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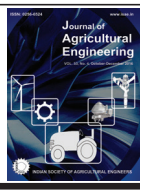

Quality Characteristics and Functional Properties of Soy Protein Isolate Prepared Using Ultrafiltration

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ABSTRACT

Soy protein isolate (SPI) were obtained through ultrafiltration using 10 kDa hollow fibre cartridge, 117.21 kPa trans-membrane pressure and 3.5 volume concentration ratio. SPI prepared by ultrafiltration possessed highest protein content (88±0.3 %) and 91.09±0.84 % nitrogen solubility index (NSI) as compared to 84±0.5 % protein content and 19.29±0.74 % NSI for acid precipitated and 85±0.5 % protein content for commercially available SPI. Ultra filtered SPI also possessed highest value of emulsion stability (ES) of 51.96±1.42 %, whereas commercially available SPI exhibited the least value of 48.38±0.015 per cent. Highest value of oil absorption capacity (OAC) was 2.15±0.04 $g.g^{-1}$ of protein for ultra-filtered, followed by 1.94±0.01 $g.g^{-1}$ of protein for commercially available, and 1.73±0.04$g.g^{-1}$ of protein for acid precipitated SPI. The hydrophilic lipophilic index (HLI) values for both ultra-filtrated and commercial SPIs were more than 2, indicating hydrophilic-lipophilic balance of proteins. However, water absorption capacity (WAC) and emulsion capacity were less for ultra-filtered sample as compared to commercially available counterpart. Commercially available SPI had highest value of 6.84±0.08 $ml.g^{-1}$ of protein, whereas it was least at 1.67±0.016 $ml.g^{-1}$ of protein for acid precipitated SPI.

Protein is obtained from both animal and plant sources. Soybean is one of the rich sources of plant protein, and contains around 40 % protein (Agarwal *et al*., 2013). Soy protein is a high-quality, complete protein that contains all essential amino acids. It provides nutritional, functional and environmental advantages over animal-based proteins (Anon, 2011).

Lusas and Riaz (1995) reported that soy proteins are mainly used as ingredients in formulated foods and as a substitute for other ingredients used in compounded foods for their functional properties, including water and fat absorption, emulsification, aeration (whipping) and for increasing total protein content and improving the essential amino acids profile. Functionally, soy protein can replace more expensive meat or dairy products without changing the taste and quality of foods. Among all the major commercially available soy proteins, isolate having about 90 % protein on dry basis has been industrially produced with wide range of functional properties for various purposes. It can be used as emulsifiers, texture enhancers, and as ingredients to increase or replace protein content in food products like bread, pastry products, beverages, and meat (Lai *et al*., 2013).

Extensive research has been done on enzymatic, mechanical and thermal modifications of soy proteins to improve the functional properties of isolate and expand its range of applications (Bernard *et al*., 2007). However, its use has been limited in food products due to undesirable flavours, reduced bio-availability, presence of high levels of phytic and nucleic acids and insolubility. Traditionally, soy protein concentrates (SPC) and soy protein isolates (SPI) are produced using extraction, heat treatment, precipitation by the addition of acid or alcohol, and centrifugation to separate the protein from the other components. The time consuming conventional method produces SPI with low functional properties, and generates

large amounts of by-products causing environmental problems. SPI produced through this method contains many phytochemicals including residual fatty acids, lecithin, phytic acid, isoflavones, saponin, pigments and minerals (Fang *et al.,* 2007). Though these components offer health benefits, but some of them are not suitable for infants and young children (Bousquet and Burney, 1993). Membrane separation technique can be adopted as an alternative method for protein concentration and purification. Junuh (2009) reported that the main undesirable components in soy bean are oligosaccharides, phytic acid and trypsin inhibitors. These components have smaller molecular weight, and can be removed by selecting proper membrane cartridge on the basis of molecular weight cut-off (MWCO) and operating parameters. Protein yield, protein quality and functional properties can be improved by applications of membrane processes. Shallo *et al.* (2001) observed that protein recovery can be increased from 17 % to 26 % by using membrane separation as compared to current commercial processes. Soy proteins produced using membrane separation process contains relatively small molecular compounds that can form a firm active internal emulsion surface producing a stable emulsion (Kim and Kim, 2015).

Over the years, ultrafiltration has received considerable interest for concentration of soy proteins as proteins are retained by the membrane, while the oligosaccharides and minerals are removed as permeate. The products with improved properties are obtained through ultrafiltration, and there are also no excessive uses of chemicals (Alibhaia *et al*., 2006). Soy protein isolate has applications as an ingredient in high protein foods, including dairy foods, as a milk replacer, nutritional supplements, meat systems, infant formulas, nutritional beverages, cream soups and sauces, snacks, etc. With the variation in molecular weight cut-off (MWCO) of ultrafiltration cartridge, the composition of end-product and hence its properties will change. Singh (2007) reported the advantages of using low molecular weight cut-off membranes to produce high quality soy protein isolate. The functional properties of soy protein isolates obtained through conventional process are lower due to repeated pH shocks. It can be improved using membrane separation technique. Research attempts show that mild processing conditions (non-use of concentrated acids or alkali and moderate temperatures) in membrane filtration for the production of soy protein isolates improved the functional properties of the products, which makes it an attractive and technologically viable solution. Therefore, an attempt was made to study the functional properties of soy protein isolate using ultrafiltration with proper filter cartridge at optimized processing parameters, and compare it with acid precipitated and commercially available counterpart.

