

Nutritional and functional properties of protein concentrate and protein isolates of foods

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1. Introduction

In recent years, scientific evidence has increased about health benefits from proteins and bioactive peptides derived from food [1]. Protein and isolated extracts from flour have been obtained, reaching a final product with 80-90% of proteins [2]. For protein extraction, different techniques have been developed among them micellization, ultrafiltration, acidic and alkaline aqueous extraction followed by isoelectric precipitation [3]. Due to low solubility in protein sources, the obtainment of protein hydrolysates and even bioactive peptides has enhanced [4].

The protein concentrates of different food products have been reported considering species like rice (75.5%) [5], amaranth (77.8 %) [6], mushroom (48.56-49.94%) [7]; for bean protein isolates of 71.9 to 75.6 % [8], chickpea (85.40 %), lentil (81.90 %), pea (88.76 %) and soy (87.58 %) [9]. On the other hand, protein sources have been added in food elaboration/production due to its physicochemical properties like water and oil absorption capacities, gelification, and foaming and emulsifying properties, which affects food protein behavior and influence in quality and organoleptic characteristics from food system [10].

The present review provides information about nutritional composition and functional properties from different protein sources from some food. Likewise, extraction process for protein concentrates, protein isolates, protein hydrolysates and bioactive peptides. The section for existing applications of protein sources is also included.

2. Protein concentrate and isolates

In recent years the health benefits of bioactive proteins and peptides derived from some foods have been scientifically demonstrated [1]. Protein concentrates and isolates from the flour of different foods have been obtained, which decrease the non-protein components in order to obtain a final product with high protein content [2]. Depending on the protein concentration on a dry basis, they are named protein concentrates, with maximum values of 65% or protein isolates up to 90% [11]. However, in certain cases they may present a low solubility or allergenicity [4].

Protein concentrates are obtained when non-protein components such as carbohydrates, soluble minerals, antinutritional factors and some low molecular weight nitrogenous compounds are eliminated from food, and removed using aqueous-alcoholic solutions (ethanol, 1-butanol, isopropyl alcohol, etc.), acidic or basic solutions. In order to obtain protein isolates, proteins are solubilized in aqueous media by adjusting pH with sodium hydroxide [12].

3. Processing protein concentrate and protein isolate

Protein extraction and isolation involves solubilization and precipitation techniques based on their physicochemical properties [11]. One of the most used techniques in protein extraction is aqueous-alkaline extraction followed by precipitation, in which the proteins are solubilized at elevated alkaline pH and precipitate at pH near to their isoelectric point (~pH 4-5) [3]. Protein extractions of *Lupinus campestris* seeds at pH 9 (30 min, room temperature) have been reported followed by precipitation at pH 4.5, obtaining protein isolates with a 93.2% protein content [13], protein concentrates from mucuna seeds from degreased flour with an organic solvent in a 1:10 w/v ratio were obtained, adjusted pH to 8 for proteins solubilization and precipitated at pH 4, the protein percentage in this concentrate was 78.3% [14]. Likewise, Protein concentrates were also obtained from cowpea seed degreased flour by bringing the pH of the aqueous solution (1:3 w/v) to 9 and then precipitating the proteins at pH 4.33 [15]. Other compounds such as sodium hexametaphosphate (HMP), carboxy methyl cellulose (CMC), ammonium sulfate [16], methanol [17] etc. can be used.

In order to obtain protein concentrates from rice bran, proteins are solubilized at pH 9 and subsequently precipitated at pH 4.5. The obtained concentrate presents of 52.46 to 58.92% protein content [18]. Butt and Batool [19] prepared protein isolates from legume seeds using alkaline conditions at pH 9.5, the obtained precipitate was suspended in water

(1:5 w/v) and centrifuged, the supernatant of the latter process was combined with the first supernatant and the pH adjusted to 4.5, centrifuged, neutralized and lyophilized, obtaining protein isolates from pigeon pea, cowpea, mung bean and pea with values of 82.95%, 89.25%, 85.46%, and 83.61%, respectively. Also, to obtain protein concentrates from the baru nuts defatted flour solution (1:20 w/v), only alkaline extraction at pH 10 was used obtaining a 93% protein content [20]. Although the method of obtaining protein concentrates by alkaline extraction followed by acid precipitation is a traditional method for extracting proteins from plant species, it presents disadvantages due to protein denaturalization comprising the high concentrations of alkaline solutions. Maillard reactions are also likely to cause intense brown color in the protein concentrates and low nutritional value of the protein [21].

