

Research Article

Amino Acid Profiling and SDS-PAGE Analysis of Protein Isolates Obtained from Nonconventional Sources

Muhammad Sibte-Abbas ¹, Masood Sadiq Butt ², Mian N. Riaz ³,
Tadesse Fikre Teferra ⁴, and Iahtisham Ul-Haq ⁵

¹Department of Food Science & Nutrition, TIMES Institute Multan, Multan, Pakistan

²National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan

³Department of Food Science and Technology, Texas A&M University, College Station, TX, USA

⁴College of Agriculture, School of Nutrition, Food Science and Technology, Hawassa University, Hawassa, Ethiopia

⁵Kausar Abdulla Malik School of Life Sciences, Forman Christian College University, Lahore, Pakistan

Correspondence should be addressed to Tadesse Fikre Teferra; tadessefikre@hu.edu.et

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Proteins play an imperative role in enhancing the nutritional status of the human body. The present study was designed to determine the molecular weight of protein isolates prepared from defatted oilseeds, i.e., sesame, flaxseed, and canola, using SDS-PAGE. The electropherogram revealed protein bands ranging from 15 to 65 kDa. Furthermore, proteins were subjected to amino acid profiling followed by calculation of amino acid score with reference to requirement for preschool children. The amino acid profiling results indicated that sesame protein isolates (SPI) exhibited the highest values for aromatic amino acids, histidine, isoleucine, and valine. However, the maximum values for sulfur-containing amino acids were depicted by flaxseed protein isolates (FPI). Moreover, the lysine content was highest in canola protein isolates (CPI). Results indicated better profile and quality of proteins, capable to meet the requirements of essential amino acids, especially for preschoolers. Moreover, the values for the protein digestibility corrected amino acid score (PDCAAS) and in vitro protein digestibility (IVPD) were also determined. Conclusively, protein isolates from defatted oilseeds exhibit better-quality proteins with a balanced amino acid profile. By potential utilization in numerous food products, these proteins can play a pivotal role in fulfilling the nutritional requirements of individuals, especially in developing economies.

1. Introduction

High-quality proteins play an imperative role in maintaining better health of an individual. Purposely, the proteins obtained from animal sources are of high quality as compared to plant sources; nevertheless, they are more expensive than vegetable proteins [1]. Owing to the high cost and comparative dearth of food with animal proteins, it has become inevitable to find some new sources of better-quality proteins [2]. In addition, the increasing cost and insufficient provision of animal proteins have diverted the interest of researchers towards some nonconventional protein sources, i.e., high-protein oilseeds [3].

The sesame (*Sesamum indicum* L.), an imperative oilseed crop belonging to the *Pedaliaceae* family, contains 25.8–26.9% protein [4]. The sesame meal acquired after oil extraction exhibits a reasonable proportion of high-quality proteins that have the ability to be potentially used as a functional ingredient in numerous food commodities and nutritional supplements [5].

The flaxseed (*Linum usitatissimum*), belonging to the *Linaceae* family and commonly known as “Alsi” in IndoPak, 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 (20%) and polyunsaturated fatty acids [6]. The defatted flaxseed meal contains about

35–40% protein, having a balanced amino acid profile, that has paved the way for its utilization in value-added food products [7].

Canola (*Brassica napus* L.) is a widely cultivated oilseed crop in Canada, and nowadays, it is grown throughout the world including different areas of the subcontinent. The extraction rate of canola oil is about 40%, and the resultant meal is a rich source of protein. The canola meal contains about 35–36% protein, exhibiting a balanced amino acid profile [6, 8].

Proteins can be separated on a molecular weight basis by using the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique [9]. The SDS-PAGE technique gives information regarding the molecular size along with intermolecular disulfide bonds of proteins. The proteins as well as their fractions are presented on an electropherogram and characterized as fingerprints [10].

