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NUTRITIONAL AND FUNCTIONAL CHARACTERIZATION OF DEFATTED OILSEED PROTEIN ISOLATES

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In present study efforts were made to elucidate the importance of oilseeds as non-conventional protein sources. Purposely, three different oilseeds *i.e.* sesame, flaxseed and canola were initially subjected to proximate and mineral composition. Afterwards, defatted oilseed meals were used to prepare protein isolates through isoelectric precipitation method. The resultant protein isolates were examined for protein content, recovery and yield. Moreover, functional properties *i.e.* water and oil absorption capacities, foaming and emulsifying properties along with nitrogen solubility index and least gelation concentration of protein isolates were also determined. The results indicated highest crude protein content in whole as well as defatted sesame. Likewise, sesame protein isolates (SPI) exhibited maximum values for crude protein content (90.14±2.37%) followed by canola protein isolates CPI (89.75±3.58) and flaxseed protein isolates FPI (86.37±3.69). Likewise, protein isolates recovery was maximum in SPI (36.86±1.22) trailed by FPI (31.59±0.98) and CPI (30.52±1.20). Similar trend was noticed for yield *i.e.* SPI (79.03±2.18), CPI (78.53±4.02) and FPI (74.61±2.93). Moreover, higher water absorption capacity was revealed in SPI followed by CPI and FPI. Similarly, maximum foaming capacity was observed in SPI tracked by FPI and CPI. Conclusively, defatted oilseed meals contain appreciable quantities of quality proteins. In the nutshell, sesame protein isolates exhibit better recovery, yield as well as functional properties in comparison with flaxseed and canola protein isolates. Therefore, these can be a potential candidate for utilization in various food formulations.

Keywords: Sesame, Flaxseed, Canola, Protein isolates, Yield, Functional properties.

INTRODUCTION

Proteins are vital components of diet helping to improve the health of individuals. Purposely, the proteins obtained from animal sources are of high quality as compared to plant sources (Salcedo-Chávez *et al.*, 2002). Though, animal proteins exhibit high quality nevertheless, they are more expensive than plant proteins. Therefore, need of the time is to explore some new and potential sources of better quality proteins. (Martínez-Flores *et al.*, 2006). Moreover, rising prices and inadequate supply of animal proteins have forced the researchers to focus on high protein oilseeds.

Food industry has utilized plant proteins primarily from grains and legumes as potential ingredients in numerous food products due to their balanced amino acid profile (Horax *et al.*, 2004). Owing to better health benefits, plant proteins are being utilized by people in routine diet especially in developed countries (Ahmed *et al.*, 2011). However, supplementation of protein via plant sources is also becoming popular in developing economies (Khalid *et al.*, 2003).

The sesame (*Sesamum indicum* L.) is an imperative oilseed crop that belongs to the Pedaliaceae family mostly cultivated in tropical areas. Globally, it is commonly known as beniseed (English), gingely (Hindi), sim sim (Arabic) and til (Urdu). The chemical composition of sesame seed revealed that it contains 25.8-26.9% protein, 2.50-3.90% fiber, 2.00-5.59% ash and 10.10-17.90% carbohydrate (Onsaard, 2012). The fat free meal obtained after oil extraction exhibits a reasonable proportion of high quality proteins that can be potentially utilized as functional ingredient in various food commodities and nutritional supplements. Moreover, sesame meal acquired after oil extraction comprises about 50% protein that is primitively used in animal feed (Iqbal *et al.*, 2006; Nunes *et al.*, 2006).

Flaxseed (*Linum usitatissimum* L.) commonly recognized as "Alsi" especially in Indopak, belongs to Linaceae family. Flaxseed is a multipurpose crop mainly cultivated for the production of oil, seed and textile fiber. It also contains an appreciable amount of high quality proteins and polyunsaturated fatty acids (Pradhan *et al.*, 2010). Generally, flaxseeds comprised of about 7.7% moisture, 20% protein, 41% fat and 28% fiber (Ganorkar and Jain, 2013). Nevertheless, flaxseed meal is among certain unexplored sources containing high quality protein for human consumption. Moreover, due to better nutritional as well as functional attributes, flaxseed is being incorporated in various food products. Resultantly, it helps to improve the overall health of individuals (Hussain *et al.*, 2012).

Canola (*Brassica napus* L.), extensively grown in Canada is now being cultivated in sub-continent. Canola contains about 40% oil, however, defatted canola meal contains about 35-36 g/100 g protein as well as 12 g/100g crude fiber contents along with some important minerals and vitamins. The protein found in canola meal exhibits balanced amino acid profile as compared to other plant based proteins (Knispel and Mclachlan, 2010). Currently, canola is being utilized in livestock as well as aquaculture feed industry (Khattab and Arntfield, 2009; Canola Council of Canada, 2014). However, owing to better nutritional profile, the defatted canola meal can be possibly utilized in numerous food commodities. Additionally, owing to their better amino acid profile, canola proteins have the ability to impart better functional attributes to the food (Yoshie-Stark *et al.*, 2008).

