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Enhanced functionality of pea-rice protein isolate blends through direct steam injection processing

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ABSTRACT

Direct steam injection (DSI) processing with pH adjustment was investigated to enhance the functionality of pea-rice protein isolate blends (PR). Protein slurries at concentration of 5% (w/w) of commercial pea and rice protein isolates in the ratio of 2:1 (w/w) across a range of steam temperatures (66–107 °C) and pH values (2 −1 1) were studied. After DSI treatment, the PR were freeze-dried to obtain the final dry protein powder. Based on protein solubility profiles, the optimal DSI processing conditions were 107 °C and pH 11. Available lysine was not reduced ($P > 0.05$) in the blend. Solubility (from 3 to 41%, at pH 7), emulsifying activity index (from 5.9 to 52.5 m^2/g), foam stability (from 68.2 to 82.8%), and oil holding capacity (from 1.8 to 4.9 g/g) values increased ($P < 0.05$) compared to the untreated PR. DSI can modify the functionality of PR without affecting the essential amino acid composition.

1. Introduction

Pea and rice are good sources of protein due to their nutritional value and availability (Boye, Zare, & Pletch, 2010). Market for pea protein is increasing rapidly and is expected to reach 34.8 million USD by 2020 due to growing consumer interest in plant protein ingredients, and relatively low cost of production of peas (Grand View Research., 2015). The production value of rice protein market was estimated at 34.3 million USD in 2015 and is also predicted to grow rapidly (Grand View Research, 2016). Protein isolates can be used to increase the nutritional value and functional properties of foods (Boye, Aksay, et al., 2010). Pea protein is considered a good source of essential amino acids with a high amount of lysine but low in methionine (Boye, Zare, et al., 2010). Rice protein, is rich in methionine and low in lysine, making it a complementary protein for pea. Combined in the proper proportions, pea and rice proteins ensure sufficient quantities of all essential amino acids needed in the human diet as recommended by United Nations (2011).

Protein extracted from dry peas and rice has great potential for use as a food ingredient (Boye, Aksay, et al., 2010; Cao, Wen, Li, & Gu, 2009). Pea protein possesses good foaming and emulsifying properties (Aluko, Mofolasayo, & Watts, 2009; Taherian et al., 2011), can form gels (Shand, Ya, Pietrasik, & Wanasundara, 2007) and bind water and oil (Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014). Rice protein is colorless and tasteless protein source, but its use in food formulations is limited due to it low solubility (Wang, Wang, Wang, & Chen, 2016). Commercially available pea and rice protein isolates have poorer functional properties than those reported above, primarily due to differences in the fractionation processes. Several authors (Aluko et al., 2009; Osen et al., 2014; Stone, Karalash, Tyler, Warkentin, & Nickerson, 2015) have related low protein solubility of isolates to more severe processing conditions such as high temperatures during spray-drying or alcohol decoloration in commercial settings.

DSI is a process that exposes the product to high temperatures for short periods of time (Lewis, Heppell, & Hastings, 2000). Use of this technology for improving functional properties of vegetable proteins, including soy, was previously proposed (Gomi, Hisa, & Soeda, 1978; Gomi, Hisa, & Soeda, 1980; Hawley, Frederiksen, & Hoer, 1972) Wang and Johnson (2001) used DSI to restore solubility properties of soy protein concentrate to approximately that of the native protein. Longer process times improved protein solubilities of soy concentrate and isolate, but the soy isolate had a darker color due to the creation of Maillard reaction products. The authors speculated that more than one biochemical mechanism contributed to the improvement of functional properties by steam injection.

In 2011, Ganjyal and others developed and patented a DSI processing method for preparing protein blends with enhanced functional and/or nutritional properties. Functionality evaluation revealed enhanced solubility, emulsification, foaming and gelling of protein treated by the DSI process. The authors speculated that the modification of electrostatic properties by adjusting pH combined with heat shock led

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to protein unfolding and the subsequent cooling period allowed for protein rearrangement. DSI was assumed to alter disulfide bonds (SS) and sulfhydryl groups (SH) to create cross-linked hybrid proteins from two or more protein sources, however there was a lack of analysis supporting this hypothesis. There is a need to better understand this process and investigate the potential mechanisms that help to enhance the functionality of the protein blends.

To exploit the potential applications of rice and pea proteins as a food ingredient, new methods to improve solubility without adversely affecting the nutritional value must be developed. Thus this study investigated high pressure DSI with pH adjustment to create modified pea:rice isolate (PR) blends with enhanced functional properties. The mechanism behind improvement of functional properties of commercial pea and rice protein isolate by DSI was investigated to help extend the use of these ingredients in wider food processing applications.

2. Materials and methods

2.1. Protein isolates

Pea protein isolate (Pisane B9), obtained from Cosucra (Warcoing, Belgium), and rice protein isolate (Oryzatein Silk80), obtained from Axiom Foods (Los Angeles, CA) were used for preparing pea-rice (PR) protein blends. All samples were stored in a dry place at room temperature (20–23 °C) in glass bottles sealed by plastic caps with liners to minimize changes in moisture content and oxidation.