Amino acids being the important building blocks of protein play an imperative role in determining the protein quality. Defatted oilseed protein isolates contain higher amounts of leucine, glutamine, arginine, and glutamic acid, while lower quantities of sulfur-containing amino acids [11, 12]. Previous studies have explicated that defatted sesame seeds contain sufficient quantities of excellent-quality proteins with a balanced amino acid profile. The data for essential as well as nonessential amino acids indicated that the highest values were observed for leucine (3.86–7.54 g/100 g) and glutamic acid (12.23–18.67 g/100 g) [13]. Moreover, flaxseed protein contains somewhat high amount of arginine, glutamic acid, and aspartic acid, while lysine, cysteine, and methionine are considered as limiting amino acids. Albumin and globulin are the major types of proteins in flaxseed with globulin fraction up to 73.4% and albumin up to 26.6% of total protein [14]. Furthermore, canola protein isolates (CPI) exhibit higher quantities of leucine, arginine, glutamine, and glutamic acid while lower quantities of sulfur-containing amino acids. The lysine content of CPI mainly depends on the methods of extraction and ranged from 5.04 to 6.34% which is almost equal to infant's requirements. Similarly, CPI contains a considerably higher quantity of threonine (4.49%–5.30%) in comparison with sesame protein isolates (3.98%) [15].

Owing to the fact that animal proteins exhibit high quality as compared to plant sources, however, high cost and insufficient supply of animal proteins demands exploration of some new and nonconventional protein sources [2]. Moreover, cost-effectiveness of food is among the basic concerns for most of the population in developing economies. People demand quality food that must be less expensive and nutritionally sound. Keeping in view the abovementioned facts, the present research project was designed to evaluate the quality of proteins obtained from defatted oilseeds (inexpensive and nonconventional protein sources). These proteins can be further utilized in numerous food formulations and can serve the purpose of fulfilling the nutritional requirements of individuals, especially infants and young children.

2. Materials and Methods

2.1. Preparation of the Raw Material. Oilseeds, i.e., sesame (TS-5), flaxseed (Chandni), and canola (Faisal canola), were procured from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. The seeds were initially cleaned and then ground to fine powder [16]. Moreover, the conventional solvent (hexane) method was employed to extract oil from the selected samples using the Soxtec system (Model: H-2 1045 Extraction Unit, Hoganas, Sweden) [17]. The resulting defatted oilseeds were dried and stored for further processes.

2.2. Protein Isolate Preparation. For preparation of protein isolates (Figure 1), defatted oilseeds were dissolved in distilled water (1/10) with pH 9.5. Furthermore, centrifugation was carried out at 4000 rpm for 20 min to separate the supernatant. Later, the pH of collected supernatant was set at 4.5 following recentrifugation, neutralization, and freeze-drying [18].

2.3. Gel Electrophoresis (SDS-PAGE). Initially, 250 μ L sample buffer was used to solubilize the protein isolate samples. In order to perform the electrophoresis on the Bio-Rad Mini-Protean 3 System (Bio-Rad Laboratories, Hercules, CA, USA), 12.5% and 4% stacking and separating gels were used, respectively. Purposely, samples' loading was performed at 10 μ L/lane. A constant voltage (60 V) was supplied for 2.5 hr to run loaded gels till the front dye moved far down the gel. Coomassie brilliant blue (CBB) was used to stain the gels, while methanol water mixture was used for destaining purpose [19].

2.4. Amino Acid Profile. The amino acid profiling was conducted at the University of Veterinary and Animal Sciences (UVAS) Lahore, Pattoki campus. Purposely, a calculated volume of the prepared supernatant was injected using Biochrom 30 + Amino Acid Analyzer [20]. However, for tryptophan, samples were hydrolyzed in the presence of Ba (OH)₂, isolated through gel filtration, and colorimetrically analyzed.

2.5. Amino Acid Score. The amino acid score was determined by following the amino acid requirement for preschoolers [21, 22].