Another method to obtain protein concentrates is acid extraction, its extraction principle is similar to alkaline extraction except that it is carried out under acidic conditions (pH ~ 4), it has been observed that proteins solubility is high in very acidic conditions, so this physicochemical property is used to dissolve proteins at low pH and then carry out precipitation by adjusting the pH value of the isoelectric point of proteins [3]. Following this method Aremu et al. [22] obtained cashew nut protein concentrates with protein values of 69.6% using an aqueous solution of cashew nut degreased flour in 1:10 w/v concentration and adjusting pH with 0.1M HCl. On the other hand, acidic extractions have been reported at pH 2, followed by isoelectric precipitations at pH 4.5, obtaining soy protein isolates [23].

On the other hand, the micellization process (salt extraction) has also been used, which is based on the "salting-in" and "salting out" of proteins. In this process, after protein extraction with a salt solution inducing protein precipitation, the precipitate is recovered by centrifugation or filtration, followed by drying [3]. Sodium sulfite (Na_2SO_3) was used to solubilize proteins (pH 10.5, 4 ° C, 1 h) and then acidify the solution to the isoelectric point of proteins (pH 4) to obtain protein isolates with 92.4% protein content [24]. In addition, it has been reported that *Lupinus campestris* protein isolates were obtained with 95.7% protein extracted from degreased flour with 0.5 M sodium chloride and pH 7 [13].

Recently, membrane technology has been successful in recovering solubilized proteins. The use of ultrafiltration and diafiltration processes has allowed obtaining protein concentrates or isolates presenting low concentrations of soluble solids with low molecular weight, such as phytates, polyphenols and glucosinolates [11]. Protein concentrates were obtained from clarified lucerne juice with a 10 kDa ultrafiltration membrane at 242 kPa pressure and 16.7 rev/s speed, however the protein content was 35% lower than the obtained under acidic and neutral conditions that was 72% and 63%, respectively [25]. However, studies reporting 98.8% protein contents in concentrates obtained from rape seed acid supernatants, using 10 kDa ultrafiltration membranes, compared to the alkaline precipitation method in which concentrates were obtained with 70.8% [26].

Likewise, proteins based on their solubility have been consecutively extracted by the Osborne Method, for example, degreased rice flour is extracted with distilled water for 4 h at room temperature and then centrifuged to obtain the "albumen" fraction (supernatant). The obtained residue was extracted with 0.1 M NaOH solution for one hour to obtain the "glutelin" rich fraction. The latter extracted residue is combined with 70% ethanol to extract prolamins. Each albumin, globulin and glutelin fraction was precipitated according to its isoelectric point, ie 4.1, 4.3 and 4.8, respectively. A higher amount of glutelins (95.63%) was obtained, followed by prolamins (92%), globulins (90.41%) and to a lesser extent albumins (78.14%) [27].

Other factor that is important consider is the ionic strength effect on protein content, for example, protein isolates from sesame seeds were obtained by extraction with water (pH 7, 35 ° C) and in the presence of three NaCl concentrations (0.2, 0.6 and 1.0 M), followed by acid precipitation at pH 4.5 (25 ° C), dialysate and freeze-dried. At concentrations of 0 and 0.2 M NaCl the protein percentage was 100%, which decreases to 93.2% and 94.6% with concentrations of 0.6 and 1.0 M, respectively [28]. On the other hand, Elsohaimy et al. [29] indicated the positive effect of the agitation period during the extraction of quinoa proteins, which increases while agitation increases, presenting a maximum of protein extractability in 120 min.

It is important to indicate that during the process of obtaining protein isolates and concentrates, partial protein denaturalization can be caused by the drying process, since an insoluble protein aggregation is present irreversibly. The most commonly used methods are spray-dried, freeze-dried and vacuum-dried. However, in a research work it has been indicated that drying lentil protein isolates by the three aforementioned methods does not alter the physicochemical characteristics and their nutritional composition, no significant differences in protein content being found in a range of protein concentration from 90.2% to 91.9%, but there was an effect on the coloration of the protein isolate [30].

