Research Article

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Effect of drying methods on the structure, thermo and functional properties of fenugreek (*Trigonella foenum graecum*) protein isolate

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Abstract

BACKGROUND: Different drying methods due to protein denaturation could alter the functional properties of proteins, as well as their structure. So, this study focused on the effect of different drying methods on amino acid content, thermo and functional properties, and protein structure of fenugreek protein isolate.

RESULTS: Freeze and spray drying methods resulted in comparable protein solubility, dynamic surface and interfacial tensions, foaming and emulsifying properties except for emulsion stability. Vacuum oven drying promoted emulsion stability, surface hydrophobicity and viscosity of fenugreek protein isolate at the expanse of its protein solubility. Vacuum oven process caused a higher level of Maillard reaction followed by the spray drying process, which was confirmed by the lower amount of lysine content and less lightness, also more browning intensity. ΔH of fenugreek protein isolates was higher than soy protein isolate, which confirmed the presence of more ordered structures. Also, the bands which are attributed to the α-helix structures in the FTIR spectrum were in the shorter wave number region for freeze and spray dried fenugreek protein isolates that show more possibility of such structures.

CONCLUSION: This research suggests that any drying method must be conducted in its gentle state in order to sustain native structure of proteins and promote their functionalities.

Keywords: amino acid content; differential scanning calorimetry; drying method; fenugreek protein isolate; FTIR analysis; functional and surface properties

INTRODUCTION

Fenugreek (*Trigonella foenum graecum*) is an important crop belonging to Fabaceae family, which is extensively cultivated in Mediterranean countries and Asia.[1] Studies have shown fenugreek seeds are a great source of plant proteins with total protein content of about 250 to 386 g kg^{-1}.[1–3] Among different ingredients, plant protein isolates are highly demanded as food ingredients due to their nutritional and functional properties.[4] Plant proteins could offer a wide range of functional properties. Their functional attributes originate from molecular size, charge distribution and three-dimensional structure. The structure–function relationships of proteins determine the way they interact with themselves and other ingredients in a complex food system.[5] Several researchers showed that different procedures including defatting solvent,[6] extraction parameters and conditions[4,5] have considerable effects on the protein content, amino acid profile, and especially the thermo and functional properties of fenugreek flour and its protein isolate. It is noteworthy that although studies have shown that the drying method has considerable effect on functional properties of different protein isolate powders including gingerbread plum seed,[7] lentil[8] and chia seed[9] protein isolates, there is no available research on this aspect for fenugreek protein isolate. In spite of the fact that drying procedure may cause formation of irreversible insoluble aggregates, it improves long-term and stable storage condition for protein isolate powders.[10] Due to their effect on protein structure and denaturation, different drying methods can alter protein functional properties. While the common commercial drying method for protein isolates is spray drying, most researchers use freeze drying to produce protein isolate powders. On the other hand, vacuum oven drying is relatively a low cost method, where drying temperature could be adjusted below the denaturation temperature (T_d) of protein isolate. So, in the present research we investigated the effect of different drying procedures including spray, freeze and vacuum oven drying on amino acid profile, protein structure, thermo and functional properties of fenugreek protein isolate (FPI).

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MATERIALS AND METHODS

Materials

Fenugreek seeds were obtained from a retail market in Esfahan, Iran. They were washed with tap water, and then milled using electrical miller (M20; IKA, Königswinter, Germany). Fenugreek seed flour was defatted using *n*-hexane by mixing at a ratio of 1:4 (w/v) and continuously stirring for 3 h at room temperature. Defatted fenugreek flour (DFF), and undefatted fenugreek flour (UDFF) were sieved by a 50 mesh sieve. The UDFF contained 347.0 g kg^{-1} protein, 57.6 g kg^{-1} lipid, 547.3 g kg^{-1} carbohydrate and 47.9 g kg^{-1} ash based on dry weight basis; while the DFF contained 514.0 g kg^{-1} protein, 12.5 g kg^{-1} lipid, 435.5 g kg^{-1} carbohydrate and 37.9 g kg^{-1} ash. All proximate chemical compositions were determined according to AOAC methods.[11] Commercial soy protein isolate was obtained from Fuji Oil Co. (Hyogo, Japan).

All the chemicals used for defatting and chemical analysis were of analytical grade. Chemicals for protein content analysis, protein solubility, coagulation and emulsifying properties were purchased from Merck KGaA (Darmstadt, Germany), and those for electrophoresis and high-performance liquid chromatography (HPLC) analysis were obtained from Bio-Rad Laboratories Inc. (Hercules, CA, USA), and Sigma chemical Co. (St. Louis, MO, USA), respectively.

Methods

Protein extraction and drying process

Protein isolate extraction was performed according to the method described by Feyzi *et al.*[3] who optimised extraction of FPI using pH 9.25 and NaCl concentration of 0.33 mol L^{-1}. In brief, DFF was added to 0.33 mol L^{-1} NaCl solution and pH was adjusted at 9.25 using 1 mol L^{-1} NaOH. The stirring period was 2 h during which the pH was adjusted, too. The slurry was centrifuged at 4500 × *g* for 30 min and the supernatant was adjusted at pH 4.5 (isoelectric pH of fenugreek protein) using 1 mol L^{-1} HCl. The protein solution was centrifuged at 4500 × *g* for 20 min in order to precipitate the protein. Deionised water and protein precipitate (2:1 v/v) were mixed thoroughly. The slurry was centrifuged for 5 min at 4500 × *g*. The final precipitate was re-solubilised in deionised water (2:1 v/v) followed by adjusting the pH at 7.2, using NaOH 1 mol L^{-1}. The final protein isolate solution was either freeze dried (Martin Christ, Osterode am Harz, Germany), at pressure of 100 mmHg and at −30 °C in a drying chamber, vacuum oven dried overnight at 50 °C and pressure of 560 mmHg (OVEN-OT53; Ovenco, Tehran, Iran), or spray dried with inlet and outlet air temperature of 160 °C and 60 °C, respectively (B290; Buchi, Osterode am Harz, Germany). All FPI powders were stored at 4 °C for further analysis.

Amino acid composition

To determine amino acid composition, a 50 mg sample from each FPIs was hydrolysed under nitrogen in 6 mol L^{-1} HCl at 110 °C for 22 h. Precolumn derivatisation was carried out using *o*-phthaldialdehyde (OPA). Chromatography was performed using a Knauer high-performance liquid chromatographic system, (Berlin, Germany) which was equipped with a gradient controller (Manager 5000), fluorescence detector (Knauer, RF-20 Axs) set at 330 nm and 450 nm as excitation and emission wavelengths, respectively, and a Dikma C18 column (250 mm × 4.6 mm, with 5 µm particle size) maintained at 30 °C. The solvents system for linear gradient elution consisted of two eluents. Solvent A was a solution of 50 mmol L^{-1} sodium acetate, water and tetrahydrofurane, with pH 7. Solvent B consisted of 300 mmol L^{-1} sodium acetate buffer, methanol and tetrahydrofuran at pH 5.[7] Amino acid contents are reported based on g kg^{-1} of protein isolate.

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectra of FPIs were obtained from KBr discs. Each disc contained an approximately 1 mg of sample and 100 mg of KBr. The spectra were recorded in the 450 to 4000 cm^{-1} range on a Spectrum One FT-IR Spectrophotometer (Bruker, Karlsruhe, Germany).

