



Characterization of Spray-Dried Hydrolyzed Proteins from Pink Perch Meat Added with Maltodextrin and Gum Arabic

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ABSTRACT

In the present study, spray-dried pink perch meat protein hydrolysate (PPH) and hydrolysate with maltodextrin and gum arabic (PPHMG) were characterized in relation to their physical, rheological, functional, antioxidant, thermal, and sensory properties. The Herschel–Bulkley model was suitable to explain the flow behavior of PPH and PPHMG emulsions. Both the samples exhibited functional and antioxidant properties. Antioxidant activities were significantly higher for PPH compared with PPHMG ($p < 0.05$). PPH and PPHMG alone did not exhibit antimicrobial activity but enhanced the activity of chitosan against pathogens. Sensory acceptability of the samples in soup revealed that PPHMG of up to 4% was highly scored without any traces of bitterness, whereas for PPH, a concentration of up to 3% was acceptable.

KEYWORDS

Fish protein hydrolysate; maltodextrin; rheological properties; functional properties; antioxidant activity; sensory acceptability

Introduction

In recent years, considerable attention has been paid to the biological activities of peptides and proteins from food sources. The importance of fish as a source of novel, biologically active substances is growing rapidly (Khora 2013). Fish proteins exhibit antioxidant and functional properties; this is demonstrated by a number of studies wherein the potential of fish protein hydrolysate as a source of antioxidants and functional components has been reported (Naqash and Nazeer 2013; Phadke et al. 2016). Enzymatic hydrolysis is a mild process that is faster and more controllable compared with mechanical and chemical treatments (Kristinsson and Rasco 2000). Use of proteolytic enzymes is often an attractive means for improving the functional properties of food proteins, without losing their nutritional value. Hydrolysates are considered as sources of peptides that are recommended for humans, but their direct use is limited due to intensely bitter taste, hygroscopicity, and likelihood of interaction with protein matrix, limiting further enrichment of the food (Rocha et al. 2009; Aishwarya Mohan et al. 2015). The efficiency of gelatin and soy protein isolate for masking the bitter taste of casein hydrolysate has been reported by Favaro-Trindade et al. (2010).

It is well-known that the drying of different materials can be achieved through the spray-drying technique (Sagar and Kumar 2010). Polysaccharides are considered ideal for use as carrier agents in spray-drying due their structural stability, abundance, and lower cost. Gum arabic, which is obtained from certain Acacia (family leguminosae) trees, is commonly used in spray-drying due to its good emulsifying properties and low viscosity in the aqueous form (Righetto and Netto 2005). The

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colossal molecular structure of polysaccharides contributes to their stability as carriers during the production and processing of encapsulated products. It also correlates positively with the particle size of the spray-dried products. Polysaccharides derived from plant, microbial, and animal sources can be utilized in the spray-drying of protein hydrolysates (Rocha et al. 2009). Crucian carp protein hydrolysate isolates were encapsulated by maltodextrin and gum arabic, evaluated for their antioxidant activities, and found to have the best oxidative protection activity at 1000 mg/kg (Taghvaei et al. 2014). Furthermore, the microencapsulation of fish oil with fish gelatin, chitosan, and maltodextrin in order to increase its stability has been reported by Pourashouri et al. (2014). Bioactive peptides can be encapsulated to mask their bitterness from the exposure of taste receptors to hydrophobic amino acid residues generated from protein hydrolysis.

It is well-recognized that rheological properties play a vital role in process design, evaluation, and modeling. These properties are sometimes measured as an indicator of product quality. Rheological data are required for calculation in any process involving fluid flow and play an important role in the analysis of flow conditions in different food-processing operations (Binsi et al. 2009).

There is increasing concern among consumers regarding the consumption of oxidative food. Lipid oxidation and peroxidation mechanism in food may lead to cardiovascular diseases, neurological abnormalities, Alzheimer's disease, etc. (Stadtman, 2006). Even though these synthetic antioxidants exhibit stronger activities compared with natural antioxidants, use of synthetic antioxidants such as butylated hydroxyl anisole (BHA) or butylated hydroxyl toluene (BHT) is under strict regulation due to the health-related hazards (Ito et al. 1986). Therefore, there is a great interest among researchers regarding protein hydrolysates to overcome the problem of lipid oxidation. Considering the adverse effects of synthetic antioxidants and low thermal stability, it is logical to substitute synthetic antioxidants with natural ones (Taghvaei and Jafari 2015). The characteristics of protein hydrolysates affect their functionality in the food system (Kristinsson and Rasco 2000; Gashti and Prakash 2016). Chitosan is known to possess antimicrobial properties against a range of bacteria. Furthermore, researchers have demonstrated the antimicrobial activity of fish protein hydrolysates.

