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Recovery of Bioactive Peptide Fractions from Rainbow Trout (*Oncorhynchus mykiss*) Processing Waste Hydrolysate

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ABSTRACT

Aims Bioactive peptides with antioxidant properties derived from fish by-products and wastes by enzymatic hydrolysis have become a topic of great interest for pharmaceutical, health food, and processing/preservation industries.

Materials & Methods This study aimed to characterize peptide fractions with antioxidative activity recovered from rainbow trout gelatin hydrolysate. Four peptide fractions (<3 kDa, 3–10 kDa, 10–30 kDa, and >30 kDa) were obtained from gelatin hydrolysate by subjecting them to centrifugal ultrafiltration using successively a 30 kDa, 10 kDa, and a 3 kDa membrane. The 10–30 kDa fraction was characterized in terms of amino acid composition. The antioxidant activity of all fractions was monitored by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) radical scavenging as well as reducing power activity.

Findings The free amino acids in 10–30 kDa fraction were dominated by Gly, Pro, Ala, and Hyp; the total hydrophobic amino acid of 10–30 kDa fraction was also 61.5%. All hydrolysate and peptidic fractions demonstrated high antioxidant activities. Moreover, 10–30 kDa fraction exhibited the highest DPPH and ABTS radical scavenging activity (2, 5, and 10 mg ml^{-1}) and reducing power (10 mg ml^{-1}) compared to other fractions ($p<0.05$).

Conclusion These results revealed the potential of peptide fractions recovered from rainbow trout skin gelatin as source of natural antioxidants for use in food products.

Keywords Bioactivity; Fish Waste; Peptidic Fractions; Rainbow Trout; Recovery

CITATION LINKS

[1] Marine bioactives as functional food ingredients: Potential to reduce the incidence ... [2] Consumer acceptance of ... [3] Functional food, Product development, marketing ... [4] Biochemical properties of fish protein ... [5] Influence of the extent of enzymatic ... [6] Thermal characterisation of gelatin ... [7] Free radical scavenging and angiotensin-I converting ... [8] Active peptides from skate (Okamejei kenojei) ... [9] National aquaculture sector ... [10] Optimization of physico-chemical properties of gelatin ... [11] Gelatin hydrolysate from blacktip shark skin prepared using ... [12] Biopolymer-coated nanoliposomes as ... [13] Functional foods development: Trends ... [14] Evaluation of the in vitro antioxidant properties of ... [15] Antioxidant properties of fractions isolated from blue shark ... [16] Purification and identification of antioxidant peptides from ... [17] Characterization and potential use of cuttlefish skin gelatin ... [18] Antioxidant properties of a radical-scavenging ... [19] Characteristic and antioxidant activity ... [20] Official methods of ... [21] Antioxidant activity of several marine skin ... [22] Production and characterization of encapsulated antioxidative protein hydrolysates from Whitemouth croaker ... [23] Antioxidant activities and functional properties of protein ... [24] Extraction and electrospinning of gelatin ... [25] Characterisation of acid soluble collagen from ... [26] Nanoencapsulation of an active peptidic ... [27] Antioxidant activities of enzymatic rapeseed ... [28] Extracting conditions for megrim (Lepidorhombus boscii) skin collagen affect functional properties ... [29] Antioxidant and free radical-scavenging activities of smooth hound ... [30] Effects of enzymatic hydrolysis on molecular structure ... [31] The role of molecular size in antioxidant activity of peptide fractions from ... [32] Effect of degree of hydrolysis on the antioxidant ... [33] Antioxidant and antimicrobial peptide fractions from squid and tuna ... [34] Purification of a radical scavenging peptide from fermented mussel sauce ... [35] Preparation and evaluation of antioxidant peptides ... [36] Functional and antioxidant properties of hydrolysates ... [37] Antioxidant activity of cod (Gadus morhua) protein hydrolysates: Fractionation and characterisation ...

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Introduction

There is an increasing knowledge that dietary origin and form of food could influence overall health.[1] Hence, a new class of food, known as functional foods, has been initiated for improving human health and/or disease risk reduction.[2,3] Due to physiological functions, with medicinal features and health profits, marine-originated bioactive constituents have good potential as functional/nutraceutical food elements.[1]

The significance of fish as a valuable source of protein and novel bioactive compounds is growing rapidly as compared to other protein sources.[4]

One of the issues that have the greatest influence on the environment is the management of fish rest raw materials (RRMs) from seafood processing plants. Recycling of marketable materials from fish wastes is a substantial waste reduction approach for the industry. Large amounts of protein-rich RRMs from the seafood industry are rejected without any effort to recovery.[5] Fish processing discard after filleting is estimated approximately 75% of the total weight, in which 30% of the waste is in the form of bones and skins, which lead to environmental pollution.[6] In the past years, the use of fish RRMs as a safe material has been of growing attention.[5]

It is described that fish skin provides the best source of gelatin due to its high availability, reducing pollution, and no religious barriers.[7] Therefore, there has been an increased need to investigate an efficient means to utilize this by-product.[8]

In this context, rainbow trout fish (*Oncorhynchus mykiss*) from the Salmonidae family are one of the valuable farmed cold water species in Iran, which its production reached 126,515 tons in 2014.[9] Rainbow trout are usually processed into skinless fillets (mainly for gefilte fish), so there is a plentiful quantity of raw skins accessible.[10] Here, a study on the properties of rainbow trout skin gelatin could be helpful in rationalizing the use of fish residuals.[10]