Functional properties

Protein solubility

Protein solubility of FPIs was determined at pH 2–10. A 15 g L^{-1} (w/v) solution of each sample was made in deionised water and treated with either 0.5 mol L^{-1} HCl or 0.5 mol L^{-1} NaOH to obtain a certain pH (2–10), then stirred for 30 min at room temperature. The slurry was centrifuged at 5000 × *g* for 15 min to separate the supernatant.[3] Protein content was determined by the Biuret method using a UV–visible spectrophotometer (UV–2601; Rayleigh, Beijing Shi, China) at 540 nm.[12] Calibration was done using bovine serum albumin (BSA) as external standard at varying concentrations from 0 to 10 mg mL^{-1}.

Coagulated protein

Percentage of coagulated protein was measured according to the method described by Kramer and Kwee.[13] A 0.2 g sample of each FPIs was dissolved in 10 mL of 0.025 mol L^{-1} citrate–phosphate buffer (pH 7.0) and stirred, then it was centrifuged. Biuret reagent was added to the supernatant, and it was stored in a dark place. The remaining supernatant was heated for 15 min at 100 °C using a water bath; after cooling the same procedure was repeated. The absorbance of both samples, before heating (A_1) and after heating (A_2), was measured at 540 nm. The percent of coagulated protein was calculated by the following equation:

$$\%\text{coagulated protein} = \left(A_1 - A_2/A_1\right) \times 100 \quad (1)$$

Water and oil binding capacity

Water and oil binding capacity (WBC and OBC) were determined using the method described by Timilsena *et al.*[9] One gram of each four samples (W_0) was weighed in a centrifuge tube. For each samples 10 mL of distilled water or sunflower oil (V_1) was added to FPI, and mixed with vortex (Reax control; Heidolph, Schwabach, Germany). Samples were allowed to stand at room temperature for 30 min, and then centrifuged at 3000 × *g* for 20 min (Centrifuge 5430; Eppendorf, Hamburg, Germany). The supernatant was decanted into a graduated cylinder, and the volume of decanted water or oil was recorded (V_2). Water or oil binding capacity was expressed as the milliliters of water or oil per gram of FPIs by following equation:

$$\text{WBC (OBC)} = \left(V_1 - V_2\right)/W_0 \quad (2)$$

Dynamic surface and interfacial tensions

Dynamic surface tension (σ) and dynamic interfacial tension (Υ) were measured applying Du Nouy ring method,[14] while the Kruss Processor Tensiometer K 100 was used.

To measure σ or Υ, a 10 mg mL^{-1} solution of each FPI was prepared in deionised water, followed by centrifugation at 7000 × *g* for 15 min. The supernatants were filtered using Whatman filter paper number 42 pore size (2.5 µm) to eliminate insoluble materials.

Foaming capacity and stability

Foaming capacity (FC) and stability (FS) were determined using the method described by Timilsena *et al.*,[9] with slight modifications. A 20.0 g L^{-1} (w/v) aqueous solution from each FPI was prepared. Solutions were then whipped vigorously by a disperser (T 25 digital ULTRA-TURRAX; IKA) at 10 000 rpm. The volume of solution before whipping and the total volume after whipping were recorded as V_0 and V_1, respectively. The foam capacity (FC) percentage was calculated as:

$$FC\ (\%) = \left[\left(V_1 - V_0\right)/V_0\right] \times 100 \quad (3)$$

Foam stability (FS) was determined as the volume of the foam after 60 min at room temperature. The total volume of the solution and foam after 60 min was recorded as V_2. FS was calculated as:

$$FS\ (\%) = \left(V_2 - V_0\right) \times 100/\left(V_1 - V_0\right) \quad (4)$$

Emulsifying properties

Emulsifying activity (EAI) and stability (ESI) indices were determined using the method of Pearce and Kinsella,[15] while emulsifying capacity (EC) was determined using the method described by Neto *et al.*[16] with slight modifications. In order to prepare samples for EAI and ESI, 0.0225 g of each FPI was weighed in a beaker, 4.5 mL phosphate buffer (pH 7) was added and the mixture was stirred [sample concentration (E) was 0.005 g mL^{-1}]. Then sunflower oil was added and the mixture was homogenised at 22 000 rpm with a disperser (T 25 digital ULTRA-TURRAX; IKA). For ESI measurement, immediately 250 μL of the homogenised solution at time zero, and then at time 15 min was pipetted, and mixed with 50 mL of 1 g L^{-1} sodium dodecyl sulfate solution. Absorbance of diluted emulsions at times zero (A_0) and 15 min (A_{15}) was recorded at 500 nm with a UV–visible spectrophotometer (UV–2601; Rayleigh, Beijing Shi, China). ESI (min) was calculated as:

$$ESI = \left[A_0/\left(A_0 - A_{15}\right)\right] \times t \quad (6)$$

where $t = 15$ min.

In order to determine EAI, 800 μL of homogenised emulsion was immediately transferred into a pre-weighed dish (A). The weight of dish and emulsion was recorded (B), and then it was placed in a 120 °C oven for 2 h. The weight of dish was recorded after drying as well (C). EAI (m^2 g^{-1}) was calculated as:

$$EAI = \left(4.606 \times A_0 \times 200\right)/\left(\Phi \times 0.005 \times 10\,000\right) \quad (7)$$

where:

$$\Phi = [(C - A) - E\,(B - C)]/[(C - A) + 0.918\,(B - C)] \quad (8)$$

For EC, a 10.0 g L^{-1} aqueous solution from each FPI was prepared, then sunflower oil was added slightly. The mixture was homogenised at 5000 rpm for 2 min with a disperser (T 25 digital ULTRA-TURRAX; IKA) to make an emulsion and then was centrifuged at 1000 × g for 2 min. The height of both emulsified layers after centrifugation (H_1) and the total contents in the tube before centrifugation (H_0) were measured. Emulsion capacity (EC) was calculated as:

$$EC\ (\%) = \left(H_1/H_0\right) \times 100 \quad (9)$$

Browning intensity

Estimation of Maillard reaction and browning intensity was performed using a UV–visiblle spectrophotometer (UV–2601; Rayleigh, Beijing Shi, China). Absorbance at 294 nm (A_{294}) is an indicator of intermediate products of Maillard reaction, while absorbance at 420 nm (A_{420}) estimates the content of the final products.[17] A solution of 1.6 mg mL^{-1} in deionised water was prepared from each sample. The solutions were centrifuged at 6500 × g for 5 min. Final supernatants were used to collect absorbences against deionised water as blank.

Colour parameters

A digital colorimeter (Choroma meter CR-410; Konica Minolta, Tokyo, Japan) was used to measure the colour of FPI samples in $L^*a^*b^*$ colour space. A cylindrical plastic dish (58 mm in diameter and 15 mm in depth) containing the same quantity of samples was placed at the light port (50 mm in diameter). The instrument was initially calibrated with a white standard tile with $L^* = 98.14$, $a^* = -0.23$, $b^* = 1.89$.

