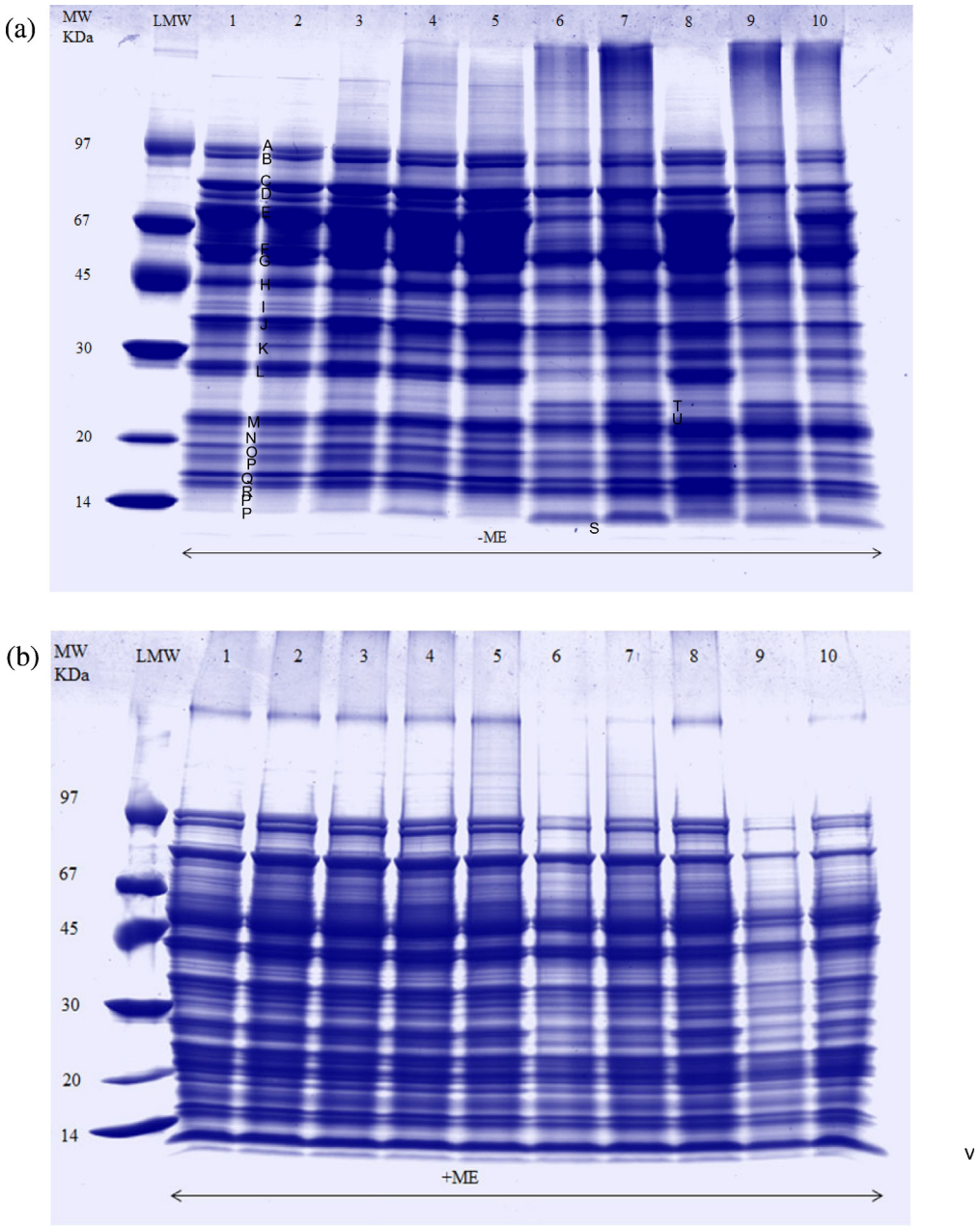

Fig. 4. SDS-PAGE of raw, dry and wet heated yellow pea flours: (a) at non-denaturing conditions with the absence of 2-mercaptoethanol (−Me); (b) at denaturing conditions with the presence of 2-mercaptoethanol (+Me). LMW: low MW markers. Lane 1–10 are 1: RYPF (raw yellow pea flour); 2: SLYP (split yellow pea); 3: ROYP (autoclaved yellow pea); 4: MNYP (micronized yellow pea); 5: ROYPF (roasted yellow pea flour); 6: CCYP (conventional cooked yellow pea); 7: MCYP (microwave cooked yellow pea); 8: GYP (germinated yellow pea); 9: GCCYP (germinated conventional cooked yellow pea); 10: GRYP (germinated roasted yellow pea).