In Indian coastal waters, pink perch (*Nemipterus japonicus*) is considered as a major bycatch from shrimp fishing and considered as commercially important fishes for the formulation of surimi and surimi seafood. The aim of this research was to study the potential of maltodextrin and gum arabic as the carrier agents in pink perch protein hydrolysate for reducing its bitter taste and to characterize the protein hydrolysates with respect to their antioxidant, rheological, functional, and physical properties.

Materials and methods

Raw material

Japanese threadfin bream, commonly known as pink perch (*Nemipterus japonicus*), were purchased from the local fish market and brought to the laboratory in iced condition (1:1 ratio of fish: ice). The average length and weight of the fish varied between 8.2 and 10.5 cm and 6.9 and 15.31 g, respectively. The fish were washed thoroughly with potable water, descaled, gutted, beheaded, and washed again. Furthermore, meat picking operation from pink perch was carried out manually. Picked meat was immediately used for further processing.

Preparation of fish protein hydrolysate

For the preparation of protein hydrolysate, picked meat was ground in a domestic grinder for 15 s in order to make a fine paste and was subjected to enzymatic hydrolysis using 1% papain (from *Carica papaya*) as an enzyme. The samples were mixed with distilled water 1:2 (w: v) and homogenized for 2 min. The conditions for hydrolysis were 1% enzyme to substrate ratio, 60 min of hydrolysis, 50 °C hydrolysis temperature, and physiological pH (6.6±0.2). Hydrolysis was performed in a shaking incubator with constant agitation.

Enzyme was inactivated using incubation at 90 °C for 15 min at the end, and the soluble fraction was collected using filter paper and used for spray-drying.

Spray-drying of fish protein hydrolysate with and without the addition of maltodextrin and gum arabic

Soluble fraction obtained by hydrolysis from pink perch meat was divided into two lots. The first lot of soluble fraction was spray-dried and stored in airtight containers for further evaluation and was coded as PPH. In the second lot, carrier materials (maltodextrin (20 g) and gum arabic (10 g)) were dissolved in the hydrolysate solution (liter). The composition of wall materials was used on the basis of preliminary studies. Then, it was homogenized using a tissue homogenizer (Polysystem PT 2100, Kinematica, AG0, Bohemia, NY, USA) at 25000 rpm under iced condition for 10 min. The samples were allowed to stabilize at room temperature for 1 h and then spray-dried using a pilot-scale spray dryer (Hemraj Pvt. Ltd, Mumbai, India) under the following condition: inlet temperature 160°C, outlet temperature 80°C, nozzle diameter 0.5 mm, air pressure 4 bar, and spray flow feed rate 15–20 mL/min. Spray-dried pink perch protein hydrolysate added with maltodextrin and gum arabic was coded as PPHMG.

Physical properties of fish protein hydrolysate

Yield of spray-dried PPH and PPHMG samples was calculated as a percentage of the spray-dried PPH and PPHMG powders obtained in relation to the wet weight of the raw material used. Morphology of the spray-dried PPH and PPHMG was observed using a PHILIPS XL-30 scanning electron microscope (Philips FEI quanta200, FEI, Hillsboro, OR, USA) at an accelerating voltage of 10 kV; the images were captured at 1000X magnification. The samples were mounted on a specimen stub with double-sided adhesive tape and subjected to gold sputter coating to render them electrically conductive. The size of the particles was also measured. The bulk density of PPH and PPHMG was calculated by dividing the mass of the powder by the volume occupied in the cylinder and reported as g/mL (Goula and Adamopoulos 2004). Hygroscopicity of the samples was expressed as g of water absorbed/100 g of dry solids (Cai and Corke 2000). The color of spray-dried PPH and PPHMG was evaluated using Hunter Lab color analyzer (ColorFlex-EZ) and reported as L^* (lightness), a^* (positive values indicate redness and negative values indicate greenness), and b^* (yellowness) values. Browning intensity of PPH and PPHMG was measured by the method as described by Ajandouz et al. (2001).

Rheological properties of fish protein hydrolysate in emulsion system

Preparation of emulsions containing PPH and PPHMG

For the rheological characterization of hydrolysates in an emulsion system, emulsions containing PPH and PPHMG were prepared as per the method described by Yin et al. (2010) with slight modifications. For the preparation of emulsions, PPH /PPHMG (6%, w/w), vegetable oil (59.35%, w/w), double-distilled water (30%, w/w), sodium chloride (3.6%, w/w), lemon juice (1%, w/w), and gum arabic (0.05%, w/w) were used. During the preparation of emulsions, ice bath was used for keeping the samples. Initially, salt was added in double-distilled water in a beaker and mixed with a homogenizer (Ultra-Turrax Ika T-25, Janke and Kunkel, Breisgau, Germany) at 13500 rpm for 2 min. Afterwards, gum arabic was added and homogenized at the same speed for 2 min. PPH or PPHMG was added to the sample, and it was subjected to homogenization for 3 min, followed by the addition of vegetable oil and homogenization for 12 min in order to form emulsion. Lemon juice was added, and the emulsion was further homogenized for 2 min. The resulting emulsions of PPH and PPHMG were used for rheological characterization.