In the developing economies, inadequate supply and high cost of animal protein has persuaded the food researchers to use proteins obtained from under-utilized sources *i.e.* oilseed meals and legumes (Enujiugha and Ayodele-Oni, 2003). However, proteins isolated from non-conventional sources must have the ability to properly interact with other food components (*i.e.*, water and lipids) to assist their incorporation in various food formulations (Khattab and Arntfield, 2009). Now, the food industries have taken initiative for the supplementation of protein isolates in numerous food products to fulfill protein requirements. The present project was designed to prepare protein isolates from defatted oilseeds *i.e.* sesame, flaxseed, canola. Purposely, whole as well as defatted oilseeds were initially subjected to proximate and mineral analyses. Moreover, the defatted oilseed samples were used to prepare protein isolates using isoelectric precipitation method. Furthermore, the resultant protein isolates were evaluated for protein content, recovery and yield. The basic objective of the present project was to elucidate the importance of oilseeds as non-conventional protein sources. The defatted oilseed protein isolates can be potentially utilized in various food formulations that can be a way forward to curtail the nutritional deficiencies among masses.

MATERIALS AND METHODS

Procurement of raw materials**:** Oilseeds *i.e.* sesame (TS-5), flaxseed (Chandni) and canola (Faisal canola) were procured from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. The chemicals and standards were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan).

Defatting of samples**:** The conventional solvent (hexane) method was employed to extract oil from the selected samples using soxtec system (Model: H-2 1045 Extraction Unit, Hoganas, Sweden) (AOAC, 2006). Resulting defatted oilseeds were dried and stored for further analyses.

Proximate and mineral analyses of whole and defatted oilseeds**:** The whole as well as defatted oilseed materials (sesame, flaxseed, canola) were analyzed for moisture, crude protein, crude fat, crude fiber, ash and NFE following the respective methods (AACC, 2000; AOAC, 2006). Moreover, the respective oilseeds were examined for mineral profile (AOAC, 2006). Purposely, Atomic Absorption Spectrophotometer (Varian AA240, Australia) was used to determine the concentrations of calcium (Method 968.08), iron (Method 985.01) and zinc (Method 991.11) after wet digestion while, sodium (Method 968.08) and potassium (Method 968.08) were estimated using Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge).

Preparation of protein isolates**:** To prepare protein isolates, the resultant defatted oilseeds were dispersed in distilled water (1/10) and pH was adjusted at 9.5 using 1 N NaOH solution. Furthermore, centrifugation was carried out at 4000 rpm for 20 min to separate the supernatant. Afterwards, the collected supernatant was adjusted to pH 4.5 using 1 N HCl for protein precipitation followed by re-centrifugation, neutralization and freeze drying at -40°C and 0.15 Torr pressure (Makri *et al.*, 2005).

Protein isolates assay

Protein content**:** The crude protein content of the prepared protein isolates was measured using Kjeltech Apparatus following the respective protocols (AACC, 2000). The protein (%) was calculated by the following formula.

Protein (%) = % Nitrogen × 5.40 (Conversion factor)

Isolate recovery**:** Oilseed protein isolates recovery was assessed as weight of protein isolates obtained after isoelectric precipitation per 100 g sample (Wang *et al.*, 1999).

Protein yield**:** Protein yield of resultant isolates was calculated by using the expression as described by Wang *et al*. (1999).

$$\text{Yield (\%)} = \frac{\text{Weight (g) of protein isolates}}{\text{Weight (g) of defatted meal}} \times \frac{\text{Protein content of protein isolates (\%)}}{\text{Protein content (\%) of defatted meal}} \times 100$$

Functional properties of defatted oilseed protein isolates

Water absorption capacity (WAC)**:** To determine water absorption capacity,3 g sample was mixed in 25 mL distilled water. The resultant solution was stirred and then centrifuged for 25 min at 3000×g ("g" denotes acceleration due to gravity). After decanting and removal of excess moisture, the resulting supernatant was reweighed. Water absorption capacity was calculated by the following formula (Kaur and Singh, 2007).

Water absorbed (g)/Sample (g)

Oil absorption capacity (OAC)**:** For oil absorption capacity, 0.5 g of sample was mixed in 6mL of corn oil in centrifuge tubes. The dispersion was stirred for 1 min to dissolve the sample in oil. After keeping for a period of 30 min, the tubes were centrifuged for 25 min at 3000 × g. The separated oil was removed and the tubes were inverted for 25 min to drain the oil prior to reweighing. The oil absorption capacity was expressed as grams of oil absorbed per gram of the sample as mL/g (Kaur and Singh, 2007).

***Foaming properties*:** To determine the foaming properties, 1g protein isolate was mixed in 50 mL distilled water that was transferred to 250 mL graduated cylinder. Foaming capacity (FC) was depicted as foam volume measured after incorporation of air current in the solution for 15 min. The final observation was made after 60 min to determine the foaming stability (FS) (Siddiq *et al.,* 2010).

***Emulsion properties*:** For the determination of emulsifying properties, 0.5 g of protein isolate was mixed in 3 mL distilled water. Afterwards, 3mL oil was added and the sample was shaken vigorously for 5 min followed by centrifugation at 2000 × g for 30 min. The emulsifying capacity (EC) (mL/100 mL) was calculated by using ratio of the height of emulsified layer to the liquid layer. Moreover, to determine the emulsifying stability (ES), the resultant emulsion was heated at 80ºC using a water bath (WNB-29, Memmerts, Germany). Later, it was centrifuged (3000 × g) and ES (mL/100 mL) was calculated as follows (Siddiq *et al.,* 2010).

$$\frac{\text{Volume of emulsifying layer} \times 100}{\text{Heated slurry}}$$

***Nitrogen Solubility Index (NSI)*:** For the determination of NSI, initially protein solutions were formed using deionized water followed by pH adjustment ranging from 2 to 12 (0.01N HCL or NaOH solutions). Further, samples were centrifuged (2000 × g) after agitation for 30 min (120 rpm, 30ºC). The supernatant was collected and its nitrogen content was measured to determine NSI (Shand *et al.,* 2007).