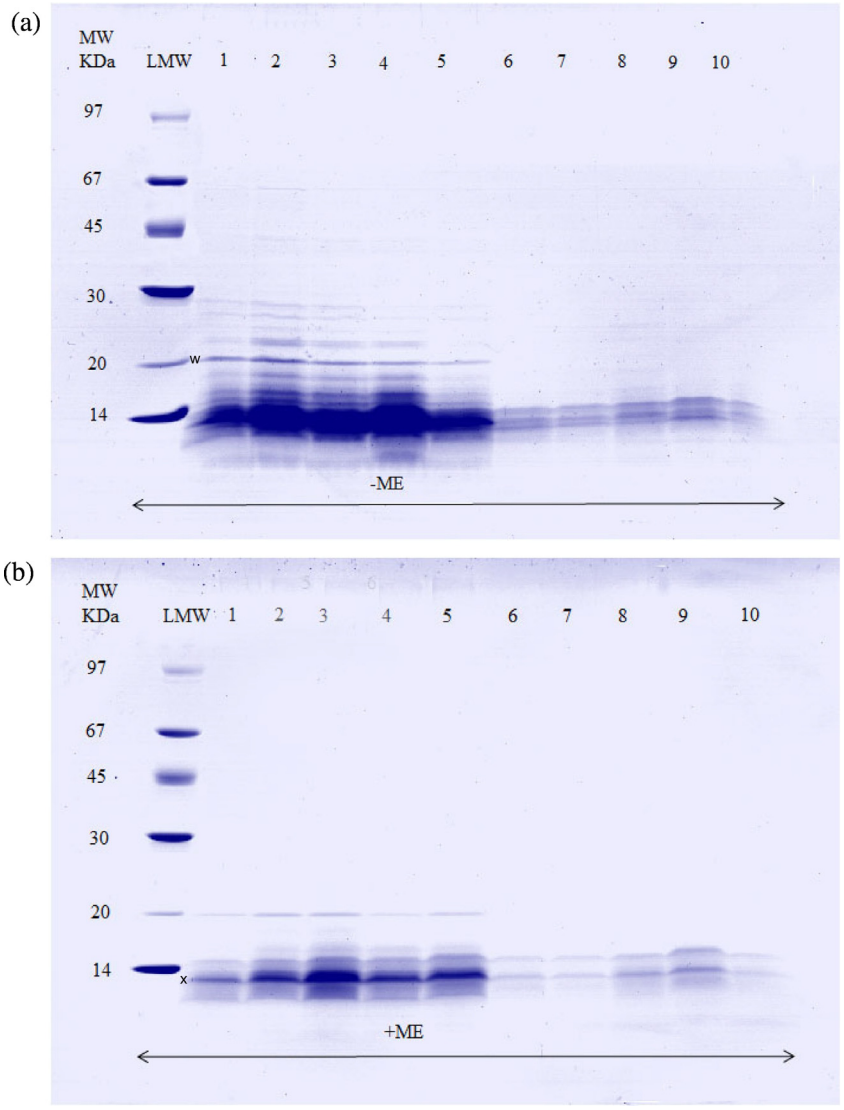

Fig. 5. SDS-PAGE of raw, dry and wet heated yellow pea flours hydrolysates: (a) at non-denaturing conditions with the absence of 2-mercaptoethanol (−Me); (b) at denaturing conditions with the presence of 2-mercaptoethanol (+Me). LMW: low MW markers. Lane 1–10 are 1: RYPFH (raw yellow pea flour hydrolysates); 2: SLYPH (split yellow pea hydrolysates); 3: ROYPH (autoclaved yellow pea hydrolysates); 4: MNYPH (micronized yellow pea hydrolysates); 5: ROYPFH (roasted yellow pea flour hydrolysates); 6: CCYPH (conventional cooked yellow pea hydrolysates); 7: MCYPH (microwave cooked yellow pea hydrolysates); 8: GYPH (germinated yellow pea hydrolysates); 9: GCCYPH (germinated conventional cooked yellow pea hydrolysates); 10: GRYPH (germinated roasted yellow pea hydrolysates).