Rheological properties were analyzed using a Controlled Stress Rheometer (CSR) (AR-1500 ex, TA Instruments, Crawley, West Sussex, UK) in the oscillatory mode. A 4-cm parallel-plate measuring geometry was used with a gap setting at 1 mm. The linear viscoelastic region was evaluated (data not given), and based on the results, 1% strain was chosen for the experiments. Each sample was placed in the temperature-controlled Pelletier plate and allowed to equilibrate to 25°C for 10 min.

Frequency sweeps and oscillation stress sweep of emulsions of PPH and PPHMG

Each sample was placed on the parallel plate, and the frequency sweep test was conducted at a constant temperature of 25°C to determine the viscoelastic properties of emulsion containing PPH and PPHMG. Shear stress was measured at varying shear rates from 0 to 200 s⁻¹. The frequency sweeps of the PPH and PPHMG were carried out at the frequency range of 1–10.0 Hz at 25°C. The elastic modulus (G'), viscous modulus (G''), and tan δ (G''/G') values were measured as a function of frequency. A plot of frequency versus G' and G'' was obtained. The slope of the regression line of G' (on a log scale) with a change in frequency was obtained to assess the viscoelastic nature of the sample (Chandra and Shamasundar 2015). Oscillation stress sweep was plotted for storage modulus for oscillation stress from 0.01 to 10 Pa.

Flow behavior (shear–stress sweep) of emulsions of PPH and PPHMG

Shear–stress sweep of PPH and PPHMG was carried out at 25°C using CSR. The measuring geometry used was a 4-cm parallel plate. The total time for each flow experiment was 2 min, 1 min for ascent (up curve) and 1 min for descent (down curve), respectively. A flow curve was obtained by plotting log shear stress compared with log shear rate values. A rheological flow model was used to predict the flow behavior based on the shear stress–shear rate data using the rheometer software. The Herschel–Bulkley model was selected as the best-fit model on the basis of standard error and correlation coefficient values.

$$\text{The Herschel-Bulkley model equation is } \tau = \tau_0 + k \gamma^n \quad (1)$$

where τ is the shear stress (Pa), τ_0 is the yield stress (Pa), γ is the shear rate (s⁻¹), k is the consistency coefficient (Pasⁿ), and n is the flow behavior index (dimensionless).

Functional properties

Solubility of the proteins for fish protein hydrolysate at different pH values ranging from 3 to 11 was determined by finding the protein content of the sample and the supernatant (Lowry et al. 1951). Emulsion activity index (EAI) and emulsion stability index (ESI) were determined according to the method of Pearce and Kinsella (1978). Foaming capacity (FC) and foam stability (FS) of PPH and PPHMG were calculated by the method described by Sathe and Salunkhe (1981). Fat absorption capacity (FAC) was measured according to the method described by Shahidi et al. (1995), and the results are expressed as g of oil absorbed per gram of protein hydrolysate.

Antioxidant properties

DPPH free radical scavenging activity (%) of PPH and PPHMG at 2 mg/ml protein concentration was performed according to the method of Yen and Wu (1999). Ferric reducing antioxidant power (FRAP) was determined by the method as described by Oyaizu (1986). The metal chelating activity of PPH and PPHMG was measured by the method of Decker and Welch (1990) at 10 mg/mL protein concentration.

Differential scanning calorimetry (DSC) analysis

DSC was used to measure the thermal transitions of PPH and PPHMG. The test was performed with Q100 DSC equipment (TA Instruments, New Castle, DE, USA), fitted with a nitrogen-based cooling system. Equipment calibration was performed with indium (T melting = 156.6°C). The samples were weighed in aluminum pans and hermetically sealed with lids for analysis; an empty pan was used as the reference. The mass of each sample pan was matched in advance with the mass of an empty reference pan to within 0.1 mg. Thermo-analytical curves were obtained by the heating/cooling rate of the sample at 10°C/min. The scanning temperatures were from 20 to 180°C. Thermal denaturation temperature and denaturation enthalpy values were calculated from thermo grams.