2.6. PDCAAS Value. The protein digestibility corrected amino acid score (PDCAAS) was calculated via true digestibility of respective protein isolates and the lowest amino acid score by the following expression [23]:

$$\text{PDCAAS (\%)} = \text{true digestibility} \times \text{lowest amino acid score.} \quad (1)$$

2.7. In Vitro Protein Digestibility (IVPD). IVPD (%) of protein isolates was determined using the procedure outlined by Aboubacar et al. [24]. For the purpose, protein

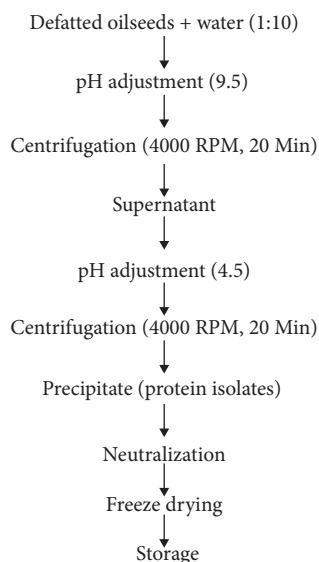


FIGURE 1: Systematic flow sheet for oilseed protein isolate preparation.

isolate samples (200 mg) were weighed into Erlenmeyer flasks and mixed with 35 mL of porcine pepsin solution (1.5 g of pepsin/L in 0.1 M KH_2PO_4 , pH 2.0). Samples were digested for 2 hr at 37°C in a shaking water bath. Digestion was stopped by adding 2 mL of 2N NaOH. Samples were centrifuged ($4900 \times g$, 4°C) for 20 min, and the supernatant was discarded. The residues were washed and centrifuged twice with 20 mL of buffer (0.1 M KH_2PO_4 , pH 7.0). Undigested nitrogen (N) was determined with a Technicon nitrogen analyzer. Digestibility was calculated as

$$\% \text{digestibility} = \frac{(N \text{ in sample} - \text{undigested } N)}{N \text{ in sample}} \times 100. \quad (2)$$

2.8. Statistical Analysis. All the abovementioned parameters were analyzed in triplicate to ensure the precision and accuracy of results, and the collected data were statistically analyzed using statistical package (Costat-2003, Co-Hort, v 6.1). Accordingly, the level of significance was estimated by analysis of variance (ANOVA) using completely randomized design (CRD) as defined by Steel et al. [25].

3. Results and Discussion

3.1. SDS-PAGE. Proteins of resultant isolates, i.e., sesame protein isolates (SPI), flaxseed protein isolates (FPI), and canola protein isolates (CPI), were characterized for their molecular weight using sodium dodecyl sulfate polyacrylamide gel electrophoresis. The electropherogram for sesame, flaxseed, canola protein isolates and the reference standard are illustrated in Figure 2. Respective isolates were recorded ranging from 15 to 65 kDa. The electropherogram also presented numerous fractions having low molecular weights. Moreover, SPI included several polypeptide bands ranging from 15 to 45 kDa, while FPI bands ranged between

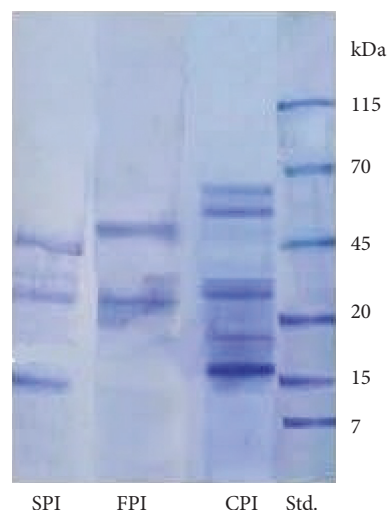


FIGURE 2: Electropherogram of oilseed protein isolates. Sesame protein isolates (SPI), flaxseed protein isolates (FPI), canola protein isolates (CPI), and standard (Std) are shown.

25 and 48 kDa. Furthermore, the CPI bands ranged from 16 to 65 kDa with fewer bands than other tested protein isolates.