4. Nutritional value of concentrate and protein isolate

Protein characterization from isolates and concentrates is of great importance. Some of the characteristics that should be considered are: surface hydrophobicity, solubility, and electrophoretic patterns that indicate both the molecular weights of proteins and possible cross-linkings. Not forgetting the thermal properties, which are useful to know the protein changes during food cooking process [31]. In addition, nutritional quality and functional properties are also characteristics that must be considered, for their application in functional foods or nutraceuticals development [32].

Protein concentrates or isolates recovered from different vegetable or animal flours have a high protein content compared to the original flours, altering the moisture, fat, ashes, fiber, and carbohydrates contents (Table 1). For example, Nassar [33] reported a protein increase from 13.62% to 62.41% and decreases in other components from

protein concentrates of prickly pear seed, indicating that carbohydrates and fats in flour are removed at a great proportion during the protein concentrate process. In contrast, higher fat content (5.84%) was found in protein concentrates from cowpea flour (1.80%), this result is due to high fat content in protein concentrates or seed isolates of legumes caused by the binding between protein and lipid as a result of lipid emulsification by proteins [15].

Table 1 Nutritional composition of protein concentrate and protein isolate.

	Sample	Moisture	Protein	Fat	Ash	Fibre	Carbohydrate
Protein concentrate							
Prickly pear seed [33]	Flour	9.03	13.62	10.43	6.47	9.23	51.11
	PC	7.16	62.41	3.57	5.31	5.29	15.79
<i>Pleurotus tuberregium</i> [45]	Flour	12.5	17.6	5.95	6.4	5.80	51.8
	PC	6.35	5.24	1.50	40.4	4.26	42.2
Bambara beans [46]	Flour	6.45	24.78	5.9	3.88	5.35	52.94
	PC	8.92	70.85	13.15	3.86	1.82	11.25
<i>Pleurotus ostreatus</i> [7]	Flour						
	PCM	7.93	24.25	1.97	7.94	9.29	56.55
	POS	7.64	25.78	2.00	7.96	8.6000	55.65
	PCMXPPOS	7.62	26.81	1.92	8.01	8.64	54.62
	PC						
	PCM	7.6	49.85	5.96	7.50	-	36.69
	POS	7.39	49.94	6.11	7.59		36.36
	PCMXPPOS	7.22	48.56	5.66	7.59		38.19
Protein isolate							
Chickpea [47]	Flour	8.1	24.7	1.5	3.7	18.8	51.3
	PI						
	Isolate-A	3.3	78.0	3.5	2.9	3.8	11.8
	Isolate-B	5.5	88.1	1.1	4.3	3.2	3.3
Lupin [48]	Flour	7.9	33.8	13.6	2.1	39.9	ND
	PI						
	Isolate A	3.4	87.4	3.2	0.7	4.0	ND
	Isolate B	9.4	83.9	1.0	0.3	4.8	
Legumes [49]	Flour						
	<i>Lathyrus clymenum</i>	11.40	21.25	1.99	2.58	41.10	19.05
	<i>Lathyrus annuus</i>	10.90	19.95	1.93	2.20	41.0	20.9
	PI						
	<i>Lathyrus clymenum</i>	4.64	82.40	1.16	0.26	9.18	Tr
	<i>Lathyrus annuus</i>	4.78	81.07	1.09	0.20	7.71	

Note: PC: protein concentrate; PI: protein isolate

Gueguen (1983) [34] mentioned that lipids are free in flours but they are trapped by proteins during the process of obtaining protein concentrates. In other investigations, protein concentrates of rice bran showed protein content in dry basis between 52.46% and 58.92% [18], 79% in protein concentrates of soybean [35], 36.42% in amaranth leaf protein concentrates [36], whereas for amaranth seeds the percentage of proteins is between 73.6% and 77.8% [6], a 75.5% in rice protein concentrates [5], and even the protein content in mesquite concentrates has been doubled to 67.9% from flour which has only 33.8% [37]. There are reports of bean protein isolates presenting 83.96-89.25% of proteins, three times more than bean flours (22.36-28.5%), considering that ashes, carbohydrate and lipid content are reduced in these isolates [38], protein content has also increased to 75.8% and 83.4% in amaranth seeds protein isolates [39]. Also, to 89.9%-94.4% was reported for protein isolates from chickpea defatted flour which contained 20.6 and 26.7% of proteins [40], for rice flour protein isolates, protein content between 79% and 91.1% was obtained [41], in beach pea isolates, the reported protein content was 85.1% and 86.6% [42].

