



Hydrolysis of peanut (*Arachis hypogea* L) protein concentrate by fungal crude protease extract: effect on structural, functional and in-vitro protein digestibility

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Revised: 18 July 2021 / Accepted: 28 July 2021 / Published online: 3 August 2021
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Abstract Peanut protein concentrates (PPCs) were subjected to hydrolysis by crude protease extract (CPE) obtained from three fungi viz; *Rhizopus oligosporus*, *Trichoderma reesei*, and *Aspergillus oryzae* and the effect on structural, functional and in-vitro protein digestibility (IVPD) properties were studied. Particle size was found significantly ($p \leq 0.05$) lower in hydrolyzed samples than un-treated samples. Fourier transform infrared spectroscopy (FTIR) spectrum of hydrolyzed samples displayed intense absorbance peaks in the wavelength ranging from 1500 to 2600 cm^{-1} . Peanut protein concentrates hydrolyzed by CPE from *R. oligosporus* showed higher surface hydrophobicity (564.18). Total sulfhydryl content was found lower in all the hydrolyzed samples whereas, reverse trend was observed for exposed sulfhydryl content. The structural changes simultaneously affected the functional and IVPD attributes of hydrolyzed PPCs. In comparison to the PPCs hydrolysed using crude extracts from *T. reesei* and *R. oligosporus*, PPCs hydrolysed by *A. oryzae* showed higher solubility, water and oil binding capacity, foaming capacity and foam stability. Higher IVPD values of 86.70% was also found in PPCs hydrolyzed with CPE of *A. oryzae*. The study established that CPE hydrolysis of PPCs has

potential for scale-up studies and may serve as a cost effective alternative to protein hydrolysis with pure enzymes.

Keywords Peanut protein concentrate · Hydrolysis · Crude protease extract · Conformational changes · Solubility

Introduction

Peanut (*Arachis hypogea* L.), a major source of edible oil and protein is considered valuable for animal and human nutrition. Defatted peanut meal containing about 30–35% protein with higher bioavailability and lower anti-nutritional factors is underutilized product of the peanut industry (Yadav et al 2012). Extraction of protein from defatted peanut meal and their applications in formulation of protein beverages can be of great significance (Malik et al 2016). Plant based protein drinks could also serve as an alternative to animal protein drinks due to some dietary restrictions and religious concerns (Jain et al 2019). Peanut protein has promising nutritional profile, however, its uses in food formulations are limited owing to lower solubility and poor emulsifying properties.

Different approaches like high-intensity ultrasound (HIUS) (Mir et al 2019); heat treatment (Mir et al 2020) and irradiation (Malik and Saini 2017) have been studied for modification of native protein structure in order to enhance the functional properties. Enzymatic hydrolysis with pure enzymes is also used for enhancing functional properties of the protein and usually preferred from the food safety point of view. Yu et al (2007) and Quist et al (2009) reported that enzymatic hydrolysis can generate lower molecular weight polypeptides and free amino acids. The lower molecular weight polypeptides are easily

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absorbed by the intestine and also possess lower allergenic effects. In addition, the biological functions like antihypertension, opioid agonists, orantagonists, immunomodulatory, antithrombotic, antioxidant, anti-cancer, and antimicrobial activities of hydrolyzed peanut proteins have also been reported by Elias et al (2008).

Though, modification with pure enzyme gives promising results with respect to protein functionality, but its uses are limited due to higher cost of pure enzymes which in turn increases the overall cost of the process and final product. To overcome these limitations, crude protease extracts obtained from different fungi can be explored and utilized for hydrolyzing different kinds of proteins. Oliveira et al (2010) reported that proteases obtained from fungi have tremendous importance due to their potential of catalyzing the hydrolysis reactions. In addition, crude protease extracts obtained from different microbial sources are preferred over pure enzymes obtained from plant and animal sources as they possess almost all the characteristics required for biotechnological applications (Gupta et al 2005). Among different microbial sources, fungal proteases exhibit higher activity and have the potential for commercial applications. Therefore, the present research was carried out with the major objectives: (1) Extraction of crude protease extracts from *R. oligosporus*, *T. reesei*, and *A. oryzae* by solid state fermentation, (2) Isolation of protein concentrate from defatted peanut flour and its enzymatic hydrolysis by crude protease extracts, and (3) Effect of enzymatic hydrolysis on the structural and functional characteristics of peanut protein concentrates.

