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Functional characteristics of protein isolates from chicken liver

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ABSTRACT

The study was conducted to isolate protein from chicken liver and characterization of its techno-functional properties. Prior to protein isolation, protein solubility test was carried out to know the highest solubility at a particular pH. The isolates obtained were freeze dried and subjected for different quality evaluation and comparison was made with the chicken liver powder. Higher protein solubility was observed at pH 1.5, 2.0 11.0 and 11.5 with significantly higher yield at alkaline pH and highest total protein content at pH 11.0. The bulk density of protein isolates was significantly lower than that of whole chicken liver powder. As compared to whole liver powder, the whiteness values were significantly higher for isolates and highest value was observed for isolate at pH 11.5 and accordingly had lowest total pigment content. The other techno-functional quality parameters such as fat absorption capacity, water absorption capacity, foaming capacity, foaming stability, emulsifying activity index, emulsifying stability index improved in all types of isolates. The ABTS and DPPH assays were also reflected significantly higher antioxidant activity in protein isolates as compared to whole liver powder. The protein isolates form poultry liver can be utilized for production of high-quality protein isolates.

Keywords: Chicken liver, Functional characteristics, pH shift, Protein isolates

The world poultry population is rapidly rising due to increased demand for high-quality protein, purchasing power, lifestyle improvements, nutritional patterns, etc. The global poultry population has grown to three times that of humans and is nearly five times to that of its five decades back values. The USA is the world's leading producer (17%) followed by China and Brazil while India ranks 6th in terms of chicken production (FAOSTAT 2020). According to India's 20th livestock census, there are 851.81 million poultry birds in the country, a sharp hike of 16.81% from the previous livestock census and poultry meat contributes 50% of total meat production in India (DAHD 2019). The rising popularity of chicken meat, and the absence of any religious taboo has contributed to the poultry industry's expansion. The chicken alone contributes 4.78 million tonnes of meat to the country's annual meat production of 9.29 million tonnes. With 40.6% of the global production in 2020, chicken meat had the most significant growth contributing to almost half of the total world meat production (FAO 2021).

Poultry, particularly with a dressing percentage of 70-72%, is one of the most carcass-yielding species among meat animals. Other than meat, the remaining 28-30 % is usually a challenge for poultry processors since appropriate disposal costs a fortune. Chicken sleeves and visceral organs are the two types of by-products that come from poultry processing. Blood is another by-product with numerous applications such as blood meal preparation, and biologicals. Visceral organs such as heart, liver without a gall bladder, and gizzard, are together known as 'giblets'. During the processing of chicken meat, the giblets are usually harvested and are treated separately, quickly chilled (due to high glycogen content). They are used for consumption purposes but are usually withheld from the preparation of high-value products like barbeque.

The liver among the giblets contains approximately 18% proteins and is one of the highest protein-containing poultry by-products. Finding out an economical and efficient method to transform the minimal value and unutilized chicken liver proteins into fruitful and productive uses is an unsolved problem yet (Xiong *et al.* 2020). Accounting for the products from chicken liver, the majority are characterized by poor techno-functional properties. Hence, there is an urgent need to utilize chicken liver comprehensively. Extraction of proteins from chicken liver is an alternative that can open a wide door for research. Liver represents an important source of protein with functional characteristics associated with different protein fractions and physico-chemical conditions such as pH, ionic strength, and specific processing cases. Several studies have been reported on the utilization of recovered proteins from fish (Nolsoe and

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Undeland 2009), chicken meat (Hrynets *et al.* 2011), and mechanically separated turkey meat (Ozimek *et al.* 1986) by acid–alkaline treatment. These studies showed that the recovered proteins exhibited improved functionalities than the conventional proteins. Thus, the application of the acid–alkaline treatment appears promising for efficient recovery of functional muscle proteins from meat by-products. However, there are few published studies on the acid–alkaline processing of chicken liver till date. On the other hand, development of novel protein sources is one of the major challenges for the present world food industry. Therefore, this study aimed to isolate proteins from chicken liver and investigate the quality characteristics of protein isolates.