Hydrolyzed gelatin has been known as the potential source of bioactive peptides with high antioxidative activity.[11] In general, biologically active peptides consist of 2–20 amino acid units,[12] molecular masses <6000 Da and have been recognized to have nutraceutical potentials. Marine bioactive peptides have been shown to display a wide range of biological functions including antihypertensive, immunomodulatory, mineral binding, hypocholesterolemic, antimicrobial, and antioxidant effects.[13] The antioxidant activity of peptides can be attributed to their specific ability to scavenge free radicals formed during peroxidation, in addition to scavenging of oxygen containing compounds, and metal chelation.[14] Synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and tert-butylhydroquinone have strong antioxidant activities, but there is an increasing worry about their potential risks to health.[15] Hence, the development of natural antioxidants, which have synergistic effects of amino acids, peptides, and proteins, has attracted considerable attention.[16]

Gelatin-derived bioactive peptides with antioxidant characteristics have become a topic of great interest for health food and processing industries.[17] Production of fish protein hydrolysate with antioxidant activities will allow increased value-added utilization of seafood and processing byproducts.[14] Fish protein hydrolysates such as skin gelatin hydrolysates from hoki (*Johnius belengerii*),[18] cobia (*Rachycentron canadum*),[19] Pacific cod (*Gadus macrocephalus*),[7] and cuttlefish (*Sepia officinalis*)[17] have been also reported to exhibit antioxidant activity.

The aims of the present study were (a) to isolate different peptide fractions from rainbow trout (*O. mykiss*) gelatin by ultrafiltration and (b) to evaluate the isolated fractions for antioxidant activities including 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) radical scavenging assays as well as reducing power.

Materials & Methods

Chemicals

Alcalase enzyme, DPPH, and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bovine serum albumin, Folin-Ciocalteu reagent (1.9-2.1 N), trichloroacetic acid (TCA), potassium persulfate, and ferric chloride were provided by Merck Chemicals Co. (Darmstadt, Germany). Sodium hydroxide and ABTS were supplied from Applichem (Darmstadt, Germany) and Biobasic Inc., (Ontario, Canada), respectively. Furthermore, potassium ferricyanide was obtained from Rankem (Haryana, India).

Collection and preparation of rainbow trout skin

Rainbow trout (*O. mykiss*) with average weight of 880.66 ± 40.55 g and average length of 37.40 ± 1.84 cm were provided by a local aquaculture farm (Amol, Mazandaran province). Fish were placed in polyethylene bag, stored in ice and rapidly transported to seafood processing laboratory within 1 h of harvesting. They were gutted, filleted with skin; the obtained skin was packaged in sealed polyethylene bag, frozen promptly, and stored at 20°C before using for gelatin extraction and further analysis.

Determination of proximate composition

The moisture, protein, ash, and fat content of skin were ascertained in accordance with the AOAC[20] official method with the analytical Nos. of 950.46, 928.08, 920.153, and 960.39, respectively. A conversion factor of 5.4 was used in calculating the protein content from the Kjeldahl nitrogen content.

Fish gelatin extraction

Cleaned rainbow trout skins were subjected to gelatin extraction following the method of Tabarestani *et al.*[10] with some modifications. Frozen skin was thawed overnight at 4°C, and then, the adhering tissues were removed. Thawed skin was cut into small pieces (1 cm × 1 cm) and then washed in cold water (~4°C). To remove non-collagenous proteins, the small pieces of skin were soaked in 0.19 N of NaOH with a skin/solution ratio of 1:7 (w/v) for 3 h, and the solution was changed every 1 h, then washed with cold distilled water. Afterward, the washed skins were then soaked in cold 0.21 N of acetic acid with a skin/solution ratio of 1:7 (w/v) and then washed by cold distilled water until neutral or slightly basic pH (pH 7–7.5) of wash water was obtained. The pretreated skins were mixed with deionized water at a ratio of 1:2 (w/v). The gelatin extraction was performed by incubating the mixture in a water bath (WNB 14, Memmert, Germany) at 50°C for 16 ± 2 h. The resulting gelatin solution was then filtered using two layers of cheesecloth. The filtrate was further filtered using a Whatman No. 41 filter paper, followed by drying the solution in freeze drying equipment (FDU-7012, Operon, South Korea).

Preparation of gelatin hydrolysates

Gelatin hydrolysis (GH) was performed according to the method described by Alemán *et al.*[21] with some modifications. The gelatin powder was dissolved in distilled water (5% w/w) and subjected to enzymatic hydrolysis using alcalase with an enzyme/substrate ratio 1:100 (w: w) in optimal conditions for enzymatic activity (pH 8, 50°C) for 4 h. During the reaction, the pH of the mixture was maintained constant by continuous addition of 1 N NaOH solution to the reaction medium. After hydrolysis, the mixture was heated in a water bath at 90 C for 10 min to inactivate the enzyme and then centrifuged at 7000× *g* for 20 min at 4°C (Universal 320 R, hettichlab, Germany). The supernatants were collected and stored at −80°C until use.

The degree of hydrolysis (DH), defined as the percent ratio of peptide bonds, broken number to the total number of peptide bonds in the protein substrate, was analyzed using TCA method. Briefly, an aliquot of the hydrolysis solution was mixed with 10% TCA, then centrifuged at 6700× *g* for 5 min at 4°C. The supernatant was used to determine the concentration of soluble proteins by Lowry method with bovine serum albumin (BSA) as the standard (0–1000 µg/ml). DH is calculated as the ratio between the amount of total protein present in the substrate and the amount of soluble proteins.[22] The resulting DH was 46.5 ± 0.34%.