2.2. Chemicals

Bovine albumin standard (BSA), Ellman's reagent, and 8-anilino-1-naphthalenesulfonic acid (ANS) were procured from Sigma-Aldrich (Saint Louis, MO, U.S.A). Coomassie Brilliant Blue was procured from Bio-Rad Laboratories (Hercules, CA, U.S.A). Acid Orange 12 was procured from Chem-Impex International Inc. (Wood Dale, IL, U.S.A). Dye propionic anhydride was procured from EMD Millipore Corporation (Billerica, MA, U.S.A). All chemicals were of reagent grade or better.

2.3. Preparation of protein blends

PR protein isolate blends were prepared by combining pea protein isolate and rice protein isolate in the ratio 2:1 w/w, to ensure sufficient quantities of methionine and lysine to meet the FAO (2011) recommendations. DSI process for preparing PR protein blends was conducted according to Ganjyal, Maningat, and Bassi (2011) (Fig. 1). Protein blends were combined with water (4 L, 5% w/w protein) and blended using a laboratory homogenizer (Model 17105 Omni-Mixer Homogenizer, Omni International, Waterbury, CT, U.S.A) while pH of the slurries was adjusted to acidic (pH 2 and 3) or alkaline (pH 9, 10, 11) conditions using either 1 M HCl or 1 M NaOH. Slurries were held for 1 h at room temperature (20–23 °C) before processing in the DSI system (EZ Heater H2010, Hydro - Thermal Corporation, Waukesha, WI, U.S.A) at temperatures of 66 °C, 80 °C, 93 °C, and 107 °C, with ± 3 °C variation during the process, and around 5 L/min flow rate. Slurries were cooled in a continuous-flow heat exchanger and then held at room temperature to cool down to approximately 23 °C. Following this the pH of the solution was readjusted to 7.0 with 1 M HCl or 1 M NaOH and then freeze dried (Unitop 600 L, The Virtis Company, Gardiner, NY, U.S.A) to target moisture contents below 7% (w.b.).

2.4. Experimental design

Fig. 1 provides the description of the experimental design and conditions used (pH, temperature). Additionally, 1 batch (1 L, 5% protein content w/w) at 2:1 PR ratio and pH 2, 3, 9, 10 was prepared without DSI processing to evaluate the influence of only pH adjustment on solubility. All batches were analyzed for protein content (n = 2) and protein solubility profiles were determined from pH 3–9 (n = 3).

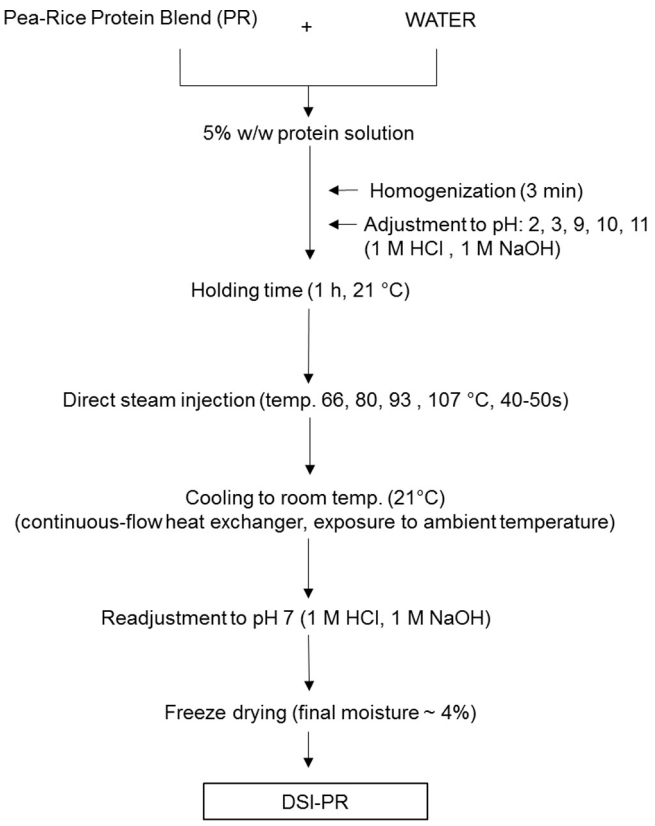

Fig. 1. Flow diagram for the direct stem injection procedure of pea:rice protein blends.

Three replicate batches of each of three treatments that resulted in the greatest solubility improvement were prepared and analyzed for biochemical and functional properties.

2.5. Chemical composition

Ash content (n = 3) was determined by dry ashing (AACCI 2000; 08-01.01a). Crude protein (n = 2) was determined using a nitrogen/protein analyzer (FP-528, Leco Corporation, St. Joseph, MI, U.S.A) (AACCI 2000; 46-30.01) with nitrogen conversion factor of 6.25.