***Least Gelation Concentration (LGC)*:** To determine least LGC, the suspensions of protein isolates 2 to 20% (w/v) were heated at 90 °C in water bath for 1 hr and then immediately cooled to 10°C under running cold water. LGC was measured as the concentration of sample when it did not slip along the inverted test tube walls. The results were determined as no (−), complete (+) or partial (±) gelation (Siddiq *et al.,* 2010).

***Statistical Analysis*:** The collected data wee statistically analyzed using Statistical Package (Costat-2003, Co-Hort, v 6.1.). Accordingly, level of significance was estimated by analysis of variance (ANOVA) using completely randomized design (CRD) as defined by Steel *et al.,* (1997).

RESULTS AND DISCUSSION

***Proximate analysis of whole and defatted oilseeds*:** The results for proximate composition of whole oilseeds (Table 1) indicated that moisture content ranged from 4.53±0.37 to 6.32±0.10% while in defatted oilseeds it varied from 7.34±0.60 to 9.37±0.15%. The maximum crude protein content was observed in sesame (22.41±0.55%) followed by flaxseed (21.62±0.38%) and canola (19.93±0.56%). Likewise, in defatted oilseeds (Table 2) maximum crude protein content was observed in sesame (40.90±1.00%). Crude fat differed significantly with value for sesame as 41.29±1.24%, canola 39.70±1.35% and flaxseed 34.99±1.42%. However, in defatted oilseeds, the crude fat was reduced to 3.97±0.12% in sesame, 2.48±0.09% in canola whilst 1.91±0.08% in flaxseed. Crude fiber ranged from 3.42±0.13 to 7.55±0.29% in whole while 7.82±0.30 to 12.81±0.50% in defatted oilseed samples. The ash content ranged from 3.05±0.11 to 5.44±0.19% and 5.30±0.18 to 7.49±0.42% in whole and defatted oilseeds, respectively. Likewise, NFE showed significant difference with values ranging from 21.74±0.50 to 27.97±1.22% in whole whilst 32.48±1.01 to 35.09±0.81% in defatted oilseeds.

Current results for proximate composition are in agreement with previous literature, though, slight variations may occur owing to varietal differences and environmental conditions. Proximate composition of sesame was also investigated by Makinde and Akinoso (2013), they stated moisture ranging from 4.18-5.41% for different sesame varieties, protein 21.94-23.64%, fat 45.63-46.09%, fiber 4.70-7.15 and ash 6.16-7.34%.

Table 1. Proximate composition (%) of whole oilseed samples

Parameter	Sesame	Flaxseed	Canola
Moisture	4.53±0.37^{c}	6.32±0.10^{a}	5.64±0.19^{b}
Crude protein	22.41±0.55^{a}	21.62±0.38^{b}	19.93±0.56^{c}
Crude fat	41.29±1.24^{a}	34.99±1.42^{c}	39.70±1.35^{b}
Crude fiber	3.42±0.13^{c}	6.05±0.38^{b}	7.55±0.29^{a}
Ash	4.27±0.24^{b}	3.05±0.11^{c}	5.44±0.19^{a}
NFE	24.08±0.75^{b}	27.97±1.22^{a}	21.74±0.50^{c}

Means sharing the same letter in a row are not significantly different; NFE= Nitrogen free extract

Table 2. Proximate composition (%) of defatted oilseed samples

Parameter	Sesame	Flaxseed	Canola
Moisture	7.34±0.60^{c}	9.37±0.15^{a}	8.26±0.27^{b}
Crude protein	40.90±1.00^{a}	36.57±0.64^{b}	34.88±0.98^{c}
Crude fat	3.97±0.12^{a}	1.91±0.08^{c}	2.48±0.09^{b}
Crude fiber	7.82±0.30^{c}	11.85±0.74^{b}	12.81±0.50^{a}
Ash	7.49±0.42^{a}	5.30±0.18^{c}	6.48±0.23^{b}
NFE	32.48±1.01^{b}	35.00±1.52^{a}	35.09±0.81^{a}

Means sharing the same letter in a row are not significantly different; NFE= Nitrogen free extract

The current findings are also in agreement with the results of Essa *et al.* (2015), they stated moisture content 8.79% for defatted sesame, protein 51.05%, fiber 18.26 and ash 6.05%. Moreover, Herchi *et al.* (2015) documented that moisture, protein, fat, fiber and ash were 5.22, 22.65, 35.10, 30.00 and 2.90%, respectively in flaxseed. Similarly, Bhise and Kaur

(2013) delineated 2.61% moisture, 38.24% crude protein, 2.71% fat and 12.24% fiber for defatted flaxseed. Likewise, Li *et al.* (2012) expounded 0.89% crude fat, 49.26% crude protein and 8.62% crude fiber while Tan *et al.* (2011) illustrated 10.24% moisture and 5.34% ash in defatted canola. In various research studies, it was stated that protein content was sensitive to light intensity, rainfall, day duration, length of growing season, temperature and agronomic practices (Bampidis and Christodoulou, 2011).