Recovery of peptide fractions from hydrolysate

A portion of hydrolysate was fractionated further by centrifugal ultrafiltration (Millipore, Billerica, MA, USA) with Millipore membranes having molecular mass cutoffs of 30, 10, and 3 kDa. The fractions were collected as follows: >30 kDa (PF>30), peptides retained without passing through 30 kDa MWCO membrane; 10–30 kDa (PF30), peptides permeating through the 30 kDa MWCO membrane but not the 10 kDa MWCO membrane; 3–10 kDa (PF10), peptides permeating through the 10 kDa MWCO membrane but not the 3 kDa MWCO membrane; and <3 kDa (PF3), peptides permeating through the 3 kDa MWCO membrane. All fractions recovered were stored at −80°C until required for further use.

Amino acid composition analysis

About 100 mg of freeze-dried sample was dissolved in 7.5 ml of 6 mol L^{-1} HCl, and the mixture was hydrolyzed in vacuum-sealed glass tubes at 110°C for 24 h. After hydrolysis, the sample was again vacuum-dried and filtered through a 0.22-mm Minisart syringe filter (Sartorius AG, Gottingen, Germany) before analysis. Amino acids were separated on a reverse phase column sphere-Image 80-5 ODS 2 (25 cm, Knauer, Berlin, Germany) using methanol-acetate buffer as the mobile phase. Ultrapure water (Milli-Q, Millipore) and HPLC-grade methanol (Merck, Darmstadt, Germany) were used for elution and a mixture of amino acids was used as the standards (Sigma-Aldrich, Inc., St. Louis, Mo., USA). The amino acid content was determined from standard curves based on peak area measurements, using EZChrom Elite software and expressed as a percentage of total amino acids in the sample.

Antioxidant activities

DPPH radical scavenging activity assay

The scavenging effects of the peptidic fractions on the DPPH free radical were measured as previously described in Taheri *et al.*[23] with some modifications. Briefly, a volume of 2 ml of the sample or ascorbic acid (400 µg ml^{-1}) as positive control was added to 2 ml of 0.16 mM DPPH in 95% ethanol. The mixture was then kept at room temperature in the dark for 30 min, and the reduction of DPPH radical was measured at 517 nm. The blank was prepared in the same manner, except that 95% ethanol was used instead of the sample. The scavenging effect of DPPH radical was expressed as follows:

Scavenging activity (%)=

$$\frac{\text{Blank absorbance-sample absorbance}}{\text{Blank absorbance}} \times 100 \quad (1)$$

ABTS radical scavenging activity

ABTS radical scavenging activity was determined according to a modified version of the method of Alemán *et al.*[21] The ABTS radical was produced by reacting ABTS stock solution (7 mM) with potassium persulfate (2.45 mM) and allowing the mixture to react in dark at room temperature for 16 h before use. The ABTS solution was diluted with distilled water to obtain an absorbance of 0.7 ± 0.02 at 734 nm. A 20 µl aliquot sample or ascorbic acid (400 µg ml^{-1}) as a positive control was mixed with 980 µl of ABTS reagent and then left to stand in the dark at 30 ºC for 10 min. The absorbance values were read at 734 nm. The ABTS scavenging activity of samples was calculated from following Eq. (2):

ABTS scavenging activity (%) = $[(A_{blank} - A_{sample})/A_{blank}] \times 100$ (2)

Where, A_{blank} is the absorbance without sample at 734 nm, and A_{sample} is the absorbance of the test solution.

Reducing power assay

The reducing power of peptide fractions was determined according to the method of Taheri *et al.*[23]. 1 mL of each sample or ascorbic acid (400 µg ml^{-1}) as positive control was added to 2.5 mL of 0.1 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. Then, samples were incubated (50 C, 20 min). After that, 2.5 mL of 10% TCA was added and they were centrifuged at 1650× g for 10 min. A 2.5 mL aliquot of the supernatant was mixed with 2.5 mL distilled water and 0.5 ml of 0.1%

(w/v) ferric chloride. The absorbance of the resulting solution was measured at 700 nm after a 30 min reaction. Higher absorbance of the reaction mixture indicated higher reducing power.

Statistical analysis

Statistical analyses were performed with SPSS statistic program (SPSS 16.0 for window, SPSS Inc., Chicago, IL, USA). All data were subjected to analysis of variance and differences between means were evaluated by Duncan's test at 5% significance level. All results are given as mean values of triplicates with indication of standard deviation unless otherwise stated.

Findings and Discussions

Proximate composition of the skin

The proximate composition of rainbow trout skin is as summarized in Table 1. In general, skin of rainbow trout had high moisture (64.3%) and protein (31.6%) contents with low ash (2.7%) and fat (1.3%) contents. Similar results were observed for Nile tilapia (Oreochromis niloticus(skin (67.7% moisture, 30.6% protein, 2.1% ash, and 1.1% fat),[24] but different to the result obtained for the Nile perch skin (68.4% moisture, 21.6% protein, 6% ash, and 6.8% fat).[25] It is a known fact that the chemical composition of skin varies with age and sex of the animal as well as the treatment of the skin on the removal from the carcass.[24]

Table 1: Proximate compositions of rainbow trout skin

Composition	Content (% wet weight)
Moisture	64.35 ± 0.8[a]
Protein	31.66 ± 1.27[b]
Ash	2.71 ± 0.53[c]
Fat	1.37 ± 0.2[c]

Different superscript letters in column indicate statistically significant difference ($P < 0.05$). Values are given as mean ± SD from triplicate determinations, SD: Standard deviation