Surface hydrophobicity

The H_0 measurement was done using the fluorescent probe 8-anilinonaphthalene-1-sulfonic acid (ANS).[18] Stock protein isolates solution (0.1 g L^{-1}) were prepared in 0.01 mol L^{-1} sodium phosphate buffer solution (pH 7.0). A series of protein solutions was prepared with concentrations ranging from 0.02 to 0.1 g L^{-1} in 0.01 mol L^{-1} phosphate buffer. An aliquot of 4 mL of these solutions was reacted with 20 μL of 0.008 mol L^{-1} ANS in 0.01 mol L^{-1} sodium phosphate buffer, and mixed by vortex. The procedure was completed by keeping each sample for 15 min in the dark, followed by reading their fluorescence intensities using a spectrofluorophotometer (RF-1501; Shimadzu, Kyoto, Japan) at wavelengths of 390 nm (excitation) and 470 nm (emission). The H_0 was determined as a slope of the linear regression between relative fluorescence intensities and protein concentrations.

Rheological characteristics

Viscosity of FPIs was determined according to the method described by Chakraborty,[19] with slight modifications. A 50.0 g L^{-1} suspension of each sample was prepared. Viscosities of samples were measured using programmable Brookfield rheometer (LVDV III–Ultra; Brookfield, Middleboro, MA, USA), with spindle ULA at room temperature. Shear rates from 1 (s^{-1}) to 54 (s^{-1}) was applied. Based on the best fitted model (newtonian, power law and Herschel Bulkley) on the experimental data, with highest R^2, different parameters were determined. It was revealed that the power law model was the most adequate one. This model is expressed as:

$$\tau = k\gamma^n \quad (10)$$

where τ is the shear stress (Pa); γ denotes the shear rate (s^{-1}); k or m represents the consistency coefficients (Pa s^n); and n stands for the flow behaviour index (dimensionless). The equation parameters, including slope of the curve (m) and flow behaviour index (n), were obtained. Also, the apparent viscosities at shear rates of 15, 30 and 50 (s^{-1}), as the selected shear rates, were determined.

Differential scanning calorimetry

Calorimetric measurement was carried out using a differential scanning calorimetry (DSC) instrument (200 F3 Maia; NETZSCH, Selb, Germany). Four milligrams of each FPI was accurately

Table 1. Amino acid composition of fenugreek and soy protein isolates (g kg^{-1})

Amino acid	FFPI	SFPI	VFPI	SPI
Aspartic acid	91.30^{b}	92.00^{b}	78.70^{c}	97.50^{a}
Glutamic acid	158.20^{a}	156.60^{a}	137.00^{a}	152.90^{a}
Histidine	15.90^{d}	27.70^{a}	26.60^{b}	20.50^{c}
Serine	32.20^{b}	49.60^{a}	45.50^{a}	44.00^{a}
Arginine	76.10^{b}	88.90^{a}	79.10^{a}	64.60^{c}
Glycine	37.90^{ab}	44.70^{a}	38.80^{ab}	36.00^{b}
Threonine	47.60^{a}	28.50^{b}	24.80^{d}	27.60^{c}
Alanine	32.00^{b}	35.20^{a}	30.90^{b}	36.10^{a}
Tyrosine	20.70^{b}	19.50^{b}	15.60^{c}	27.60^{a}
Methionine	5.900^{b}	ND	ND	7.600^{a}
Valine	39.10^{a}	30.40^{c}	26.60^{d}	37.70^{b}
Phenylalanine	28.40^{d}	35.20^{b}	30.80^{c}	39.50^{a}
Isoleucine	40.40^{b}	34.80^{b}	39.70c	43.40a
Leucine	63.00^{a}	52.80^{b}	55.10^{c}	58.00^{b}
Lysine	54.30^{a}	49.60^{ab}	40.10^{b}	51.60^{a}
Acidic	249.50 ± 0.07^{a}	248.60 ± 0.17^{a}	215.70 ± 0.09^{b}	250.40 ± 0.04^{a}
Basic	146.30 ± 0.06^{b}	166.20 ± 0.01^{a}	145.90 ± 0.68^{b}	136.70 ± 0.02^{b}
Hydrophobic	218.80 ± 0.01^{b}	198.40 ± 0.34^{c}	173.00 ± 0.04^{d}	224.30 ± 0.01^{a}
Charged hydrophilic	385.80 ± 0.03^{b}	404.80 ± 0.16^{a}	371.50 ± 0.58^{c}	387.10 ± 0.05^{b}
Uncharged hydrophilic	138.40 ± 0.00^{a}	142.20 ± 0.87^{a}	124.70 ± 0.37^{ab}	111.80 ± 0.06^{b}
Total	743.00 ± 0.15^{a}	745.50 ± 0.16^{a}	669.30 ± 0.13^{b}	744.60 ± 0.08^{a}

FFPI, Freeze dried fenugreek protein isolate; SFPI, Spray dried fenugreek protein isolate; VFPI, Vacuum oven dried fenugreek protein isolate; SPI, Soy protein isolate.
Acidic: aspartic acid, glutamic acid; Basic: lysine, arginine, histidine; Hydrophobic: alanine, isoleucine, leucine, methionine, phenylalanine, valine; Charged polar: basic and acidic amino acids; Uncharged polar: glycine, serine, tyrosine, threonine.
ND, not determined.
Different letters in each row indicate significant differences ($P < 0.05$).

weighed into aluminium pans. The pans were hermetically sealed and heated at temperatures ranging from 20 to 200 °C with the scan rate of 10 °C min^{-1}. Transition temperatures (T_o, onset temperature of denaturation; T_d, maximum temperature of denaturation; T_e, end set temperature of denaturation) and denaturation enthalpy (ΔH, area below the curve in J g^{-1} of protein isolate) were measured.[7]

Electrophoresis pattern

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was carried out according to the method described by Laemmli,[20] with slight modifications that Feyzi *et al.* conducted for FPI.[3] A vertical slab gel of 1 mm thickness containing 12% acrylamide separating gel and 4% stacking gel and Bio-Rad Mini-protean electrophoresis system (Mini-Protean tetra Cell; Bio-Rad Laboratories) was used. Samples were prepared under two conditions: non-reduced (in the absence of 2-mercaptoethanol) and reduced (in the presence of 2-mercaptoethanol). FPIs were placed in sample buffer at room temperature, following heating at 85 °C in a water bath. Then samples were loaded onto the gel and electrophoresis was performed at a constant voltage of 100 V. Gels were stained with Coomassie brilliant blue R-250 (Sigma Chemical Co.) staining solution for 12 h, and destained for 48 h in 1% (v/v) acetic acid and scanned by a laboratory scanner. A prestained protein ladder (CinnaGen Co. Tehran, Iran) with a molecular weight of 10–245 kDa was used as standard. The molecular weights and relative amounts of each protein band were approximated by matching with the migration patterns of the ladders using Total Lab 120 software (Nonlinear Dynamics Ltd, Newcastle-upon-Tyne, UK). Finally, differences between lanes of samples were evaluated using the Minitab 16 software (Minitab Inc., State College, PA, USA).

Statistical analysis

All reported data for different parameters were an average of triplicate observations, and were subjected to analysis of variance (ANOVA) using SPSS Statistical Software version 16. Tukey's multiple range test was used to compare means. Significance level was equal to $P < 0.05$.