Materials and methods

Materials

Commercially available de-oiled peanut cake (protein content, 36.5%, db) were procured from the local market of Ludhiana, Punjab, India from a local supplier. The cake was pulverized in a laboratory grinder in order to reduce particle size and passed through 60 mesh (0.250 mm) standard sieve. Defatted flour was dried in hot air oven for 2 h at 40–45 °C to remove any residual solvent, thereafter used for the preparation of protein concentrate. All chemicals used in the experiments were of analytical grade and purchased from Sigma Aldrich, MO, USA. Papain was obtained from Hi Media Laboratories Pvt Ltd, Mumbai, India. Cultures of *T. reesei*, *A. oryzae* and *R. oligosporus* were obtained from Bio-process Engineering Laboratory, ICAR- Central Institute of Post-Harvest Engineering and Technology (ICAR-CIPHET), Ludhiana, India.

Methods

Preparation of peanut protein concentrate

Peanut protein concentrate was prepared according to the method of Yu et al (2007) using alkali extraction and isoelectric precipitation method. Defatted peanut flour was mixed with water in the ratio of 1/10 (w/v), and pH of the mixture was adjusted to 10.0 with 1.0 N sodium hydroxide (NaOH). The suspension was stirred at room temperature (30 ± 2 °C) for 2 h and centrifuged at 3000 g for 15 min. Supernatant was collected and the pH was adjusted to 4.5 (iso-electric point) with 1.0 N hydrochloric acid (HCl). The precipitates obtained were centrifuged at 3000 g for 15 min. The supernatant was discarded and the precipitate was neutralized, freeze dried and stored under refrigerated conditions for further use.

Determination of chemical characteristics of peanut protein concentrate

Chemical characteristics of native PPCs, such as moisture, protein, ash, fat and crude fiber content were determined according to the standard methods of AOAC (2006). The carbohydrate content was estimated by subtracting the sum of percentage of moisture, crude fat, crude protein and ash from 100.

Preparation of crude protease extract using different fungal cultures

Crude protease extract (CPE) from *R. oligosporus*, *A. oryzae* and *T. reesei* was prepared by the method described by Su et al (2011) with some modifications. Wheat bran (20 g), defatted peanut flour (8 g), calcium chloride (0.028 g) and deionized water (28 mL) were mixed and autoclaved at 121 °C for 30 min in a conical flask. Spores from each of the isolates were harvested following inoculation on PDA plates and incubation at 30 °C for 72 h. The plates were removed from the incubator followed by harvesting of spores using sterile water. The spores count was determined with a hemocytometer and the spore concentration of 1×10^8 spores/mL was used for enzyme production (Sandhu et al 2011). The sterilized mixture was cooled, inoculated individually with 2 mL spore suspension of *R. oligosporus*, *A. oryzae* and *T. reesei* and incubated at 30 °C for 68 h. After 3 days of incubation, 100 mL of 0.2 M phosphate buffer (pH 7.0) was added to each of the flasks and vortexed for 30 min. The mixture was centrifuged at 10,000 g for 30 min at 4 °C. The obtained supernatant (CPE) was stored for further use.

Determination of protease activity

The proteolytic activities of CPE and papain were determined by the procedure as described by Su et al (2011). Crude protease extracts showed protease activity of 15,478–17,522 USP units/mg in comparison to papain (30,000 USP units/mg).

Enzymatic hydrolysis of PPC with crude fungal protease extracts

Hydrolysis of PPC was carried out as per the method described by Silvestre et al (2012) with minor modifications. The PPCs were dissolved in distilled water to make 12.5% (w/v) solution and pH was adjusted to 6.5 using 2% citric acid solution for optimum activity of CPE. The solution was allowed to hydrate at 30 °C for 2 h. Thereafter, the temperature of the solution was increased to 50 °C. Crude protease extract (0.75%, w/w of protein) from each of the fungal source was added separately and the solution was continuously stirred for 1 h. The pH was maintained at 6.5 using 1 N NaOH solution during hydrolysis process. At the end of hydrolysis, the enzyme was inactivated by heating the mixture in a water bath at 85 °C for 10 min. Hydrolysates obtained were centrifuged at 5000 g for 20 min at 20 °C and pellets were collected, freeze dried and stored for further analysis. Papain (0.37%, w/w of protein) was used in place of CPE for hydrolyzing peanut protein concentrates. Peanut protein concentrates (PPCs) hydrolyzed with crude protease extracts of *R. oligosporus*, *A. oryzae* and *T. reesei* were designated as HPPCR, HPPCA and HPPCT. Peanut protein concentrates hydrolyzed with papain were designated as HPPCPs, while native peanut protein concentrates were designated as NPPC.