Table 2: Amino acid composition of PF30 peptide fraction

Amino acid	Residues/100 residues
Aspartic acid (Asp)	3.49
Glutamic acid (Glu)	7.4
Serine (Ser)	5.47
Histidine	1.91
Glycine (Gly)	31.04
Threonine (Thr)	2.58
Arginine (Arg)	5.24
Alanine (Ala)	9.43
Proline (Pro)	10.29
Tyrosine (Tyr)	0.11
Methionine (Met)	2.13
Valine (Val)	2.87
Phenylalanine (Phe)	1.73
Isoleucine (Ile)	1.66
Leucine (Leu)	2.4
Lysine (Lys)	1.55
Hydroxyproline (Hyp)	7.35
Amino acids (Pro + Hyp)	17.64
∑ Hydrophobic[a]	61.55

[a]Gly, Ala, Pro, Met, Val, Phe, Ile, Leu

Amino acid composition analysis

The amino acid profile of the PF30 is shown in Table 2. The results showed that the most abundant amino acids are Gly (31%), Pro (10.2%), and Ala (9.4%), followed by Glu and Hyp. High concentration of Gly in gelatin hydrolysate and peptide fractions has been reported in previous studies.[17] As described in Table 1, the total hydrophobic amino acids PF30 contribute up to 61.5% of total amino acids. Jridi *et al.*[17] have reported similar hydrophobic amino acid level for cuttlefish (S. officinalis) skin gelatin hydrolysate (58.5%). According to Mosquera *et al.*,[26] the antioxidant activity of protein hydrolysates has been positively linked to the high content of hydrophobic amino acids. Furthermore, Mendis *et al.*[18] have suggested that the presence of hydrophobic amino acids in the peptide sequences in jumbo squid skin gelatin contributed greatly to its antioxidant properties. Hydrophobic amino acids have been observed in several antioxidant peptide sequences.[21] In addition, it is thought that acidic amino acids such as Glu and Asp play an important role in the antioxidant ability owing to the presence of excess electrons that can be donated during interactions with

free radicals; also the presence of Met, His, Lys, and Tyr has been shown to contribute greatly to the potency of antioxidant peptides.[27] The total level of amino acid residues (Pro and Hyp) was estimated at 17.6%. The stability of collagen and gelatin has been described to be proportional to the total content of pyrrolidine amino acids, given that it is the Pro + Hyp rich zones of the molecules that are most likely included in the formation of junction zones stabilized by hydrogen bonding.[28]

Antioxidant activities

DPPH radical scavenging activity assay

DPPH is a stable radical that gives a strong absorption band at 517 nm. When DPPH radicals meet a proton-donating substrate like antioxidant, the radicals would be scavenged and the absorbance is decreased.[29] Figure 1 shows the DPPH radical scavenging activity of the gelatin hydrolysate (GH) and peptidic fractions at various concentrations. The results revealed that all hydrolysates were able to scavenge DPPH radicals and the GH at lower concentrations (i.e. 1 and 2 mg ml^{-1}) had a lower scavenging activity than peptidic fractions which was similar to the result of Girgih *et al.*[14] who studied the antioxidant activity of cod protein hydrolysate and peptide fractions. The DPPH scavenging effects (%) of ascorbic acid, which was used as positive control was about 69.6% at a concentration of 400 µg/ml, which was close to DPPH scavenging activity of 10 mg $m^{l-}1$ of PF30. Furthermore, there was a positive correlation between the concentration (1–10 mg ml^{-1}) and DPPH radical scavenging activity (7.44–67.77%). The result showed that PF30 exhibited highest DPPH radical scavenging activity in comparison to other samples at all concentrations ($P < 0.05$) except for 1 mg ml^{-1} concentration. Furthermore, the highest DPPH radical scavenging activity was obtained for 10 mg ml^{-1} PF30 which was able to scavenge almost 67.7% of DPPH free radicals. Bamdad *et al.*[30] demonstrated that a critical peptide size is required to display the excellent scavenging capability and medium-sized peptides may play an important role in antioxidant activity. Furthermore, according

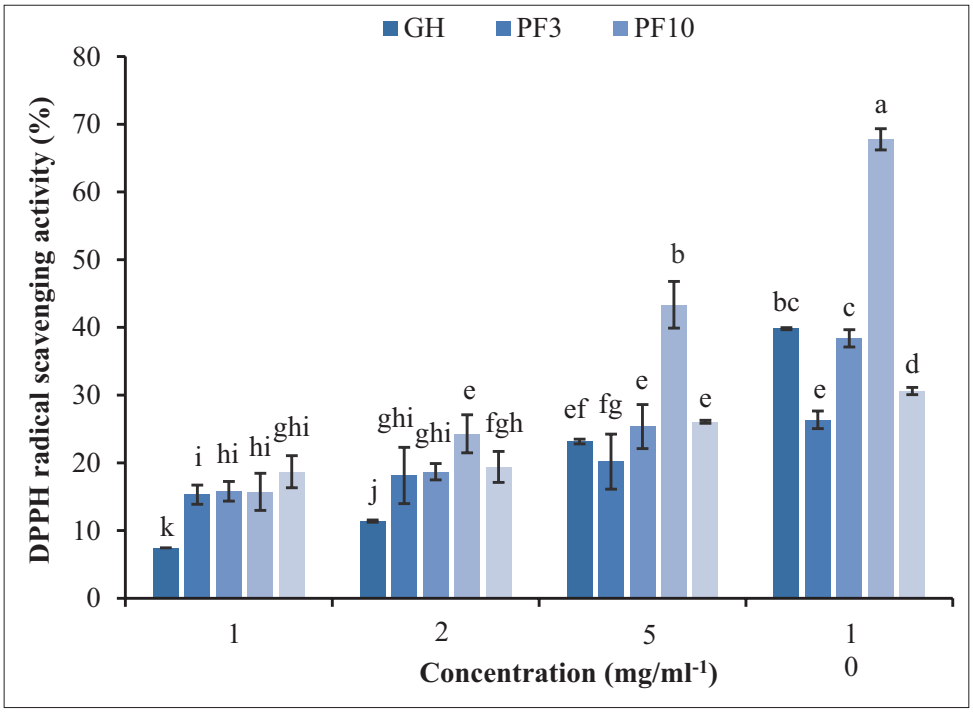

Figure 1: DPPH radical scavenging capacity of rainbow trout gelatin hydrolysate and its membrane ultrafiltration peptide fractions. Bars (mean ± standard deviation, $n = 3$) with different letters have mean values that are significantly different at $P < 0.05$