Gatehouse, Tyler, and Boulter (1980) and Barac et al. (2010). Convicilin was found for all the processed samples, where the intensity in GCCYP was the lower. The variations in the electrophoretic patterns for raw field peas and those subjected to various dry heat treatments (ROYP, MNYP and ROYPF) were very subtle, differences were mostly presented in terms of band intensities, *i.e.*, more intensified bands appeared at ~52 kDa and ~65 kDa for ROYP, MNYP, ROYPF compared with raw peas (RYPF), which were further identified by LC/ESI-MS/MS as subunits of pea vicilin and legumin, respectively. On the other hand, apparent divergence was noticed on the electrophoretic profile of pea protein subjected to hydrothermal (CCYP and MCYP) and germination-heat treatments (GCCYP and GRYP), as shown in lane 6, 7, 9 and 10 respectively, in comparison with that of peas before processing (RYPF) as shown in lane 1 (Fig. 4a). These four treatments caused partial degradation of dominant polypeptides and their aggregation into smaller molecular weight fractions. Especially it was noticed that the intensities of band D and E, which were identified as convicilin and legumin subunits, respectively, (Table 6), decreased significantly due to hydrothermal treatments. As the same amount of proteins of raw and processed peas was loaded for each sample on the electrophoretic gel, the fainter bands thus indicated the decreased intensities on the SDS-PAGE profile. This was in accordance with their significantly higher IVPD values as discussed earlier that these four samples had the most pronounced improvement of nutritional quality, which could possibly due to the thermal degradation of the major storage proteins as shown in their electrophoretic profile. The band at ~65 kDa (legumin subunit) was also affected by the presence of β-Me which were dissociated by the breakage of a single disulfide bond into an acidic subunit of ~40 kDa and a basic subunit of ~20 kDa as appeared in Fig. 6b. This was in accordance with those reported by Gueguen, Chevalier, Barbot, and Schaeffer (1988).

Band F (~52 kDa), G (~50 kDa), and J (~32 kDa) were identified as subunits of vicilin (*Pisum sativum*). The results were corroborated by those reported previously that pea vicilin is heterogeneous and different gene encoding is believed to produce the group of vicilin polypeptides of ~50 kDa and ~30 kDa (Barac et al., 2010; Spencer, Chandler, Higgins, Inglis, & Rubira, 1983). Specifically, the vicilin with a MM of 50 kDa (as shown in band of Fig. 4a) was confirmed as the prominent protein in peas and was devoid of disulfide bonds between subunits, which could be verified in their SDS-PAGE profile under reducing

Table 6
Protein identification of yellow field peas by LC/ESI-MS/MS analysis.

BID[a]	Protein identification	T.Mr[b] (kDa)	Accession No.
A	LOX 3_Pea seed linoleate 9S-lipoxygenase-3 [*Pisum sativum*] (Garden pea)]	98	P09918
B	LOX2_Pea seed linoleate 9S-lipoxygenase-2 [*Pisum sativum* (Garden pea)]	97	P14856
C	PEA Alpha-dioxygenase [*Pisum sativum* (Garden pea)]	73	Q5GQ66
D	PEA Convicilin [*Pisum sativum* (Garden pea)]	72	Q9M3X6
E	PEA legumin (minor small) [*Pisum sativum* (Garden pea)]	65	O24294
F	PEA Vicilin [*Pisum sativum* (Garden Pea)]	52	P13918
G	PEA Vicilin 47 k [*Pisum sativum* (Garden Pea)]	50	D3VNE1
H	PEA Alpha-galactosidase 1[*Pisum sativum* (Garden Pea)]	45	Q5ZP79
I	PEA legumin K (Fragment) [*Pisum sativum* (Garden Pea)]	40	P05693
J	PEA vicilin (Fragment) [*Pisum sativum* (Garden Pea)]	32	P02855
K	PEA Provicilin (Fragment) [*Pisum sativum* (Garden Pea)]	32	P02855
L	PEA Lectin [*Pisum sativum* (Garden pea)]	30	P02867
M	PIP22_PEA Kunitz-type trypsin inhibitor-like 2 protein [*Pisum sativum* (Garden Pea)]	24	O82711
N	PEA Albumin −2 [*Pisum sativum* (Garden Pea)]	26	P08688
O	PEA Albumin −2 [*Pisum sativum* (Garden Pea)]	26	P08688
P	PEA 18.1 kDa class I heat shock protein [*Pisum sativum* (Garden Pea)]	18	P19243
Q	PEA ABA-responsive protein ABR 17 [*Pisum sativum* (Garden Pea)]	17	Q06931
R	PEA Albumin-1A [*Pisum sativum* (Garden Pea)]	14	P62926
	PEA Albumin-1D [*Pisum sativum* (Garden Pea)]	14	p62929
S	PEA Seed trypsin/chymotrypsin inhibitor IVA (Fragment) [*Pisum sativum* (Garden Pea)]	11	Q41065
T	PIP22_PEA Kunitz-type trypsin inhibitor-like 2 protein [*Pisum sativum* (Garden Pea)]	24	O82711
U	PEA Albumin-2 [*Pisum sativum* (Garden Pea)]	26	P08688
V	PEA Albumin-1A/E [*Pisum sativum* (Garden Pea)]	14	P62926
W	PEA Albumin −2 [*Pisum sativum* (Garden Pea)]	26	P08688
X	PEA legumin L1 beta chain (Fragment) [*Pisum sativum* (Garden Pea)]	8	Q7M1N3
	PEA Seed trypsin/chymotrypsin inhibitor IVA (Fragment) [*Pisum sativum* (Garden Pea)]	11	Q41065