RESULTS AND DISCUSSION

Amino acid composition

Aspartic acid and glutamic acid are the main amino acids in plant proteins,[7] which results in acidic isoelectric point of plant proteins such as FPI.[2,3] According to Table 1, the drying method had a significant effect ($P < 0.05$) on acidic amino acids of FPIs. In general, different groups of amino acids in Table 1 such as acidic, charged hydrophilic, uncharged hydrophilic and hydrophobic ones as well as total amino acid content of VFPI were significantly lower than the others ($P < 0.05$).

It has been proved that Ser, Lys, Cys and Met are more sensitive to alkaline pH, higher extraction temperature and longer extraction time compared to the other amino acids, and among them Cys showed the highest sensitivity.[21] Also, Iwe *et al.*[22] reported that Lys, Arg, Trp, Cys, Met and His are the most heat sensitive amino acids, especially Lys, which is an indicator of the Maillard reaction. These findings are in accordance with a lower Lys level in VFPI, which confirms the occurrence of the Maillard reaction. During vacuum oven drying, despite the presence of little oxygen in the early stage

Table 2. Surface and functional properties, browning intensity, colour parameters and rheological characteristics of fenugreek protein isolates

Property	FFPI	SFPI	VFPI	SPI
Surface and functional properties				
Coagulated protein (%)	3.17 ± 0.07^{a}	7.27 ± 0.50^{a}	4.17 ± 0.18^{a}	4.17 ± 0.06^{a}
WBC (mL g^{-1})	2.70 ± 0.14^{b}	2.50 ± 0.20^{b}	2.30 ± 0.14^{b}	5.95 ± 0.21^{a}
OBC (mL g^{-1})	6.60 ± 0.28^{a}	3.60 ± 0.05^{b}	1.85 ± 0.30^{c}	5.75 ± 0.21^{a}
σ (mN m^{-1})	46.69 ± 0.94^{b}	46.08 ± 0.28^{b}	49.68 ± 0.54^{a}	49.88 ± 0.69^{a}
Υ (mN m^{-1})	8.96 ± 0.50^{b}	8.77 ± 0.18^{b}	9.23 ± 0.19^{b}	14.00 ± 0.69^{a}
FC (%)	60.00 ± 0.31^{ab}	78.80 ± 0.7^{a}	46.00 ± 0.89^{b}	36.00 ± 0.81^{b}
FS (%)	86.43 ± 1.05^{a}	76.16 ± 1.11^{a}	45.38 ± 0.61^{b}	14.63 ± 0.09^{c}
EAI (m^2 g^{-1})	93.28 ± 0.86^{ab}	178.90 ± 0.90^{a}	80.40 ± 0.63^{ab}	22.40 ± 0.48^{c}
EC (%)	28.89 ± 0.95^{a}	30.00 ± 0.57^{a}	31.11 ± 0.14^{a}	27.78 ± 0.57^{a}
ESI (s)	22.23 ± 0.80^{b}	18.24 ± 0.18^{b}	45.69 ± 0.33^{a}	23.43 ± 0.01^{b}
H_0	182.90 ± 1.50^{c}	230.67 ± 1.60^{b}	249.54 ± 1.70^{ab}	257.98 ± 0.94^{a}
Browning intensity				
Absorbance at 294 nm	0.49 ± 0.01^{c}	0.57 ± 0.02^{b}	0.62 ± 0.01^{a}	0.36 ± 0.01^{d}
Absorbance at 420 nm	0.15 ± 0.03^{c}	0.17 ± 0.05^{b}	0.18 ± 0.07^{a}	0.14 ± 0.01^{d}
Colour parameters				
L^*	83.495 ± 0.91^{b}	80.11 ± 0.43^{c}	36.30 ± 0.75^{d}	87.23 ± 0.14^{a}
a^*	-1.35 ± 0.01^{a}	-1.22 ± 0.10^{a}	6.48 ± 0.30^{b}	19.80 ± 0.73^{a}
b^*	24.60 ± 0.08^{a}	23.70 ± 0.24^{a}	16.40 ± 0.30^{c}	19.80 ± 0.73^{b}
Rheological characteristics				
m	0.2133 ± 0.89^{a}	0.0122 ± 0.01^{b}	0.1163 ± 0.02^{ab}	0.0136 ± 0.01^{b}
n	0.767 ± 0.08^{b}	1.394 ± 0.00^{ab}	1.074 ± 0.11^{ab}	2.016 ± 0.51^{a}
Apparent viscosity (cP) at shear rate of 15 s^{-1}	1150.00 ± 2.20^{b}	852.70 ± 0.68^{b}	2170.70 ± 3.68^{a}	1905.41 ± 3.68^{ab}
Apparent viscosity (cP) at shear rate of 30 s^{-1}	922.60 ± 1.37^{ab}	405.22 ± 0.03^{b}	1771.60 ± 1.24^{ab}	2339.61 ± 4.43^{a}
Apparent viscosity (cP) at shear rate of 50 s^{-1}	785.50 ± 0.94^{ab}	347.17 ± 0.07^{b}	1077.10 ± 0.74^{ab}	1556.80 ± 0.62^{a}

FFPI, freeze dried fenugreek protein isolate; SFPI, spray dried fenugreek protein isolate; VFPI, vacuum oven dried fenugreek protein isolate; SPI, soy protein isolate; WBC, water binding capacity; OBC, oil binding capacity; σ, dynamic surface tension; Υ, dynamic interfacial tension; FC, foaming capacity; FS, foam stability; EAI, emulsifying activity index; EC, emulsifying capacity; ESI, emulsifying stability index; H_0, surface hydrophobicity.
Results are given as the mean ± SD of triplicates.
Different letters in each row indicate significant differences ($P < 0.05$).

and little moisture in the later stage, the Maillard reaction could not be totally avoided.[7] According to our analysis, Met was not detected in the SFPI and VFPI samples. Moreover, the content of Lys and Met in SFPI were less than FFPI, which could be attributed to the progression of the Maillard reaction at high temperature and the oxidation of Met at elevated temperature in the presence of oxygen, respectively. A lower L^* value in SFPI, beside the higher a^* value (Table 2) compared with FFPI confirm these results.