Determination of degree of hydrolysis

The degree of hydrolysis (DH), defined as the percent ratio of the number of peptide bonds broken (h) to the total number of peptide bonds in the substrate (h_{tot}), was determined with the o-phthalaldehyde (OPA) as described by Nielsen et al (2001). The assay was carried out by mixing 0.4 mL of a 0.45 μ filtered sample (or control) and 0.3 mL OPA reagent. After vortex for 5 s and incubation at room temperature (30 ± 2 °C) for exactly 2 min, the absorbance of the mixture was measured in UV spectrophotometer (Model: UV-2700, Shimadzu, Tokyo, Japan) at 340 nm. L-serine (0.9516 meq/L) was used as positive control and distilled water as negative control. Calibration curve of L-serine was prepared in distilled water. The DH was calculated by the following equation:

$$\text{Serine} - \text{NH}_2 = \frac{(A_{340\text{sample}} - A_{340\text{Blank}}) / (A_{340\text{Standard}} - A_{340\text{Blank}}) \times 0.915 \text{ meqv/I} \times S \times D}{\times (P/V)} \quad (1)$$

where serine-NH₂ is meqv serine in NH₂/g protein; S is sample volume in litre; D is dilution volume; P is protein content in sample volume; V is the sample volume in assay; α , β and h_{tot} constants for casein are 1.039, 0.383 and 8.2, respectively.

$$\text{DH} (\%) = h/h_{\text{tot}} \times 100 \quad (2)$$

where $h = (\text{Serine-NH}_2 - \beta) / \alpha$ meqv/g protein.

Determination of Particle size distribution (PSD)

Particle size analyzer based on laser light scattering ((Model Horiba Partica LA-950V2, Japan) was used for the determination of particle size and surface area of native and hydrolyzed samples. The particle size distributions (PSDs), i.e., particle size at 10% (Dv10), median diameter and 90% (Dv90) of the volume distribution were calculated from the graph. The specific surface area (cm^2/cm^3) was also calculated.

Fourier transform infrared (FTIR) spectroscopy

Effect of enzymatic hydrolysis of peanut protein concentrate on FTIR spectra was recorded on ALPHA, BRUKER (Laser class 1, Germany) using the compressed potassium bromide pellets. Moisture free protein concentrate samples were mixed with dry potassium bromide in the ratio of 1:100, and compressed in a hydraulic press to form transparent pellets. The spectra were recorded in the transmittance mode at 4000–400 cm^{-1} region.

Total and exposed sulfhydryl contents

Total and exposed sulfhydryl groups of native and hydrolyzed peanut protein concentrates were determined according to the method of Yin et al (2009).

Surface hydrophobicity (H0)

Fluorescent probe (8- anilinonaphthalene-1-sulfonic acid, ANS) was used for the determination of surface hydrophobicity of native and hydrolyzed PPCs using the established procedure of Wagner et al (2000). The protein surface hydrophobicity was obtained by calculating the initial slope of the fluorescence index versus the protein concentration plot.

Determination of functional characteristics of peanut protein concentrates

Solubility of native and hydrolyzed PPCs was determined according to the method described by Achouri et al (2012) at 30 ± 2 °C. Water binding capacity was determined using the method described by Beuchat (1977). The method of Chakraborty (1986) was adopted for the determination of oil binding capacity.

Foaming capacity and foam stability

The method described by Fekria et al (2012) was used for measuring foaming capacity (FC) and foam stability (FS) of protein samples with some modifications. Dispersions were prepared by dissolving sample (2.0 g) in water (100 mL). The solutions were whipped with a mixer blender for 5 min at high speed. After blending, the content was shifted into a cylinder and the foam volume was recorded. At different time intervals (10, 20, and 30 min), changes in volume was recorded. Percentage FC and FS were calculated according to the following equations:

$$FC (\%) = \frac{V_w - V_{uw}}{V_{uw}} \times 100 \quad (3)$$

where V_w is the volume of whipped sample and V_{uw} is the volume of un-whipped sample.

$$FS (\%) = \frac{V_t}{V_i} \times 100 \quad (4)$$

where V_t is the change in volume of foam with time and V_i is the initial volume of foam.

Determination of in-vitro protein digestibility (IVPD)

The method of Hsu et al (1977) was followed for the determination of in-vitro protein digestibility of native and hydrolyzed peanut protein concentrates with some modifications. Water suspensions of 25 mL containing peanut protein concentrate (6.25 mg/mL) were prepared and the pH was maintained at 8.0 using 0.1 N NaOH. Multi-enzyme solution (cocktail) was prepared by mixing 1.6 mg/mL Trypsin, 3.1 mg/mL Chymotrypsin, and 1.3 mg/mL Protease. The cocktail was maintained in an ice bath and the pH was adjusted to 8 using 0.1 N NaOH. From this cocktail, 2.5 mL was taken and added to protein suspension already maintained at 37 °C in a water bath shaker. The pH drop after 10 min was measured by a calibrated digital pH meter (Model: Five Easy FEP20, Mettler-Toledo, Switzerland). Casein protein was used as a standard. The IVPD was calculated according to the following equation:

$$IVPD (\%) = 210.46 - 18.10Xf \quad (5)$$

where Xf is the pH value of the solution after 10 min of enzymatic digestion.