[a] BID: spot ID, where the letters from A to X represent for the band selected from the electrophoresis band (Figs. 4 and 5) for LC/ESI-MS/MS analysis.
[b] T.Mr: theoretical molecular weight.

conditions in Fig. 4b. Band G was found to be missing in lane 6, 7, 9 and 10, indicating that these four treatments caused degradation of dominant vicilin polypeptides and their aggregation into smaller polypeptides. Band H was confirmed as pea α-galactosidase (*Pisum sativum*), with a MM of 45 kDa. The finding was supported by Sharma and Sharma (1977) who have separated two molecular species of α-galactosidase with apparent MMs of 134 kDa and 43 kDa from chickpeas. Band E and I were assigned to legumin subunits, with MM of 65 kDa and 40 kDa, respectively. The observation was confirmed by Barac et al. (2010), Urbano et al. (2005), and Martínez-Villaluenga et al. (2008). The intensity of legumin subunit with a MM of 40 kDa (band I) was apparently decreased for CCYP, MCYP, GCCYP, and GRYP, and was particularly obvious for GCCYP and GRYP. According to Martínez-Villaluenga et al. (2008), germination improves the nutritional quality of legumes by degrading the globulin fraction of pea sprouts along with a decrease in proteins with molecular weights between 50– 45 kDa (no processed vicilin) and 43– 35 kDa (legumin).

Band N and O, with MM of 26 kDa, were identified as subunits/polypeptides of albumin-2 (*Pisum sativum*). This observation was supported by previous researchers (Higgins et al., 1987) that pea albumin 2 (PA2: Mr ~26 kDa) was a major compound of the albumin fraction and had two closely related components (PA2a and PA2b) as evidenced on the SDS gel electrophoresis and chromatography on DEAE-sephacel. New band (as shown in band U of Fig. 4a) around ~26 kDa appeared on the electrophoretic pattern in lane 7, 8, 9, and 10 (Fig. 4a) which corresponded to MCYP, GYP, GCCYP, and GRYP, respectively, were identified as pea albumin subunit. According to Croy, Hoque, Gatehouse, and Boulter (1984), the larger protein of albumin has a MM of 53 kDa and consists of two ~25 kDa subunits, whereas the smaller albumin protein has a MM of 48 kDa and consists of two ~24 kDa subunits. Our observation indicated that the above four treatments (*i.e.*, MCYP, GYP, GCCYP, and GRYP) facilitated the degradation of albumin proteins and led to the appearance of smaller subunits. Band N of albumin fraction was missing in lane 6, 7, 9 and 10. Martínez-Villaluenga et al. (2008) also observed that the albumin fraction were significantly decreased in the germinated peas on the SDS-PAGE profiles for different pea cultivars. The band P (18 kDa) was identified as pea heat shock protein, which was confirmed earlier (DeRocher & Vierling, 2003; Lee, Pokala, & Vierling, 1995) that HSP18.1 and HSP 17.7 represent class I and class II proteins from pea, respectively. The heat shock proteins were normally absent from peas unless induced by heating at temperatures above 30 °C, which could be also caused by milling and dehulling during processing of peas. Band V, as observed on the electrophoretic pattern under reducing conditions (Fig. 4b) was assigned as albumin subunits 1A and 1E, with MM of ~14 kDa (Fig. 4b). This finding was confirmed by those results reported earlier (Alonso et al., 2000b) with albumin fraction found at MM of 14 kDa in peas. It was also noticed that more bands between 22– 25 kDa appeared in Fig. 4b due to the breakage of disulfide bonds of albumin into smaller subunits in the presence of β-Me. The observation was supported by Croy et al. (1984) who isolated and purified two closely related major albumin proteins, where the larger protein (designated PMA-L) has a MM of ~53 kDa and consists of two 25 kDa MM subunits, and the smaller fraction, PMA-S, has a MM of ~48 kDa and contains two 24 kDa MM subunits.