2.5.1. Amino acid composition

Amino acid composition was determined at an external laboratory (AAA Service Laboratory, Damascus, OR, U.S.A). A Hitachi L8900 amino acid analyzer, Hitachi column (part# 855-4516), and Hitachi complex of buffers (Hitachi, Ltd, Tokyo, Japan) was used according to methods of Moore and Stein (1948). Proteins (n = 2) were hydrolyzed in 6 N HCl containing 2% phenol at 110 °C for 22 h (Roach and Gehrke, 1970) dried in a speed-vacuum system to 150 mTorr and re-suspended to a concentration of 10 mg material per mL of sample buffer, diluted 1:50 and 50 µL was injected onto the analyzer. Norleucine was included as an internal standard. Postcolumn derivatization with ninhydrin was used to visualize the peaks. Proline was detected at a wavelength of 440 nm, while the remaining amino acids were detected at 570 nm.

2.5.2. Available lysine content

Available lysine content was determined using propionic anhydride as the blocking agent (Aalaei, Rayner, Tareke, & Sjöholm, 2016). The concentration of available lysine was determined using a standard curve prepared from a stock solution of Acid Orange 12 dye at an absorbance of 475 nm. The standard curve ranged from 0.0048 to 0.0150 mg dye/mL buffer. The concentration of lysine was calculated from the difference in presence and absence of blocking agent.

2.6. SDS-PAGE

Total and soluble protein fractions collected during solubility testing were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing and non-reducing conditions according to Laemmli (1970) with slight modifications, using a Mini PROTEANw3 system, pre-cast gels (4–15% gradient), and reagents from Bio-Rad Laboratories (Hercules, CA, U.S.A). Isolates were dispersed in Laemmli sample buffer (65.8 mM Tris-HCl, 26.3% (w/v) glycerol, 2.1% SDS, 0.01% bromophenol, pH 6.8), heated for 5 min in 95 °C, and cooled to 21–23 °C. For reducing condition 5 µL of β-mercaptoethanol was added to samples. Aliquots (20 µL, 0.1% protein content) were loaded into wells and electrophoresis was run at constant voltage of 300 V. Gels were stained using Coomassie Brilliant Blue. Precision Plus Protein Standards (Bio-Rad Laboratories, Hercules, CA, U.S.A) were used as molecular weight markers.

2.7. Functional properties

2.7.1. Protein solubility profiles

Solubility of protein blends was determined at seven different pH values (3–9) according to Jiang, Xiong, and Chen (2010) with slight modifications. Blends (250 mg) were suspended in 50 mL of 50 mM sodium citrate buffers (pH 3, 4, 5, 6) or 50 mM sodium phosphate buffers (pH 7, 8, 9). Final pH was readjusted if necessary with 1 M NaOH or 1 M HCl. The slurries were stirred for 1 h at room temperature (20–23 °C) and centrifuged at 1467*g* for 15 min at 21 °C (Eppendorf 5810, Germany). The protein concentration in the supernatant was measured according to Biuret method (Jennings, 1961), using BSA stock solution as a standard. Percent solubility was calculated by dividing protein content of the supernatant by total protein content of the isolate determined by using a nitrogen/protein analyzer (FP-528, Leco Corporation, St. Joseph, MI, U.S.A) following AACCI method 46-30.01 with nitrogen conversion factor of 6.25.

2.7.2. Emulsifying properties

Emulsifying activity index (EAI) and emulsifying stability index (ESI) of protein blends were measured according to Pearce and Kinsella (1978) with slight modifications. Six milliliters of 0.5% protein dispersion prepared in 50 mM phosphate buffer (pH 7) and 2 mL of pure corn oil (Wesson, Natural Corn Oil) were homogenized in homogenizer (Ultra-Turrax TP 18/10S1, Janke & Kunkel, Saufen, Germany) at the highest setting for 1 min. A 50 µL emulsion sample was taken from the bottom of the tube at 0 and 10 min after homogenization and diluted in 10 mL 0.1% sodium dodecyl sulfate solution. The absorbance of the emulsion was measured at 500 nm with UV/Visible Spectrophotometer (Ultrospec 4000, Pharmacia Biotech Braunschweig, Germany) using plastic cuvettes (1 cm path length).

EAI and ESI were calculated by using the following equations:

$$\mathrm{EAI}(m^2/g) = \frac{2 \cdot 2.303 \cdot A_o \cdot DF}{c \cdot \varphi \cdot 10000}$$

$$\mathrm{ESI}(\mathrm{min}) = \frac{A_o}{\Delta A} \cdot t$$

where, A_o is the absorbance of the diluted emulsion immediately after homogenization, DF is the dilution factor (×200), c is the concentration of protein (g/mL) in aqueous phase before emulsion formation, φ is oil volume fraction of the emulsion, ΔA is the change in absorbance between 0 and 10 min, and t is the time period (10 min).