Statistical analysis

The data presented in tables and figures are the mean of three replicates. Error bars represent deviation. Data were statistically analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range tests ($p \leq 0.05$) was used to determine significant differences among the mean values using Statistica software version 7.0.

Results and discussion

Chemical composition

Moisture content of protein concentrate was 5.09%, which is important from the microbiological point of view. Protein content was 72.08%. Total ash and insoluble ash content were 10.07 and 0.28%, respectively. Higher ash content depicts the presence of minerals in protein concentrate, while the insoluble ash generally refers to silica content. Crude fibre and fat content were 0.68 and 0.83%, respectively. Carbohydrate obtained by the difference method was found to be 10.97%.

Degree of hydrolysis (DH)

Peanut protein concentrates was subjected to CPE hydrolysis for 1 h. Degree of hydrolysis increased with increase in time (Fig. 1a). At 60 min, the highest DH value of 16.4% was found in PPCs hydrolysed with CPE from *A. oryzae* followed by CPE from *R. oligosporus* (15.6%) and CPE of *T. reesei* (15.0%). The DH value for samples hydrolysed with papain was 15.9% and comparable with other CPE hydrolysed samples. This indicates that the CPE from all three fungal sources contained protease enzymes, which hydrolysed the protein samples. Higher values (43.4%) of DH in defatted peanut meal hydrolysed with CPE from *Aspergillus* has also been reported by Su et al (2011). Hydrolysis through CPE might be associated with the presence of cellulolytic enzymes that could possibly decreased the viscosity of the reaction mixture and enhanced the protein dissolution and finally DH. The *Aspergillus oryzae* strain used in this study produces a consortium of enzymes comprising of cellulases, xylanases and pectinases (Sandhu et al 2011). Higher DH value observed in case of CPE of *A. oryzae* in comparison with CPE of *R. oligosporus* and *T. reesei* could also be due to

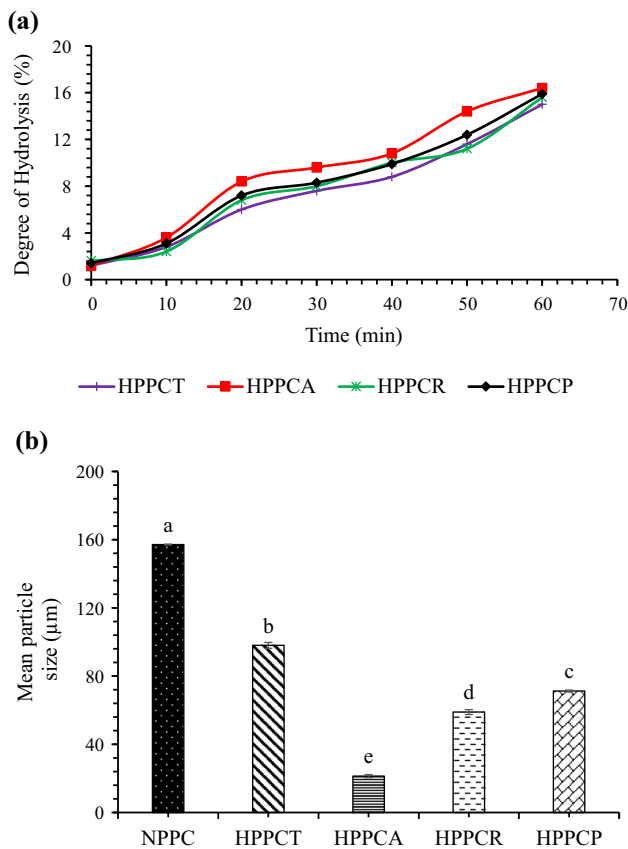


Fig. 1 Effect of crude protease extract enzymatic hydrolysis on degree of hydrolysis (a) and particle size (b) of peanut protein concentrate. [HPPCT = hydrolyzed peanut protein concentrate with crude protease extract of *Trichoderma reesei*, HPPCA = hydrolyzed peanut protein concentrate with crude protease extract of *Aspergillus oryzae*, HPPCR = hydrolyzed peanut protein concentrate with crude protease extract of *Rhizopus oligosporus* and HPPCP = hydrolyzed peanut protein concentrate with papain]

the presence of larger expansion of hydrolytic genes in *A. oryzae* as reported by Vishwanatha et al (2009).