Conclusively, tested oilseeds as sesame, flaxseed and canola had good nutritional profile with respect to protein, fat and fiber. Furthermore, these are accessible and exhibit quality protein that can be replaced with dietary animal protein. It is evident from the present investigation that oilseeds are nutritionally favorable in terms of protein availability.

Mineral profile of whole and defatted oilseeds**:** The results (Table 3 and 4) indicated that sodium was maximum in whole and defatted canola as 651.70±21.44 and 776.73±17.89 mg/100 g, respectively followed by sesame (76.30±6.26 and 133.88±4.18 mg/100 g), while minimum was observed in flaxseed (30.36±0.50 and 52.69±2.29 mg/100 g). Likewise, the results for potassium were in subsequent manner for whole canola (1048.50±29.51 mg/100 g), flaxseed (824.12±14.32 mg/100 g) and sesame (549.91±13.40 mg/100 g). However, for defatted samples maximum potassium was observed in flaxseed (1430.14±42.54 mg/100 g) followed by canola (1249.65±51.69 mg/100 g) and sesame (964.89±30.52 mg/100 g). Similarly, for calcium the results were 1226.05±41.82, 1146.25±34.48 and 195.09±7.94 mg/100 g for canola, sesame and flaxseed, respectively. However, maximum value for calcium was observed for sesame (2011.25±61.07 mg/100 g) followed by canola (1461.27±78.99 mg/100 g) and flaxseed (338.55±12.28 mg/100 g). Iron was in higher concentration in whole and defatted canola as 22.51±0.87 and 26.83±1.47 mg/100 g, respectively. as compared to sesame and flaxseed. However, zinc was higher in whole as well as defatted sesame (5.62±0.31 and 9.86±0.59 mg/100 g) followed by flaxseed and canola.

Earlier, Obiajunwa *et al.* (2005) and Essa *et al.* (2015) delineated that calcium is the major mineral in sesame seed. Likewise, Ogungbenle and Onoge (2014) described that whole and defatted sesame seeds contain 87.21 and 59.88 mg/100 g Na, 61.37 and 63.42 mg/100 g Ca, 7.29 and 7.26 mg/100 g Fe and 19.29 and 17.29 mg/100 g Zn, respectively. Similarly, Zebib *et al.* (2015) explicated that calcium ranged from 1172.08-1225.71 mg/100 g in sesame, whilst minimum ranges were documented for iron (10.2-10.75 mg/100 g) and zinc (4.23 - 4.45 mg/100 g).In earlier research studies, Katare *et al.* (2012) and Hussain *et al.* (2008) explained that K and Ca were prevailing in flaxseed whilst, Na, Fe and Zn were in lower concentration. Later, Bernacchia *et al.* (2014) delineated that flaxseed contain 831 mg/100 g K, 236 mg/100 g Ca, 27 mg/100 g Na, 5.0 mg/100 g Fe and 4.0 mg/100 g Zn. According to Acikgoz and Deveci (2011)**,** canola exhibited essential minerals like potassium, calcium, iron and zinc as 3.06, 2.65, 23.96 and 2.95 mg/100 g, respectively. Likewise, Khajali and Slominski (2012)**,** documented that defatted canola revealed essential minerals like sodium, potassium and calcium as 0.08, 1.17 and 0.67%, respectively.

Protein isolates recovery, crude protein and protein yield**:** Oilseeds, mainly utilized for oil extraction purpose, are also a vital source of high quality proteins that can be extracted by isoelectric precipitation with substantial yield. Purposely, protein isolates of the selected oilseeds were evaluated for their recovery, protein content and yield. The mean values for these parameters have been presented in Table 5.

Maximum protein isolates recovery (36.86±1.22g/100 g) was depicted in sesame protein isolates (SPI) followed by 31.59±0.98 g/100 g in flaxseed protein isolates (FPI). However, the lowest protein isolates recovery (30.52±1.20g/100 g) was noticed in canola protein isolates (CPI). Likewise, maximum crude protein (90.14±2.37%) was recorded in SPI trailed by CPI (89.75±3.58%) and FPI (86.37±3.69%). Similarly, the highest protein yield was noted in SPI (79.03±2.18%) whilst, 78.53±4.02%for CPI. Nonetheless, the lowest yield was observed in FPI (74.61±2.93%).

Current findings for recovery of oilseed protein isolates are in conformity with the outcomes of Gandhi and Srivastava (2007) indicating 29.20% recovery for SPI. Likewise, Kaushik *et al.* (2016) explicated 12.10-20.29% FPI recovery. However, the current findings for CPI are in contrast with the work of Tan *et al.* (2011). They elucidated 71.49% recovery for CPI. The results for crude protein are in agreement with the findings of Essa *et al.* (2015), delineated 92.43% crude protein in SPI. Similarly, Kuhn *et al.* (2014) documented 68.53% crude protein in FPI.