2.7.3. Foaming properties

Foaming properties of the protein blends was determined according to Stone et al. (2015) with slight modifications. Protein dispersions (1% (w/w), based on protein content) were prepared with 50 mM sodium phosphate buffer (pH 7) and stirred overnight at 4 °C. Protein dispersions (15 mL) were foamed using an Omni Macro Homogenizer at 8000 rpm (Omni International, Marietta, GA, U.S.A) with a 20 mm saw tooth generating probe for 5 min and transferred to a 100 mL graduated cylinder. The foam volume was measured at time zero (V_0) and after 30 min (V_{30}). Foaming capacity (FC) and foaming stability (FS) were calculated by using the following equations:

$$\%\mathrm{FC} = \frac{V_0}{15} \times 100\%$$

$$\%\mathrm{FS} = \frac{V_30}{V_0} \times 100\%$$

2.7.4. Oil holding capacity

Oil holding capacity was determined as per Stone et al. (2015) and defined as the amount of oil absorbed by 1 g protein.

2.8. Surface hydrophobicity

Surface hydrophobicity (H_o) of the protein blends was determined according to Haskard and Li-Chan (1998). Fluorescence intensity was measured at 370 nm (excitation) and 470 nm (emission) using Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek, U.S.A). The initial slope (S_0) of fluorescence intensity against protein concentration (mg/mL) was plotted by linear regression and used as an index of H_o.

2.9. Sulfhydryl group and disulfide bond contents

The free and total SH and SS contents of the protein blends were determined as described by Tang and Sun (2010). The absorbance of the protein solutions was measured at 412 nm with UV/Visible Spectrophotometer (Ultrospec 4000, Pharmacia Biotech Braunschweig, Germany). The SH and total SH content (µmol/g) was calculated using the molar absorptivity of 3-carboxylate 4-nitrothiophenolate ion. The SS content (µmol/g) was calculated by subtracting the free SH content from the total SH content and dividing the result by 2.

2.10. Statistical analysis

Results were expressed as means with standard deviation. Data was analyzed using one-way analysis of variance (ANOVA). The least significant difference test, LSD Fisher, was performed using Minitab 17 (Minitab Inc., State College, PA, U.S.A) when treatment effects were significant ($P < 0.05$).

3. Results and discussion

3.1. Preliminary study results

Preliminary studies were conducted to evaluate the effect of DSI processing temperature and pH on the solubility profiles from pH 3–9 (data not shown). Solubilities of PR blends increased as DSI temperature was increased, with maximum solubility observed at 107 °C. In addition, solubilities were highest when PR blends were adjusted to alkaline pH values prior to DSI processing, as compared to pH 2 and 3. Based on these results, a DSI injection temperature of 107 °C at 9 (DSI-PR-pH9), 10 (DSI-PR-pH10), and 11 (DSI-PR-pH11) were selected for further study.

3.2. Chemical composition

The protein and ash content of PR blends after direct steam injection at 107 °C and three pH values were determined (Table 1). Protein contents did not differ among protein isolates and averaged 88.7% (dry weight basis). All subsequent chemical and functional tests were normalized for protein content. Ash ranged from 4.33% (PR) to 6.13%

Table 1
Ash, protein, available lysine, free and total sulfhydryl group (SH) and disulfide bond (SS) content and surface hydrophobicity (H_o) of pea:rice protein blends after direct steam injection at 107 °C and pH values of 9, 10, 11 (DSI-PR-pH9, DSI-PR-pH10, DSI-PR-pH11), and untreated blend (PR).

Sample	Ash (%)[1]	Protein (%)[2]	Available lysine (mg/g)[1]	Free SH (μmol/g)[1]	Total SH (μmol/g)[1]	SS bonds (μmol/g)[1]	H_o (a.u.)[1]
PR	4.33 ± 0.6^b	88.27 ± 0.0^a	61.04 ± 0.73^a	22.40 ± 0.80^b	46.89 ± 1.58^a	12.25 ± 1.07^c	32982 ± 3410^a
DSI-PR-pH9	5.59 ± 0.7^a	89.09 ± 1.2^a	60.41 ± 1.61^a	21.50 ± 1.16^b	47.87 ± 1.31^a	13.19 ± 0.60^b	26429 ± 3274^b
DSI-PR-pH10	5.48 ± 0.7^a	89.03 ± 2.0^a	59.97 ± 0.53^a	23.60 ± 1.11^a	46.50 ± 1.73^a	11.45 ± 0.93^c	23582 ± 2188^c
DSI-PR-pH11	6.13 ± 1.0^a	88.48 ± 1.0^a	61.53 ± 1.31^a	16.52 ± 1.19^c	47.35 ± 1.41^a	15.42 ± 0.86^a	19673 ± 1979^d

Means in the same column not sharing the same letter (a–b) are different ($P < 0.05$). Mean ± standard deviation;
[1] n = 3,
[2] n = 2.