MATERIALS AND METHODS

Chemicals and reagents: Fine chemicals such 2, 2'-azino- bis (3- ethyl bcnzthiazolinc- 6- sulphonic acid (ABTS) and 2, 2-Diphenyl-1-Picryl hydrazyl (DPPH) were procured from Hi-media Laboratories Pvt Ltd, Maharashtra. All other chemicals used in the experiments were of analytical grade and obtained from Hi-media™, Sigma-Aldrich™, Merck™, and CDH™ etc. All solutions used in the experiment were prepared using double distilled water at room temperature unless mentioned.

Chicken liver: Chicken liver was procured from Post-Harvest Technology Division, ICAR- Central Avian Research Institute (CARI), Izatnagar, and local markets of Bareilly. The gall bladder was removed carefully and the liver was transported to the laboratory under hygienic conditions. The procured liver was cleaned, washed, packed in low density polyethylene (LDPE) bags and stored under deep freezing conditions (-20°C).

Protein solubility test: The protein solubility test was done by the method described by Kim *et al.* (2003) with slight modification. Frozen chicken liver was finely minced using food mixer grinder (Butterfly, India) and was suspended in chilled distilled water (1:50 w/v), followed by homogenization for 2 min using Ultra-Turrax T25 tissue homogenizer. The homogenate was divided equally (30 mL each) into 22 tubes and pH adjustments was done ranging from 1.5 to 12 with an increment of 0.5, using 2N HCl and 2N NaOH. The homogenate was centrifuged at 8000 rpm for 10 min using refrigerated centrifuge (446 High speed centrifuge, HERMLE Labortechnik, Wehingen, Germany). Supernatant was collected and the soluble protein content was determined.

Isolation of chicken liver proteins: Based on the solubility of liver protein, two pH from acidic side - 1.5 and 2.0, and two from alkaline side - 11.0 and 11.5 were selected for large-scale production of protein isolates for further experiments. Fresh chicken liver was cleaned and finely minced in a mixer grinder (Butterfly, India) and was immersed in chilled (4°C) distilled water (1:6 ratio w/v), followed by homogenisation for 5 min in Ultra -Turrax T25 tissue homogeniser at 13,500 rpm. The homogenate was divided into four equal parts for extraction. Two parts were adjusted to acidic pH and the rest two to alkaline pH using 2N hydrochloric acid and 2N sodium hydroxide solution, respectively. The pH adjusted homogenate were kept at room temperature for 4 h with continuous agitation and centrifuged using refrigerated centrifuge at 8000 rpm for 20 min. The supernatant was collected carefully to avoid mixing of any lipids and connective tissue into it. Isoelectric precipitation was done by adjusting the pH of supernatant to 5.5 by addition of either 2N HCl or 2N NaOH followed by centrifugation using refrigerated centrifuge at 8000rpm for 20 min. The supernatant was discarded and the precipitate was collected, weighed and was subjected to lyophilization using laboratory freeze drier (FD5508 Floor-Model Freeze dryer -85ºC, Ilshin Biobase, Maxwellstraat 11 ede, Netherlands) for approximately 24 h and finally milled to get fine powder. The protein isolate powders were packed in air tight containers, labelled and stored at -20°C for further analysis. The fresh chicken liver was also dried and used for comparison in the experiment.

Characterization of protein isolates from chicken liver: The prepared protein isolates were characterized for its physical & functional properties and compared with whole chicken liver powder.

Proximate composition: The proximate composition of chicken liver was performed initially followed by the estimation of moisture, protein, fat and ash percentage of isolates, following the AOAC (2000) protocols.

Bulk density: Bulk density estimation (Foh *et al.* 2011) was done which can determine the storage capacity of the prepared products.

Instrumental colour parameters: Hunter colour lab (Mini ScanEZ, 4500L, USA) was used for analysis of different colour characteristics of raw liver powder and isolates.