Nonetheless, a trivial difference in the movement was observed in electrophoretic bands of SPI, FPI, and CPI. Certain variations may be attributed to structural as well as compositional changes in proteins along with their interaction with salt. The present findings are in conformity with the outcomes of Vemuri et al.; they delineated the molecular weight between 15 and 50 kDa for sesame proteins [26]. Earlier, a group of scientists explicated that the molecular weight of sesame ranged from 45 to 66.2 kDa [27]. Recently, Chen et al. observed the molecular weight of sesame proteins to be ranging from 09 to 47 kDa at different pH levels [28]. Moreover, SDS-PAGE for major fractions of flaxseed proteins was studied, and molecular weights were observed between 13 and 19 kDa [29]. Likewise, in another research exploration, the molecular weight of flaxseed proteins was demarcated in the range of 10–50 kDa [30]. Furthermore, canola protein isolates exhibited proteins with the molecular weight ranging from 12 to 16 kDa [31]. Similar results (10–29 kDa) were reported for the molecular weight of canola proteins via SDS-PAGE in another study [32]. Moreover, it has been delineated that canola protein isolates exhibit 8 major bands in the range of ~4.5–66.4 kDa [33].

3.2. Amino Acid Profile of Defatted Oilseed Protein Isolates.

The mean values for essential and nonessential amino acids of oilseed protein isolates are given in Tables 1 and 2. These isolates exhibit better amino acid profile as the protein quality mainly depends on essential amino acids. The maximum lysine content was recorded in CPI as 2.60 ± 0.09 g/100 g followed by FPI (1.62 ± 0.07 g/100 g), while SPI showed the lowest value of 1.48 ± 0.04 g/100 g.

Data regarding essential amino acids of sesame protein isolates (SPI) showed the values for aromatic amino acids (phenylalanine + tyrosine) as 3.36 ± 0.11 , leucine as

4.39 ± 0.11, sulfur-containing amino acids (methionine + cysteine) as 1.59 ± 0.05, and valine as 4.95 ± 0.19 g/100 g, respectively. Likewise, for FPI, the maximum values were observed for leucine (4.37 ± 0.22 g/100 g) and aromatic amino acids (3.30 ± 0.90 g/100 g), while the minimum values (1.67 ± 0.04 and 1.96 ± 0.03 g/100 g) were noted for histidine and tryptophan, respectively. Moreover, CPI exhibited the maximum value for leucine (4.30 ± 0.23 g/100 g) followed by aromatic amino acids (2.89 ± 0.15 g/100 g), valine (2.69 ± 0.05 g/100 g), and threonine (2.38 ± 0.08 g/100 g).

Means pertaining to nonessential amino acids of oilseed protein isolates indicated that the highest value for alanine (3.30 ± 0.08 g/100 g) was observed in FPI, while the lowest value (2.66 ± 0.11 g/100 g) was observed in CPI. Glutamic acid was ranged from 9.70 ± 0.33 to 12.70 ± 0.76 g/100 g in the tested protein isolates, while serine ranged from 2.51 ± 0.11 to 3.17 ± 0.05 g/100 g. The highest value for arginine was noted in FPI (7.49 ± 0.18 g/100 g), while the lowest value was observed in CPI (3.91 ± 0.06 g/100 g). Moreover, SPI and FPI exhibited higher aspartic acid levels as 5.87 ± 0.29 and 5.86 ± 0.08 g/100 g in contrast to CPI (0.51 ± 0.02 g/100 g). Furthermore, glycine ranged from 2.82 ± 0.13 to 3.09 ± 0.12 g/100 g in tested oilseed protein isolates.

The present results regarding amino acid composition of SPI are in accordance with the outcome of previous research exploration that illustrated histidine (2.25 g/100 g), isoleucine (4.85 g/100 g), leucine (7.57 g/100 g), lysine (5.06 g/100 g), threonine (4.85 g/100 g), and valine (5.44 g/100 g). Similarly, for nonessential amino acids, the values were recorded for alanine (2.83), arginine (7.45), aspartic acid (9.88), glutamic acid (16.54), glycine (2.06), and serine (6.62 g/100 g) [34].