(DSI-PR-pH11). Increases in ash content between the control and DSI treatments were caused by the addition of acid and base during pH adjustment before and after DSI processing. This corresponds to an increase in salt concentration of 1.3–1.9% which may be sufficient to affect overall solubility of the proteins (Jiang et al., 2010). It has been reported that pH shifting, by subjecting proteins to very high or low pH values and then back to neutral pH, may lead to alterations in protein functional (Jiang et al., 2010). However, our preliminary studies indicated that pH adjustment of the PR protein slurries without subsequent DSI processing had no effect on solubility profiles. It is possible that the protein denaturation and aggregation that occurred during the commercial isolate preparation process reduced the sensitivity of the proteins to pH adjustment.

3.2.1. Amino acid composition

Essential amino acids (EAAs) and branched-chain amino acids (BCAAs) content of PR protein blends after DSI were compared with the recommended amino acid pattern for children and older children (FAO, 2011), casein (FAO., 1981), whey protein, rice protein (Kalman, 2014), and pea protein (Cosucra., 2015) (Table 2). DSI at 107 °C and pH values of 9, 10, and 11 did not change the amino acid composition as compared to the PR control ($P > 0.05$). To the best of our knowledge, the influence of DSI in high pH conditions, on amino acid composition of PR protein blends has not been investigated. Previous studies reported that extrusion processing of pea protein slurries (60% moisture) at 140 °C (Osen et al., 2014) and soy protein slurries (60% moisture) at 160 °C (MacDonald, Pryzbyszewski, & Hsieh, 2009) had no effect on the amino acid composition.

Amino acid analysis confirmed that the 2:1 PR blend after DSI processing contained the expected amount of methionine and lysine (~16 mg/g protein and ~ 60 mg/g protein, respectively). These concentrations are similar to the FAO (2011) recommended values for a complete protein: 16.0 mg methionine/g protein and 57.0 lysine mg/g protein.

Additionally, PR protein blends had high concentrations of BCAAs, about 200 mg/g protein, which surpassed the concentration of BCAAs in casein but were lower than found in whey protein. BCAA (leucine, isoleucine, and valine) are involved in anabolic processes in protein metabolism. They are thought to prevent protein from degradation and promote protein synthesis, thus muscle growth, after physical activity (Blomstrand, Eliasson, Karlsson, & Köhnke, 2006). All treatments contained about 60 mg lysine/g protein and no differences were observed between the control and any DSI blend (Table 1). Although not found in the present study, high temperature and alkali treatment have been reported to create cross-linked amino acids such as lysinoalanine, which can reduce protein digestibility and bioavailability (Friedman, 1996).

3.3. SDS-PAGE

Protein bands were observed on top of the stacking gel and streaking was observed in the resolving gel in PR control indicating high molecular weight aggregates and proteins with poor solubility (Fig. 3). These high molecular weight insoluble aggregates were present in both R and NR conditions, were not disrupted by either SDS or β-mercaptoethanol (Guo et al., 2015). After DSI processing, smaller amounts of protein were observed at the top of both the stacking and resolving gels, suggesting disruption of these high molecular weight aggregates and the formation of smaller aggregates. Under reducing conditions, the protein patterns of DSI blends at pH 9, 10 and 11 were identical. No differences in protein bands were observed on SDS-PAGE between total and soluble protein fractions. Various pea protein and rice proteins were identified on the gel based on molecular weight and previous reports. Two major fractions of pea protein, albumin and globulin, were observed. Pea albumin was identified at 20 kDa. Pea globulins include vicilin, legumin, and convicilin (Roy, Boye, & Simpson, 2010). Pea vicilin is composed of three subunits (Barać et al., 2011) observed at 30, 35, and 50 kDa. Vicilin lacks cysteine, cannot form disulfide bridges, thus there was no differences between R and NR conditions. Legumin is a hexameric protein that consists of two subunits, acidic (α) at 40 kDa and basic (β) at 24 kDa, both of which were visible when electrophoresis was performed under R conditions. In NR conditions legumin α- and β-subunits were covalently linked by disulfide bonds (Taherian et al., 2011) and identified at 60 kDa in PR, DSI-PR-pH9, and DSI-PR-pH10. Pea legumin was not

Table 2
Essential amino acid (EAA) and branched-chain amino acid (BCAA) concentration (mg/g protein) of pea:rice protein blends (2:1 w/w) after direct steam injection at 107 °C and pH values of 9, 10, 11 (DSI-PR-pH9, DSI-PR-pH10, DSI-PR-pH11), and untreated blend (PR) (n = 3), compared with recommended amino acid pattern (FAO, 2011), casein (FAO, 1981), commercial pea protein isolate (Cosucra, 2015), whey protein, and commercial rice protein isolate (Kalman, 2014).