Total pigment content (TPC): Raw liver powder and protein isolates (100 mg each) were mixed in 5 mL of acid-acetone having 90% acetone, 8% deionized water and 2% HCl. The mixture was stirred and vortexed for few min and allowed to stand at room temperature for 1 h. Whatman filter papers (grade 1) were used for filtration of extracts and absorbance of the filtrate was noted at 640 nm using Genesys 10S UV-VIS Spectrophotometer (A_{640}) using acid-acetone as blank. TPC was determined the formula:

Total pigment content (ppm)=$A_{640} \times 680$ (Lee *et al.* 1998)

Haem content (mg Mb/g) =$17.18 \times A_{640}$ (Hrynets *et al.* 2011)

Water absorption capacity (WAC) and Fat absorption capacity (FAC): WAC and FAC were determined (Ogunwolu *et al.* 2009) to define the nature of prepared isolates as a component of foods. Briefly, the dispersions of each protein isolate and raw liver powders were prepared in distilled water and soyabean oil (100 mg/mL) for WAC and FAC, respectively and weighed (W_1), followed by vortexing for 1 min and the mixture was centrifuged at 4000 rpm for 20 min. The supernatant was drained off and the centrifuge tubes were weighed (W_2), WAC and FAC were determined according to the formula:

WAC & FAC= W_2-W_1/ Sample weight (g)

Weight of water or oil absorbed per gram of sample was expressed as water absorption capacity and fat absorption capacity respectively.

Emulsifying activity index (EAI) and Emulsifying stability index (ESI): EAI and ESI of the isolates were calculated to determine the emulsion properties (Ogunwolu *et al.* 2009). Briefly, the isolates (210 mg) were mixed with distilled water (21 mL) and soyabean oil (7 mL). The resultant dispersion was homogenized at 20000 rpm for 1 min. From bottom of the tube, a 50 µL aliquot was taken at 0 min and 10 min of homogenization and was mixed with 5 mL of 0.1% SDS solution. Absorbance of the solution was taken at 500 nm.

$$EAI\ (m^2/g) = 2.33 \times A_0 \text{ (Hrynets } et\ al. \text{ 2011)}$$

$$ESI\ (min) = A_0 * \Delta t / \Delta A$$

Where, $\Delta t = T_{10} - T_0$; $\Delta A = A_0 - A_{10}$

Foaming capacity (FC) and Foaming stability (FS): Foaming capacity and foaming stability was determined using method described by Foh *et al.* (2011) to assess the functionality as food ingredient.

Water activity (a_w): Water activity was determined by digital water activity meter (4TE Dew Point water activity meter, Aqua lab).

2-2-Azinobis-3-ethylbenthiazoline-6-sulphonic acid [ABTS] radical scavenging activity: The spectrophotometric analysis of ABTS+ radical scavenging activity was estimated by method of Salami *et al.* (2009). ABTS radical cation (ABTS+) was produced by reacting ABTS+ solution (7 mM) with equal volume of 2.45 mM Potassium persulphate ($K_2S_2O_8$) and allowing the mixture to stand in the dark at room temperature for about 16 hs before use. The lyophilised isolate powder was reconstituted (100 mg/mL) in phosphate buffer (pH 8). Prior to use, the stock solution was diluted to get an absorbance of 0.70 at t_0 (t=0 min) and equilibrated at 30°C exactly 6 min after initial mixing. About 1 mL of ABTS+ working solution was mixed with 10 µL of samples and absorbance was measured after 20 min (A_{20}) at 734 nm in Genesys 10S UV-VIS spectrophotometer. The ABTS+ activity was calculated by using the formula:

$$ABTS\ activity\ (\%\ inhibition) = [(0.7 - A_{20})/0.7] \times 100$$

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity: The ability to scavenge DPPH radical by the sample was estimated by method described by Brand-Williams *et al.* (1995) with minor modifications. The lyophilized isolate powder was reconstituted (100 mg/mL) in phosphate buffer (pH 8) and was used for the test. Briefly, 1 mL of DPPH reagent (100 µM) was mixed with 0.25 mL of 0.1M Tris-HCl buffer (pH 7.4) and 25 µL of isolate sample in test tubes. The content was gently mixed and the absorbance was measured at 517 nm using Genesys 10S UV-VIS Spectrophotometer at 0^{th} min. (A_0) and 20 min (A_{20}). Ethanol was used as the blank. The DPPH inhibition activity was calculated as the decrease in absorbance using following equation:

$$DPPH\ activity\ (\%\ inhibition) = 100 - [(A_{20}/A_0) \times 100]$$

RESULTS AND DISCUSSION

Protein solubility test: The results of protein solubility test showed that chicken liver proteins were maximum soluble at extreme pH, both in acidic (pH 1.5 and 2) as well as in alkaline side (pH 11 and 11.5), giving rise to a rough V-shaped curve (Fig. 1). Lowest solubility was observed at pH 5.5 which is also the isoelectric point of the majority of muscle proteins. Compared to acidic condition, alkaline extraction resulted in slightly higher solubility for liver proteins. The findings were in accordance with the findings of Xiong *et al.* (2016) in chicken liver and Xue *et al.* (2019) in goose liver. At isoelectric pH, the interaction between proteins and water is replaced by protein-protein interaction and hence precipitation occurs. Further acidification and alkalization can increase the charges of proteins from chicken liver leading to repulsion between protein molecules and hence, more interaction with water molecules causing increased solubilization. In the present study, maximum solubility was observed at extreme pH conditions; however it was significantly higher in alkaline pH condition. A similar trend in the solubility of mechanically separated turkey meat was also reported by Hrynets *et al.* (2011). In chicken liver, an increase in recovery yield was attributed to an increase in solubilization pH. The solubility was highest for pH 11 than 11.5 and 12 and this may be due to the exposure of much more hydrophobic groups at the higher pH value.

Proximate composition of freeze-dried chicken liver and protein isolates: The percentage moisture protein, fat and ash in dried chicken liver were 10.78±0.35, 68.80±1.98, 12.1±0.052 and 5.95±0.05, respectively. The protein content of dried isolates in acid and alkaline treatment ranges between 81.6% at pH 1.5 to 90.4% at pH 11 and the protein content of alkaline extraction was found be significantly higher when compared to acidic, but all isolates had significant differences in protein content as compared to raw liver (Table 1). The values of fat and fibre were significantly lower as compared to that of raw liver. In the present study, it was observed that the protein contents of chicken liver protein isolates were more than 80% and

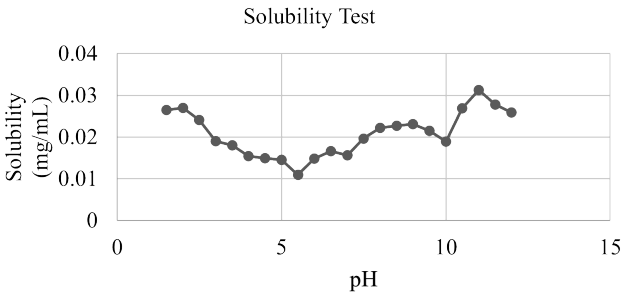

Fig. 1. Graphical representation of protein solubility of chicken liver

Table 1. Proximate composition of freeze-dried chicken liver and protein isolates (Mean±SE)

Parameter	Protein isolation pH				Liver powder
	1.5	2	11	11.5	
Moisture (%)	2.95±0.08^{a}	2.93±0.07^{a}	2.82±0.09^{a}	2.83±0.06^{a}	10.78±0.35^{b}
Protein (%)	81.69±0.64^{c}	84.97±2.42^{bc}	90.48±1.68^{a}	89.59±1.72^{ab}	68.80±1.98^{d}
Fat (%)	5.73±0.12^{b}	5.61±0.27^{b}	4.62±0.19^{c}	4.95±0.046^{c}	12.1±0.052^{a}
Ash (%)	5.53±0.12^{b}	4.32±0.25^{c}	2.79±0.19^{e}	3.64±0.05^{d}	5.95±0.05^{a}

Mean ± S.E,$^{a, b}$ mean values within column with different superscripts are significantly different ($p<0.05$)

highest was 90.4% at pH 11.0. Similar findings were also reported in tilapia protein isolates (Pires *et al.* 2012).

