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Physico-chemical and functional properties of Rainbow trout fish protein isolate

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Abstract

Fish protein isolate contains myofibrillar proteins extracted from the fish muscle, can be used as an ingredient for production of value added and ready-to-eat products based on functionality. Rainbow trout fish protein isolate was prepared by pH shift method and studied for functional properties. The rainbow trout fish protein isolate (RTFPI) had 3.5% moisture, 75.6% protein, 2.4% fat and ash content of 4.0%. The RTFPI had bulk density of 0.58 g/mL and brownish yellow colour. Oil and water holding capacity was 0.14 and 2.2 mL/g, respectively. The emulsifying activity index of RTFPI at pH 3, 5 and 7 was 281.0, 207.3 and 535.0 m^2/g respectively, while as the emulsifying stability index was 11.3, 4.2 and 7.0 min at pH 3, 5 and 7 respectively. The RTFPI had foaming capacity and stability of 13.2% and 90% at pH 7 while as foam did not form at pH 3 and 5. Protein solubility curve of RTFPI was U-shaped with minimum solubility at pH 5.

Introduction

Globally, fish provide 15% of the total dietary intake of animal protein for 4.3 billion people. In 2010, the global consumption of fish was 18.6 kg per capita (FAO, 2012). Trout fish are found in both freshwater and saltwater and belong to the Salmoninae subfamily of the family Salmonidae. The rainbow trout *(Oncorhynchus mykiss)* is native only to the rivers and lakes of North America, west of the Rocky Mountains, but its value as a hard-fighting game fish and tasty meal has led to its introduction throughout the world. Rainbow trout possesses a characteristic rainbow stripe and the rose-coloured gill plate, but may be silver-coloured as well. Rainbow trout have fins entirely without spines, and have a small adipose fin along the back, near the tail. Rainbow trout is low in fat and calories compared to other meats. A 3-ounce serving of cooked rainbow trout contains 130 calories, 22 g of protein, 4 g fat, 1 g saturated fat and 30 mg of Sodium. They average about 20 to 30 inches (51 to 76 cm) long and around 8 pounds (3.6 kg), but can grow as long as 4 feet (1.2 m) and weigh up to 53 pounds (24 kg) (National geography). In fiscal 2009-2010, fish production in Jammu and Kashmir touched 19,000 tons, generating over 2 crore Indian rupees revenue. Although rainbow trout is used as food fish, it can also be utilized for preparation of proteins isolates which can be utilized as functional ingredient in many food products. Fish protein isolate is a protein concentrate prepared from fish muscle without retaining the original shape of the fish muscle and is used as raw material for the production of value added products or as ingredient in food industry. The aim of the study was to prepare protein isolate from rainbow trout fish muscle by pH shift method and evaluate it for functional properties.

Material and Methods

Sample collection

Farmed rainbow trout fish weighing 350 ± 30 g were supplied by Government Trout Fish Farm, Dachigam, Srinagar - India. They were euthanized by beating the head with a wooden stick and used within one hour. All the reagents used in this study were of analytical grade from Hi media, CDH.

Preparation of fish protein isolate

The trout fish was cut into fillets of suitable size after evisceration, cleaning and deboning. The fillets were then ground in a domestic mixer (Sujata, India) to get a uniform paste. The paste was diluted in the ratio of 1:10 by the addition of double distilled water and adjusted to pH of 10.5 with 0.2 N NaOH and 0.2 N HCl solutions. The pH adjusted slurry was then stirred for one hour over a magnetic stirrer at room temperature (25°C) and then centrifuged at 10,000 × g at 5°C for 10 min. The top fatty layer and the bottom sediment were discarded, while as the middle liquid portion containing solubilised proteins was collected.

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The pH of the liquid portion was adjusted to 5.5 with a pH meter (Hanna pH 213, Woonsocket RI -USA). The slurry was centrifuged at 10,000 × g at 5°C for 10 min. The top liquid portion was discarded while the pellets obtained at the bottom were collected and adjusted to a pH of 7 with 0.2 N NaOH and 0.2 N HCl. It was then dried in vacuum oven (RIC-29E, Reliable Instruments Co., India) at 35°C and was named as Trout Fish proteins isolate (RTFPI).