The current outcomes of amino acids for FPI are in accordance with previously described results. The values for leucine and methionine were observed as 4.92 and 2.88 g/100 g, respectively. The values were also noticed for histidine (6.96), isoleucine (6.26), lysine (2.70), phenylalanine (4.29), and threonine (6.94 g/100 g). For nonessential amino acids, the results ranged from 3.43 g/100 g for proline to 13.97 g/100 g for arginine. The values were also observed for alanine (6.55), aspartic acid (6.57), glutamic acid (8.47), glycine (2.19), and serine (6.76 g/100 g) [35].

The present results for amino acid profile of CPI are supported by the outcomes reported earlier. The values were explicated for histidine (3.48%), isoleucine (5.27%), leucine (8.58%), lysine (5.04%), threonine (4.37%), methionine (2.14%), and valine (5.52%). Likewise, nonessential amino acids ranged from 5.32% for serine to 26.97% for glutamic acid. The values were also observed for alanine (4.86%), arginine (6.67%), aspartic acid (8.15%), and glycine (5.95%) [36].

Deficiency of these essential amino acids in diet prevents normal growth and metabolic activities [37]. Furthermore, essential amino acids cannot be synthesized by the body; therefore, these are mandatory to be supplied through diet. Oilseed protein isolates can be obtained with high protein contents, lack of impurities and exhibiting appropriate sensory attributes [38]. Keeping in view the aforementioned

TABLE 1: Essential amino acids (g/100 g protein) of defatted oilseed protein isolates.

Amino acid	SPI	FPI	CPI
ARM*	3.36 ± 0.11 ^a	3.30 ± 0.90 ^a	2.89 ± 0.15 ^b
Histidine	1.65 ± 0.05 ^a	1.67 ± 0.04 ^a	1.39 ± 0.05 ^b
Isoleucine	2.31 ± 0.08 ^a	2.12 ± 0.05 ^b	2.29 ± 0.09 ^a
Leucine	4.39 ± 0.11	4.37 ± 0.22	4.30 ± 0.23
Lysine	1.48 ± 0.04 ^c	1.62 ± 0.07 ^b	2.60 ± 0.09 ^a
Threonine	2.41 ± 0.16	2.49 ± 0.08	2.38 ± 0.08
SAA**	1.59 ± 0.05 ^b	2.00 ± 0.06 ^a	1.36 ± 0.04 ^c
Tryptophan	0.98 ± 0.01 ^b	1.96 ± 0.03 ^a	0.85 ± 0.02 ^b
Valine	2.83 ± 0.11 ^a	2.60 ± 0.15 ^b	2.69 ± 0.05 ^b

Means having the similar letter in a row do not differ significantly. *Aromatic amino acid (phenyl alanine + tyrosine). **Sulfur-containing amino acid (methionine + cysteine). SPI = sesame protein isolates, FPI = flaxseed protein isolates, and CPI = canola protein isolates.

TABLE 2: Nonessential amino acids (g/100 g protein) of defatted oilseed protein isolates.

Amino acid	SPI	FPI	CPI
Alanine	3.23 ± 0.03 ^a	3.30 ± 0.08 ^a	2.66 ± 0.11 ^b
Arginine	7.43 ± 0.28 ^a	7.49 ± 0.18 ^a	3.91 ± 0.06 ^b
Aspartic acid	5.87 ± 0.29 ^a	5.86 ± 0.08 ^a	0.51 ± 0.02 ^b
Glutamic acid	12.52 ± 0.07 ^a	12.70 ± 0.76 ^a	9.70 ± 0.33 ^b
Glycine	3.05 ± 0.15 ^a	3.09 ± 0.12 ^a	2.82 ± 0.13 ^b
Serine	3.01 ± 0.13 ^a	3.17 ± 0.05 ^a	2.51 ± 0.11 ^b

Means with the similar letter in a row are momentarily alike. SPI = sesame protein isolates, FPI = flaxseed protein isolates, and CPI = canola protein isolates.

amino acid profile, oilseed protein isolates can be potentially utilized in numerous food preparations. These proteins and their amino acids are essential components of food in order to provide better growth and maintenance to the body.