Particle size

Particle size of native and hydrolyzed peanut protein concentrates is shown in Fig. 1b. It is clear from the figure that enzymatic hydrolysis caused significant changes in the particle size of PPCs. Native PPCs has particle size of about 157.12 µm which was significantly ($p \leq 0.05$) higher than the particle size of hydrolyzed samples. Among hydrolyzed PPCs, the lowest particle size of 21.35 µm was observed in the samples hydrolyzed with CPE produced by *A. oryzae*, and the highest particle size of 98.04 µm was observed in the samples hydrolyzed with CPE obtained from *T. reesei*. Papain hydrolyzed samples showed higher particle size than samples hydrolyzed with CPE from *A. oryzae* and *R. oligosporus* but the values were lower than the samples hydrolyzed with CPE from *T. reesei*. The

possible reasons for the decline in particle size could be owing to enzymatic hydrolysis, which might have induced strong electrostatic repulsion between the newly created particles. The newly formed particles may have less chances to aggregate which in turn is responsible for the lower values of particle size for hydrolyzed samples. Tang et al (2019) also observed reduction in particle size of egg yolk powder after enzymatic hydrolysis. Moreover, the results of particle size also justify the results of foaming capacity, showing higher values for PPCs with lower particle size (Table 1).

Fourier transform infra-red spectroscopy (FTIR) properties

Changes in secondary structure due to crude protease enzymatic hydrolysis of peanut protein concentrates was recorded by FTIR spectroscopy (Fig. 2). The results depict that small absorbance peaks were visible in the wavelength ranging from 500 to 100 cm^{-1} , which corresponds to N–H, C–N and C = O amide bonds. The intensity of absorbance peaks slightly increased from 800 to 1200 cm^{-1} , which corresponds to the deformation in the NH_3 groups of peanut protein concentrates probably due to the free amino group of lysine and the presence of three asymmetrical CH_3 bending, symmetrical CO_3^{-1} stretching, and ring vibrations attributed to free amino acids i.e. valine, glutamic acid and aromatic amino acid i.e. phenylalanine (Pavia et al 2008). The zone ranging from 1600 to 1800 cm^{-1} known as Amide-I (C = O bond) region is the most vulnerable zone which depicts the changes in the secondary structure of protein molecules. In our results, we observed a sharp decline in the peaks for all the samples, however the peaks were more intense in case of hydrolyzed samples compared to the native. This clearly indicates that changes in the secondary structure was due to crude protease hydrolysis. Pavia et al (2008) reported that structural changes corresponding to this region mainly arises due to changes in β -sheet and α -helix of protein, which is mainly caused due to C–H stretching. Other intense peaks were also observed in the wavelength ranging from 2000 to 2600 cm^{-1} , which further confirms the dominance of structural changes in hydrolyzed samples than unhydrolyzed. Some minor absorbance peaks were also observed in the wavelengths ranging from 2700 to 3500 cm^{-1} , which correspond to hydrogen bonding, N–H stretching and C–H bending of amide bonds. Similar peaks were observed for the samples hydrolyzed with papain. The validity of FTIR is further corroborated by the results of surface hydrophobicity and SH-groups showing the presence of structural changes owing to hydrolysis. These structural changes profoundly affected different functional

Table 1 Effect of crude protease extract enzymatic hydrolysis on surface hydrophobicity, SH groups, foaming properties and in-vitro protein digestibility of peanut protein concentrate

Sample	Surface hydrophobicity	Total SH ($\mu\text{mol}/\text{mg}$)	Exposed SH ($\mu\text{mol}/\text{mg}$)	Foaming capacity (%)	Foam stability (%)			In-vitro protein digestibility (%)
					10 min	20 min	30 min	
NPPC	213.51 \pm 1.96 ^e	8.78 \pm 0.01 ^a	1.11 \pm 0.02 ^c	22.33 \pm 1.24 ^e	52.92 \pm 0.91 ^e	45.40 \pm 1.23 ^c	30.18 \pm 0.60 ^e	70.11 \pm 1.83 ^c
HPPCT	442.07 \pm 2.26 ^b	6.54 \pm 0.02 ^c	3.35 \pm 0.01 ^a	33.66 \pm 2.05 ^d	67.70 \pm 0.92 ^d	53.73 \pm 2.06 ^d	48.07 \pm 0.82 ^d	83.65 \pm 2.08 ^b
HPPCA	340.64 \pm 1.44 ^d	7.33 \pm 0.02 ^b	2.56 \pm 0.01 ^b	58.70 \pm 1.68 ^a	88.28 \pm 0.93 ^a	83.46 \pm 1.42 ^a	77.48 \pm 1.44 ^a	86.69 \pm 0.78 ^b
HPPCR	564.18 \pm 2.46 ^a	8.01 \pm 0.02 ^a	1.88 \pm 0.01 ^c	47.31 \pm 1.25 ^b	76.25 \pm 0.54 ^b	73.28 \pm 0.93 ^b	67.13 \pm 1.98 ^b	84.33 \pm 0.82 ^b
HPPCP	392.23 \pm 2.94 ^c	5.45 \pm 0.29 ^d	3.56 \pm 0.17 ^a	41.33 \pm 1.24 ^c	73.07 \pm 1.00 ^c	61.52 \pm 0.86 ^c	57.66 \pm 0.89 ^c	94.62 \pm 0.59 ^a