Table 3. Mineral composition (mg/100 g) of whole oilseed samples

Mineral	Sesame	Flaxseed	Canola
Sodium	76.30±6.26^{b}	30.36±0.50^{c}	651.70±21.44^{a}
Potassium	549.91±13.40^{c}	824.12±14.32^{b}	1048.50±29.51^{a}
Calcium	1146.25±34.48^{b}	195.09±7.94^{c}	1226.05±41.82^{a}
Iron	9.45±0.36^{b}	4.15±0.26^{c}	22.51±0.87^{a}
Zinc	5.62±0.31^{a}	3.37±0.12^{b}	2.78±0.10^{c}

Means sharing the same letter in a row are not significantly different

Table 4. Mineral composition (mg/100 g) of defatted oilseed samples

Mineral	Sesame	Flaxseed	Canola
Sodium	133.88±4.18^{b}	52.69±2.29^{c}	776.73±17.89^{a}
Potassium	964.89±30.52^{c}	1430.14±42.54^{a}	1249.65±51.69^{b}
Calcium	2011.25±61.07^{a}	338.55±12.28^{c}	1461.27±78.99^{b}
Iron	16.59±1.07^{b}	7.21±0.34^{c}	26.83±1.47^{a}
Zinc	9.86±0.59^{a}	5.85±0.15^{b}	3.31±0.04^{c}

Means sharing the same letter in a row are not significantly different

Table 5. Protein content, recovery and yield of oilseed protein isolates

Oilseeds	Protein isolate recovery (g/100g defatted oilseed)	Crude protein (%)	Protein yield (% defatted oilseed protein)
SPI	36.86±1.22^{a}	90.14±2.37^{a}	79.03±2.18^{a}
FPI	31.59±0.98^{b}	86.37±3.69^{c}	74.61±2.93^{c}
CPI	30.52±1.20^{c}	89.75±3.58^{b}	78.53±4.02^{b}

Means sharing the same letter in a column are not significantly different; SPI= Sesame protein isolates; FPI= Flaxseed protein isolates; CPI= Canola protein isolate

Likewise, Karaca *et al.* (2011) depicted crude protein as 75.31% for CPI. The current results are for protein isolates yield are supported with the findings of Das *et al.* (2009), they noticed considerable sesame protein isolates yield. Further, Ho *et al.* (2007), expounded 66.8% yield for FPI. Similarly, Akbari and Wu (2015), depicted 56.20% yield for CPI.

Functional properties of protein isolates

***Water and oil absorption capacities*:** Water and oil absorption capacities indicate the amphiphilic nature of protein isolates. The conformational attributes of protein and their interfacial tension affect the water absorption capacity (WAC). Moreover, oil absorption capacity (OAC) helps in flavor preservation, improves mouthfeel as well as emulsion characteristics of the food commodities (Escamilla-Silva *et al.,* 2003). Results indicated that SPI showed highest WAC 2.12±0.08 mL/g followed by CPI and FPI *i.e.* 1.47±0.06 and 1.43±0.03 mL/g, respectively. Likewise, highest OAC was revealed by SPI 3.11±0.12 mL/g trailed by FPI 2.77±0.18 mL/g and CPI 1.14±0.07 mL/g (Fig. 1).

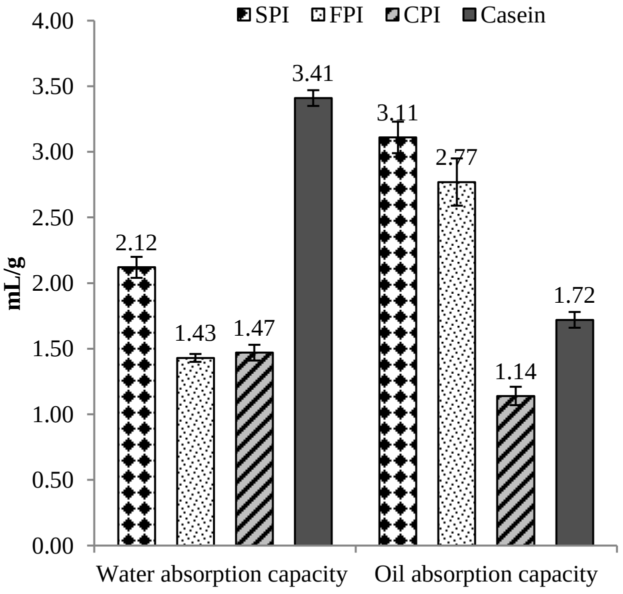

Figure 1. Water and oil absorption capacity of oilseed protein isolates

The instant results are comparable with those of Essa *et al.* (2015) who observed 1.30 g/g water holding capacity and 3.07 g/g oil holding capacity for sesame protein isolates. Likewise, Demirhan and Özbek (2013) delineated 2.67 g/g water holding capacity and 1.21 mL/g oil holding capacity for SPI. Similarly, Kaushik *et al.* (2016) documented 3.90 g/g water holding capacity and 2.60 g/g fat absorption capacity for FPI. Likewise, the *et al.* (2014) presented results for water & oil holding capacity of FPI and CPI as 4.2 & 6.5 mL/g and 7.8 & 7.0 mL/g, respectively. Later, Gerzhova *et al.* (2015) expounded 1.30 & 1.11 g/g water and fat absorption capacity for CPI in respective manner.

The high WAC of sesame protein isolates can be attributed to the presence of polar amino acids at protein-water interface. However, conformational changes in protein may result in lower WAC in canola protein isolates. The oil binding characteristics of protein isolates depict their efficiency to contact with oil molecules. In present research, SPI developed strong oil binding as compared to FPI and CPI; might be owing to the existence of more non-polar side chains that bind with hydro-carbon chains leading to improved oil absorption. However, decreased oil absorption is possibly attributable to the occurrence of large proportion of hydrophilic groups on the protein molecules.