Bulk density: Bulk density (BD) is the desirable characteristic of food ingredients that aids in packaging and transportation. The results showed that acidic extraction yielded slightly bulkier isolates than alkaline but both isolates were significantly lighter than raw liver powder (Table 2). However, the differences in bulk density of both the isolates were not similar. Similarly, the proteins of the catfish isolates had a bulk density of 0.64 g/mL (Haryati *et al.* 2020) but another study reported that the BD of catfish isolates was as low as 0.338g/mL (Kumarakuru *et al.* 2018). Rainbow trout fish protein isolates has a bulk density of 0.58 g/mL (Lone *et al.* 2015) and the fresh meat concentrate from tilapia fish was having BD value of 0.34 g/mL (Foh *et al.* 2011). The tapped bulk densities of milk protein concentrate powder tends to increase with decrease in protein content.

Instrumental colour profile: The current study showed a significant difference between the whiteness values of acidic and alkaline hydrolysates. Alkaline hydrolysates were significantly lighter in colour than acidic isolates. All isolates had significantly differed from the values of raw liver also. Each liver paste sample was measured in triplicate to obtain the lightness value (L*), redness value (a*) and yellowness value (b*) (Xiong *et al*. 2020). Tian *et al.* (2017) also reported that acid and alkali isolate from common carp prepared using the pH-shift approach dramatically raised the product's *L** and *b** values while lowering it's *a** value, making it significantly whiter than fresh muscle. The elimination of pigments during the pH-shift process is likely to have caused this color improvement. The drop in *a** values also demonstrate that, due to changes in protein structure, hemoglobin is separated from other proteins during the protein isolation process at severe pH conditions. Because of modifications in protein structure, the protein hemoglobin is segregated from other proteins. The haem proteins unfold, get oxidized and denatured, and turn brown after being adjusted with acid and base on the isoelectric point, contributing to the enhanced yellow of the final product. Xiong *et al.* (2020) also reported the colour values of normal liver as *L** values of 46.32, *a** values of 23.93 and *b** values 11.55. The isolates had significantly high whiteness values compared to raw liver. Alkaline isolate had high *L** and whiteness compared to acid isolates.

Total pigment content: The total pigments in protein isolates recorded in the current study showed a clear

Table 2. Properties and functional characteristics of protein isolates at different pH and chicken liver powder (Mean±SE)

Parameter	Protein isolation pH				Chicken liver powder
	1.5	2	11	11.5	
Bulk density (g/mL)	0.33±0.0006^{b}	0.32±0.001^{b}	0.26±0.006^{c}	0.265±0.008^{c}	0.78±0.0042^{a}
Instrumental colour profile L*	76.81±0.64^{c}	70.33±0.81^{d}	83.94±0.94^{b}	92.8±0.70^{a}	45.88±0.72^{e}
a*	18.48±0.52^{b}	17.67±0.88^{b}	7.73±0.37^{c}	4.598±0.35^{d}	24.0±0.68^{a}
b*	22.14±0.72^{a}	20.71±0.696^{ab}	19.398±0.61^{b}	20.63±0.549^{ab}	11.63±0.57^{c}
Whiteness	62.92±0.40^{c}	59.63±0.498^{d}	73.57±0.65^{b}	77.61±0.58^{a}	39.61±0.41^{e}
Total pigment content (ppm)	150.96±0.86^{c}	172.04±0.66^{b}	99.17±2.33^{d}	95.09±1.36^{d}	183.6±2.41^{a}
Haem content (mg Mb/g)	3.81±0.022^{c}	3.81±0.016^{b}	2.50±0.059^{d}	2.40±0.034^{d}	4.35±0.061^{a}
Water absorption capacity (g/g)	2.46±0.08^{c}	2.54±0.086^{bc}	2.79±0.08^{b}	3.34±0.041^{a}	1.83±0.086^{d}
Fat absorption capacity (g/g)	3.78±0.102^{c}	3.79±0.115^{c}	4.21±0.085^{b}	5.07±0.067^{a}	3.05±0.075^{d}
Foaming capacity (%)	33.33±2.11^{b}	38.33±3.07^{b}	93.33±2.10^{a}	90±3.65^{a}	5±2.24^{c}
Foaming stability (%)	15±2.236^{c}	18.33±3.07^{c}	50±3.65^{a}	31.67±3.07^{b}	1.67±1.05^{d}
Emulsifying activity index (m^2/g)	1.39±0.024^{d}	1.51±0.616^{c}	1.82± 0.034^{a}	1.72±0.035^{b}	0.68±0.002^{e}
Emulsifying stability index (min)	43.18±1.41^{c}	51.50±2.93^{b}	60.34±3.08^{a}	47.86±1.21^{bc}	24.12±0.91^{d}
Water Activity	0.33±0.001^{c}	0.26±0.001^{e}	0.40±0.002^{b}	0.30±0.002^{d}	0.49±0.002^{a}