Fourier transform infrared spectroscopy

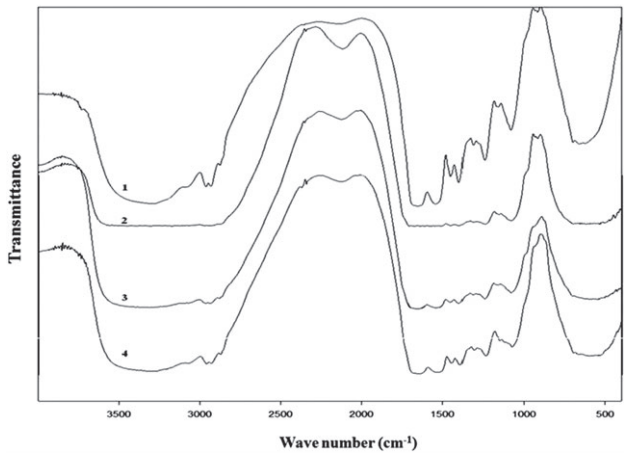
Fourier transform infrared spectroscopy (FTIR) measurement of secondary structure in proteins highlights the mechanism of protein aggregation and stability.[23,24] The bands at 1600–1700 cm^{-1} are associated with amide I in protein vibrations, which have been widely used to reflect the secondary structure of proteins.[25] While presence of peak in the region of 1650.7 to 1655 cm^{-1} (Fig. 1) in all samples shows the α-helix structure,[25–27] shorter wavenumbers in the region of 1650.7, 1651 and 1652.42 cm^{-1}, of FFPI, SFPI, and VFPI compared to 1655.04 cm^{-1} for SPI confirms the greater helix structure length in FPIs in comparison with SPI.[25] Moreover, this finding shows that freeze and spray drying methods increased the probability of helix structure in polypeptide chain length of FPI rather than vacuum oven drying method.[25] Also, there was a weak band at 1615.61 cm^{-1} in the FTIR spectrum of SFPI. This band could be attributed to a β-sheet structure,[25] especially the intermolecular ones.[28,29] This may occur due to the conversion of helix structures to β-sheet structures, as more stable ones, in the cooling step after the drying process.[29,30] It has been reported that the extent of the α-helix structure of proteins may reduce during heat treatment, while a new band at 1615 cm^{-1} may appear due to the formation of intermolecular β-sheet structures.[29] It has been stated that the appearance of a new sharp or broad band at 1615 cm^{-1} may show protein aggregations.[28] SFPI showed a weak band in this region which could not be the evidence of aggregiations. In addition, high protein solubility (Fig. 2) and low enthalpy of SFPI (1.86 J g^{-1}, Table 3) confirm the absence of intramolecular interactions between β-sheet structures. According to what Kudre *et al.*[31] reported, the main secondary structures of legume seed proteins are α-helix, random coil and β-turn. In this study we have observed α-helix structures in all samples.

Bands related to amide II were observed for all samples between 1517 and 1550 cm^{-1}, which are related to β-sheet structures.[25,28] In all FPIs and SPI a weak band was found at higher frequencies (1530–1550 cm^{-1}) than the ones for anti-parallel β-sheet structures. These bands are related to parallel β-sheet structures.[25]

Functional properties

Protein solubility

Figure 2 shows the protein solubility of FPIs and SPI at pH 2–10. Although there was no significant difference ($P < 0.05$) between protein solubility of SFPI (4.89 mg mL^{-1}) and FFPI (4.86 mg mL^{-1}), FFPI showed more solubility in extreme acidic (pH 2) and alkaline (pH 10) media. VFPI had lower protein solubility (3.45 mg mL^{-1}), followed by SPI (2.37 mg mL^{-1}). All samples had a U-shaped



1. FFPI: Freeze dried fenugreek protein isolate
2. SFPI: Spray dried fenugreek protein isolate
3. VFPI: Vacuum oven dried fenugreek protein isolate
4. SPI: Soy protein isolate.

Figure 1. FTIR spectrum of fenugreek and soy protein isolates.

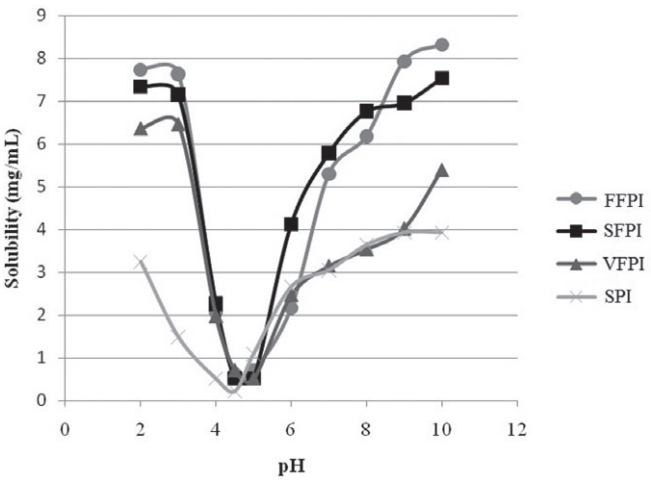


Figure 2. Protein solubility profile of fenugreek and soy protein isolates at pH 2–10. FFPI, freeze dried fenugreek protein isolate; SFPI, spray dried fenugreek protein isolate; VFPI, vacuum oven dried fenugreek protein isolate; SPI, soy protein isolate.

protein solubility trend against pH with the least amount at pH 4.5. However, vacuum oven drying caused more protein denaturation, so less protein solubility occurred in VFPI compared to other FPIs. A result similar to this was observed for soy protein isolate,[32] lentil protein isolate using freeze, spray and vacuum oven dryers;[8] while the spray drying resulted in higher protein solubility of soy protein isolate and freeze drying resulted in better protein solubility of lentil protein isolate.

Since the spray dryer outlet air temperature (60 °C) was less than denaturation temperature of the SFPI (61.90 °C, Table 3), high protein solubility is resulted. Also, lower protein solubility of VFPI is in agreement with its less charged and uncharged hydrophilic amino acids content (Table 1) and its higher H_0 (Table 2) compared to the two other produced FPIs.

Coagulated protein

Coagulated protein percentage shows the amount of total soluble proteins which are coagulated after heating at 100 °C. There was no significant difference ($P < 0.05$) between coagulated protein percentage of all samples (Table 2), but lower amount of it in FFPI shows the positive effect of gentle drying process on better protein solubility. A lower amount of coagulated protein and higher protein solubility is desired in nutritional additives or ingredients in breakfast drinks, but higher protein coagulation is highly required in creating firm gels,[13] and stable foam formation.[33] Although FFPI had little lower coagulated protein in comparison with SFPI, their foaming properties were comparable (Table 2).

Water and oil binding capacity

According to Table 2, WBC of SPI was significantly higher than FPIs ($P < 0.05$). This could be due to higher content of total carbohydrate compounds and lower amount of lipid content in SPI compared to FPI. This finding is similar to what we measured in our previous study.[3] WBC is required for gel formation or viscosity promotion through water uptake in food stuffs such as confectionary and bakery products. WBC of all FPIs in the present research was higher than what was reported about fenugreek protein isolate using freeze drying method (1.68 mL g^{-1}),[2] while it was comparable with chia seed protein isolates dried with the same methods (2.1–2.9 g g^{-1}).[9]

Amongst four samples, FFPI and SPI had the most OBC. No significant difference ($P < 0.05$) was observed between OBC of these two samples (Table 2). This observation is in agreement with higher content of hydrophobic amino acids in SPI followed by FFPI in this study (Table 1). Moreover, SFPI had higher amount of both hydrophobic amino acids and OBC compared to VFPI.

In contrast to our finding, Timilsena *et al.*[9] observed higher OBC for vacuum oven dried chia seed protein isolate followed by spray and freeze dried samples; while Amza *et al.*[7] found no difference between OBC of freeze and vacuum oven dried gingerbread plum seed protein isolates.

Dynamic surface and interfacial tensions

Data in Table 2 are the average surface tensions of 10 points during the time that σ and Y reach an equilibrium. The lowest σ belonged to FFPI and SFPI, while the highest belonged to VFPI and SPI. These results confirm higher efficiency of FFPI and SFPI in migration and reorientation at the water–air interface, as surface active agents. These explanations are in accordance with the result of FC in the same table.