The fat absorption mechanism includes physical entrapment of oil. Therefore, oil absorption capacity can be influenced by various factors like, particle size, moisture content and microstructure. Furthermore, different protein composition & quantity of non-polar amino acids along with conformational changes and starch-protein-lipid binding may cause variations in oil retention attributes of oilseed proteins (Lazou and Krokida, 2010).

***Foaming capacity and stability*:** FC and FS play imperative role in determining the functional characteristics of proteins. Moreover, higher water solubility, flexibility and the ability of protein to become part of cohesive film at the air-water interface help in the formation of better foam (Cano-Medina *et al.,* 2011). The FC represents relative increase in the volume of protein solution by the incorporation of air. Nonetheless, FS indicates the ability of food molecules to retain air in the form of bubbles. It is estimated either by the reduction or separation of foam volume from food over a short time period (Boye *et al.,* 2010).

The present results indicate that SPI showed highest FC 18.51±0.60 mL followed by FPI *i.e.,* 14.13±0.52 mL, however, lowest FC was depicted by CPI 12.29±0.53 mL (Fig. 2). Likewise, maximum FS was noticed in SPI 46.98±0.90 min and minimum in CPI 35.46±1.19 min while FPI indicated FS as 39.87±1.43 min (Fig. 3).

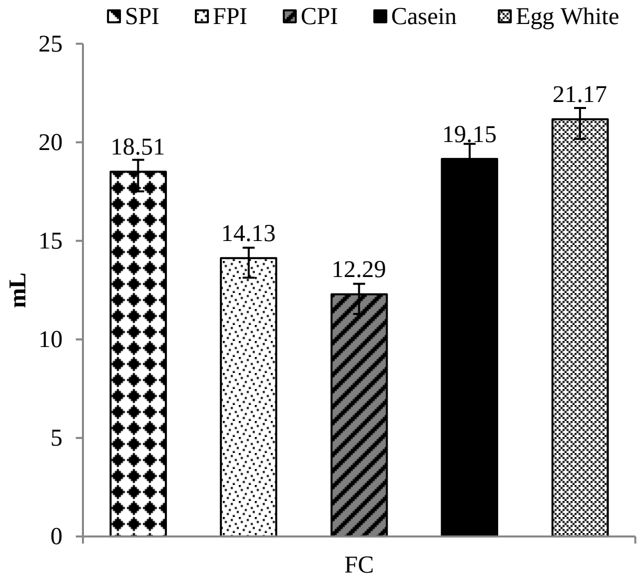

Figure 2. Foaming capacity of oilseed protein isolates

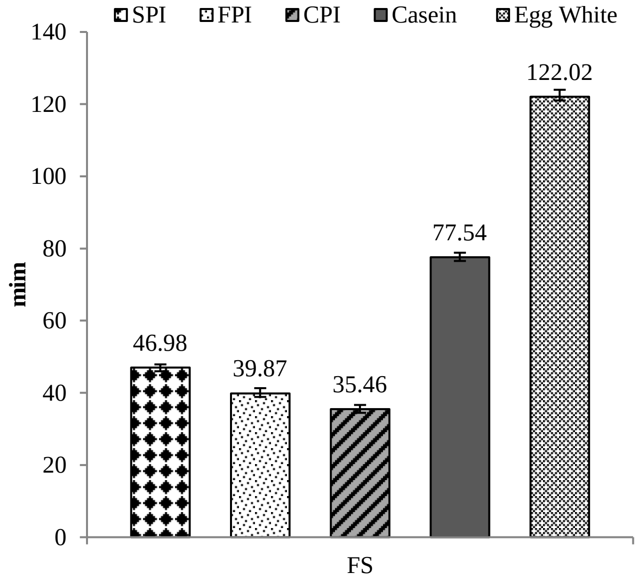

Figure 3. Foaming stability of oilseed protein isolates

The highest FC was noticed in SPI due to upsurge in foam hydration and stable molecular layer formation at water & air interface. Nonetheless, CPI showed low FC as the disulfide bonds are denatured resulting in decreased flexibility. Previously, Alamanou and Doxastakis (1997) explained that the protein isolation process also affects the degree of denaturation. In a research investigation, Demirhan and Özbek (2013) revealed that sesame cake protein hydrolysates exhibit 45.2% FC and 31.5 mL FS. Similarly, Onsaard *et al.* (2010) expounded foaming capacity and stability of sesame protein concentrates as 58% and 14 min, respectively. Contrarily, Ogungbenle and Onoge (2014) noticed 6.53% foaming capacity and 3.25% foaming stability in sesame protein concentrates. The results of present study regarding FC and FS of FPI are in accordance with the outcomes of Hussain *et al.* (2008), reported 17.40 mL FC and 9.00 mL FS in partially defatted flaxseed flour. Likewise, Martínez-Flores *et al.* (2006) revealed 12% FC and 83.3% FS in flaxseed protein concentrates. Similar results were obtained by Gerzhova *et al.* (2015) for the foaming properties of canola protein isolates. They observed 57.83% FC and 18% FS for CPI.