3.3. Amino Acid Score of Defatted Oilseed Protein Isolates.

The amino acid score of defatted oilseed protein isolates was associated with the reference pattern required for preschool children. The respective amino acid scores have been given in Table 3. Sesame protein isolates (SPI) revealed relatively a better essential amino acid score as than FPI and CPI. Oilseed protein isolates exhibited good-quality proteins, ensuring the provision of required amount of essential amino acids for preschoolers [22].

Lysine was found as a limiting amino acid in oilseed protein isolates, i.e., SPI, FPI, and CPI. The protein score of oilseed protein isolates was noted as 28.46, 31.15, and 50.00 for SPI, FPI, and CPI, respectively. In the present study, several essential amino acids in oilseed protein isolates explicated good-quality protein that can be recommended for human utilization [39, 40].

Nutritional proficiency of proteins is estimated by their ability to fulfill human amino acid requirements. The amino acid score clearly indicates the existence of different essential amino acids in the samples as compared to the reference pattern. In a recent research investigation, the amino acid profile of different oilseeds like soybean, rapeseed, and

TABLE 3: Amino acid score for defatted oilseed protein isolates.

Amino acid	SPI	FPI	CPI
ARM*	73.04	71.74	62.83
Histidine	91.67	92.78	77.22
Isoleucine	74.52	68.39	73.87
Leucine	69.68	69.37	68.25
Lysine	28.46	31.15	50.00
SAA**	63.60	80.00	54.40
Threonine	89.26	92.22	88.15
Tryptophan	140.00	280.00	121.43
Valine	69.02	63.41	65.61
Protein score	28.46	31.15	50.00
LAA***	Lys	Lys	Lys

* Aromatic amino acid (phenylalanine + tyrosine). ** Sulfur-containing amino acid (methionine + cysteine). *** Limiting amino acid. SPI = sesame protein isolates, FPI = flaxseed protein isolates, and CPI = canola protein isolates. The values for Lysine have been presented in bold to indicate that it is the Limiting Amino Acid.

canola were studied to assess their nutritional performance [40]. They delineated that the tested oilseeds were rich in lysine, leucine, and proline that can satisfy the human needs for essential amino acids. It was also described earlier that oilseeds, legumes, cereals, and their products exhibit appreciable quantities of quality protein comprising adequate essential amino acids. Moreover, the heat treatment can lower the quality of protein by affecting the lysine content during processing [41].

3.4. Protein Digestibility Corrected Amino Acid Score (PDCAAS). The PDCAAS is estimated by the ratio between the first limiting amino acid in sample protein and the respective amino acid in the reference pattern [42]. The present results indicated significant variations among different protein isolates (Table 4) depicting variation in amino acid content and digestibility of protein isolate samples. The PDCAAS results revealed that the maximum value in CPI was $35.17 \pm 1.31\%$, followed by FPI ($22.58 \pm 0.66\%$) and SPI ($21.98 \pm 1.22\%$).

The PDCAAS is a method to assess the quality of protein requiring description of limiting amino acid and true digestibility. Moreover, the PDCAAS method for protein quality determination is a way to assess the ability of proteins to fulfill human requirements of essential amino acids [43]. Previously, the PDCAAS value of 61% was revealed for extruded flaxseed meal (EFM) [44]. Likewise, the PDCAAS values for rapeseed and mustard protein isolates were noted as 83% and 79%, respectively [45]. Later, it was documented that animal proteins exhibit relatively better quality than plant proteins due to rich amino acid profile ranging from 92% to 100% [46].