to Alemán *et al.*,[21] radical scavenging capacity could not be well correlated with the molecular weight distribution of the whole hydrolysate. Cheung *et al.*[31] suggested that some factor such as substrate, protease type, hydrolysis conditions, peptide composition, molecular size of the peptides, and sequence might also influence the radical scavenging activity. A literature review demonstrated that the antioxidant activity of protein hydrolysates was related to the DH since DH greatly influenced the molecular weight and amino acid residue composition of protein hydrolysate, and further influenced the antioxidant activity;[32] there was a tendency for increasing antioxidant activity with increasing DH. The protein DH in the present study was relatively high (46.5%), which is similar to the results described by Gómez-Guillén *et al.*[33] for the hydrolysates of tuna skin gelatin (47.52%) and remarkably higher than the DH values for marine fish skin gelatins documented in the literature[21]. Furthermore, The high level of DPPH free radical scavenging activity of protein hydrolysates is associated with a high amount of hydrophobic amino acids or peptide hydrophobicity (61.5% in this study).[34] The results of the present study suggest that PF30 probably contained more peptides than the other peptidic fractions, which are electron donors that could react with free radicals to convert them to more stable products and terminate the radical chain reaction.[17]

ABTS radical scavenging activity

Oxidized ABTS generates a colored cation radical, which shows strong absorbance at 734 nm.[35] Figure 2 showed all hydrolysate exhibited ABTS free radical scavenging activities to different extents in a concentration-dependent manner. According to results, with increasing concentrations, the scavenging effects of hydrolysate on ABTS radical increased until about 74.7% in 10 mg/ml in PF30. The ABTS radical scavenging effects (%) of 400 μg/ml ascorbic acid were about 79.2%, which was comparable to ABTS scavenging activity of 10 mg/ml of PF30. Furthermore, the scavenging activity of PF30 was higher than that of other hydrolysate and its four fractions at in concentration of 2, 5, and 10 mg ml^{-1}. All peptidic fractions showed higher ABTS radical scavenging ability than their parent hydrolysates (GH) at the test concentrations, demonstrated that the fractionation of GH resulted in fractions with better antioxidant activity than their parent hydrolysates. The abilities of all hydrolysate to quench the ABTS radical reflected the capacities of them to donate electrons or hydrogen atoms to inactivate this radical species.[35] It seems the plenty of hydrophobic amino acids in gelatin peptides sequences be responsible for the higher antioxidant impacts, owing to an increase of their solubility in lipids.[26]

Reducing power assay

Reducing power is related to the ability of compound to donate an electron to free radicals making them into more stable compounds. By this electron donation, an oxidized antioxidant molecule can be regenerated.[36] Depending on the reducing power of each hydrolysate, the yellow color of the solution shifts to different shades of green and blue. There is a direct correlation between antioxidant activities and reducing power of certain bioactive constituents, as reported in various studies.[29]

As shown in Figure 3, the reducing power was found to be very low for all the fractions and exhibited a concentration dependency. Reducing power of all hydrolysates increased with increasing concentrations (1–10 mg/ml), which was corresponded to result of Jridi *et al.*[17] Furthermore, the higher activity was found in followed by PF30 when the concentration was increased to 10 mg/ml (absorbance of 0.19 at 700 nm). Furthermore, none of the fractions were as efficient as ascorbic acid as a positive control, which showed an absorbance of 1.34 when assayed at a concentration of 400 μg/ml. The increase in reducing power for PF30 may be ascribed to the exposure of electron-dense amino acid side chain groups such as polar or charged moieties.[30] Similar results were observed by Bamdad *et al.*[30] who stated that the peptide fractions, with