Composition

Moisture (925.10), protein (984.13), fat (920.85) and ash (923.03) contents were determined according to the methods of AOAC (1990) procedures.

Bulk density (BD)

Bulk density was measured as a ratio of mass to volume according to the method of Wani *et al*. (2013).

Colour

The colour values of the TFPI samples were observed by a Hunter Colour Lab (MiniScan XE Plus, Model No. 45/0-L, Hunter Associate Laboratory Reston, USA). The instrument was calibrated with black and white tile before colour measurement. The 'L' value indicates the lightness, 0–100 representing dark to light. The 'a' value gives the degree of the red–green colour, with a higher positive 'a' value indicating more red. The 'b' value indicates the degree of the after standardizing with white and black tiles.

Water holding capacity (WHC)

WHC of TFPI was determined by the method of Diniz and Martin (1997), with slight modification. 0.5 g of sample (dry basis) was taken in pre weighed centrifuge tube; 10 mL of distilled water was added to it and vortexed (Labnet, Labnet International Inc., USA) for 30 sec. The dispersion was allowed to stand at room temperature (25°C) for 30 min and then centrifuged at 2800 × g for 25 min. The supernatant was decanted carefully. Difference between the weight of the centrifuge tube with sample after decanting the supernatant and the initial weight of the centrifuge tube with sample is noted. The results were reported as gram of water absorbed per gram of protein sample.

Oil holding capacity (OHC)

OHC of TFPI was determined as the volume of edible oil held by 0.5 g of material according to the method of Shahidi *et al.* (1995). 0.5 g of sample (dry basis) was taken in pre weighed centrifuge tube; 10 mL of mustard oil was added to it and vortexed for 30 sec. The dispersion was allowed to stand at room temperature (25°C) for 30 min and then centrifuged at 2800 × g for 25 min. The supernatant was decanted carefully. Difference between the weight of the centrifuge tube with sample after decanting the oil and the initial weight of the centrifuge tube with sample is noted. The results were reported as gram of oil absorbed per gram of protein sample.

Emulsifying activity index (EAI) and emulsion stability index (ESI)

EAI was determined according to the method of Pearce and Kinsella (1978), with minor modifications. For emulsion formation, 45 mL of 0.2% (w/v) protein isolate solutions and 15 mL of mustard oil were homogenized in a beaker using home blender for 3 min. Fifty micro-liters of emulsion were taken from the bottom of the homogenized emulsion, immediately after homogenisation, and diluted (1:100, v/v) in 0.1% (w/v) SDS solution. After shaking in a vortex mixer for 5 seconds, the absorbance of dilute emulsions was read at 500nm (HITACHI U-2900, Hitachi Corporation, Tokyo). The absorbance measured immediately (A0) and 10 min (A10) after emulsion formation were used to calculate the emulsifying activity index (EAI) and the emulsion stability index (ESI).

$$\text{EAI}\ (m^2/g) = \frac{2 \times 2.303 \times A_0 \times DF}{c \times \Phi \times (1-\theta) \times 10{,}000}$$

Where DF is the dilution factor (100), c is the initial concentration of protein (g/mL), Φ is the optical path (0.01 m), Θ is the fraction of oil used to form the emulsion (0.25), and A_0 is the absorbance of diluted emulsions respectively. Measurements were performed in at least three times.

$$\text{ESI} = \frac{A_0}{A_0 - A_{10}}$$

Foaming capacity and foam stability (FC and FS)