The dynamic interfacial tension of water–oil (Υ_0) in the present study was 19.25 mN m^{-1}. Protein isolates with better surface activity at boundary layer of water–oil will decrease interfacial area of water–oil through their migration into the interface. This causes more reduction in interfacial tension (Υ), while provides more interfacial pressure ($\Upsilon - \Upsilon_0$). FPIs showed non-significant ($P < 0.05$) behaviour in Υ (Table 2). SPI with higher Υ in comparison with FPIs showed inferior behaviour in increasing interfacial pressure. These findings are in accordance with the result of EAI and EC (Table 2).

Foaming properties

According to Table 2, SFPI had the highest foaming capacity (FC), followed by FFPI and VFPI; while the foam stability (FS) of FFPI and SFPI was comparable. Higher protein solubility (Fig. 2) and lower σ (Table 2) of SFPI and FFPI indicate that these samples are flexible enough to migrate into the interface of water–air and encapsulate the air bubbles. Also, these two samples have higher amount of hydrophobic amino acids content compared to the VFPI. Positive correlation between protein solubility and

FC in globular proteins occurs, since they may be difficult to denature, migrate to and reorient in the interface rapidly which are required for significant foaming capacity. Thus, high protein solubility facilitates this progress.

Better FC and protein solubility was observed for spry dried peanut protein concentrate than vacuum oven dried sample.[34]

Significantly lower amount of FC and FS of SPI ($P < 0.05$) in comparison with FPIs may be as a consequence of lower protein solubility, higher hydrophobic amino acids and H_0. All these together may result in hydrophobic interaction between protein molecules which reduces structural flexibility.

Emulsifying properties

Since proteins are amphiphilic in nature, they simultaneously could migrate to water–oil interface, which are immiscible and thermodynamically unstable,[35] creating a layer around oil droplets which prevents coalescence and creaming. Amongst different FPIs, the SFPI had the highest EAI (230.90 $m^2\ g^{-1}$), while FFPI and VFPI had relatively comparable EAI (Table 2). Similar to our study, it was observed that spray dried soy protein isolate had higher EAI, followed by vacuum oven and freeze dried samples.[32] Although SFPI could provide the most active surface at the interface layer or around oil droplets, the volume of oil which was emulsified (EC) by SFPI was comparable and in the same range with other two FPI powders. In the other words, higher interface area will not necessarily decrease the interfacial tension and increase EC. This might occur since Υ is measured until the equilibration (no change in Υ); while the EAI is basically measured at specific times which there might be no equilibrium in the system containing proteins.

Our results showed that the lower protein solubility of VFPI and its high H_0 resulted in the best protein–protein and protein–oil interactions through hydrophobic patches which in conclusion caused the best ESI. Hu *et al.*[32] observed that vacuum oven dried soy protein isolate (at 60 °C for 48 h) had the lowest ESI and H_0. These observations confirm the possibility of protein aggregations and inappropriate efficiency of protein isolate to create stable film around oil droplets over time. So, the more gentle the drying process, the better interfacial properties could be achieved.

Browning intensity

Browning intensity and occurrence of Maillard reaction was estimated using A_{294} and A_{420} (Table 2). Intermediate Maillard reaction products are colourless which show A_{294}, while final Maillard reaction products are brown polymers and absorb at 420 nm.[17,36] VFPI contained the most Maillard reaction products, since it showed the highest absorbance at both wave lengths. This result is in agreement with the lowest Lys amount of VFPI in comparison with other FPIs (Table 1). Because aldehydes could react with amine compounds especially Lys as an indicator of Maillard reaction.[22,37] Moreover, SPI followed by FFPI showed the least absorbances and Maillard progression (Table 2).

Colour parameters

Fenugreek seeds contain five flavonoids including vitexin, tricin, naringenin, quercetin and tricin-7-*O*-β-D-glucopyranoside.[38] Table 2 shows that commercial SPI had lighter colour than FPIs. Amongst FPIs, FFPI had the lightest colour with L^* value of 83.49, followed by SFPI and VFPI with L^* values of 80.11 and 36.30, respectively. Lower L^* of VFPI implicates that vacuum oven drying would have led to reaction of amine compounds with aldehydes via Maillard reaction to form dark pigments (melanoidins).[37] This finding is in accordance with the lowest amount of Lys content in VFPI (Table 1), and the highest absorbance at 294 nm and 420 nm which confirm the browning intensity (Table 2).

The least a^* value belonged to FFPI followed by SFPI which indicates an intense green component. In contrast, VFPI had a higher a^* value which is related to a red component. Also, FFPI with the highest b^* value had the most yellow nature, while SFPI and VFPI had lower b^* value, respectively.

In conclusion, the least darkness, which shows the lowest degree of protein modification via the Maillard reaction and colourant destruction, occurred in the freeze drying method. This finding is in accordance with the highest level of Lys (Table 1), and the least colourless and brown products of FFPI (Table 2). Similar findings were reported for gingerbread plum seed protein isolate which was dried with vacuum oven and freeze dryers, except that the vacuum oven dryer resulted in more b^* value.[7] Timilsena *et al.* [9] also observed the highest L^* and a^* values for spray dried chia seed protein isolate, whilst the highest b^* value was reported for vacuum oven dried sample.

Hexane defatted fenugreek flour had L^*, a^*, and b^* values of 89.85, −2.92, and 2.44, respectively, in our previous study.[6] Comparing these values with those presented in Table 2, it is concluded that protein extraction and drying process caused no considerable changes in L^* and a^* parameters, except for vacuum oven drying which significantly decreased L^* and increased a^* parameter. The b^* value was highly increased in comparison with the flour, regardless what drying method was used.

Surface hydrophobicity

In this study, FFPI had significantly ($P < 0.05$) the lowest H_0 (182.90) (Table 2), followed by SFPI (230.67), VFPI (249.54) and SPI (257.98). The value of H_0 of FFPI was comparable with the hexane defatted fenugreek flour (183.29) in our previous study.[6] This finding demonstrates non-severe and mild denaturing effect of optimised protein extraction and gentle freeze drying process on the structure of fenugreek protein isolate and so the H_0 value. Lower H_0 of FFPI and SFPI compared with VFPI is in accordance with their higher protein solubility (Fig. 2), and their surface properties including FC, FS and EAI (Table 2).

H_0 shows the available hydrophobic sites for ANS binding,[39] and it does not necessarily show the structure flexibility. For surface functional properties flexible protein structure is required to provide rapid migration, reorientations and highly exposed hydrophobic pockets and hydrophilic sites for interactions. From these results, it is noteworthy that there is no direct relationship between H_0 and surface functional properties.

A higher value of H_0 in vacuum oven dried protein samples was reported for chia seed protein isolate.[9] In contrast, Hue *et al.*[32] found that freeze dried soy protein isolate had higher H_0, followed by spray dried and vacuum oven dried samples.

Rheological characteristics

The power law model was applied to fit shear stress–shear rate plots of 50.0 g L^{-1} protein isolate solutions. All the samples exhibited non-newtonian behaviour, as the flow behaviour index or '*n*' parameter of the equations was not equal to 1 (Table 2). FFPI was the only sample which had the shear thinning behaviour since its '*n*' parameter was less than 1 (equal to 0.767), also its apparent viscosity through all shear rates of 15, 30 and 50 s^{-1} decreased gradually. In contrast, SFPI, VFPI and SPI had shear thickening behaviour with '*n*' parameter equal to 1.394, 1.074, and 2.016, respectively.