Antimicrobial properties

The antimicrobial activities of PPH and PPHMG alone and in combination with chitosan were evaluated using the agar well diffusion method (Bauer et al. 1996) by measuring the inhibitory zone diameter (mm).

Preparation of vegetable soup fortified with fish protein hydrolysate

Sweet corn was purchased from the local market and boiled in a pressure cooker. Similarly, vegetable stock was prepared using carrot, onion, tomato, garlic, and pepper powder. Sweet corn vegetable soup was prepared by adding crushed boiled corn and a little butter to the vegetable stock with a pinch of pepper powder, green peas, carrot, corn, and salt. Fish protein hydrolysate (FPH) powder with or without the addition of maltodextrin and gum arabic was added at a known concentration to the sweet corn vegetable soup.

Sensory analysis

FPH powder with or without the addition of maltodextrin and gum arabic was added at 2, 3, and 4% concentration to the sweet corn vegetable soup, and the samples were analyzed for sensory properties such as appearance, color, taste, flavor, bitterness, and overall acceptability using the hedonic scale by the method of Amerina et al. (1966). Sweet corn soup without the addition of FPH served as the control. The panelists received samples (at 2, 3, and 4% concentration of the FPH soup with and without the addition of maltodextrin and gum arabic) served at random in paper cups. In each test, the panelists were instructed to rank the samples in an increasing order according to the sensory properties. In between sampling, panelists were instructed to rinse their mouth with water.

Statistical analysis

The experimental data was analyzed by SPSS, version 16.0 (SPSS Inc., St. Louis, MO, USA). The analyses, except for the sensory tests, were carried out in triplicate, and the results are expressed as mean \pm SD. Furthermore, Student's t-test was used to compare the means at a 5% level of significance.

Results and discussion

Physical properties

Yield

The yield of spray-dried PPH and PPHMG samples was 6.13 and 6.57%, respectively. Typically, yield ranged between 10 and 15% of the raw material (Quaglia and Orban 1990). Lower yields of PPH and PPHMG may have been due to significant loss during spray-drying. Yields in the range of 4.6–9.5%

of hydrolysates produced from the pink perch frame waste using papain have been reported (Phadke et al. 2016). Yield of spray-dried tuna waste protein hydrolysates was 3.9% of the raw material (Parvathy et al. 2016).

Bulk density

To optimize the process parameters of packaging, distribution, processing, and storage, it is important to have knowledge of food density. The bulk density is defined as the mass of the solid particles and includes moisture divided by the total volume occupied by the particles, surface moisture, and all the pores, closed or open to the surrounding atmosphere, and is generally used to characterize the final product obtained by drying (Johanson 2005; Kurozawa et al. 2009). PPH exhibited significantly higher bulk density compared with PPHMG ($p < 0.05$) (Table 1). Bulk density shows variation with fineness of particles. Weaning foods formulation requires low bulk density (Kamara et al. 2009). Higher bulk density of PPH can be due to enzymatic hydrolysis as a result of filling the spaces between larger particles by smaller particles. In the case of PPHMG, the bulk density was lower; this may be due to the increasing feed concentration during spray-drying (Goula and Adamopoulos 2004). Decreased bulk density with added maltodextrin was also reported by Goula and Adamopoulos (2010). This may be attributed to minimal thermoplastic particles not sticking due to the addition of maltodextrin (Goula and Adamopoulos 2010).

Hygroscopicity

In general, protein hydrolysates are hygroscopic in nature, so an attempt was made by checking the influence of the addition of maltodextrin and gum arabic to the PPH during spray-drying. Significantly higher hygroscopicity was observed for PPHMG compared with PPH ($p < 0.05$) (Table 1). Maltodextrin and gum arabic combination was not effective in the reduction of hygroscopicity of the hydrolysate. As the hygroscopicity is affected by the concentration of maltodextrin in the solution (Kurozawa et al. 2009), it can be said that the concentration of maltodextrin in PPHMG may not be sufficient to reduce the hygroscopicity.

Color

Color influences the overall acceptability of food products and is affected by several factors such as species, processing, fat content, moisture, light, temperature, hemoglobin, myoglobin, and new protein ingredients in food formulations (Bueno-Solano et al. 2008).

As can be seen in Table 1, the L^* , a^* , and b^* values of PPH were significantly higher than those of PPHMG ($p < 0.05$). The differences in color may be attributed to the differences in the composition of PPH and PPHMG.

Browning intensity

The browning intensity of PPH and PPHMG was evaluated in order to assess the color of hydrolysates due to spray-drying and the addition of maltodextrin and gum arabic. Browning intensity as

Table 1. Physical properties of spray-dried PPH and PPHMG.