	Recommended amino acid pattern	Casein	Whey protein	Pea protein isolate	Rice protein isolate	PR	DSI-PR-pH9	DSI-PR-pH10	DSI-PR-pH11
Thr	27.00	29.70	72.00	39.00	27.99	39.02 ± 0.21	39.55 ± 0.21	39.14 ± 0.10	39.62 ± 0.05
Val	42.00	43.00	58.00	50.00	44.69	57.30 ± 0.01	56.92 ± 0.54	56.60 ± 0.07	55.75 ± 0.01
Ile	31.00	34.50	58.00	45.00	33.81	47.39 ± 0.33	47.56 ± 0.24	46.97 ± 0.22	46.84 ± 0.16
Met	16.00	17.80	19.00	11.00	22.79	16.54 ± 0.34	15.93 ± 0.05	16.11 ± 0.25	15.29 ± 0.01
Leu	66.00	60.70	102.00	84.00	62.03	90.07 ± 0.26	90.76 ± 0.13	90.06 ± 0.33	89.39 ± 0.07
His	18.00	18.60	22.00	25.00	16.99	26.02 ± 0.15	26.16 ± 0.08	25.83 ± 0.11	25.51 ± 0.21
Lys	57.00	51.80	96.00	72.00	21.20	59.87 ± 0.99	62.26 ± 0.17	59.91 ± 0.12	60.70 ± 0.01
BCAA	157.00	147.00	256.00	201.00	117.04	197.33	200.58	196.94	196.93

Thr. threonine; Val, valine; Ile, isoleucine; Met, methionine; Leu, leucine; His, histidine; Lys, lysine; BCAA, branched-chain amino acid (Leu + Val + Ile).
Mean ± standard deviation; n = 2.

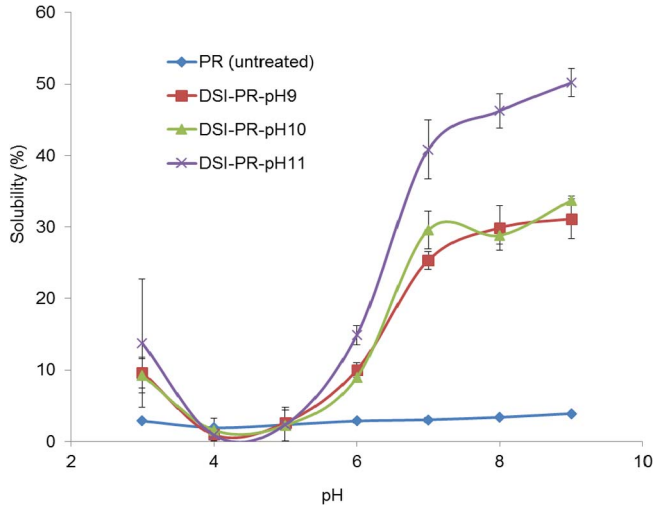

Fig. 2. Solubility profiles of pea:rice 2:1 ratio protein blends after direct steam injection at 1072 °C and pH values of 9, 10, 11 (DSI-PR-pH9, DSI-PR-pH10, DSI-PR-pH11) compared to untreated blend (PR). Error bars indicate standard deviation, n = 3.

observed under NR conditions in DSI-PR-pH11.

Rice proteins are poorly soluble and difficult to extract by electrophoresis. Acidic (α) and basic (β) subunits of the alkaline soluble glutelin protein with molecular masses of 45 kDa and 21 kDa, respectively (Amagliani, O'Regan, Kelly, & O'Mahony, 2016), were observed in R conditions in all samples. Rice glutelin subunits polymerize through disulfide bonds creating macromolecular complexes ranging from 64 to 500 kDa (Van Der Borght et al., 2006). Rice glutelin was not observed in DSI-PR-pH11 under NR conditions.

Pea legumin and rice glutelin create complexes via disulfide bonds. Neither the 70 kDa band attributed to rice glutelin nor the 60 kDa band of pea legumin were observed when processed by DSI at pH 11, although these bands were visible in DSI-PR-pH9 and DSI-PR-pH10 treatments. Jiang et al. (2010) reported that extreme alkaline pH treatment (pH 12) promoted cleavage of native disulfide bonds between α- and β-subunits in soy glycinin. Osen et al. (2014) reported the absence of legumin on SDS-PAGE gels under NR condition when pea protein isolate was extruded at 60% moisture content and 140 °C. The high temperature, high shear and/or high pH during DSI processing may have disrupted the native legumin and glutelin subunit structure which might have led to disulfide interchange reactions and creation of new polymers connected via disulfide bonds. More research is needed to determine if disulfide crosslinking and conformation changes led to the formation of smaller, more soluble macromolecules unable to enter the SDS-PAGE gel.