Mean ± S.E

variation among acid and alkaline conditions. The total pigment content in both the isolates was significantly lower than that of raw liver powder and lowest value was recorded in alkaline-aided isolates at pH 11.5. The highest pigment content was recorded in protein isolates at pH 2. These findings were also supported by findings of instrumental colour profile wherein more whiteness was observed in alkaline isolates. Xiong *et al.* (2016) also reported significantly higher total haem concentration in acid treated isolate as compared to alkaline treatment of chicken liver. The lower total pigment content in the alkaline treatment was closely related to the lower total haem level as well as the myoglobin concentration. It could be because alkaline treatment removes more haem proteins than acidic.

Water & fat absorption capacity (WAC & FAC): In the current study, the water & fat absorption capacity of alkaline-treated isolates were recorded significantly higher than that of acid-treated isolates and both isolates had significantly higher WAC than raw liver powder. Also, the FAC is found to be higher compared to the WAC which may be due to the exposure of more hydrophobic amino groups as compared to hydrophilic ones. The existence of polar amino acids, which can attract water molecules, is what gives proteins their capacity to bind water. It has been also reported that the absorption of water in food matrix depends upon the amount of protein in a food. Therefore, foods having higher protein content will have high WAC. Zou *et al.* (2017) reported that the FAC value liver protein isolate was 1.9 g/g and the ultrasound treatment markedly boosted the readings. The ability of cape hake fish proteins to absorb fat was reported to be 4.67g/g. It was shown that cat fish hydrolysates had an oil absorption capacity of 4.08 g/g of proteins. The use of a high pH (11.0) for isolating protein may be connected to this high oil absorption ability.

Foaming capacity and foaming stability (FC and FS): The results of the current study showed that the foaming capacity and foaming ability were improved and were significantly higher for protein isolates of alkaline condition compared to that of acidic condition and also both isolates had significantly higher ($p<0.05$) FC and FS values than the raw liver powder. Proteins isolate under acidic pH conditions had significantly lower foaming capacity (FC) and foam stability (FS) than proteins recovered under alkali conditions, which demonstrated a pH-dependent behavior.

According to findings of Pezeshk *et al.* (2021) the FC of trout proteins considerably increased following ultrasonic therapy at the various alkaline pH values in comparison to the control and postulated that the overall improvement in FC of the recovered proteins shown after ultrasonic treatment may be due to the partial denaturation of proteins at the air-liquid interface and the unwinding of protein molecular structure. According to Lone *et al.* (2015), the isolated fish proteins from rainbow trout did not exhibit any foaming ability or foaming stability at pH 3 or 4 but were 13.2% and 90%, respectively, at pH 7. The lower solubility and higher hydrophobicity of rainbow trout isolates may account for its lower FC and FS values. In barley protein, the foaming capacity was least at isoelectric pH and tends to improve towards both extremes of pH conditions (Yalcin *et al.* 2008).