Emulsion capacity and stability**:** Protein exhibits better tendency to form emulsions by facilitating their formation and improving the stability. Moreover, proteins from plant sources help in the production of required physicochemical attributes in various emulsions. The emulsifying ability of protein is attributed to its hydrophobic as well as hydrophilic structure. Furthermore, protein reduces the oil-water interfacial tension and its electrostatic repulsion mechanism assists in the stabilization of oil droplets, thus facilitating the emulsion formation (Brewer *et al.*, 2016).

The present results indicated that the maximum emulsifying capacity (EC) was recorded in SPI 81.36±2.19% followed by FPI 73.24±2.50% whereas, minimum in CPI 65.40±3.13% (Fig. 4). Emulsion stability (ES) refers to the ability of protein isolate to create resistance against emulsion breakdown. The results revealed higher stability in SPI 78.69±1.08% while, lower in FPI 75.08±3.22% and CPI 71.97±2.50% (Fig. 4).

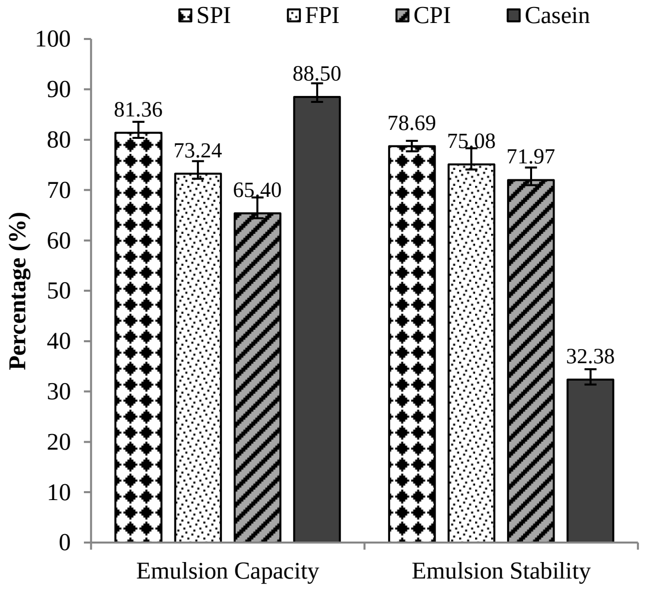

Figure 4. Emulsion capacity and stability of oilseed protein isolates

The lowest emulsifying capacity of CPI may be attributed to fewer hydrophobic residues on the surface of protein. Resultantly, the oil droplets diffused in continuous aqueous phase. Protein denaturation may enhance the emulsifying properties owing to increased elasticity and hydrophobic surface. Furthermore, the emulsion properties of proteins can be influenced by molar mass, hydrophobicity, conformational stability and some physicochemical factors like pH, temperature & ionic strength (Lam and Nickerson, 2013).

The instant findings are in conformity with the outcomes of Ogungbenle and Onoge (2014), estimated 27.43% EC and 30.50% ES for sesame protein isolates. In another research investigation, Khalid *et al.* (2003) found 70.00% emulsion activity (EA) and 70.02% emulsion stability (ES) for sesame seed proteins. Likewise, Rabetafika *et al.* (2011) reported 63 & 59% EC and 81 & 70% ES at pH 4 & 9, respectively for FPI. Previously, Martínez-Flores *et al.* (2006) expounded 84.8% EC at pH 6 whilst 88.4% ES at pH 8 for flaxseed proteins. Similarly, Stone *et al.* (2014) delineated 63.34% EC and 76.00% ES for CPI. Likewise, Teh *et al.* (2014) documented 50% emulsion activity (EA) and 100% emulsion stability (ES) for CPI. The emulsion properties (EC & ES) are the momentous attributes of food proteins that play imperative role in the stabilization of food system. Previous research investigations have proven that protein rich materials exhibit better emulsion properties hence can potentially be utilized as functional ingredient in various food products like mayonnaise, cake batter and salad dressings (Akubor, 2003).

Nitrogen solubility index (NSI)**:** Solubility is mainly dependent on physicochemical attributes of protein affecting functional properties like foaming, gelling and emulsification capacity. The nitrogen solubility of defatted oilseed protein isolates was pH dependent as shown in Fig. 5. The lowest nitrogen solubility 7.32-23.43% was observed at pH 4.0 might be due to isoelectric region. Furthermore, an increasing trend for solubility was noticed on either side of pH *i.e.* acidic and basic. Moreover, a noticeable rise in nitrogen solubility was detected till pH 8.0 where it showed an index of 34.46 to 54.31%. A progressive increase was noticed up to pH 12.0, where nitrogen solubility index ranged from 62.51 to 82.56%.These results are supported by the outcomes of previous research studies. Earlier, Bandyopadhyay and Ghosh (2002) delineated 55.97% protein solubility for sesame protein isolates at pH 7. Similarly, Karaca *et al.* (2011) observed 40% nitrogen solubility index (NSI) for flaxseed protein isolates. Whilst, Gerzhova *et al.* (2015) explicated that nitrogen solubility index for canola protein isolates ranged from 8.09-56.82% at various pH levels. It was also noticed that alkali caused disaggregation and dissociation of proteins that generally helps to improve protein solubility (Hojilla-Evangelista *et al.*, 2009). Previous research investigation has proven that nitrogen solubility index (NSI) determines protein solubility primarily caused by protein dispersion in solvent.