MATERIALS AND METHODS

Soy Protein Extraction

Defatted soy flour (DSF), used as the starting material for extraction of soy protein and all chemicals were purchased from local market. Commercially available soy protein isolate was procured from M/S Sonic Biochem Extractions Ltd., Indore (M.P.), India. Chemical acid precipitation method was used for the preparation of commercial SPI for improved functional properties by M/S Sonic Biochem. The experiments for ultra-filtered SPI were conducted at the Centre of Excellence on Soybean Processing and Utilization, Central Institute of Agricultural Engineering, Bhopal.

Extraction of protein from defatted soy flour was performed in purified water at pH 9 (adjusted with 0.2 M NaOH) and at 50 ºC with a solid/liquid ratio of 1/10, using a mechanical stirrer (Make Jyoti, model JSI-555, rpm range: 0 to 2000) for one and half hours. Solid-liquid separation was performed in a centrifuge (Remi instruments-model K-70, India) at 10,000 g for 20 min at 15 °C temperature (Sinha et al., 2010). The supernatant obtained was used as a feed to the ultrafiltration after pre-filtration through a Millipore micro filtration unit (cellulose nitrate membrane filters with 47 mm diameter and 5.0 µ size). For conducting the experiments, 75 g of defatted soy flour (particle size: 330 µm) was taken, and 550 ml extract was obtained after centrifugation. Total solid content of the extract was recorded as 7 ± 1 per cent.

Soy Protein Isolate Preparation

Ultrafiltration method

Preliminary study was conducted to identify the process parameters of ultrafiltration. Membrane modules (1-30 kDa), trans membrane pressure (82.74-151.69 kPa), diafiltration (0-2 batch type) and volume concentration ratio (3-4) were selected as independent variables; and permeate flux and rejection factor were the dependent parameters. Temperature, pH and solid content of feed were kept constant.

Optimization was done with Design Expert software using response surface methodology (Box Benhken

design) through a separate study with 46 runs (6 central points). Optimized process parameters were used for producing SPI by ultrafiltration in the present study. A detail of statistical design is given in Table 1. Optimized conditions obtained were: Ultrafiltration membrane cartridge: 10 kDa; Trans membrane pressure: 117.21 kPa; Volume concentration ratio: 3.5 and Diafiltration one time (batch type) at 69 % of pump capacity. Optimized conditions were used for analysing the quality characteristics of SPI.

Hollow fibre-type ultrafiltration cartridge (10 kDa; GE Healthcare, model UFP-10-C-4MA, USA) was used with maximum trans-membrane pressure of 2 $kg.cm^{-2}$ and a volume concentration ratio of 3.5 for getting a concentrate with 16 ± 2 % total soluble solids. Filtration was carried out in retentate recycle mode with 1-2 $ml.min^{-1}$ feed rate. Concentrate obtained from ultrafiltration was dried using a spray drier (Yamato mini spray dryer, model: ADL 31; Yamato Scientific Co. Ltd., Tokyo, Japan; water evaporation: 1,300 $ml.h^{-1}$; temperature range: 40 °C to 220 °C) with inlet temperature of 180 °C, outlet temperature of 85 °C and atomizing air pressure of 2.25 $kg.cm^{-2}$ to obtain SPI powder. This was stored under refrigerated condition for further analysis.

Acid precipitation process (at iso-electric point)

The pH of crude protein extract obtained from extraction was lowered to 4.5 (general isoelectric point of soy-protein) by adding 1 M HCl. The precipitated protein was separated from the soy whey by centrifugation at 10,000 g for 10 min at 15 °C. The precipitate was washed twice by dispersing in de-ionised water (pH: 4.5) and centrifuged at 10,000 g for 10 min. The washed precipitate was finally dispersed in de-ionised water (10 % w/v) and the pH adjusted to 7 using 1 M NaOH. This constituted the main SPI solution, which was fed to the spray drier (Yamato mini spray dryer, model: ADL 31; Yamato Scientific Co. Ltd., Tokyo, Japan) with inlet temperature of 180 °C, outlet temperature of 85 °C and atomizing air pressure of 2.25 $kg.cm^{-2}$ for getting SPI in powder form. Powdery SPI was stored under refrigerated conditions.

Determination of Quality Characteristics

Moisture, protein, fat and ash contents of DSF, ultra-filtered SPI, acid precipitated SPI and commercially available SPI were estimated as described below using standard methods.

Estimation of moisture content

The moisture content of SPI was determined by gravimetric method. A sample was taken in a petri dish and the initial weight ($W_{initial}$) was measured by an electronic balance (Citizen, cy304, Range: 10mg to 320 g, least count: 0.1 mg). The sample was kept in a hot air oven maintained at a temperature of 105 °C for 12 h, and the weight of the dried sample was taken (W_{final}).

Moisture content was expressed as the percentage change in weight (Ranganna, 1995).

$$\text{Moisture content (\%)} = \frac{\text{Winitial–Wfinal}}{\text{Wfinal}} \times 100 \quad \ldots (1)$$

Estimation of protein

Protein estimation was done according to the method described by Ranganna (1995).