Physical Property	Sample	
	PPH	PPHMG
Bulk Density (g/ml)	0.23 ± 0.00 ^a	0.12 ± 0.00 ^b
Hygroscopicity (g of water/100 g of sample)	8.44 ± 1.21 ^b	10.95 ± 0.92 ^b
Color		
L^* (Lightness)	94.75 ± 0.01 ^a	91.75 ± 0.01 ^b
a^* (Greenness)	-1.21 ± 0.01 ^b	-0.75 ± 0.01 ^a
b^* (Yellowness)	8.68 ± 0.01 ^a	5.56 ± 0.01 ^b
Browning Intensity	0.03 ± 0.00 ^a	0.06 ± 0.00 ^b

Different lower case letters indicate significant difference between two samples ($p < 0.05$) $n = 3$

indicated by absorbance was found to be significantly higher for PPHMG as compared to PPH ($p < 0.05$) (Table 1). This may be due to the addition of gum arabic, which gives a brownish coloration to PPHMG upon spray-drying. Furthermore, hydrolysis leads to aldehyde formation and aldehyde interacts with free amino groups. Hydrolysis at 50°C causes the muscle lipids to undergo oxidation process (Elavarasan and Shamasundar 2016).

Rheological properties of fish protein hydrolysate in emulsion system

Frequency sweep

Frequency sweep results for PPH and PPHMG emulsions are presented in Figure 1. The G' (an elastic or storage modulus) and G'' (a viscous or loss modulus) of the emulsion samples containing PPH and PPHMG were determined as a function of frequency (ω) at a fixed temperature of 25°C. The storage modulus of PPH was higher than the loss modulus throughout the frequency range studied. The slope of the G' curve for PPH was steep, followed by that of PPHMG. Slope indicates the strength of the network with the applied frequency. Frequency sweep provides a rheological description of the products regarding their behavior during storage and application (Chandra and Shamasundar 2015). The results revealed an increase in the storage modulus values of PPH with increased frequency (Figure 1). The slope of the G' values as a function of the frequency sweep was 0.3 and 0.01 for PPH and PPHMG, respectively. Lower values indicated a strong gel network as a result of higher stability at the junction zones (Chandra and Shamasundar 2015).

Oscillation sweep

Decreased values of storage modulus were observed, along with increase in oscillation stress from 0.01 to 10 Pa (Figure 2). The breakdown of structure started at low oscillatory stress in both the samples, indicating low stability of the emulsions of PPH and PPHMG. The G' value of PPHMG was higher at the initial stress of 0.01 Pa. Breakdown of structure started at 0.5 Pa oscillation stress in both the samples.

Flow properties (shear-stress sweep) of emulsions of PPH and PPHMG

The rheological models were tested for the up curve and down curve data. The flow behavior model parameters of samples yield stress, consistency coefficient, and flow behavior index are given in Table 2. The yield stress of PPHMG was higher than that of PPH, but it was not significantly higher, which indicated PPHMG had greater resistance to flow and higher intermolecular attraction.

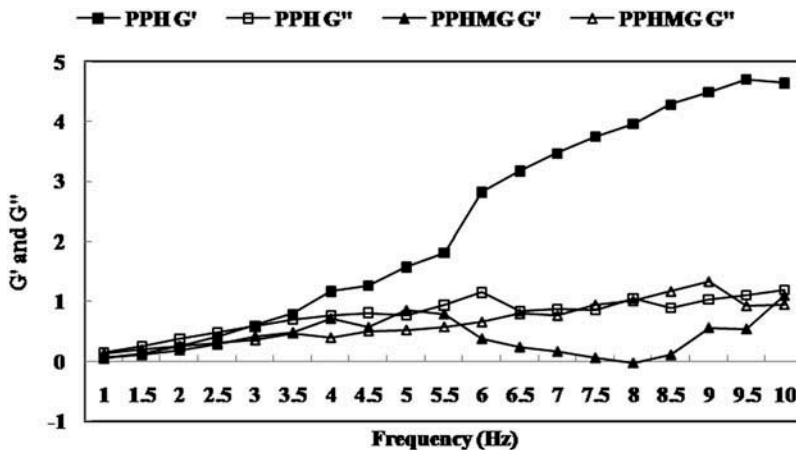


Figure 1. Frequency sweep for PPH and PPHMG.