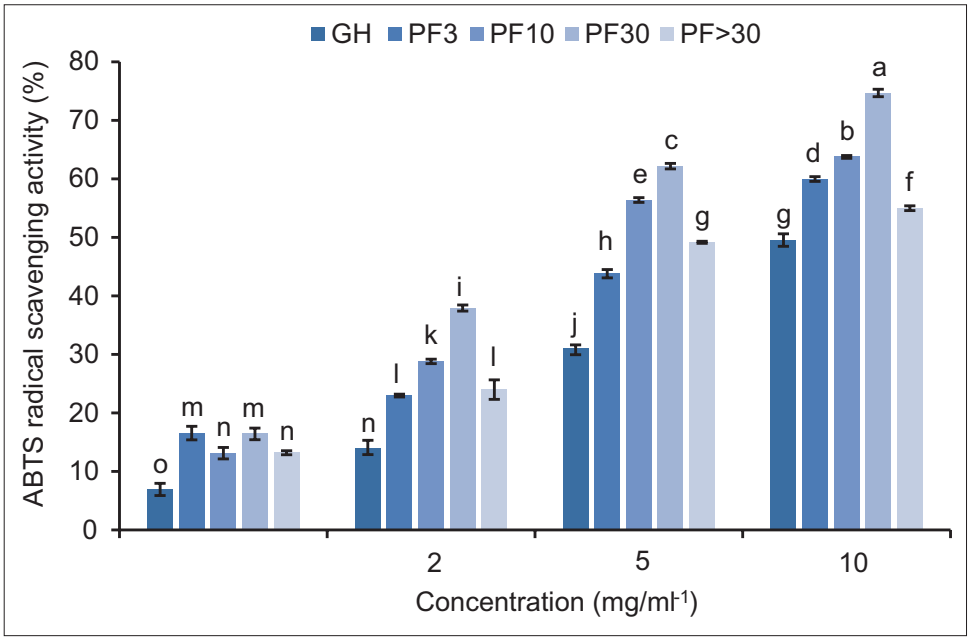

Figure 2: 2,2-azino bis(3-ethylbenzthiazoline)-6-sulfonic acid radical scavenging capacity of rainbow trout gelatin hydrolysate and its membrane ultrafiltration peptide fractions. Bars (mean ± standard deviation, n = 3) with different letters have mean values that are significantly different at $P < 0.05$

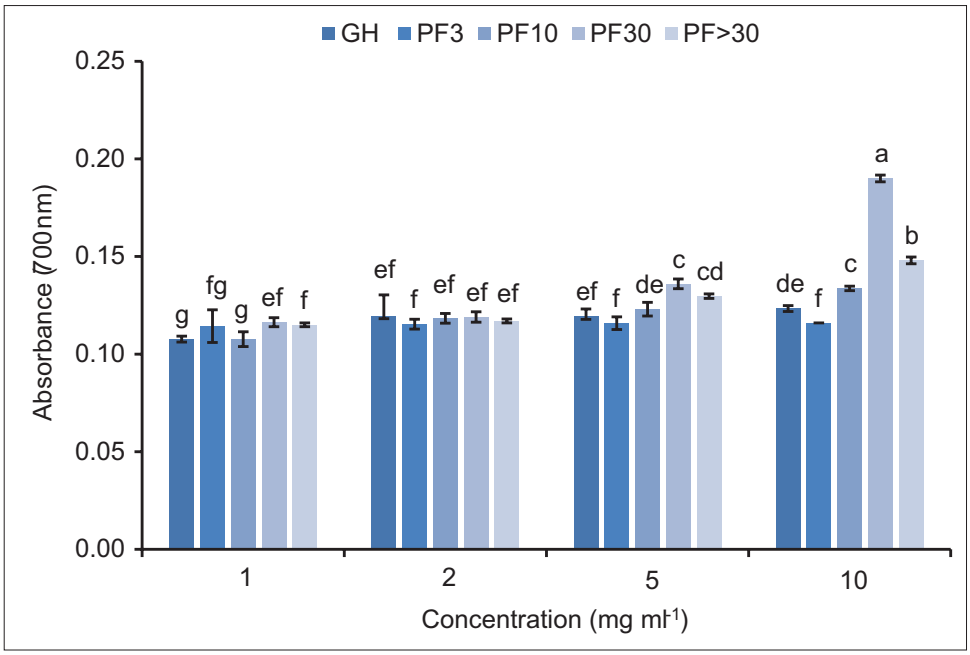

Figure 3: Reducing power assay of rainbow trout gelatin hydrolysate and its membrane ultrafiltration peptide fractions. Bars (mean ± standard deviation, $n = 3$) with different letters have mean values that are significantly different at $P < 0.05$

the relatively higher molecular weight peptides of the medium-sized range group displaying greater activity. A high correlation between certain amino acid residues and the antioxidant capacity of peptides has been observed in some research.[37] The difference of the activities that samples presented may be related to the amino acid composition and

the peptides size.[36]

Conclusion

This study determined the antioxidant activity of fractionated (<3 kDa, 3–10 kDa, 10-30 kDa, and >30 kDa) rainbow trout gelatin hydrolysate, which was prepared enzymatically using alcalase. All GH and its ultrafiltration fractions were found to possess effective *in vitro* antioxidant properties. The fractionated GH had better antioxidant activity than their parent hydrolysates and may provide a better alternative as an ingredient to formulate antioxidant foods when compared to the unfractionated GH. PF30 was generally the most effective scavengers of DPPH and ABTS free radicals but had weaker reducing power when compared to other peptide fractions. The above results show that fish skin, an industrial by-product, can be exploited by enzymatic hydrolysis producing hydrolysates with antioxidant activity. Such hydrolysates may be used in functional foods to alleviate high blood pressure, as well as to increase the products shelf life. However, in vivo availability, potency, and safety must be determined before the products can be used for therapeutic purposes. Further, works should be done to isolate and identify the specific peptides in rainbow trout gelatin hydrolysates that are responsible for the overall antioxidative capability.