Half gram of the sample was weighed into the digestion tubes of the digester (KEL PLUS Automatic Macro Six Sample Auto sequencing PC Compatible System,capacity: 250 ml; Model KES 06L VA

Table 1. Optimization of ultrafiltration process parameters Box Behnken design

Independent parameter	Levels		
	-1	0	1
Ultrafiltration membrane modules (kDa)	1	10	30
Trans membrane pressure (PSI)	12	17	22
Volume concentration ratio (VCR)	3	3.5	4
Diafiltration (batch)	0	1	2
Flow rate (% pump capacity)	55	65	75
Dependent parameters: Permeate flux, Protein content in retentate, Protein rejection			

DLS), and two heaped spatulas of digestion mixture (potassium sulphate and copper sulphate) were added to each tube. Ten ml of concentrated H_2SO_4 was also added, and the samples digested until the contents of the tubes were sea green in colour.

Each tube along with the digested sample was transferred into a distillation chamber (Pelicon Kelplus, Make: Pelican Equipments, Model: KELPLUS Classic-DX VATS (E); Single sample automatic system), and 20 ml of 2 % boric acid with 1-2 drops of the mixed indicator was placed in the collecting conical flask to trap the liberated ammonia. The unit was furnished with 40 % NaOH and distilled water to facilitate operation. Distillation was done for 5 min, and the ammonia was collected and trapped by the boric acid. The boric acid turned from reddish-pink to green as it collected the ammonia. The green coloured boric acid was then titrated against 0.1 N HCl until its colour turned to pink. A blank was run simultaneously. The titre values obtained were incorporated in Eq. 2 to obtain the per cent nitrogen present in the sample, which in turn was multiplied by the factor 6.25 to obtain the per cent protein.

$$\text{Per cent protein content} = \frac{(V_A - V_B) \times 0.1 \times 14.001 \times 6.25)}{W} \quad \ldots (2)$$

Where,

V_A = Sample titre value, ml,

V_B = Blank titre value, ml, and

W = Sample weight, g.

Estimation of fat

Estimate of fat was done in a Soxhlet apparatus (SoxTRON, Model: Sox 6– Six samples Automatic Fat/oil Solvent Extraction System with auto time temperature Microprocessor Programming control Unit) in which fresh solvent continuously remained into contact with the material to be extracted over a period of time. One gram of the sample was weighed into a thimble and plugged with fat-free cotton wool. The thimble was placed in the automatic Soxhlet Apparatus attached to a pre-weighed flask and extracted with anhydrous ether for about 3 h. Thereafter, the flask was retrieved from the apparatus with as little solvent in it as was possible. It was then transferred into an oven to evaporate the remaining solvent, leaving behind only the residue or extract. The flask was then cooled in a desiccator, and then weighed to estimate the fat.

Fat content was then calculated as (Ranganna, 1995):

$$\text{Fat content (\%)} = \frac{\text{Weight of residue (g)}}{\text{Weight of sample taken (g)}} \times 100 \quad \ldots (3)$$

Estimation of ash

The total ash content was determined by weighing about 3-5 g of the sample in a previously heated and cooled silica/porcelain dish (AOAC, 1980). The sample was carefully charred on a flame, and then heated in a muffle furnace maintained at 650°C for 3 h or until white ash was obtained.

Ash content was calculated as:

$$\text{Ash (\%)} = \frac{\text{Weight of the residue after ashing}}{\text{Weight of the sample}} \times 100 \quad \ldots (4)$$

Determination of Functional Properties

Water absorption capacity

Water absorption capacity (WAC) was determined according to the method of Beuchat (1977) with some modifications. Approximately 0.5 g of soy protein isolate was taken in a 50 ml centrifuge tube, and 6 ml of distilled water was added to that. The tube was agitated on a vortex mixer (Labnet International VX-200; 0-2850 rpm; continuous mode of operation) for 1 min at high speed (2000 rpm). The sample was left to settle for 30 min, and then centrifuged at 5000 g for 20 minutes.

WAC (expressed as ml of water per g of protein) was calculated as:

$$\text{WAC} = \frac{\text{Volume of water added (ml)} - \text{Volume of supernatant (ml)}}{\text{Weight of protein (g)}} \quad \ldots (5)$$

Oil absorption capacity

Oil absorption capacity (OAC) was determined according to the method of Chakraborty (1986) with some modifications. Soy protein isolate sample of 0.5 g was taken in a 50 ml centrifuge tube, and 6 ml of soy oil was added to that. The tube was agitated on a vortex mixer (Labnet International VX-200; 0-2850 rpm; continuous mode of operation) for 1 minute at high speed (2000 rpm). The sample was left for 30 min to settle down, and then centrifuged at 5000 g for 20 minutes. OAC (expressed as gram per gram of protein)

was determined as:

$$OAC = \frac{\text{Weight of tube with sediment (g)} - \text{Weight of tube with dry sample (g)}}{\text{Weight of dry sample (g)}} \quad \ldots(6)$$

Hydrophilic/lipophilic index

Hydrophilic/lipophilic index (HLI), defined as the relative affinity of powder to water and oil, was determined as the ratio of WAC to OAC (De Kanterewicz *et al*., 1987; Njintang *et al*., 2001).