One of the researchers groups expounded that net negative charge on protein is increased at higher pH values resulting in the dissociation of its aggregates (Tomotake *et al.*, 2002). However, the carboxyl and amino groups are protonated as -COOH and -NH, respectively at lower pH value that generally results in positive charge.

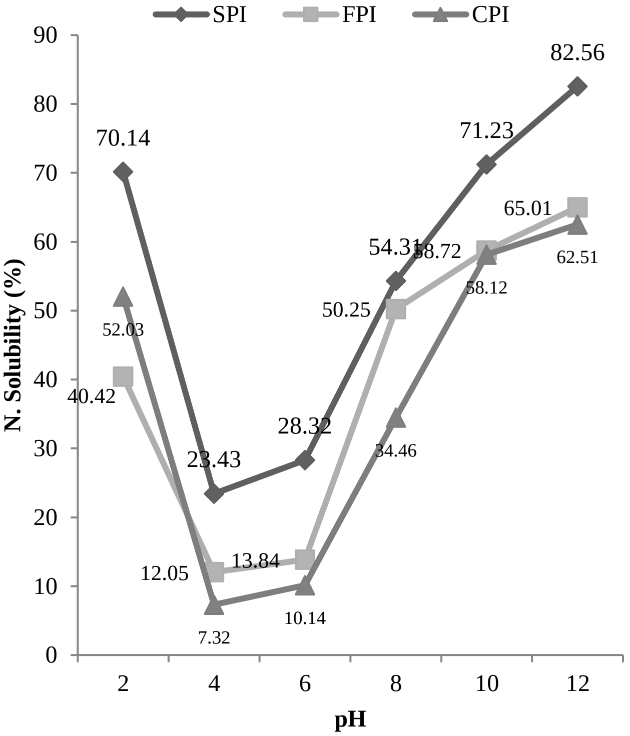

Figure 5. Nitrogen solubility index of oilseed protein isolates

Moreover, the amino groups disassociate into $-NH_2$ and $-H^+$ with increase in pH causing the protein to be negatively charged due to $-COO^-$ group. Nevertheless, a gradual rise in pH causes a few carboxyl groups to dissociate into $-COO^-$ and $-H^+$ (Yemisi and Kayode, 2007; Nicole *et al.*, 2010).

Solubility of protein isolates is influenced by processing conditions. Previous studies have indicated highest protein solubility at low acidic and high basic pH values. Nevertheless, lowest solubility was noticed at pH values near isoelectric point. Nitrogen solubility of protein isolates and concentrates can be increased by hydrolysis and physicochemical modifications (Boye *et al.*, 2010).

Least Gelation Concentration (LGC)**:** The gelation ability of proteins is typically stated in terms of least gelation concentration. LGC is a qualitative attribute that determines least protein concentration required to form gel. Furthermore, this gel must not slide along the inverted test tube walls owing to the formation of self-supporting network (Rai *et al.*, 2014). Gel formation of oilseed protein isolates occurs at a temperature higher than protein denaturation.

The results indicated that SPI exhibited high least gelation concentration 16% followed by FPI 15% and CPI 14% (Table 4). Gelation ability was observed from 12 to 14% concentration of protein isolates, whilst, a stable and strong gel was detected from 16% concentration to onward. Furthermore, lower concentration solution of protein isolates showed higher liquid phase. Soy protein revealed a sticky tendency at 12% concentration; however, a stable gel was noticed at 16%. Moreover, protein denaturation and gel strength caused lesser LGC for canola protein isolates.

Least gelation concentration relies on certain characteristics like viscosity, elasticity and plasticity. The gel forming ability of protein gives structural matrix that helps in water binding. The variations in gelling ability of different protein isolates were due to the differences in their protein, lipid and carbohydrate contents. Moreover, LGC plays imperative role in food system by contributing towards texture and rheology of end product (Nicole *et al.*, 2010).

Previously, Fekria *et al.* (2012) explicated 6.0% least gelation concentration for defatted sesame seeds. Likewise, Singer *et al.* (2011) elucidated 11% gelation for flaxseed. However, for canola protein isolates 14.9-15.7% LGC was indicated by Nithiyanantham *et al.* (2013). Earlier, Cheng *et al.* (2009) explained that protein-protein interaction of isolates at isoelectric point affects the gelation ability as there is no net charge on protein molecules.

Table 4. Least gelation concentration of oilseed protein isolates

Conc. (%)	SPI	FPI	CPI	Soy protein
2	(−)	(−)	(−)	(−)
4	(−)	(−)	(−)	(−)
6	(−)	(−)	(−)	(−)
8	(−)	(−)	(−)	(−)
10	(−)	(−)	(±)	(−)
12	(±)	(±)	(±)	(±)
14	(±)	(±)	(+)	(±)
16	(+)	(+)	(+)	(+)
18	(+)	(+)	(+)	(+)
20	(+)	(+)	(+)	(+)
LGC	16	15	14	16

Gelation levels: (−) no, (±) partial, (+) complete gel; SPI= Sesame protein isolates; FPI= Flaxseed protein isolates; CPI= Canola protein isolates

***Conclusion*:** The outcomes of current study indicated that oilseed protein isolates are rich in quality protein and exhibit remarkable functional properties that can be explored in the food systems. The protein isolates can be successfully incorporated into bakery products. Nevertheless, their possible effectiveness depends on functional properties that ultimately affect sensory attributes of the food.

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