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References

1. Lordan S, Ross RP, Stanton C. Marine bioactives as functional food ingredients: Potential to reduce the incidence of chronic diseases. Mar Drugs. 2011;9(6):1056-100.
2. Honkanen P. Consumer acceptance of (marine) functional food. In: Luten JB, editor. Marine functional food. wageningen: Wageningen Academic Pub; 2009. pp. 141-54.
3. Siro I, Kápolna E, Kápolna B, Lugasi A. Functional food, Product development, marketing and consumer acceptance--A review. Appetite. 2008;51(3):456-67.
4. Jafarpour SA, Shabanpour B, Shirvani Filabadi S. Biochemical properties of fish protein isolate (FPI) from Silver carp (Hypophthalmichthys molitrix) by application of acid-alkali processes compared to traditional prepared surimi. ECOPERSIA. 2013;1(3):315-27.
5. Wasswa J, Tang J, Gu XH, Yuan XQ. Influence of the extent of enzymatic hydrolysis on the functional properties of protein hydrolysate from grass carp (Ctenopharyngodon idella) skin. Food Chem. 2007;104(4):1698-704.
6. Rahman MSh, Al-Saidi GhS, Guizani N. Thermal characterisation of gelatin extracted from yellowfin tuna skin and commercial mammalian gelatin. Food Chem. 2008;108(2):472-81.
7. Ngo DH, Ryu B, Vo TS, Himaya SW, Wijesekara I, Kim SK. Free radical scavenging and angiotensin-I converting enzyme inhibitory peptides from Pacific cod (Gadus macrocephalus) skin gelatin. Int J Biol Macromol. 2011;49(5):1110-6.
8. Ngo DH, Ryu B, Kim SK. Active peptides from skate (Okamejei kenojei) skin gelatin diminish angiotensin-I converting enzyme activity and intracellular free radical-mediated oxidation. Food chem. 2014;143:246-55.
9. Food and Agriculture Organization of the United Nations. National aquaculture sector overview, Iran (Islamic Republic of) [Internet]. Rome: Food and Agriculture Organization of the United Nations; 2005 [Cited 2017; Mar]. Available from: http://www.fao.org/fishery/countrysector/naso_iran/en.
10. Tabarestani HS, Maghsoudlou Y, Motamedzadegan A, Mahoonak AS. Optimization of physico-chemical properties of gelatin extracted from fish skin of rainbow trout (Onchorhynchus mykiss). Bioresour Technol. 2010;101(15):6207-14.
11. Kittiphattanabawon P, Benjakul S, Visessanguan W, Shahidi F. Gelatin hydrolysate from blacktip shark skin prepared using papaya latex enzyme: Antioxidant activity and its potential in model systems. Food Chem. 2012;135(3):1118-26.
12. Ramezanzade L, Hosseini SF. Nikkhah M. Biopolymer-coated nanoliposomes as carriers of rainbow trout skin-derived antioxidant peptides. Food Chem. 2017;234:220-9.

13. Betoret E, Betoret N, Vidal D, Fito P. Functional foods development: Trends and technologies. Trends Food Sci Technol. 2011;22(9):498-508.
14. Girgih AT, He R, Hasan FM, Udenigwe ChC, Gill TA, Aluko RE. Evaluation of the in vitro antioxidant properties of a cod (Gadus morhua) protein hydrolysate and peptide fractions. Food Chem. 2015;173:652-9.
15. Weng W, Tang L, Wang B, Chen J, Su W, Osako K, et al. Antioxidant properties of fractions isolated from blue shark (Prionace glauca) skin gelatin hydrolysates. J Funct Foods. 2014;11:342-51.
16. Kumar NS, Nazeer RA, Jaiganesh R. Purification and identification of antioxidant peptides from the skin protein hydrolysate of two marine fishes, horse mackerel (Magalaspis cordyla) and croaker (Otolithes ruber). Amino Acids. 2012;42(5):1641-9.
17. Jridi M, Lassoued I, Nasri R, Ayadi MA, Nasri M, Souissi N. Characterization and potential use of cuttlefish skin gelatin hydrolysates prepared by different microbial proteases. Bio Med Res Int. 2014;2014:461728.
18. Mendis E, Rajapakse N, Kim SK. Antioxidant properties of a radical-scavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. J Agric Food Chem. 2005;53(3):581-7.
19. Yang JI, Ho HY, Chu YJ, Chow CJ. Characteristic and antioxidant activity of retorted gelatin hydrolysates from cobia (Rachycentron canadum) skin. Food Chem. 2008;110(1):128-36.
20. Association of Official Analytical Chemists. Official methods of analysis, 17th Volume, Washington, DC: Association of Official Analytical Chemists; 2000; p. 112.
21. Alemán A, Giménez B, Montero P, Gómez-Guillén MC. Antioxidant activity of several marine skin gelatins. LWT Food Sci Technol. 2011;44(2):407-13.
22. Da Rosa Zavareze E, Telles AC, El Halal SL, da Rocha M, Colussi R, de Assis LM, et al. Production and characterization of encapsulated antioxidative protein hydrolysates from Whitemouth croaker (Micropogonias furnieri) muscle and byproduct. LWT Food Sci Technol. 2014;59(2):841-8.
23. Taheri A, Sabeena Farvin KH, Jacobsen C, Baron CP. Antioxidant activities and functional properties of protein and peptide fractions isolated from salted herring brine. Food Chem. 2014;142:318-26.
24. Songchotikunpan P, Tattiyakul J, Supaphol P. Extraction and electrospinning of gelatin from fish skin. Int J Biol Macromol. 2008;42(3):247-55.
25. Muyonga JH, Cole CGB, Duodu KG. Characterisation of acid soluble collagen from skins of young and adult Nile perch (Lates niloticus). Food Chem. 2004;85(1):81-9.
26. Mosquera M, Giménez B, da Silva IM, Boelter JF, Montero P, Gómez-Guillén MC, et al. Nanoencapsulation of an active peptidic fraction from sea bream scales collagen. Food Chem. 2014;156:144-50.
27. He R, Girgih AT, Malomo SA, Ju X, Aluko RE. Antioxidant activities of enzymatic rapeseed protein hydrolysates and the membrane ultrafiltration fractions. J Funct Foods. 2013;5(1):219-27.
28. Montero P, Gómez-Guillén MC. Extracting conditions for megrim (Lepidorhombus boscii) skin collagen affect functional properties of the resulting gelatin. J Food Sci. 2000;65(3):434-8.
29. Bougatef A, Hajji M, Balti R, Lassoued I, Triki-Ellouz Y, Nasri M. Antioxidant and free radical-scavenging activities of smooth hound (Mustelus mustelus) muscle protein hydrolysates obtained by gastrointestinal proteases. Food Chem. 2009;114(4):1198-205.
30. Bamdad F, Wu J, Chen L. Effects of enzymatic hydrolysis on molecular structure and antioxidant activity of barley hordein. J Cereal Sci. 2011;54(1):20-8.
31. Cheung IW, Cheung LK, Tan NY, Li-Chan EC. The role of molecular size in antioxidant activity of peptide fractions from Pacific hake (Merluccius productus) hydrolysates. Food Chem. 2012;134(3):1297-306.
32. You L, Zhao M, Cui C, Zhao H, Yang B. Effect of degree of hydrolysis on the antioxidant activity of loach (Misgurnus anguillicaudatus) protein hydrolysates. Innov Food Sci Emerg Technol. 2009;10(2):235-40.
33. Gómez-Guillén MC, López-Caballero ME, AlemánA, López de Lacey A, Giménez B, Montero P. Antioxidant and antimicrobial peptide fractions from squid and tuna skin gelatin. Le Bihan E, editor. kerala: Trans world Research Network; 2010. 89-115. Available from: http://digital.csic.es/bitstream/10261/40679/1/CAPITULOS_DE_LIBROS302952[1].pdf.
34. Rajapakse N, Mendis E, Jung WK, Je JY, Kim SK. Purification of a radical scavenging peptide from fermented mussel sauce and its antioxidant properties. Food Res Int. 2005;38(2):175-82.
35. Wang B, Li ZR, Chi CF, Zhang QH, Luo HY. Preparation and evaluation of antioxidant peptides from ethanol-soluble proteins hydrolysate of Sphyrna lewini muscle. Peptides. 2012;36(2):240-50.
36. Morales-Medina R, Tamm F, Guadix AM, Guadix EM, Drusch S. Functional and antioxidant properties of hydrolysates of sardine (S. pilchardus) and horse mackerel (T. mediterraneus) for the microencapsulation of fish oil by spray-drying. Food Chem. 2016;194:1208-16.
37. Farvin KS, Andersen LL, Otte J, Nielsen HH, Jessen F, Jacobsen C. Antioxidant activity of cod (Gadus morhua) protein hydrolysates: Fractionation and characterisation of peptide fractions. Food Chem. 2016;204:409-19.