Emulsification Properties

Emulsifying capacity (EC) and emulsion stability (ES) are important emulsification properties considered in the study.

Emulsifying capacity

EC was determined according to the method of Yatsumatsu *et al*., (1972) with slight modifications. About 1 g of soy protein isolate was taken in a 50 ml centrifuge tube, and 10 ml each of distilled water and soy oil was added to that. This mixture was hand shaken for 1 min at moderate speed (800 rpm). Obtained emulsion was centrifuged at 5000 rpm for 5 minutes.

EC was calculated as:

$$EC\ (\%) = \frac{\text{Height of emulsified layer (cm)}}{\text{Height of whole layer in centrifuge tube (cm)}} \times 100 \quad \ldots(7)$$

Emulsion stability

For determining emulsion stability, the emulsion was heated at 80 °C for 30 min, and subsequently cooled with tap water for 15 minutes. The obtained emulsion was centrifuged at 5000 rpm for 5 minutes.

ES was expressed as:

$$ES\ (\%) = \frac{\text{Height of emulsified layer (cm)}}{\text{Height of whole layer in centrifuge tube (cm)}} \times 100 \quad \ldots(8)$$

Nitrogen solubility index

Nitrogen solubility index (NSI) was determined using the method suggested by Karki *et al*. (2009) with some modifications. About 0.5 g of sample was mixed with 20 ml distilled water. Extraction was done for 120 min in a magnetic stirrer at 120 rpm. Extracted sample was centrifuged at 275 g for 10 minutes. Supernatant was collected and analysed for nitrogen content using Micro Kjeldahl apparatus (Digestion unit: KEL PLUS Automatic Macro Six Sample Auto sequencing PC Compatible System (250 ml capacity) Model KES 06L VA DLS (Value Added Data Logger Version); Distillation unit: Pelicon KELPLUS Make: Pelican Equipments Model: KELPLUS CLASSIC-DX VATS (E) Single sample Automatic system).

Nitrogen soluble index was calculated as:

$$NSI\ (\%) = \frac{\%\ \text{water soluble nitrogen}}{\%\ \text{total nitrogen}} \times 100 \quad \ldots(9)$$

Statistical Analysis

All experimental trials in the study were replicated five times, and the average values reported.

Statistical analysis was done using SAS 9.3 software. ANOVA was performed to compare the effects of different SPI producing methods (acid precipitation, ultrafiltration and commercially available) on various functional properties. LSD was run as post-hoc test to compare the significant difference between the selected methods at 5 % level of confidence.

RESULTS AND DISCUSSION

Fouling and Flux Decline Characteristics of Ultrafiltration

During the initial stages of process (for first 30 min), the flux was constant at 10±1 $l.m^{-2}.h^{-1}$. Subsequently, a sharp decline in flux was observed. The flux was observed as 5±1 $l.m^{-2}.h^{-1}$ towards the end of filtration process. This decline in flux was due to fouling of the hollow fibre membranes.

The observations were in agreement with the findings of Marshall and Daufin (1995) and de Morais Coutinho *et al.* (2009), who explained that the permeate flux versus time curve and the rapid flux decrease in the initial period was attributed mainly to concentration polarization occurring very rapidly.

Quality Characteristics

Proximate analysis of DSF, ultrafiltered SPI, acid precipitated SPI and commercially available SPI are given in Table 2. SPI prepared by ultrafiltration possessed highest protein content (88±0.3 %) compared to acid precipitated SPI and commercially available SPI (84±0.5 % and 85±0.5 %, respectively).

The results were similar to the findings of Junuh (2009), who reported that the protein content in soy protein

Table 2. Proximate analysis of soy protein isolates prepared with different methods

Parameter	DSF	Ultrafilterd SPI	Acid precipitated SPI	Commercially available SPI
Protein, %	56	88±0.3	84±0.5	85±0.5
Moisture, % (w.b.)	5.5±0.1	5.7±0.03	6.8±0.02	6.0±0.2
Fat, %	0.45±0.01	0.40±0.02	0.40±0.025	0.45±0.02
Ash, %	6.77±0.11	4.40±0.03	4.60±0.02	4.50±0.02

Table 3. WAC, OAC, HLI and NSI of SPI samples

SPI sample	WAC, $ml g^{-1}$ of protein	OAC, $g.g^{-1}$ of protein	HLI	NSI, %
Ultra filtered SPI	$5.12±0.04^{b}$	$2.15±0.04^{c}$	$2.39±0.018^{b}$	$91.09±0.84^{c}$
Commercially available SPI	$6.84±0.08^{c}$	$1.94±0.01^{b}$	$3.55±0.012^{c}$	$56.61±1.53^{b}$
Acid precipitated SPI	$1.67±0.016^{a}$	$1.73±0.04^{a}$	$0.96±0.013^{a}$	$19.29±0.74^{a}$

Note: $^{a, b, c}$ indicates the treatments are significantly differ from each other at a level of significance of 1 %.

from soy milk was more when prepared with membrane separation processes. Also, protein recovery was high with membrane separation (47 %) as compared to acid precipitation (36 %).