3.4. Surface hydrophobicity, sulfhydryl group and disulfide bond contents

All PR protein blends contained about 47 μmol of total SH groups/g protein (Table 1). In PR control, 22.4 μmol/g of SH groups were in free form and 12.25 μmol/g were as disulfide linkages. The free SH content (16.5 μmol/g) was lowest ($P < 0.05$) and SS bond content (15.4 μmol/g) was highest ($P < 0.05$) in DSI-PR-pH11. The H_o of DSI-PR blends decreased ($P < 0.05$) as pH of the treatment increased, with the lowest H_o observed at pH 11. The magnitude of changes in free SH, SS bonds contents, and H_o was dependent on treatment pH, and may partially explain the higher solubility of DSI-PR-pH11.

Conditions during production of the commercial protein isolates might lead to protein denaturation, and exposure of hydrophobic residues, thus creating aggregates with reduced protein solubility (Aluko et al., 2009; Liang & Tang, 2013). Changes in free SH, SS bonds contents and H_o indicate that DSI treatment induced alteration in three-dimensional conformation of PR proteins. Protein in their native form fold into the lowest possible free energy state such that hydrophobic residues are buried inside and hydrophilic residues are exposed on the surface allowing interaction with water. The decrease in H_o with DSI processing and pH adjustment suggests that the surface of the proteins became more hydrophilic and may explain the increased solubility.

Disulfide bonds link α- and β-subunits of pea legumin (Liang & Tang, 2013), and α- and β-subunits of the major rice protein, glutelin (Cao et al., 2009). Monahan, German, and Kinsella (1995) reported that at alkaline pH, proteins become negatively charged inducing electrostatic repulsion, thus altering protein conformation and aggregation properties. The high shear and temperature in combination with high pH during DSI may have resulted in oxidation of free thiol groups, leading to the creation of new intra- or intermolecular disulfide bonds when the proteins refolded on cooling.

3.5. Functional properties

3.5.1. Protein solubility profiles

Solubility of the PR control was less than 4% and was insensitive to changes in pH (Fig. 2). DSI treatment partially restored the typical U-shape solubility profile expected of native pea and rice proteins. The solubility of all DSI-PR protein blends was lowest at pH 4–5, near the isoelectric point (IP) of pea and rice proteins (Barać et al., 2011; Cao et al., 2009). At pH 3, below the IP, solubility of all DSI blends was 14% or less. In general, DSI-PR-pH11 was more soluble than DSI-PR-pH9 and DSI-PR-pH10 at pH 6 and above. Solubility of DSI-PR-pH11 ranged from 14.9% at pH 6–50.1% at pH 9. DSI blends were most soluble at pH 9, which follows the typical solubility profile of native rice (Cao et al., 2009), and pea (Barać et al., 2011) protein. These results are in agreement with those of C. Wang and Johnson (2001) who reported that DSI at 154 °C for 11–42 s improved the solubility of soy protein isolates. The authors speculated that disruption of insoluble protein aggregates by high-shear mixing during DSI may lead to hydrodynamic transformations that improve solubility. Guo et al. (2015) hypothesized that protein solubilization during longer heating times was attributed to changes in noncovalent interactions and/or disulfide bonds leading to the formation of more soluble aggregates.

3.5.2. Emulsifying properties

The emulsifying activity index (EAI) of PR protein blends was improved ($P < 0.05$) by DSI treatment and increased with pH (Table 3). The DSI-PR-pH11 had the highest EAI at 52.5 m^2/g and was about 10-fold greater than the control. The emulsifying stability index (ESI) of PR blends ($P < 0.05$) decreased from 22 min for the control to 11–12 min for DSI treatments, and was independent of pH. The poor emulsifying ability of commercial pea (Barać et al., 2011) and rice (Wang et al., 2016) protein has been related to low protein solubility and high H_o. The ESI is related to molecular size of protein and protein flexibility (McClements, 2015). Higher solubility and lower surface hydrophobicity have been previously correlated with improved emulsifying activity but decreased stability (Stone et al., 2015).

3.5.3. Foaming properties

The FC of PR blend (264%) was not affected by DSI ($P > 0.05$) (Table 3). FS of PR blends was improved by DSI treatment ($P < 0.05$), increasing from 68% in control to 84% in DSI-PR-pH10, but did not differ by pH of DSI treatments. FC of proteins depend on protein solubility and molecular flexibility, which allows proteins to migrate to the surface of an air bubble and unfold to form films at air/liquid interfaces. Globular protein such as those in pea and rice are less suitable for foam formation than flexible, highly soluble protein, such as egg and meat proteins (Xiong, 1997).