Table 3. Thermal properties of FPIs

Property	FFPI	SFPI	VFPI	SPI
T_o (°C)	34.58 ± 0.01^c	51.40 ± 0.01^b	78.40 ± 0.10^a	27.11 ± 0.01^d
T_d (°C)	64.75 ± 0.02^b	61.90 ± 0.01^c	92.30 ± 0.11^a	61.80 ± 0.05^c
T_e (°C)	97.67 ± 0.04^c	65.90 ± 0.03^d	152.90 ± 0.08^d	150.01 ± 0.10^c
ΔH (J g^{-1})	19.02 ± 0.20^a	18.60 ± 0.08^a	6.63 ± 0.08^a	0.76 ± 0.10^b

T_o, onset temperature; T_d, denaturation temperature; T_e, end set temperature; ΔH, denaturation enthalpy (J g protein isolate^{-1}); FFPI, freeze dried fenugreek protein isolate; SFPI, spray dried fenugreek protein isolate; VFPI, vacuum oven dried fenugreek protein isolate; SPI, soy protein isolate.
Results are given as the mean ± SD of triplicates.
Different letters in each row indicate significant differences ($P < 0.05$).

Viscosity of 50.0 g L^{-1} solution of all FPIs in this study was more than the one of 75.0 g L^{-1} solution of peanut concentrate and soy protein isolate.[34] Also, except for SFPI, all other FPIs in the present research had higher apparent viscosity than fenugreek flours defatted by different solvents at a shear rate of 30 s^{-1}.[6] This shows that although different constituents such as carbohydrates, which are higher in flours than protein isolates, influence water binding and viscosity promoting, but the network that protein molecules create through different interactions cause more resistance against shear rates and break down.

Differential scanning calorimetry

Thermal properties of FPIs are presented in Table 3. According to what we obtained in FTIR spectra (Fig. 1), FPIs especially FFPI and SFPI resulted in more α-helix structures, as ordered ones, compared to SPI. This confirms the higher denaturation enthalpy (ΔH) of FPIs compared with SPI, since ΔH correlates with the extent of ordered secondary structure of a protein.[40] Also, high H_0 of SPI compared to FPIs (Table 2) shows that although SPI contains high amounts of hydrophobic amino acids (224.0 g kg^{-1}), these hydrophobic residues did not result in a thermal resistance core through hydrophobic interactions.

Denaturation temperature (T_d) of VFPI was more than FFPI (Table 3), which indicates higher thermostability of VFPI.[32] This observation is similar to what was reported for vacuum oven dried and freeze dried ginger bread and grass pea protein isolates by Amza *et al.*[7] and Feyzi *et al.*,[41] respectively. This may occur due to the presence of more thermal susceptible structures including α-helixes in FFPI. In addition, a higher possibility of the Maillard reaction in VFPI through covalent bonds may result in the highest T_d.

Electrophoresis pattern

Predominant protein fractions, also the most disulfide bonds containing protein fraction in fenugreek seeds are albumins and globulins, respectively.[42] The electrophoresis pattern of FPIs and SPI in the presence and absence of 2-mercaptoethanol is shown in Fig. 3. The SDS-PAGE results showed that the number of bands in all samples was more in the presence of 2-mercaptoethanol. This phenomenon has been observed for FPI in our previous study,[3] and chia seed protein isolate,[9] as well. Under reducing conditions, due to the breakdown of intramolecular disulfide bonds and the transformation of globular proteins to non-globular ones the protein molecules are unfolded. Therefore, they reveal less mobility and appear as higher molecular weight.

The number of visible bands and their colour intensity in VFPI were less than two other samples which show its lower protein solubility. But, the absence of high molecular weight band near to stacking gel in the SDS-PAGE pattern of all FPIs in this research suggests that none of drying methods had severe denaturing effect on protein molecules which ends in protein aggregations. This observation is confirmed by the absence of bands which assert intermolecular interactions and protein aggregations in FTIR analysis (Fig. 1). Similar findings were observed in a previous study on grass pea protein isolates which were dried using freeze and vacuum oven dryers.[41]

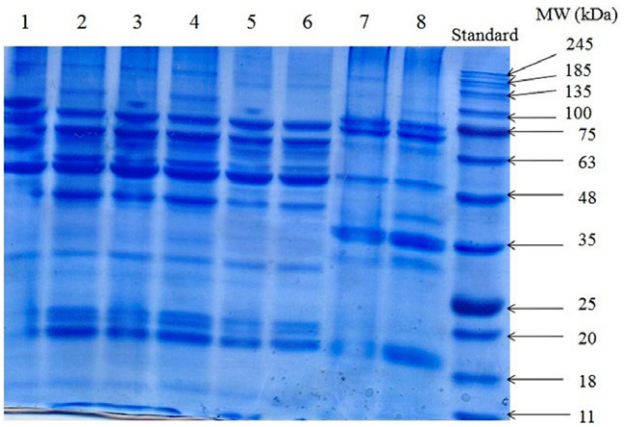


Figure 3. SDS-PAGE pattern of fenugreek and soy protein isolates. Lane 1, freeze dried fenugreek protein isolate in the absence of 2-mercaptoethanol; Lane 2, freeze dried fenugreek protein isolate in the presence of 2-mercaptoethanol; Lane 3, spray dried fenugreek protein isolate in the absence of 2-mercaptoethanol; Lane 4, spray dried fenugreek protein isolate in the presence of 2-mercaptoethanol; Lane 5, vacuum oven dried fenugreek protein isolate in absence of 2-mercaptoethanol; Lane 6, vacuum oven dried fenugreek protein isolate in presence of 2-mercaptoethanol; Lane 7, soy protein isolate in the absence of 2-mercaptoethanol; Lane 8, soy protein isolate in the presence of 2-mercaptoethanol.

CONCLUSIONS

The results of this research suggest that freeze and spray drying methods resulted in comparable protein solubility, coagulated protein percentage, WBC, and a few surface functional properties in FPIs. Vacuum oven drying caused darker colour, pigment destruction and the Maillard reaction in FPI based on browning intensity estimation and Lys content. Also, low protein solubility and poor surface functional properties were observed in VFPI. But, the absence of high molecular weight bands in the SDS-PAGE profile and better protein solubility than commercial SPI prove a promising function of VFPI in some food stuffs. Finally, this research

suggests that any drying method must be conducted in its gentle state in order to promote protein functionalities.