Some antinutritional proteins were also identified by LC/ESI-MS/MS. Band L was assigned to be pea lectin, with a MM of 30 kDa. It could be seen that the intensity of Band L was significantly decreased in lane 6, 7, 9 and 10, which corresponded to CCYP, MCYP, GCCYP, and GRYP, respectively (Fig. 4a). The observation indicated that hydrothermal treatments and combined germination/heating could significantly reduce lectin content, which agreed with those reported previously (Mubarak, 2005). Lectin from legumes with a MM of 30 kDa was also reported earlier (Sjoeholm & Oestergaard, 2005). Band S was assigned as the protease (trypsin/chymotrypsin) inhibitor with a MM of 11 kDa, which was in good agreement with those observed by Barac et al. (2010) who found minor band of 11.5 kDa on the electrophoretic patterns of pea proteins. The bands M and T were confirmed to be pea Kunitz-type typsin inhibitor protein, with MM of ~24 kDa. This finding was supported by previous results reported by Barać and Stanojević (2005). It was also noticed that the intensity of band S was slightly increased by dry heating for samples including ROYP, MNYP and ROYPF and was significantly increased for CCYP, MCYP, GCCYP, and GRYP. According to Onyeike, Abbey, and Anosike (1991), not all subunits of trypsin inhibitors are equally sensitive to heat, the trypsin inhibitor subunits