The physicochemical processes used in the different obtaining methods for isolates and proteinaceous compounds such as alkaline extraction-isoelectric precipitation, micellization, acid extraction, as well as the use of high temperatures and/or organic solvents to degrease the sample before protein extraction can cause protein denaturalization, affecting both the solubility and yield of protein concentrates and isolates [43]. [3 Valor nutricional/Pedroche et al 2004 AP.pdf](#). Regarding to yield, Wani et al. [44] reported 35.15% and 38.27% for protein isolates from watermelon seed flour, whereas in protein concentrates the yield was lower than 25.21 % and 27.41%, also indicating that dry basis protein content was from 71.38 to 83.79%. In another study, a protein content of 75.8% in amaranth protein isolates was reported on extraction at pH 8.5 and precipitation at pH 5.0, on the other hand, by increasing the extraction pH to 9.0 and lowering the pH of precipitation to 4.5, the protein content increased to 83.4% [39].

It is important to mention that the objective of removing undesirable components during the process of obtaining protein products is essential to improve their nutritional quality and to know their potential as a functional ingredient [39].

5. Functional properties of protein concentrate and protein isolate

Functional properties are physicochemical properties of proteins and affect their organoleptic characteristics and quality, interfering in the behavior and appearance of food from its preparation to its storage [50, 51]. Proteins can be used as structural stabilizers in foods, such as emulsifiers or foaming agents and even for forms or stabilizing gels [52]. Therefore the functionality of a protein will allow us to know the type of product in which it would be used and plays an important role in consumer acceptance [53].

5.1 Solubility

The amino acid that conforms the proteins presents a prevailing charge at different pHs which determines their solubility. Figure 1 shows the possible configurations of the functional groups present in the amino acids of a protein, in the region of the isoelectric point predominates a molecule called "ion zwitterion" (I), where there is a balance in the positive and negative charges minimizing the electrostatic repulsion and as a consequence, a reduction in protein solubility. With acidic pH values lower than the isoelectric point, the cation III predominates, whereas in an alkaline medium the anion II takes precedence. In both cases, there is an improvement in electrostatic repulsion and higher solubility at pH 2 and pH 11, respectively [54].

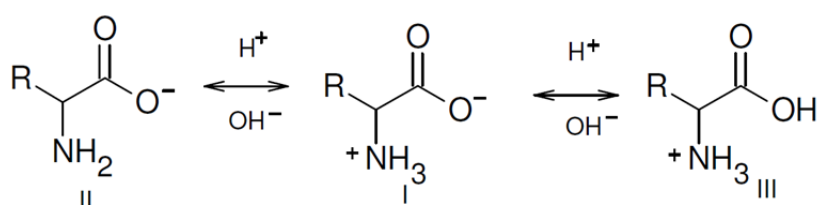


Fig. 1 Prevalent charge on proteins at different pH.

For the obtaining process for oat bran protein concentrates in acid medium, solubility percentages of smaller proteins (4.7 to 7.3%, with pH 5) have been reported compared to those obtained in alkaline medium (pH 9) reaching up to 89.3% in protein solubility [55]. In another study, under acidic conditions at pH 2, the protein concentrates of foxtail millet and soybeans had a solubility of 40%, which decreased by about 20% when the values were pH 4 and pH 5, however at pH 6, solubility increased between 50% and 80% [56]. Similarly, for soybean and faba beans protein isolates, the maximum solubility was pH 8 and pH 9, and low solubility between pH 4 and 6, because the proteins of most legumes precipitate at pH 4 [57].