$n = 3$, results are expressed as mean \pm standard deviation, samples with different superscript letter in the same column differ significantly ($p \leq 0.05$) on a particular property. [NPPC = native peanut protein concentrate, HPPCT = hydrolyzed peanut protein concentrate with crude protease extract of *Trichoderma reesei*, HPPCA = hydrolyzed peanut protein concentrate with crude protease extract of *Aspergillus oryzae*, HPPCR = hydrolyzed peanut protein concentrate with crude protease extract of *Rhizopus oligosporus* and HPPCP = hydrolyzed peanut protein concentrate with papain]

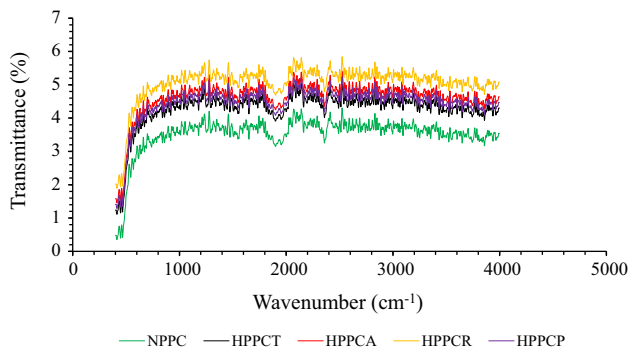


Fig. 2 Effect of crude protease extract enzymatic hydrolysis on FTIR spectra of peanut protein concentrate. [NPPC = native peanut protein concentrate, HPPCT = hydrolyzed peanut protein concentrate with crude protease extract of *Trichoderma reesei*, HPPCA = hydrolyzed peanut protein concentrate with crude protease extract of *Aspergillus oryzae*, HPPCR = hydrolyzed peanut protein concentrate with crude protease extract of *Rhizopus oligosporus* and HPPCP = hydrolyzed peanut protein concentrate with papain]

characteristics like solubility, WBC and OBC and foaming capabilities (Table 1).

Total and exposed SH content

The data showing the effect of CPE hydrolysis on the total and exposed SH content of PPCs are presented in Table 1. Higher total SH content (8.78) was found in native PPCs than hydrolyzed PPCs whereas, the lowest total SH content (6.54) was found in PPCs hydrolyzed with CPE obtained from *T. reesei*. Higher values for exposed SH content were observed in the hydrolyzed samples than native one. The values for total and exposed SH content of the samples hydrolyzed with papain were 5.45 ± 0.29 and 3.56 ± 0.17 , respectively. Native PPCs showed exposed SH values of 1.11, whereas PPCs hydrolyzed with CPE of *T. reesei*, *A. oryzae* and *R. oligosporus* showed exposed SH

values of 3.35, 2.56 and 1.88, respectively. SH groups are important functional parameters, which indicates about the strength of secondary and tertiary structure, and also reflect the extent of denaturation. Decline in the total SH content might be owing to the oxidation of disulfide bonds occurred due to the structural modifications. The structural changes occurred in PPCs due to hydrolysis were evident from the results of surface hydrophobicity (Table 1). These structural changes are associated with the improvement in other functional characteristics like solubility, water and oil binding capacity and foaming properties. Liu and Kuo (2016) reported that majority of -SH groups of native proteins are hidden inside and poorly accessible regions of the polypeptide chain, and usually do not come in contact with Ellman's reagent (DTNB). They proposed that changes in SH groups may be considered as an indicator of degree of unfolding in proteins. Enzymatic hydrolysis might have resulted in the unfolding of protein structure thereby exposing SH groups which ultimately increased the accessibility of DTNB to reaction sites.

Surface hydrophobicity (H_0)

The results for the surface hydrophobicity of native and hydrolyzed PPCs are presented in Table 1. The data shows that surface hydrophobicity of hydrolyzed PPCs was significantly ($p \leq 0.05$) higher than that of native PPCs. Among hydrolyzed PPCs, the highest surface hydrophobicity value of 564.18 was found in PPCs hydrolyzed with CPE obtained from *R. oligosporus*, and the lowest value of 340.64 was found in PPCs hydrolyzed with CPE obtained from *A. oryzae*. Peanut protein concentrates hydrolyzed with papain showed surface hydrophobicity value of 392.23. Zhao et al (2012) reported that enzymatic hydrolysis can unfold the structure of protein molecules, and thus

exposes hidden hydrophobic groups. However, it must be noted that higher surface hydrophobicity of protein is detrimental for other functional characteristics, such as solubility. Once unfolding takes place, hydrophobic groups are exposed to a larger extent and the exposed hydrophobic groups can self-aggregate resulting in the decline in solubility of protein molecules. In the present study, we observed higher surface hydrophobicity of PPCs hydrolyzed with CPE of *R. oligosporus*. The solubility of this sample was also found significantly lower than other hydrolyzed samples, which may be due to the aggregation of already exposed hydrophobic groups.