Functional Properties

Water absorption capacity

Significant difference ($p<0.0001$) was observed in water absorption capacity (WAC) of soy protein isolates (SPI), (Table 5). Commercially available SPI had highest value of WAC (6.84±0.08 $ml.g^{-1}$ of protein), whereas acid precipitated SPI had least WAC of 1.67±0.016 $ml.g^{-1}$ of protein. The WAC value of ultrafiltered SPI was 5.12± 0.04 $ml.g^{-1}$ of protein (Table 3). Fig. 1 shows the distribution of WAC values of tested SPIs. Denaturation of protein caused due to the processing methods adopted might also be one of the reasons for variations in WAC (Jovanovich *et al.,* 2003) with the effect of temperature. Butt and Batool (2010) had conducted a study to evaluate the functional properties of composite flour and reported that the observed variation in WAC of different flours might be due to their different protein concentration and their degree of interaction with water. These reasons also might be the causes of variation in WAC of SPIs.

The variation in WAC might affect other functional properties such as swelling, viscosity, gelation, emulsification, and foaming. This might be due to partial denaturation of soy protein during extraction and subsequent drying (Zeyas, 1997). Seena and Sridhar (2005) mentioned that WAC is a critical property of proteins in viscous foods such as soups, doughs, custards and baked products as these foods absorb water without protein dissolution, and thereby provides proper thickening and viscosity of end product. Since sensory properties are directly related to water retention, it is desirable to have soy proteins with higher value of WAC. Wagner and Anon (1990) reported that SPI with higher WAC might be well suited for making beverages due to more protein solubility. The observed values of WAC for ultrafiltered SPI indicated possibility for use of the SPI in bakery products like bread and cake. Mao and Hua (2012) reported that high WAC of defatted walnut flour makes it a potential ingredient for the meat, bread, and cakes industries.

Oil absorption capacity

Highest oil absorption capacity (OAC) was observed for ultra-filtered SPI (2.15±0.04 $g.g^{-1}$ of protein), followed by commercially available SPI (1.94±0.01 $g.g^{-1}$ of protein) and acid precipitated SPI (1.73±0.04 $g.g^{-1}$ of protein), Table 3. The OAC was found to be insignificantly different at $p\leq0.0001$ (Fig. 2). Table 5 shows the ANOVA for OAC, which indicated the significance of the model for OAC values. Manak *et al.* (1980) also reported OAC of ultra-filtered SPI to be higher than the commercial soy isolate. According to Jitngarmkusol *et al.* (2008), the major chemical component affecting OAC was protein that was composed of both hydrophilic and hydrophobic parts. Non-polar amino acid side chains can form

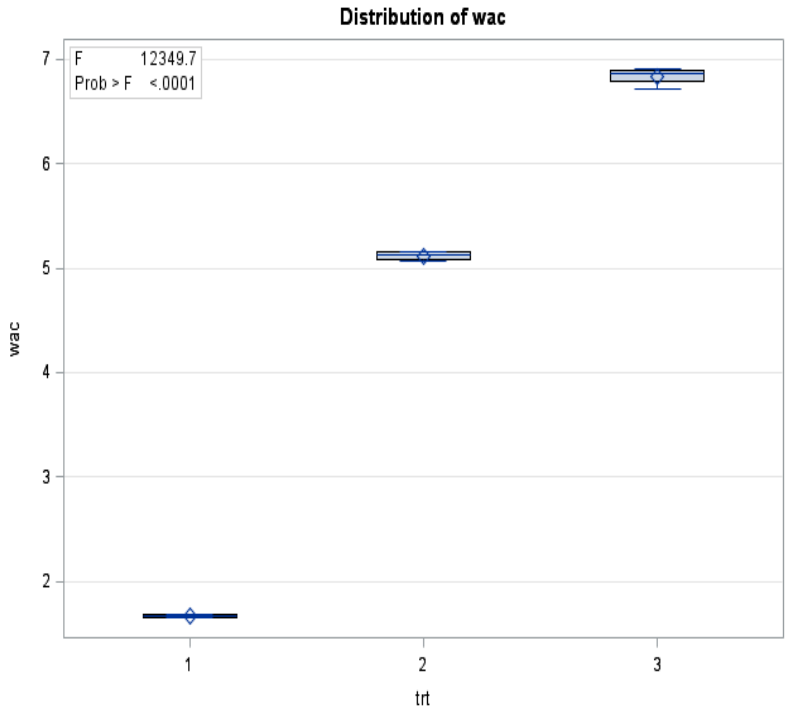

Fig. 1: WAC (%) of SPIs : (1) isoeletic precipitated SPI, (2) Ultrafiltered SPI., (3) Commercially available SPI