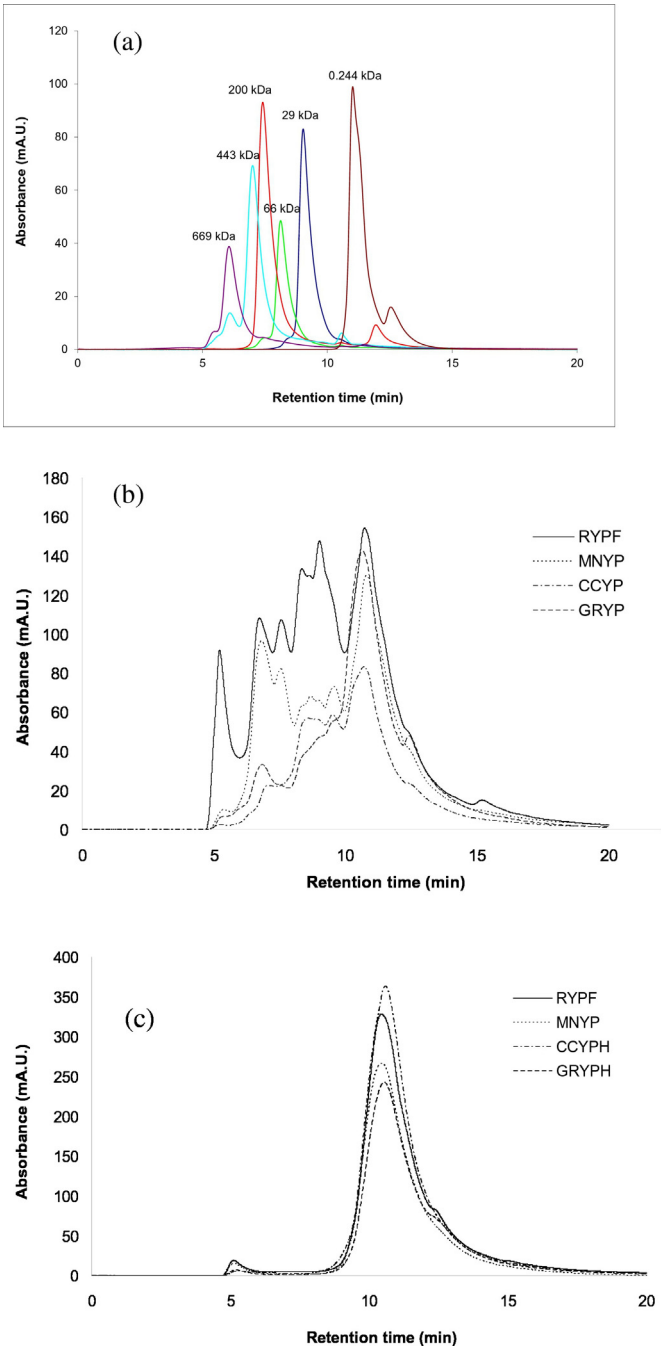

Fig. 6. Size exclusion high performance liquid chromatography patterns (SEC-HPLC): (a) standards; (b) raw and processed samples before hydrolysis; (c) raw and processed samples after hydrolysis; where RYPF (raw yellow pea flour); RYPFH (raw yellow pea flour hydrolysates); MNYP (micronized yellow pea); MNYPH (micronized yellow pea hydrolysates); CCYP (conventional cooked yellow pea); CCYPH (conventional cooked yellow pea hydrolysates); GRYP (germinated roasted yellow pea); GRYPH (germinated roasted yellow pea hydrolysates).

with molecular weight up to 10 kDa are more heat-stable than those inhibitors with MM >20 kDa. Indeed, heating probably has led to the breakage and fragmentation of hydrophobic and hydrogen bindings between soluble protease inhibitor and other subunits which enabled the availability of the band at 11 kDa (band S in Fig. 4a) in heat processed samples. The observation of SDS-PAGE profile correlated well with the TIA values obtained in this study (Fig. 1) which was slightly decreased after dry heat treatments, whereas was significantly reduced after hydrothermal treatments. The hydrothermal processing had more harsh effect than dry heating, which could explain the appearance of more visible bands at 11 kDa (band S) in hydrothermally cooked samples compared with those subjected to dry treatments. Similar result was also found for band T which was assigned as Kunitz-type trypsin inhibitor protein (Fig. 4a). According to Barać and Stanojević (2005), protease inhibitors from BBI and KTI family could undergo self-aggregation in non-reducing condition and may interact with subunits of other proteins. The result suggested that band T of the raw pea protein was the soluble fraction of trypsin inhibitor aggregates, hydrothermal treatments further denatured the protease inhibitor proteins by forming the aggregation of trypsin inhibitor subunits and resulted in intensified bands at MM of 24 kDa and 11 kDa (Fig. 4a). The relatively high content of the soluble inhibitors as observed on SDS-PAGE profile and the low trypsin inhibitor activity determined earlier indicated that most of the subunits in processed peas were existing in partially disrupted conformation and inactive form. The finding was supported by Barać and Stanojević (2005) who found that the microwave cooked soybeans (1–2 min) also had relatively high content of protease inhibitor protein aggregates and low residual activity compared with the raw seeds. It was also noticed that the intensity of band S from lane 1 to lane 10 was increased under reducing conditions on the SDS-PAGE profile (Fig. 4b) compared with those tested under non-reducing conditions (Fig. 4a). According to Zavodszky et al. (2001), the exhibited high degree of stability of some trypsin inhibitor subunits was due to the existence of disulfide bonds in their molecules.

3.10. Compositional characterization of protein hydrolysates of raw and processed peas based on SDS-PAGE and LC/ESI-MS/MS analyses