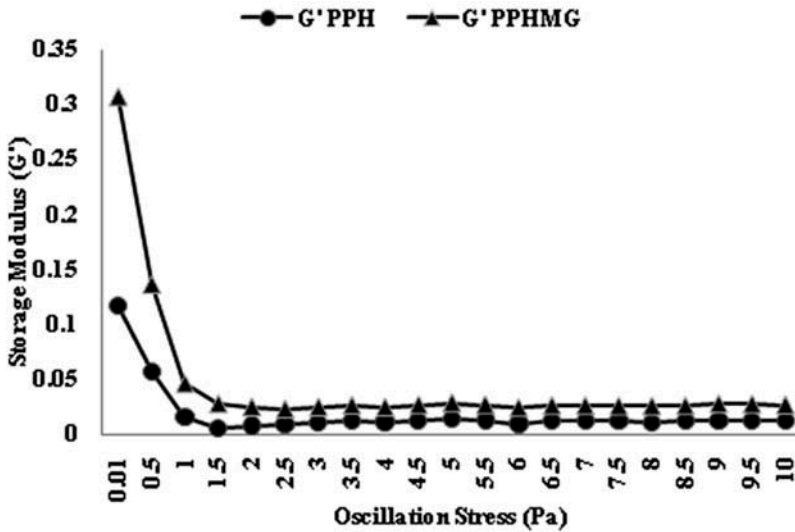


Figure 2. Oscillatory stress sweep for PPH and PPHMG.

Table 2. Herschel–Bulkley model parameters for emulsions containing PPH and PPHMG at 25°C.

Sample	Yield stress (τ) Pa	Consistency Index (k) Pa.s	Flow rate index (n)	Thixotropy, Pa.s ⁻¹	Regression Coefficient (R^2)
PPH	0.26	5.94×10^{-4}	1.27	24.76	0.98
PPHMG	0.30	5.18×10^{-3}	0.98	44.55	0.99

The consistency coefficient value obtained by the Herschel–Bulkley model for PPH was lower than that of PPHMG. It is the value of shear stress at a shear rate value of 1 s^{-1} . This higher k value is indicative of complete opening up of the polypeptide chain to random chain molecules, leading to increased viscosity (Chandra and Shamasundar 2015).

The flow profile of PPH and PPHMG is given in Figures 3 and 4, respectively. The flow behavior index is the value of the gradient of the straight line in a plot of shear rate and viscosity (log value). The flow behavior index of PPHMG was lower than 1, indicating its pseudo-plastic and non-Newtonian

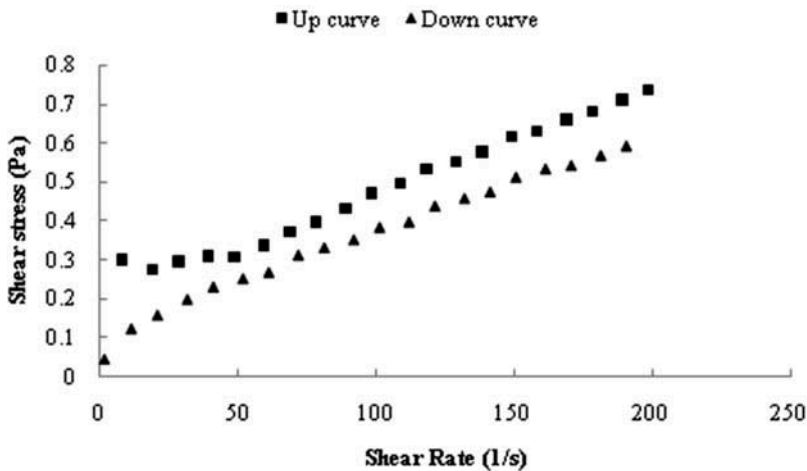


Figure 3. Flow profile of PPH.

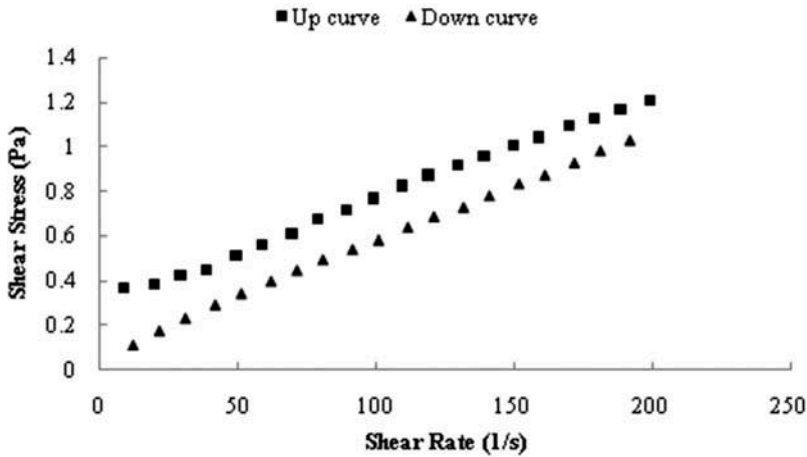


Figure 4. Flow profile of PPHMG.

nature; however, that of PPH alone was observed to be close to 1, indicating the Newtonian nature of the emulsion.