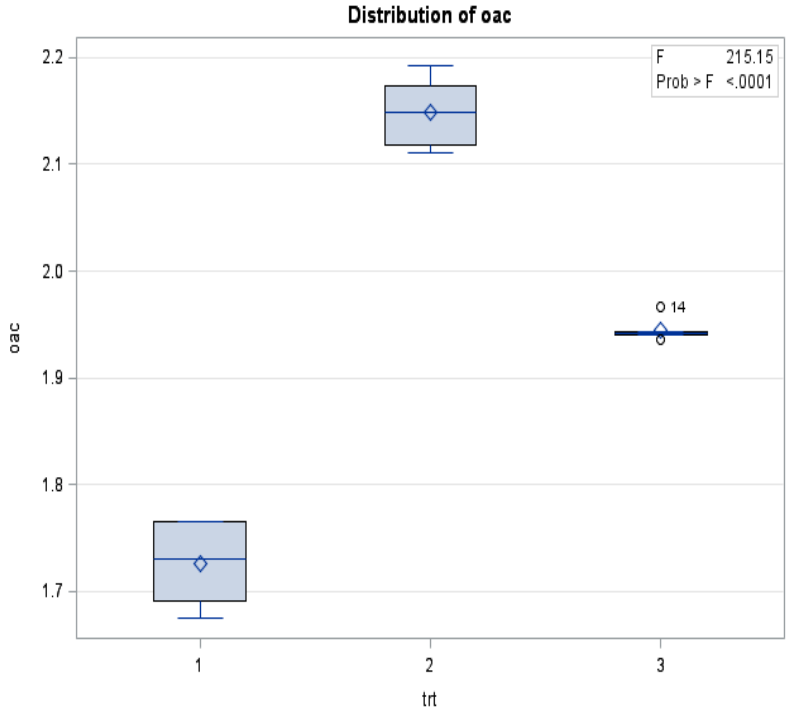

Fig. 2: OAC (%) of SPIs : (1) isoeletic precipitated SPI, (2) Ultrafiltered SPI., (3) Commercially available SPI

hydrophobic interaction with hydrocarbon chains of lipids. Butt and Batool (2010) had also observed that protein possesses both hydrophilic and lipophilic properties, and it can interact with both oil and water. Low OAC of acid precipitated SPI might be due to the presence of large proportion of hydrophilic and polar amino acids on the surface of the protein molecules (Sathe *et al*., 1982). As reported by Ulloa *et al*. (2017), nonpolar amino acid side chains of proteins can form hydrophobic interactions with lipid hydrocarbon chains that affect the oil binding capacity. The mechanism of fat/oil absorption can be explained as the physical entrapment of oil.

Fat/oil absorption capacity is a critical determinant of flavour retention. A high oil absorption capacity of protein is required in the formulation of ground meat and of replacements and extenders for use in products such as doughnuts, baked goods, frankfurters, sausages and soups (Singh *et al*., 2008). From the observed values of OAC, it could be concluded that ultrafiltered SPI is more lipophilic in nature, and can be used for bakery products. SPI with higher oil absorption capacity can be used for the preparation of frankfurters, sausages, meat patties etc. (Singh *et al*., 2008). Mao and Hua (2012) had reported that high oil absorption capacity of walnut protein isolate and walnut protein concentrate make them good ingredients in cold meat industry, particularly for sausages, where the protein usually bridge the fat and water in order to obtain good products.

Hydrophilic/lipophilic index

HLI is an indication of hydrophilic-lipophilic balance of proteins (Griffin, 1949). Application of proteins in surfactants and emulsifiers can be decided based on HLI value.

Hydrophilic/lipophilic index (HLI) values for ultra-filtered, commercially available and acid precipitated SPIs were 2.39±0.018, 3.55±0.012 and 0.96±0.013, respectively (Table 3). HLI values of SPIs have significant difference (Table 5).

Emulsification capacity increased with increasing HLI to reach a maximum when HLI was nearly two when the protein absorbed two times more water than oil per unit weight (De Kanterewicz *et al*., 1987). Hence, from the above obtained results, SPIs produced by ultrafiltration can be used as good emulsifying agent in food items. It could also be concluded that commercially available SPI is more hydrophilic in nature, whereas acid precipitated SPI is lipophilic.

Emulsification Properties

There was significant difference between the emulsion

Table 4. Emulsifying properties of SPI samples

	Emulsifying property	
	EC (%)	ES (%)
Ultra filtered SPI	53.25±0.009^{b}	53.26±0.012^{b}
Commercially available SPI	56.38±0.011^{c}	48.38±0.015^{a}
Acid precipitated SPI	46.67±0.011^{a}	48.94±0.011^{a}

Note: $^{a, b, c}$ indicates the treatments are significantly differ from each other at a level of significance of 1 %.
ES (%) of commercially available SPI and acid precipitated SPI are not significantly different.

Table 5. ANOVA of functional properties

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
WAC	2	69.252584	34.626292	12349.7	<.0001
OAC	2	0.4492515	0.2246257	215.15	<.0001
HLI	2	16.279826	8.1399128	3869.35	<.0001
EC	2	363.37598	181.68799	149.07	<.0001
ES	2	47.155024	23.577512	15.15	0.0005
NSI	2	12894.769	6447.3846	5363.46	<.0001

capacity (EC) and emulsion stability (ES) values of SPIs obtained from different methods (Table 5). From Table 4 it can be seen that commercial SPI had highest EC (56.38±1.06 %), followed by ultrafiltered SPI (53.25±0.009 %) and acid precipitated SPI (46.67±0.011 %). Ultrafiltered SPI had highest ES (51.96±1.42 %), and commercially available SPI exhibited least (48.38±0.015 %). Figure 3, 4 shows the variation in EC and ES values of SPIs studied. Increasing emulsion capacity, emulsion stability and fat binding during processing are primary functional properties of protein in such foods as comminuted meat products, salad dressing, frozen desserts and mayonnaise (Chandra *et al*., 2015). Seena and Sridhar (2005) also reported that superior emulsifying properties of legumes are desired to make milk like beverages and meat analogues. The effect of emulsifying agents on dough and baked products is based on their reaction with the starch–protein–fat–water system as strength of the gluten structure and handling characteristics of dough as well as gas retention is improved and staling is delayed (Aaron and Harold, 1985). Hence SPI produced through ultrafiltration can be used for the above-mentioned applications.