The SDS-PAGE profile of the protein hydrolysates of raw and processed peas were also analyzed under reducing (Fig. 5a) and non-reducing conditions (Fig. 5b). All the processed pea protein digests showed the presence of major bands with MM estimated between 10 and 30 kDa, confirming the effectiveness of enzymatic cleavage of pea proteins. Minor differences were observed for different processed pea digests. Various peptide bands at MM between 20 and 30 kDa were observed for RYPFH, SLPFH, ROYPH, MNYPH, and ROYPH. However in the case of hydrolysates obtained by hydrothermal treatments (CCYPH and MCYPH), and combined germination and heating (GCCYPH and GRYPH), the peptide bands at MM between 14 and 30 kDa were missing after the action of hydrolysis with multiple enzymes. This observation was in accordance with the results obtained in this study that the samples including CCYP, MCYP, GCCYP and GRYP exhibited the significantly higher *in vitro* protein digestibility (Table 3). The results, to some extent, indicated that these four treatments facilitated a higher degree of hydrolysis of pea protein by altering their tertiary and quaternary structures which thus led to overall improvements in its susceptibility to enzymes by leaving a larger number of peptide bonds being cleaved and the availability of a higher number of hydrolysis sites in the processed pea samples. Similar observations have also been found in previous literatures (Rajamohamed, Aryee, Hucl, Patterson, & Boye, 2013; Xin, Boye, Barbana, Simpson, & Prasher, 2012). However, since the peptide profile of the digestive fraction of raw and processed pea proteins obtained in this study might be different from that of the real *in vivo* protein digestion process, so the results are somewhat controversial. Future studies would be of interest by comparing the peptide profiles of the raw and processed peas using different *in vitro* methods including the pH-drop/multi-enzyme (trypsin-α-chymotrypsin-peptidase), sequential (pepsin-pancreatin) and simulated gastric and intestinal (pepsin-trypsin-α-chymotrypsin) digestion techniques (SGID). The hydrolysate at band W was identified as pea albumin-2. In the presence of β-Me, a different peptide profile was observed characterized by a disappearance of the major bands suggesting that these polypeptides were linked by disulfide bridges (Fig. 5b). Band x, the most intense band observed in the electrophoresis patterns as shown in Fig. b, was confirmed to be legumin L1 beta fragment and/or pea trypsin/chymotrypsin inhibitor fragment according to LC/ESI-MS/MS analysis.

3.11. SEC-HPLC profiles

The raw and processed peas and their hydrolysates were also analyzed by SEC-HPLC and the chromatographic profiles are selectively shown in Fig. 6. Relatively similar chromatographic profiles were observed for RYPF, SLYP, ROYP, MNYP, and ROYPF. This was in accordance with their electrophoretic profiles showing similar patterns. The chromatogram of raw peas showed generally six peaks of MM ranging from 669 kDa correspond to higher aggregates to 0.244 kDa. Compared with raw and dry heated peas (ROYP, MNYP, and ROYPF), samples including CCYP, MCYP, GCCYP, and GRYP exhibited heterogeneous patterns on their SEC-HPLC profile which also agreed with their electrophoretic profile with the significant reduction of proteins with higher molecular weight (Fig. 4a). The SEC-HPLC profile of their hydrolysates showed different peptide patterns which generally exhibited a monophasic profile with retention times between 8.7– 17.8 min, a reflection of the action of multiple of enzymes involved and peptides produced by hydrolysis.

4. Conclusion

Basic knowledge of the nutritional profile, *in vitro* digestibility and techno-functional properties of pulses such as yellow field peas, as well as their changes that occur following different types of processing treatments, could ensure the agri-food industry to improve the process efficiency with enhanced quality of field peas. The results presented clearly show that the protein digestibility of field peas was improved by various heat treatments through inactivating the antinutrients (including tannin and trypsin inhibitor) and by denaturing the proteins allowing greater access of gastrointestinal enzymes for hydrolysis. Specifically, among all samples, CCYP, MCYP, GCCYP, and GRYP exhibited the significantly higher *in vitro* protein digestibility with significantly lower trypsin inhibitor activity and tannin content. The higher IVPD results of these samples were also consistent with their electrophoretic observation that the peptide bands at MM between 14 and 30 kDa were missing after the action of hydrolysis with multiple enzymes. Meanwhile, the digestible starch contents were also found to be significantly higher for CCYP, MCYP, GCCYP, and GRYP compared with raw and other processed field peas, which was further supported by their DSC results, their lower pasting properties, starch damage result, and the scanning electron microscopic observations with pronounced changes occurred proving that the starch gelatinization and breakdown of the cell walls during processing has made starch granules more susceptible to amylolytic enzymes for these samples. The information on the enhanced nutritional profile and the following techno-functional changes in these processed samples, may be of interest to relevant industries targeting specific pulse-based food product development. Future studies by using different *in vitro* methods including the pH-drop/multi-enzyme (trypsin-α-chymotrypsin-peptidase), sequential (pepsin-pancreatin) and simulated gastric and intestinal (pepsin-trypsin-α-chymotrypsin) digestion techniques (SGID) would be also useful to determine the differences in their peptide profiles of raw and processed field peas.

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