5.2 Water and oil absorption capacity

Water absorption capacity (WAC) and oil absorption capacity (OAC) are functional properties of food proteins that are important in the development of new products, particularly for flavor fixation, as well as in the development of oxidative rancidity during storage [58]. In particular WAC from a protein isolate determines the interaction degree for water and protein solubility, influencing surface tension, binding energy, temperature, pH, ionic force, vapor pressure, among other physicochemical factors [59, 60]. In the case of protein isolates, values for WAC between 97% and 163% for legume seeds has been found, where pigeon pea isolates showed lower WAC, followed by cowpea, and peas and mung bean with higher WAC [61]. Ogundele et al. [62], indicated that protein isolates from melon seeds showed high WAC (2.67-3.5 g/g) due to their high protein content and consequently a high hydrolysis capacity, on the other hand OAC of these protein isolates (2.24-3.14 g/g) was higher in comparison to flour (0.84-1.4 g/g). Similar behavior was found for cashew nut protein isolates, which presented higher OAC (4.42 ml oil/g) than protein concentrate (3.32 mL oil/g), but both products showed higher values than flour (2.05 mL oil/g) [63]. Proteins containing higher non-polar functional groups are more hydrophobic and have an important role in oil absorption because they show greater binding to proteins, such binding is achieved between the lipid chains with the non-polar amino acid side chains [64], furthermore the conformational structure that proteins adopt is also important [65], so hydrophilic and hydrophobic proteins behavior affects OAC [66]; the increase of WAC also causes physical retention by capillarity in the new structures by aggregation of formed proteins [67].

5.3 Gelation

The critical factor for gel formation is protein concentration, not requiring a high solubility to form the gel [67]. Other important factors are electrostatic and hydrophobic interactions, hydrogen and disulfide bonds, and protein aggregation. In addition, the degreasing process favors gels formation with low lipid content and high protein content and as a consequence, the decrease in the least gelation concentration (LGC) [68]. If the LGC is lower, the gelation capacity of the protein ingredient is higher [69]. For example, cashew nut degreased flour showed 6.5% of LGC, 10.0% for protein concentrates and 13.5% for protein isolates [63].

In contrast, flour and protein fractions obtained from rice had a LGC of 4% and 5%, respectively [27]. Variations in gelling properties are due to the proportion of different constituents such as proteins, lipids and carbohydrates in flours [70]. In addition, the gel properties are influenced by several factors, such as pH, their nature, electrolytes and proteins concentration [71].

5.4 Foam properties

Foam is a colloidal system formed by tiny air bubbles dispersed in an aqueous continuous phase called lamella. Bubbles formation and stability is achieved by incorporating an agent capable of reducing the surface tension between the phases. Proteins are good foam stabilizers because they act at the air:water interface, where the hydrophobic residues of the amino acids present in the polypeptide chain are oriented towards the interior of the bubble and the hydrophilic part oriented toward the aqueous phase [72]. A good foaming capacity has been linked to proteins flexibility with reduced surface tension and with a high protein concentration [73]. For example, Segura-Campos et al. [74] evaluated the foaming capacity respecting to pH of bean protein concentrates, the lowest foam capacity was at pH 4 and higher under alkaline conditions (pH 8), indicating that it may be due to an increase in protein net charge which weakens hydrophobic interactions by improving their flexibility and enhancing foaming. Foam stability decreases in neutral pH regarding to time (0.5 min, 5 min, 30 min, and 120 min), but increases in acidic and alkaline pH.

In other investigations, protein isolates from cashew nut shell showed minimum foaming capacity at pH 3 (28.65%) whereas in alkaline conditions it was 83.45-86.51%, and for acidic conditions at pH 2 it increased to 60.13%. A similar behavior was observed for its stability, at pH 3 it was 33.89% increasing to 83.75% at pH 11, attributed to proteins solubility increases when pH is above the isoelectric point [75]. Similar results have been found in protein products of peanut, at a time of 0 min, flour and protein concentrates presented low foaming capacity corresponding to 28% for flour and 26.5% for concentrates, in contrast for protein isolates of 50%. After 30 and 60 min it does not decrease significantly in each product, which indicates that they are good foam stabilizers, and that higher protein concentration, foaming capacity and foam stability also increases [76]. For a protein to be a good foaming agent it must meet two basic requirements: 1) the ability to absorb at the water-oil interface and 2) the ability to undergo conformational changes at the interface [77].

On the other hand, it has been reported that the protein concentrate of edible fungus *Pleurotus tuber-regium* presented greater foam stability compared to flour [46]. In addition, foaming capacities depend on proteins and other components such as carbohydrates, which are present in flours [78]. Also, the ability of a protein to form or stabilize foam will depend on the type of protein and the degree of denaturalization, the presence of calcium ions, pH, temperature and whipping methods [79].