Solubility

The results in Fig. 3a indicate that in comparison with native PPC, the solubility of hydrolysed PPCs was significantly ($p \leq 0.05$) higher. Among hydrolysed samples, PPCs hydrolysed with CPE obtained from *A. oryzae* displayed higher solubility. Peanut protein concentrates

hydrolysed with papain showed solubility value of 61% and did not differ significantly ($p \leq 0.05$) with the samples hydrolysed with CPE from *T. reesei*. Improved solubility of hydrolysed proteins may be owing to the release of soluble peptides from insoluble aggregates or precipitates which favour the ionization of carboxylic and amino groups of amino acids. Zhao et al (2011) also observed higher solubility in peanut protein isolates hydrolyzed with commercial Alcalase enzyme. This may be associated with the fact that enzymatic hydrolysis results in the unfolding of secondary and tertiary structure of protein and increases the exposure of hydrophilic groups which ultimately improves hydration of protein molecules and thus solubility. Enzymatic hydrolysis might have also promoted the interaction between hydrophilic groups and water molecules due to the reduction in the peptide size, which increased the solubility.

Water binding capacity

The effect of CPE hydrolysis on the water binding capacity of PPCs is shown in Fig. 3b. The native PPCs has water binding capacity value of 1.11 g/g only. Significant ($p \leq 0.05$) increase in the water binding capacity of the samples was observed after hydrolysis. Higher water binding capacity value of 1.93 g/g was observed for the samples hydrolyzed with CPE obtained from *A. oryzae* (Fig. 3b), which was higher than the value (1.83 g/g) for samples hydrolyzed with papain. Improvement in water binding capacity of hydrolyzed PPCs is closely related to solubility. The structural changes which were evident from the results of surface hydrophobicity and SH groups may be the main contributors for the improvement in functional characteristics. The structural changes were owing to limited amount of unfolding, which occurred during hydrolysis. Once unfolding took place, the buried hydrophilic groups might have been exposed to the polar environment, which ultimately increased the solubility. Solubility is the pre-requisite for other functional properties, a small increment in solubility would affect other functional properties.

Oil binding capacity

Effect of CPE hydrolysis on the oil binding capacity of PPCs is shown in Fig. 3b. Similar trend was observed for oil binding capacity as for water binding capacity of hydrolyzed samples. Native PPCs showed lower oil binding capacity value (1.84 g/g), whereas hydrolyzed PPCs showed comparatively higher oil binding capacity. Higher oil binding capacity value of 3.04 g/g was found in PPCs hydrolyzed with CPE of *Rhizopus oligosporus* followed by PPCs hydrolyzed with *A. oryzae* and *T. reesei* (Fig. 3b).

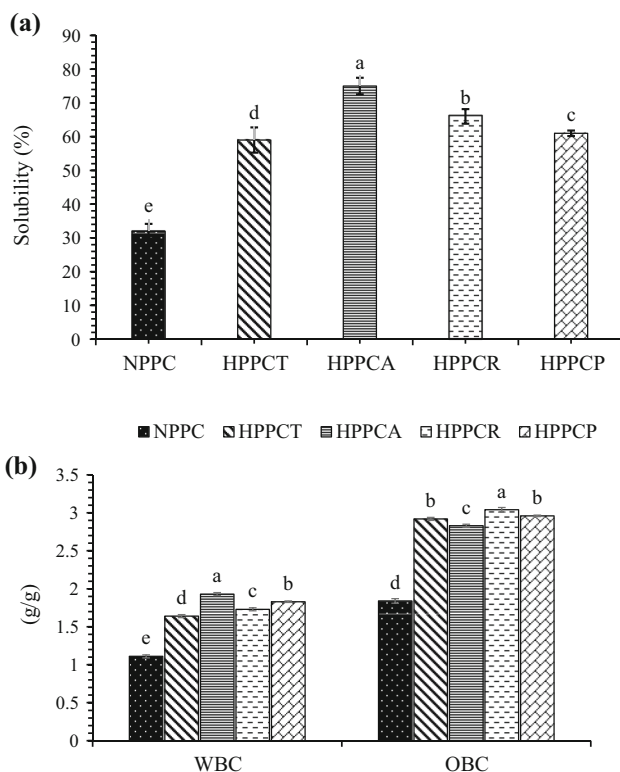


Fig. 3 Effect of crude protease extract enzymatic hydrolysis on solubility (a), water binding and oil binding capacity (b) of peanut protein concentrate. [NPPC = native peanut protein concentrate, HPPCT = hydrolyzed peanut protein concentrate with crude protease extract of *Trichoderma reesei*, HPPCA = hydrolyzed peanut protein concentrate with crude protease extract of *Aspergillus oryzae*, HPPCR = hydrolyzed peanut protein concentrate with crude protease extract of *Rhizopus oligosporus* and HPPCP = hydrolyzed peanut protein concentrate with papain]