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REFERENCES

1 Leela NK, Shafeekh KM, Fenugreek, in *Chemistry of Spices*, ed. by Parthasarathy VA, Chempakam B, Zachariah TJ. CAB International, London, UK, pp. 242–259 (2008).
2 El Nasri NA and El Tinay AH, Functional properties of fenugreek (*Trigonella foenum graecum*) protein concentrate. *Food Chem* **103**:582–589 (2007).
3 Feyzi S, Varidi M, Zare F and Varidi MJ, Fenugreek (*Trigonella foenum graecum*) seed protein isolate: extraction optimization, amino acid composition, thermo and functional properties. *J Sci Food Agric* **95**:3165–3176 (2015).
4 Tomé AS, Pires C, Batista I, Sousa I and Raymundo A, Protein gels and emulsions from mixtures of Cape hake and pea proteins. *J Sci Food Agric* **95**:289–298 (2014).
5 Joshi M, Adhikari B, Aldred P, Panozzo JF, Kasapis S and Barrow CJ, Interfacial and emulsifying properties of lentil protein isolate. *Food Chem* **134**:1343–1353 (2012).
6 Feyzi S, Varidi M, Zare F and Varidi MJ, A comparison of chemical, structural and functional properties of fenugreek (*Trigonella foenum graecum*) protein isolates produced using different defatting solvents. *Int J Biol Macromolec*; http://dx.doi.org/10.1016/j.ijbiomac.2017.06.101. Article (in press).
7 Amza T, Amadou I, Zhu K and Zhou H, Effect of extraction and isolation on physicochemical and functional properties of an underutilized seed protein: Gingerbread plum (*Neocarya macrophylla*). *Food Res Int* **44**:553–559 (2011).
8 Joshi M, Adhikari B, Aldred P, Panozzo JF and Kasapis S, Physicochemical and functional properties of lentil protein isolates prepared by different drying methods. *Food Chem* **129**:1513–1522 (2011).
9 Timilsena YP, Adhikari R, Barrow CJ and Adhikari B, Physicochemical and functional properties of protein isolate produced from Australian chia seeds. *Food Chem* **212**:648–656 (2016).
10 Vojdani F, Solubility, in *Methods of Testing Protein Functionality*, ed. by Hall GM. St Edmundsbury, Blackie Academic & Professional: London, UK, pp. 11–60 (2006).
11 Association of Official Analytical Chemists (AOAC), *Official Methods of Analysis*, 13th edition. AOAC, Washington, DC (1990).
12 Owusu-Apenten RK, *Food Protein Analysis Quantitative Effects on Processing*, 1st edition. Marcel Dekker, New York (2002).
13 Kramer A and Kwee WH, Functional and nutritional properties of tomato protein concentrates. *J Food Sci* **42**:207–212 (1977).
14 Du Nouy P, A new apparatus for measuring surface tension. *J Gen Physiol* **1**:152 (1919).
15 Pearce KN and Kinsella JE, Emulsifying properties of proteins: evaluation of a turbidimetric technique. *J Agric Food Chem* **26**:716–723 (1978).
16 Neto VQ, Narain N, Silva JB and Bora PS, Functional properties of raw and heat processed cashew nut (*Anarca-duim occidentale*, L.) kernel protein isolate. *Nahrung/Food* **45**:258–262 (2001).
17 Tu ZC, Hu YM, Wang H, Huang XQ, Xia SQ and Niu PP, Microwave heating enhances antioxidant and emulsifying activities of ovalbumin glycated with glucose in solid-state. *J Food Sci Technol* **52**:1453–1461 (2013).
18 Horax R, Hettiarachchy N, Kannan A and Chen P, Protein extraction optimization, characterization, and functionalities of protein isolate from bitter melon (*Momordica charantia*) seed. *Food Chem* **124**:545–550 (2011).
19 Chakraborty P, Coconut protein isolate by ultrafiltration, in *Food Engineering and Process Applications*, vol. 2, ed. by LeMeguer M and Jelen P. Elsevier Applied Science Publishers, New York, pp. 308–315 (1986).
20 Laemmli UK, Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* **227**:680–686 (1970).
21 De Groot AP and Slump P, Effects of severe alkali treatment of proteins on amino acid composition and nutritive value. *J Nutr* **98**:45–56 (1968).
22 Iwe MO, Zuilichem DJ, Ngoddy PO and Lammers W, Amino acid and protein digestibility index of mixtures of extruded soy and sweet potato flours. *Lebensm.-Wiss. u.-Technol* **34**:71–75 (2001).
23 Li-Chan ECY, Vibrational spectroscopy applied to the study of milk proteins. *Le Lait* **87**:443–458 (2007).
24 Withana-Gamage TS, Wanasundara JPD, Pietrasika Z and Shand PJ, Physicochemical, thermal and functional characterisation of protein isolates from Kabuli and Desi chickpea (*Cicer arietinum*L.): a comparative study with soy (*Glycine max*) and pea (*Pisum sativum* L.). *J Sci Food Agric* **91**:1022–1031 (2011).
25 Carbonaro M and Nucara A, Secondary structure of food proteins by Fourier transform spectroscopy in the mid-infrared region. *Amino Acids* **38**:679–690 (2010).
26 Kong J and Yu S, Fourier transform infrared spectroscopic analysis of protein secondary structures. *Acta Biochim Biophys Sinica* **39**:549–559 (2007).
27 Barth A and Zscherp C, What vibrations tell us about proteins. *Q Rev Biophys* **35**:369–430 (2002).
28 Guerrero P, Kerry JP and de la Caba K, FTIR characterization of protein–polysaccharide interactions in extruded blends. *Carbohydr Polym* **111**:598–605 (2014).
29 Murayama K and Tomida M, Heat-induced secondary structure and conformation change of bovine serum albumin investigated by Fourier transform infrared spectroscopy. *Biochemistry* **43**:11526–11532 (2004).
30 Fang Y and Dalgleish DG, The conformation of α-lactalbumin as a function of pH, heat treatment and adsorption at hydrophobic surfaces studied by FTIR. *Food Hydrocoll* **12**:121–126 (1998).
31 Kudre TG, Benjakul S and Kishimura H, Comparative study on chemical compositions and properties of protein isolates from mung bean, black bean and bambara groundnut. *J Sci Food Agric* **93**:2429–2436 (2013).
32 Hu XZ, Cheng YQ, Fan JF, Lu ZH, Yamaki K and Li LT, Effects of drying method on physicochemical and functional properties of soy protein isolates. *J Food Process Pres* **34**:520–540 (2010).
33 Butt MS and Batool R, Nutritional and functional properties of some promising legumes protein isolates. *PJN* **9**:373–379 (2010).
34 Yu JM, Ahmedna M and Goktepe I, Peanut protein concentrate: Production and functional properties as affected by processing. *Food Chem* **103**:121–129 (2007).
35 McClements DJ, *Food Emulsions. Principles, Practice and Techniques*, 2nd edition. CRC Press, New York (2005).
36 Kim JS and Lee YS, Antioxidant activity of Maillard reaction products derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of heating time. *Food Chem* **116**:227–232 (2009).
37 Alais C and Linden G, *Food Biochemistry*. Ellis Horwood, London (1991).
38 Shang M, Cai S, Han J, Li J, Zhao Y, Zheng J *et al.*, Studies on flavonoids from fenugreek (*Trigonella foenum-graecum*). *China J Chinese Materia Medica* **23**:614–616 (1998).
39 Alizadeh-Pasdar N and Li-Chan ECY, Comparison of protein surface hydrophobicity measured at various pH values using three different fluorescent probes. *J Agric Food Chem* **48**:328–334 (2000).
40 Koshiyama I, Hamano M and Fukushima D, A heat denaturation study of the 11S globulin in soybean seeds. *Food Chem* **6**:309–322 (1981).
41 Feyzi S, Milani E and Golimovahhed QA, Grass pea (*Lathyrus sativus* L.) protein isolate: study of extraction optimization, protein characterizations, structure and functional properties. *Food Hydrocoll* **74**:187–196 (2018).
42 Sauvaire YD, Baccou JF and Kobrehel K, Solubilization and characterization of fenugreek seed proteins. *J Agric Food Chem* **32**:34–47 (1984).