The PDCAAS is a simple method of protein quality determination and is advantageous owing to its ease and direct association with human protein requirements. The reference pattern depicts minimum quantity of amino acids required for proper growth and maintenance of body tissues. The PDCAAS is calculated by using three different protein quality assessment parameters, i.e., profile of essential amino acids, their digestibility, and ability to fulfill children's

TABLE 4: PDCAAS and *in vitro* protein digestibility (IVPD) of oilseed protein isolates.

Oilseed protein isolates	PDCAAS (%)	IVPD (%)
SPI	21.98 ± 1.22^c	87.57 ± 4.41^c
FPI	22.58 ± 0.66^b	85.41 ± 2.04^d
CPI	35.17 ± 1.31^a	82.13 ± 2.86^e
Soy		91.35 ± 3.12^b
Casein		95.42 ± 2.68^a

Means with different letters in a column are not momentarily alike. SPI = sesame protein isolates, FPI = flaxseed protein isolates, and CPI = canola protein isolates.

requirements [47]. According to the authenticated technique, the PDCAAS values, i.e., 1.00 or 100%, indicate that protein provides adequate quantity of essential amino acids for children as well as adults [1].

The PDCAAS is extensively used and an approved method for protein quality evaluation of plant-based foods, especially infant formulations. Previously, the World Health Organization (WHO) adopted an alternate method to estimate protein quality in comparison with the PDCAAS. This method is used to evaluate amino acid scores for 2- to 5-year-old children [22].

Finally, the PDCAAS imparted relatively good protein with improved digestibility. The differences in methods of PDCAAS determination have expounded that reference amino acid score affects the PDCAAS value for a product. The accuracy of PDCAAS is recommended by the WHO to determine the protein quality in numerous commodities [48].

3.5. In Vitro Protein Digestibility (IVPD). IVPD is a key factor in determining the availability of amino acids. Therefore, it plays an imperative role in nutritional quality assessment of food proteins. The mean values indicated that the highest value of *in vitro* protein digestibility was recorded for SPI ($87.57 \pm 4.41\%$), followed by FPI ($85.41 \pm 2.04\%$) and CPI ($82.13 \pm 2.86\%$). However, for soy, the IVPD was observed as $91.35 \pm 3.12\%$ while $95.42 \pm 2.68\%$ for casein (Table 4). Soy and casein are considered as reference proteins. Increase in IVPD mainly depends on elimination of antinutritional factors as well as denaturation of protein during cooking or its exposure to enzymatic action.

The instant findings are in accordance with earlier outcomes that demonstrated 79.50% *in vitro* protein digestibility for canola flour, 80.60% for sesame protein isolates, and 68.0% for flaxseed protein isolates [49]. Later, it was illustrated that the flaxseed meal and sesame seed meal exhibited 83.90% and 81.40% IVPD, respectively [50]. Similarly, 77.9% IVPD was reported in raw brown sesame seed and 85.7% in roasted sesame seed [51].

4. Conclusions

The current evaluation of defatted oilseed protein isolates indicated that these contain proteins with a wide range of molecular weight (15–65 kDa) along with a balanced amino

acid profile and score. Likewise, the PDCAAS and IVPD indicated sufficient availability of quality proteins for the human body in comparison with the reference pattern. Conclusively, defatted oilseed proteins can play an imperative role in improving the nutritional status as well as health and well-being of individuals, especially in developing and underdeveloped economies of the world. Furthermore, these protein isolates can be potentially utilized as ingredients in various food commodities that will certainly help to uplift the nutritional attributes of the products.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

The current research study presented in this manuscript is a part of the PhD research project/thesis of the main/first author (Muhammad Sibt-e-Abbas); hence, a preprint is available in the HEC repository. (<http://pr.hec.gov.pk/jspui/handle/123456789/8378>). The researchers intended to get the result of their research study published in a peer reviewed journal, therefore, decided to submit it here. Proper citations for the preprint have also been made [16].

Conflicts of Interest

All authors declare that they have no conflicts of interest.

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