Emulsifying activity index (EAI) and Emulsifying stability index (ESI): It was observed that the emulsifying activity index of alkaline isolate was significantly higher than the acidic counterparts. The emulsion capacity of isolates was significantly higher ($p<0.05$) than raw liver powder. The highest EAI was found at pH 11 and the lowest was found at pH 1.5. The emulsion stability index (in min) was also following the same trend as emulsifying capacity. According to Xue *et al.* (2019), the EAI and ESI of proteins treated at pH 11.0 were noticeably greater than those of control, whereas for pH 11.5 solubilized proteins, they did not differ appreciably for goose liver proteins. The results were in accordance with the findings of Xiong *et al.* (2016) in which the alkali-recovered chicken liver proteins had much stronger emulsifying activity and stability than their acid-recovered counterparts, with the pH 11.0 treatment having the highest emulsifying capacity. Additionally, increased reactive sulfide groups, in proteins encourage the creation of disulfide bonds, which aids in the development of a more stable and dense interfacial membrane. Therefore, the increased reactive sulfide contents of goose liver proteins caused by isoelectric solubilisation/precipitation (ISP) could be partially responsible for the higher EAI and ESI values (Xue *et al.* 2019). The decreased emulsifying stability of acid treatments would, however, be explained by the over-denaturation of proteins.

The emulsifying capacity of isolates varies greatly and is dependent on the raw materials used, such as albumin (72.92%), skipjack egg protein concentrate (81.65%), casein (90.73%), and catfish protein isolates, which had an emulsifying capacity of 152.11% (1.52 g/mL) (Haryati *et al.* 2020).

Water activity (a_w): The current study shows that the water activity of raw liver powder was significantly higher than all other isolates. Among isolates, the highest water activity was found for pH 11 and the lowest was found for pH 2. Every pH had significantly ($p<0.05$) different values. The freeze-dried pea protein isolates having moisture content of 2.86% had water activity 0.179 (Tontul *et al.* 2018).

Antioxidant properties of isolates: The protein isolates from chicken liver were also subjected to antioxidant assay using ABTS and DPPH assay (% inhibition) and were compared with chicken liver powder. In ABTS assay, the % inhibition was 92.48±0.12 and 87.33±0.43 for protein isolates at pH 1.5 and 11.0, respectively, and these values were significantly higher than the liver powder (28.6±0.28%). In the DPPH assay also similar trends were observed, however, the % inhibition values were very low as compared to the ABTS assay (Table 3). Unlike all other techno-functional properties, significant antioxidant activity was shown by acidic isolates compared to alkaline isolates.

The protein isolation process by the pH-shift method

Table 3. Antioxidants properties of protein isolates at different pH and chicken liver powder (Mean±SE)

	1.5	11	Raw liver
ABTS	92.48±0.12^{A}	87.33±0.43^{B}	28.6±0.28^{C}
DPPH	12.39±0.93^{A}	5.91±0.45^{B}	1.32±0.14^{C}

Mean ± S.E,^{A,B} mean values within column with different superscripts are significantly different ($p<0.05$)

exposes the native protein to extreme pH which may cause break down of some of the proteins and the resultant products may have some charged molecules to scavenge the free radicals during the *invitro* assay. The chicken liver protein isolates also exhibited significantly higher antioxidant activity in ABTS and DPPH assay as compared to the whole liver powder. These results were in concurrence with the results of Zhao *et al.* (2021) and Kumar *et al.* (2016), in which they reported the antioxidant properties of milk protein isolates and hydrolysates. Similarly, soy protein isolates incorporated in packaging films exhibited antioxidant properties in ABTS and DPPH assay (Adilah *et al.* 2018).

Chicken liver is the most valuable source of protein amongst poultry by-products and hence isolation of proteins can be taken into consideration as a cheap and effective method for sustainable food industry alteration. Among acidic and alkali solubilization, alkaline method was found to be more economic while considering the recovery rate and protein content. Techno functional properties also proved the significance of isolation. Except for bulk density, and total pigments all other properties have shown higher values for isolates, compared to raw liver. Hence, the production of isolates from chicken liver is recommended which can provide a concentrated protein source that can act as a food ingredient from a low-cost byproduct.

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