For the determination of foaming capacity 2.0 g (dry weight basis) of rainbow trout protein isolate was dispersed in 100 mL of distilled water. The pH of the suspensions was adjusted to 3, 5 and 7 using pH meter (Hanna pH 213, Woonsocket RI -USA) and transferred to graduated cylinders of 500 mL capacity. The suspensions were homogenized for 2 min at maximum speed using a domestic homogenizer (Phillips, India). The foam volume was noted immediately after 2 min. Foam stability was determined by measuring the fall in the volume of the foam after every 30 min. Foaming capacity and foam stability was calculated using the following equations:

$$FC = \frac{\text{Volume after whipping} - \text{Volume before whipping}}{\text{Volume before whipping}} \times 100$$

$$FS = \frac{(\text{Foam volume after time t} \times 100)}{\text{Initial foam volume}}$$

Solubility of trout fish protein isolate

Solubility of protein isolates was determined by adjusting the pH of 0.1% protein suspensions to 2, 3, 4, 5, 6, 7, 8, 9 and 10 with 0.1N HCL or 0.1N NaOH using pH meter. The suspensions were stirred for an hour at room temperature and then centrifuged at about 10000 × g for 5 min. Supernatants were taken and soluble protein was estimated by the method Lowry (Lowry *et al.*, 1951) and measuring the absorbance at 540 nm (HITACHI U-2900 UV/VIS, Hitachi Corporation, Tokyo).

Statistical analysis

All measurements were performed in triplicate for each lot, and the mean values ± standard deviations were reported for each case.

Results and Discussion

Composition

Moisture content of RTFPI was 3.5% ± 0.42. Foh *et al*. (2010) reported moisture content of 3.7% for FMMC (freshly minced meat concentrate) of Tilapia fish. Protein content of RTFPI was 75.61% ± 0.95. Protein content of 82.39 - 94.7% has been reported in fish protein isolates (Foh *et al*., 2010andLiu *et al.*, 2009). Lipid content of TFPI was 2.35% ± 0.89. Lipid content of FMMC of Tilapia fish as reported by Foh *et al.* (2010) was 1.81%. Phawakat *et al*. (2011) reported 0.12% lipid content in fish protein isolate from red tilapia. Ash content of TFPI was 4%.

Bulk density (BD)

Bulk density signifies the behavior of a product in dry mixes and is an important parameter that can determine the packaging requirement of a product. Also, it varies with the fineness of particles. High bulk density is unfavorable for the formulation of weaning foods, where low bulk density is required (Kamara *et al*., 2009). Bulk density of RTFPI was 0.58 g ± 0.01 mL^{-1}. Bulk density of FMMC of tilapia fish was 0.34 g mL^{-1} as reported by Foh *et al*. (2010).

Colour

Colour influences the overall acceptability of food products. The colour of protein isolates depends on processing conditions, the kind of raw material used and fish freshness. *L, a* and *b* colour values for RTFPI were 44.08 ± 1.95, 14.19 ± 0.93 and 42.10 ± 1.17, respectively. The colour values reported by Omana *et al.* (2010) for FMMC of tilapia fish were *'L'* = 71.85 and *b* = 17.22. Shaviklo *et al.* (2010) reported *'L' 'a'* and *'b'* values of 70.6, -1.91 and 5.5 for fish protein isolate. The lower value of 'L' and higher values for 'a' implies that the product was brown. The colourandwhiteness of fish protein isolate can in part depend on connective tissue that can increase the lightness; the retention of lipids that can influence yellowness values: co-precipitation of heme proteins which affect redness and denaturation and oxidation of haemoglobin that causes a yellow-brownish colour in products. High redness values could be attributed to heme proteins in the final product (Kristinson *et al*., 2005).

Water holding capacity (WHC)

Proteins have both hydrophilic and hydrophobic properties therefore, can interact with water and oil in foods (Butt and Batool, 2010). The functional properties of proteins in food system broadly depend on the water-protein interaction. WHC is affected by pH and ionic strength (i.e. salt). WHC reflects the extent of denaturation of the protein. WHC of TFPI was 2.2 mL g^{-1}. The WHC of FMMC of tilapia fish was 2.47 mL g^{-1} as reported by Foh *et al.* (2010).