3.5.4. Oil holding capacity

Oil holding capacity increased after DSI treatment from 1.76 g/g protein (control) to 4.90 g/g protein ($P < 0.05$) (Table 3). Oil holding

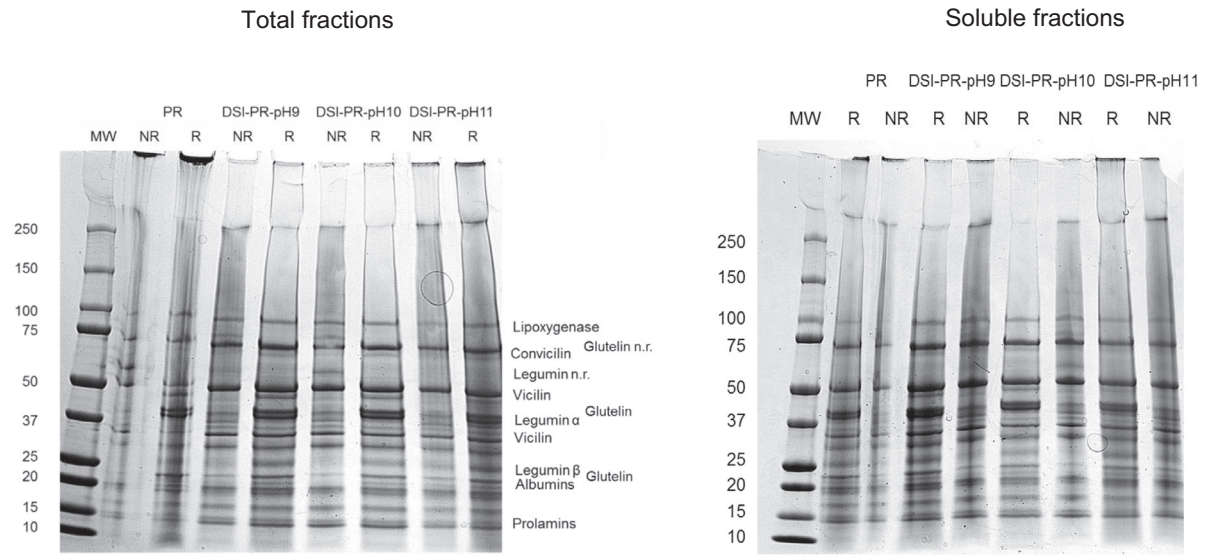

Fig. 3. SDS-PAGE pattern of total and soluble protein fraction under reducing (R) and non-reducing (NR) conditions of pea:rice protein blends after direct steam injection at 107 °C and pH values of 9, 10, 11 (DSI-PR-pH9, DSI-PR-pH10, DSI-PR-pH11), and untreated.

Table 3
Functional properties of pea:rice protein blends after direct steam injection at 107 °C and pH values of 9. 10. 11 (DSI-PR-pH9. DSI-PR-pH10. DSI-PR-pH11) compared to untreated blend (PR). FC, foaming capacity; FS, foaming stability; T1/2 foam half-life; EAI, emulsifying activity index; ESI, emulsifying stability index; OHC, oil holding capacity.

	EAI (m^2/g)	ESI (min)	FC (%)	FS (%)	OHC
PR	5.9 ± 0.4^{d}	22 ± 3.1^{a}	264.4 ± 16.8^{ab}	68.2 ± 3.4^{b}	1.76 ± 0.58^{b}
DSI-PR-pH9	44.1 ± 1.3^{c}	12 ± 0.4^{b}	256.3 ± 19.2^{ab}	81.5 ± 3.3^{a}	4.90 ± 0.60^{a}
DSI-PR-pH10	48.3 ± 0.7^{b}	11 ± 0.2^{b}	248.2 ± 17.6^{b}	84.0 ± 5.7^{a}	4.61 ± 0.46^{a}
DSI-PR-pH11	52.5 ± 1.0^{a}	12 ± 0.2^{b}	270.4 ± 15.7^{a}	82.8 ± 5.6^{a}	4.24 ± 0.38^{a}

Means in the same column not sharing the same letter (a, b) are different (m 0.05).
Mean ± standard deviation; n = 3.

capacity of proteins is an important functional property which improves mouth feel and flavor in applications such as meat replacers (Kinsella & Melachouris, 1976). Disruption of the large aggregates as observed on SDS-PAGE may have increased the surface area of protein available to bind oil.

4. Conclusions

The study demonstrated the potential use of DSI processing for improving the functional properties of blended PR isolates without affecting the essential amino acid composition. This can provide an appRoach and Gehrke to extend the use of these ingredients for numerous food applications. Although more work is needed, our results suggest that changes in solubility and functional properties may be due to conformational changes in protein during DSI processing induced by high temperature and alkaline pH. Future studies should examine the sensory profile of PR blend before and after DSI treatment in potential protein beverage and other related applications.

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Author Contributions

E. Pietrysiak designed the study, conducted experiments, completed statistical analysis, interpreted results, and drafted the manuscript as part of her M.S. thesis. G. Ganjyal, and D. Smith conceptualized and designed the study, supervised data collection, interpreted results, and edited the manuscript. B. Smith reviewed the experimental design and methods, interpreted results and edited the manuscript.

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