Figure 6 exhibits the variations in ES with HLI values of the SPIs. ES value is higher for SPI with higher HLI. Njintang *et al.* (2001) also observed that with increase in ES, HLI increases. They reported that composite flours with high HLI possessed better emulsion stability than those with lower HLI. Proteins were surface active agents that stabilized the emulsion by creating electrostatic repulsion on oil droplet surface (Makri *et al*., 2005). Kato *et al*. (1983) found that surface hydrophobicity of proteins increased with increasing temperature, and increase in surface hydrophobicity correlated linearly with EC and ES. High emulsifying properties of soy protein obtained by ultrafiltration were probably due to its high phospholipid (lecithin) content, and high solubility. Protein-lipid and protein-water interactions were the major factors of emulsion formation and affect the appearance, colour, texture and yield of finished products.

Nitrogen solubility index

SPI produced through ultrafiltration showed highest value (91.09±0.84 %) of nitrogen solubility index (NSI), whereas it was least (19.29±0.74 %) for acid precipitated SPI (Table 3). NSI values varied

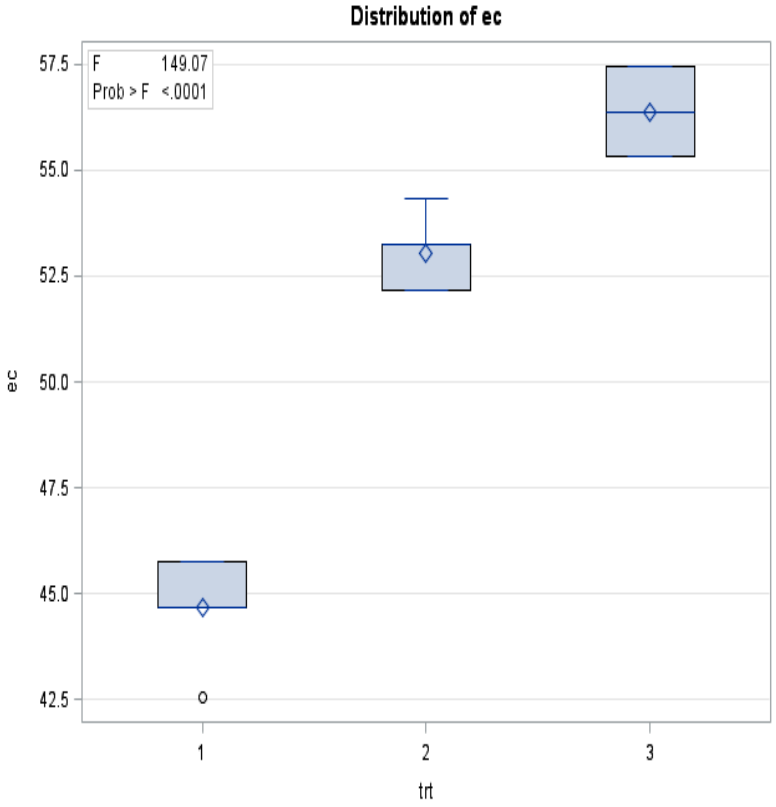

Fig. 3: EC (%) of SPIs : (1) isoeletic precipitated SPI, (2) Ultrafiltered SPI., (3) Commercially available SPI

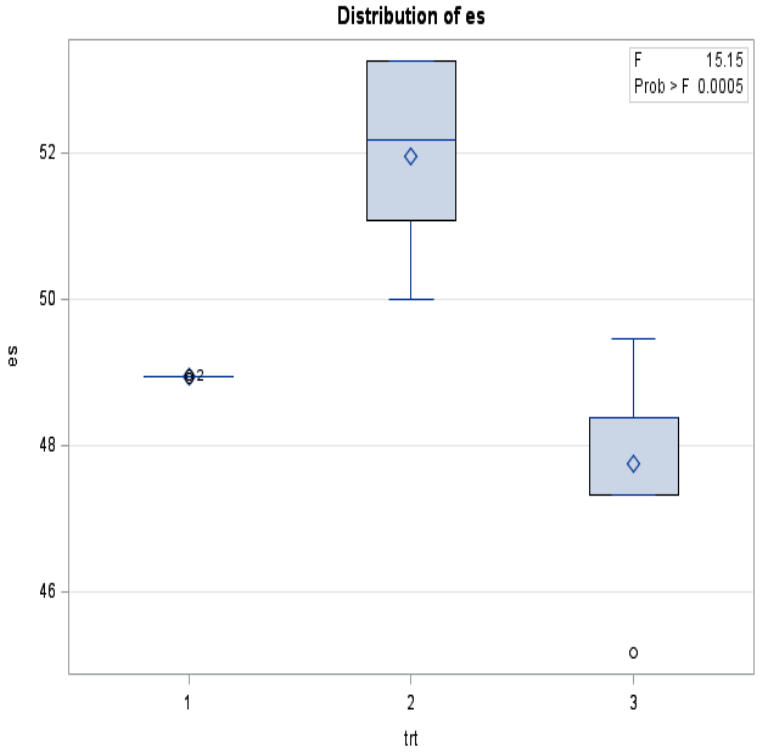

Fig. 4: ES (%) of SPIs : 1: isoeletic precipitated SPI, 2: Ultrafiltered SPI., 3: Commercially available SPI