5.5 Emulsion properties

According to Mena-Casanova and Totosaus [80], the emulsion capacity (EC) measures the ability of soluble proteins to migrate to the water-oil interface, so it must be considered that solubility and conformation of proteins are affected by environmental conditions such as ionic strength and pH. The determination of EC depends on the water-oil interface and requires a large amount of emulsifier to stabilize the emulsion and the droplets size produced during stirring. Also, other parameters have to be taken into account to measure the ability of the protein to form an emulsion, among them is the emulsion activity index (EAI) and the emulsion stability index (ESI). EAI is measured as the interfacial area stabilized per mass unit of protein based on the emulsion turbidity and the change in turbidity of that diluted emulsion measures the ESI per unit time [81, 82].

The emulsion activity indexes of protein isolates from sesame seeds were 16.8 m²/g, higher than those obtained in protein isolates of soybean (12.2 m²/g). However, for sesame protein isolates this activity was improved at pH 5 (20.7 m²/g) and decreased at pH 2 (13.0 m²/g), these results confirm that the emulsifying properties are pH dependent [83]. Another study in peanut protein isolates showed higher emulsion stability indexes at alkaline pH (7 and 9) and at acidic pH (pH 3) [84]. Similar behavior was observed in flours, concentrates and walnut protein isolates, where the emulsion activity was higher at pH 2 and 12, and decreased at the isoelectric point of proteins (pH 4.5), but at all pH the flour presented the higher values compared to concentrate and protein isolate [85].

On the other hand, surface protein concentration between flour and coriander protein concentrates has been determined, the latter had low protein concentrations per surface area (mg/m²) compared to flour, and this is attributed to the fact that flour contains non-protein components, which reduce electrostatic repulsions between proteins [86]. In conclusion, the difference in total protein composition and non-protein contribute to the emulsifying properties of protein products, that is, the proteins decrease surface tension and promote electrostatic repulsions between them, whereas some carbohydrates increase the system viscosity to stabilize the emulsion [87]. A protein capacity to improve the formation and stabilization of emulsions is important for food industry applications such as cakes, coffee bleaches and frozen desserts [88].

6. Hydrolysates and Peptides bioactives

As previously mentioned, protein isolates have limitations in the food industry, which is why protein hydrolysis processes have been carried out, aiming at the peptide bond breakdown and consequently the generation of smaller peptides or amino acids release. To achieve the bond rupture, chemical methods with the addition of acids or bases, or biological with enzymes application are necessary [2]. This latter method has an advantage in comparison to the others, since enzymes are selective and specific for the breaking of a certain bond, it is carried out in moderate temperature and pH conditions, and maintains the nutritive value because there is no degradation of separate components, while the amino acids arginine and cysteine are destroyed in alkaline and acidic hydrolysis and even tryptophan is eliminated [89].

When obtaining protein hydrolysates, certain functional characteristics such as decrease in viscosity, greater agitation capacity, dispersion and increase in solubility are favored, advantages those are desirable to be incorporated into food products. In addition, the fundamental property of hydrolysates is the degree of hydrolysis, which determines its possible use, is defined as the percentage of breakage of peptide bonds in relation to the original protein and is grouped in: hydrolysates with low hydrolysis degree (between 1% and 10%) for the improvement of functional properties; Hydrolysates with a variable hydrolysis degree to be used as flavorings, and finally, hydrolysates with hydrolysis degree greater than 10%, in specialized feed [90].

Through enzymatic hydrolysis, peptides can be generated, which are specific protein fragments and have the characteristic of being inactive within the sequence of the original protein from where they were obtained and once released they present bioactivity. The molecular weight of peptides is less than 6 kDa and formed from 2 to 20 amino acids [91]. The potential of bioactive peptides from animal or vegetable origin makes it attractive as an ingredient in food and pharmaceutical industries. Bioactivity has been found in peptides obtained from the hydrolysis of milk, egg and other proteins [92].

In addition, several biological activities have been shown in peptides and hydrolysates, such as anti-inflammatory activity [93], antioxidant [94], antihypertensive [95], antibacterial [96, 97, 98], inhibitory activity of angiotensin-I converting enzyme (ACE I) [99, 100], and immunomodulatory [101].

In conclusion, protein concentrates and isolates from plant, animals, and even fungi represent a good source of high quality proteins and important functional properties making them attractive for food processing. Also the production of hydrolysates and peptides with biological activities are important for the food and pharmaceutical industries.

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