Oil holding capacity (OHC)

The OHC of proteins is important functional property as it improves the mouthfeel and retains flavor in a food. The high oil absorption is essential in the formulation of food systems like sausages, cakes batters, and mayonnaise and salad dressing (Butt and Batool 2010). The OHC depend on the amount of non-polar amino acids in the side chain and structure of the proteins. Oil Holding Capacity of TFPI was 1.43 mL g^{-1}. OHC of FMMC as reported by Foh *et al.* (2010) was 2.43 mL g^{-1}. This might be due to lower concentration of non-polar amino acids in protein molecules.

Emulsifying activity index and emulsion stability index

Proteins are surface active agents that can form and stabilize the emulsion by creating electrostatic repulsion on oil droplet surface (Makri *et al*. 2005). The ability of proteins to form emulsion is important owing to the interactions between proteins and lipids in the food systems. EAI of TFPI was 281.0, 207.3 and 535.0 m^2/g, while as the ESI was 11.30, 4.17 and 7.0 min at pH 3, 5 and 7 respectively.

Foaming capacity and foam stability

Foamability is an important functional property

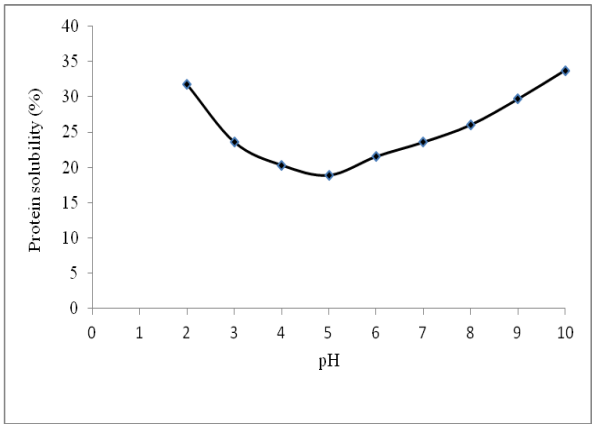

Figure 1. Solubility curve of trout fish protein isolate

of proteins by which proteins form a flexible cohesive film to entrap air bubbles. Although meat-based foam products (meat mousses) are scarce, they are available in different parts of the world. Proteins that rapidly unfold and adsorb at the freshly formed air/liquid interface during bubbling exhibit improved foamability (Damodarn, 1997). Foams are important in food formulations. Foam expansion is mainly related to the solubility of proteins, balance between flexibility and rigidity of proteins at the air water interface, hydrophobicity, pH, ionic strength, temperature etc. The RTFPI did not reveal any foaming capacity and stability at pH 3 and 4. However, at pH 7 FC and FS was 13.2% and 90% respectively.FC of FMMC of tilapia fish was 90.3% and FS ranged from 90.17 to 52.63% as reported by Foh *et al*. (2010). The lower FC and FS values of RTFPI might be due to low solubility and more hydrophobicity.

Protein solubility

Protein solubility at various pH values serves as a useful indicator of how well RTFPI would have performed if they were incorporated into food systems and also the extent of protein denaturation because of heat and chemical treatment at different pH (Horax 2004). Besides, many functional properties of proteins depend on the ability of proteins to go into solution. Solubility of proteins depends on temperature, pH, ionic strength, ratio of hydrophilicity and hydrophobicity. Figure 1 shows the solubility of TFPI between pH 2 and 10. The solubility values were higher at both low and high pH levels then at the isoelectric point (pH 5.5). At isoelectric point the net charge of the proteins are minimized resulting in aggregation of proteins. Foh *et al*. (2010) reported that the solubility curve of FMMC exhibited U-shape and all proteins had solubility above 80% at acidic pH.

Conclusion

Protein isolates are the most refined form of protein products containing the greatest concentration of protein. Trout fish protein isolates were brown in colour with good WHC and OHC. RTFPI also displayed good emulsifying properties while as foaming capacity was poor. Protein isolates may be used in the development of new class of formulated foods with enhanced nutritional properties.

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