Papain hydrolysed samples had oil binding capacity value of 2.96 g/g, which was comparable with the samples hydrolyzed with CPEs obtained from all three fungal sources. The possible reasons for the improvement in oil binding capacity could be attributed to the changes in the secondary structure of protein molecules because of enzymatic hydrolysis. The non-polar side chains of proteins might have been exposed during hydrolysis, which bounded fat molecules. Higher surface area of the protein particles and the exposure of hydrophobic groups due to unfolding of chains might be the main reasons for improvement of oil binding capacity.

Foaming capacity and foam stability

The data for the effect of crude protease enzymatic hydrolysis on the foaming capacity and foam stability of PPCs are presented in Table 1. Native PPCs showed foaming capacity value of 22.33% only, which was significantly ($p \leq 0.05$) lower than the foaming capacity of hydrolyzed PPCs. Among hydrolyzed PPCs, the highest foaming capacity value of 58.70% was observed in samples hydrolyzed with CPE obtained from *A. oryzae* followed by *R. oligosporus* (47.31%), papain (41.33%) and *T. reesei* (33.66%). Foams were more stable in *A. oryzae* hydrolyzed samples followed by *R. oligosporus*, papain and *T. reesei*. Improvement in foaming capacity and foam stability might be owing to lower particle size and higher solubility (Fig. 3a). This reconfirms the exposure of hydrophobic groups after hydrolysis. Higher foam stability may be attributed to lower particle size of hydrolyzed PPCs as mentioned previously in this manuscript. Panyam and Kilara (1996) observed that enzymatic hydrolysis of protein results in lower molecular weight peptides as well as brings some structural modifications. The structural modifications expose the hidden hydrophobic groups, which improve the surface properties of protein.

In-vitro protein digestibility (IVPD)

The data pertaining to the effect of enzymatic hydrolysis on the in-vitro protein digestibility are presented in Table 1. It can be observed that the IVPD of native PPC was comparatively lower (70.11%) than IVPD of hydrolyzed PPCs. Papain hydrolyzed samples showed IVPD value of 94.62%. Among CPE hydrolyzed samples, the higher IVPD value of 86.70% was found in samples hydrolyzed with CPE obtained from *A. oryzae* followed by *R. oligosporus* and *T. reesei*. Vishwanatha et al (2009) reported that *A. oryzae* has the higher expansion of hydrolytic genes (135 proteinase genes) and also possesses more secretory proteinase genes that function in acidic pH too. This could largely be responsible for significantly higher IVPD of PPC

hydrolyzed with CPE obtained from *A. oryzae*, compared to PPCs hydrolysed by CPEs from the other two fungi. Microbial crude protease obtained from *A. oryzae* are non-toxic due to its long history of industrial uses and also due to the absence of expressed sequence tags for the genes responsible for aflatoxin production. It's worth mentioning here that *A. oryzae* is used in the preparation of different fermented foods and has been accorded GRAS status by USFDA. Improvement in IVPD of hydrolyzed samples is due to the fact that proteases catalyze the hydrolysis of peptide bonds, which in turn converts proteins into peptides of smaller size and free amino acids. Lower molecular weight peptides and free amino acids can be easily digested by the small intestine and also possess lower allergenic effects. Su et al (2011) also observed that CPE obtained from *A. oryzae* possesses comparatively higher nutritional quality than other commercially available pure proteases.

Conclusion

It is evident from the study that hydrolysis using crude protease extracts is an effective approach for improving the functional as well as in-vitro digestibility of peanut protein concentrate. The CPE obtained from three fungal sources positively affected the different structural and functional characteristics of PPCs. Crude protease extracts obtained from *A. oryzae* was more effective in improving the functional and digestibility profile of PPCs in comparison to other two fungal CPEs. Therefore, this study has set a platform for conducting more comprehensive studies on the use of CPEs from *A. oryzae* for hydrolysis of complex protein isolates and concentrates from a variety of sources in order to improve the functional and nutritional characteristics of such proteins besides offering a cost effective solution.

Acknowledgements The authors are highly thankful to National Agricultural Science Fund (NASF), New Delhi, India for providing funds for the study. Authors also acknowledge Bio-process Engineering Laboratory, ICAR-CIPHET Ludhiana, India for providing microbial cultures used in the study.

Declarations

Conflict of interest The authors declare that there is no conflict of interest in the present study.

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