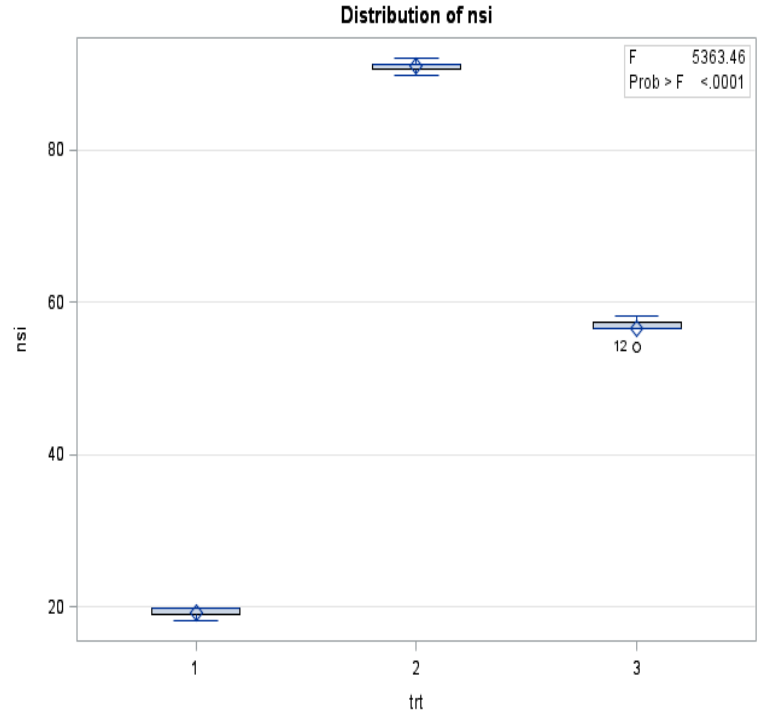

Fig. 5: NSI (%) of SPIs: (1) isoeletic precipitated SPI, (2) Ultrafiltered SPI., (3) Commercially available SPI

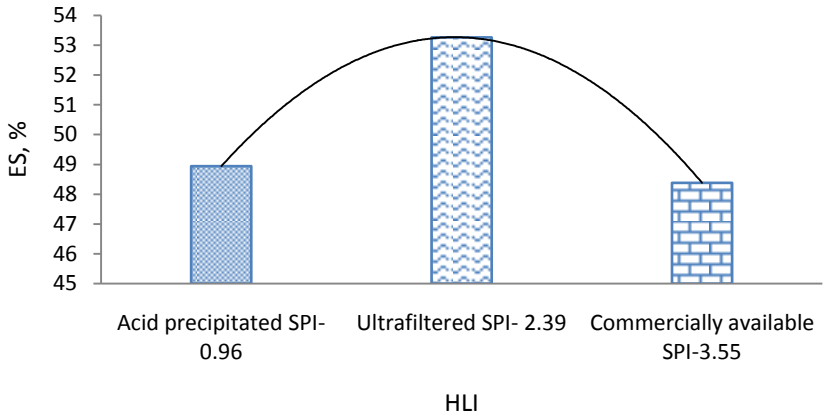

Fig. 6: Changes in ES with HLI

significantly with processing methods (Fig. 5). NSI was significant at $p<0.0001$ (Table 5). This finding was in agreement with Lopez de Ogara *et al*. (1992), who reported that solubility of soy protein isolate decreased with increase in temperature due to the denaturation of proteins. NSI is a measure of solubility of protein and its high value (more than 80 %) is desirable for applications like emulsification, whipping and film formation. Low NSI produced low solubility, and was used for applications where limited emulsifying properties and protein-protein interaction were desired. It was reported that NSI values of soy protein ranged from 25-90 % (Aaron and Harold, 1985). Hence, ultra-filtered SPI may be recommended for the above-mentioned uses.

CONCLUSIONS

Processing methods play a vital role for obtaining soy protein isolate (SPI) with improved functional properties. The present study concluded that SPI prepared by ultrafiltration possessed highest protein content (88±0.3 %), highest value (91.09±0.84 %) of NSI, emulsion stability (51.96±1.42 %), and highest OAC value of 2.15±0.04 $g.g^{-1}$ of protein as compared to commercially available and acid precipitated SPI. SPI is a potential alternative for many animal-based proteins as a supplement or replacer. Soy protein isolates are being used in many food formulations because of its functional properties rather than nutritional aspects. Ultrafiltration technique being an eco-friendly and potential alternative method to produce SPIs with improved functional properties without compromising the nutritional qualities can, therefore, be recommended as potential method for obtaining SPI without heat treatment and with higher functional properties.

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