Variation in τ , n , and k values may be observed due to the addition of maltodextrin and gum arabic in PPHMG. Variation in these values has been reported by several authors (Sathivel et al. 2005; Yin et al. 2010). The PPHMG samples exhibited a higher yield stress value compared with PPH and thereafter showed a shear thinning behavior. It has been recognized that the shear thinning behavior represents an irreversible structural breakdown, and the decrease in viscosity occurs as a result of molecular alignment that takes place within such a substance (Glicksman, 1969).

Scanning electron microscopic (SEM) structure

SEM pictures of PPH and PPHMG revealed that the PPHMG samples exhibited separated, uniformly sized particles with a shrunken nature, resulting in reduced particle size. PPH samples exhibited hydrocolloidal structure (Figure 5). Particle size of the PPH samples varied from 4.05 to 17.3 μm , and that of PPHMG ranged from 5.12 to 10.0 μm . The spray-drying process produces varied-size particles upon drying (Carneiro et al. 2013). PPHMG showed concavities in the globular structure, which could be due to the rapid evaporation of liquid droplets during spray-drying, as well as due to the particles of maltodextrin and gum arabic in composition (Subtil et al. 2014). Continuous particle external surface without cracks was observed in PPHMG, ensuring the retention of maltodextrin and

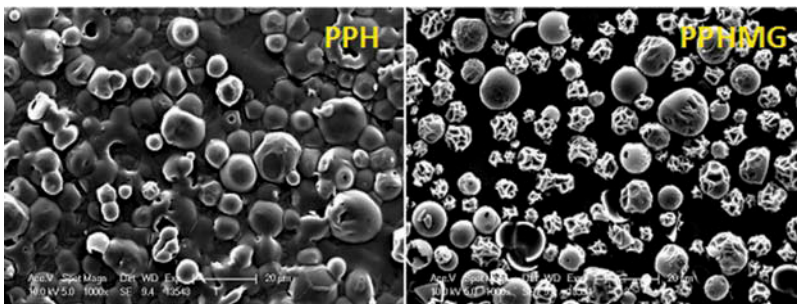


Figure 5. Scanning electron micrographs of spray dried PPH and PPHMG.

gum arabic. Uniform walls in PPHMG express proper dissolution of maltodextrin and gum arabic with protein hydrolysate before spray-drying.

Functional properties

Solubility

Hydrolyzed fish proteins contain a mixture of free amino acids, di-, tri-, and oligo-peptides, which increase the number of polar groups and solubility (Kristinsson and Rasco 2000). The solubility of PPH and PPHMG exhibited variations at different pH (Table 3). The protein solubility of PPH and PPHMG samples was higher, ranging between 84.91 and 95.93% at different pH values. The higher range of solubility of proteins in solution may be due to the ionic interactions promoting protein–water reactions. Upon hydrolysis, proteins get degraded to smaller-sized peptides, leading to higher solubility values (Gbogouri et al. 2004; Taheri et al. 2013). Minimum protein solubility of 86.54 ± 0.69% was recorded at pH 5 for PPH samples, whereas the PPHMG samples exhibited minimum solubility of 84.91 ± 0.61% at pH 7.0. There are several reports available wherein the minimum solubility of fish protein hydrolysates have been reported at different pH values (Shahidi et al. 1995; Gbogouri, et al. 2004; Klompong et al. 2007; Phadke et al. 2016). The low pH values where PPH and PPHMG exhibited lower solubility values could be their isoelectric points. Variations in the solubility values could be attributed to their increased net charge, with variation in pH above and below the isoelectric points promoting hydrophobic interactions, resulting in aggregation (Sorgentini and Wagner, 2002).

Fat absorption capacity (FAC)

FAC in terms of the physical entrapment of oil by PPH and PPHMG was evaluated, and the values are given in Table 3. PPH exhibited higher FAC, but there was no significant difference in FAC of PPH and PPHMG ($p > 0.05$). Similar observations for protein hydrolysates from different fish have been reported by several authors (Wasswa et al. 2007; Taheri et al. 2013). This could be attributed to the bulk density, as higher bulk density results in higher fat absorption (Kinsella 1976), and the PPH samples exhibited higher bulk density when compared with PPHMG. The oil absorption capacity of the hydrolyzed proteins not only influences the taste of the product but is also applicable in meat products, bakery products, etc. (Nakai 1983; Idouraine et al. 1991). PPH and PPHMG therefore could be used in various applications.

Table 3. Functional and antioxidant properties of spray-dried PPH and PPHMG.