بازیابی فراکسیون‌های پپتیدی زیست‌فعال از هیدرولیزات دورریز در عمل‌آوری ماهی قزل‌آلای رنگین‌کمان (*Onchorhynchus mykiss*)

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چکیده

اهداف: پپتیدهای زیست‌فعال دارای خاصیت ضداکسیدانی استخراج‌شده از باقی‌مانده‌های ماده خام و ضایعات ماهی حاصل از هیدرولیز آنزیمی، یکی از موارد مورد علاقه صنعت غذا دارو، غذاهای سالم و صنایع فرآوری و نگهداری هستند.

مواد و روش‌ها: این تحقیق با هدف تعیین اجزای پپتیدی با فعالیت ضداکسیدانی بازیابی‌شده از ژلاتین هیدرولیزشده پوست ماهی قزل‌آلای رنگین کمان انجام شده است. ۴ جزء پپتیدی (۳>، ۱۰-۳، ۳۰-۱۰ و ۳۰<) با جزءبندی ژلاتین هیدرولیزشده توسط فرآیند الترافیلتراسیون سانتریفیوژی با غشاهای ۳، ۱۰ و ۳۰ کیلودالتون به‌دست آمد. ترکیب اسیدآمینه جزء ۳۰-۱۰ کیلودالتون تعیین شد. فعالیت ضداکسیدانی همه اجزا توسط روش‌های مهار رادیکال‌های DPPH و ABTS و همچنین روش قدرت کاهندگی تعیین شد.

یافته‌ها: اسیدآمینه‌های غالب جزء پپتیدی ۳۰-۱۰ کیلودالتون شامل گلایسین، پرولین، آلانین و هیپسیدین بود. اسیدآمینه‌های آب‌گریز جزء پپتیدی۳۰-۱۰ کیلودالتون ۶۱/۵% بود. تمام نمونه‌ها شامل ژلاتین هیدرولیزشده و اجزای پپتیدی، فعالیت ضداکسیدانی بالایی را نشان دادند. همچنین جزء پپتیدی ۳۰-۱۰ کیلودالتون، فعالیت مهار رادیکال‌های DPPH و ABTS (در غلظت ۲، ۵ و ۱۰ میلی‌گرم بر میلی‌لیتر) و قدرت کاهندگی (در غلظت ۱۰ میلی‌گرم بر میلی‌لیتر) بالایی را در مقایسه با سایر اجزا از خود به نمایش گذاشت ($p<0/05$).

نتیجه‌گیری: این نتایج نشان‌دهنده ظرفیت اجزای پپتیدی به‌دست‌آمده از ژلاتین پوست ماهی قزل‌آلای رنگین‌کمان به عنوان منبعی از ضداکسیدان طبیعی جهت استفاده در محصولات غذایی است.

کلیدواژه‌ها
بازیابی؛
ضایعات ماهی؛
قزل‌آلای رنگین‌کمان؛
فراکسیون‌های پپتیدی؛
زیست‌فعالی

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