Property	Sample	
	PPH	PPHMG
Protein solubility (%) at different pH		
pH 3.0	93.93 ± 0.71 ^b	92.38 ± 0.35 ^a
pH 5.0	86.54 ± 0.69 ^a	91.96 ± 0.06 ^b
pH 7.0	94.05 ± 0.53 ^b	84.91 ± 0.61 ^a
pH 9.0	95.33 ± 0.12 ^b	92.39 ± 0.31 ^a
pH 11.0	95.93 ± 0.56 ^b	93.16 ± 0.41 ^a
Fat absorption capacity (g of oil absorbed per gram of protein hydrolysate)	2.09 ± 0.06 ^a	1.95 ± 0.00 ^a
Emulsion activity index (m ² /g)	8.72 ± 0.12 ^a	76.26 ± 4.69 ^b
Emulsion stability index (min)	76.26 ± 4.69 ^b	63.61 ± 0.56 ^a
Foaming capacity (%)	66.67 ± 5.77 ^a	68.33 ± 2.89 ^a
Foam stability (%)	25.00 ± 5.00 ^a	28.33 ± 2.89 ^a
DPPH free radical scavenging activity (%)	66.16 ± 0.11 ^b	49.75 ± 0.11 ^a
Ferric reducing antioxidant power (A ₇₀₀)	1.41 ± 0.00 ^b	1.09 ± 0.00 ^a
Metal chelating activity (%)	26.40 ± 0.32 ^b	25.10 ± 0.07 ^a

Different lower case letters indicate significant difference between two samples, $p < 0.05$; $n = 3$

Emulsifying properties

The results of the emulsifying properties of PPH and PPHMG are presented in Table 3. PPHMG exhibited significantly higher EAI compared with PPH. However, ESI for PPH was significantly higher than that of PPHMG ($p < 0.05$), indicating the good functionality of proteins to incorporate in the food system as an active ingredient. Higher solubility results in rapid diffusion and adsorption of PPHMG at the interface than PPH, which may result in higher emulsion activity in PPHMG. The differences in EAI and ESI could be attributed to factors such as blending speed, protein source, temperature, pH, type of oil added, and water content (Linder et al. 1996). Difference in the emulsifying properties of hydrolysates may also be due to the differences in hydrophobicity (Gauthier et al. 1993).

Foaming properties

The foaming properties of protein hydrolysates are governed by the transportation, penetration, and reorganization of molecules at the air–water interface by the rapid migration, unfolding, and reorganization of protein molecules at the air–water interface (Wilde and Clark 1996; Martin et al. 2002; Klompong et al. 2007). The foaming properties of hydrolysates indicated no significant difference in the FC as well as FS exhibited by PPH and PPHMG ($p > 0.05$) (Table 3).

Antioxidant properties

DPPH free radical scavenging activity

Both the samples exhibited the ability to scavenge free radicals (DPPH free radical scavenging activity) (Table 3). DPPH free radical scavenging activity of PPH was significantly higher compared with PPHMG ($p < 0.05$). PPH and PPHMG could contain electron donors that enable them to react with free radicals for conversion into more stable products and terminate the radical chain reaction. The results obtained were in accordance with several studies, such as the DPPH free radical-scavenging activity of pink perch frame waste hydrolysate (Phadke et al. 2016) and yellow stripe trevally protein hydrolysates (Klompong et al. 2007). The differences in the DPPH free radical scavenging activity of PPH and PPHMG could be related to the presence of carbohydrates in PPHMG. This could have an influence on the properties by limiting/inhibiting the antioxidant activity of peptides in PPHMG. Hence, peptides in PPHMG could be less available to react with DPPH.

Ferric reducing antioxidant power

The ability of PPH and PPHMG to donate electrons for reducing Fe^{3+} to Fe^{2+} ions was evaluated and was found to be good (Table 3). FRAP of PPH was significantly higher compared with PPHMG ($p < 0.05$). The FRAP was determined at 10 mg/mL protein concentration of the sample, and it varied depending on the sample concentration. The results are in agreement with those found in the literature (Klompong et al. 2007; Phadke et al. 2016). Furthermore, the presence of carbohydrates in PPHMG could have attributed to less reduction in Fe^{3+} .

Metal chelating activity

Transition metal ions such as Fe^{2+} and Cu^{2+} can catalyze the generation of reactive oxygen species that oxidize unsaturated lipids. The chelating activity of peptides in hydrolysate could decrease lipid oxidation (Naqash and Nazeer 2013). In the present study, the activity of PPH and PPHMG to chelate metal ions was lower when compared with other published studies (Table 3). The metal chelating activity of PPH was significantly higher compared with that of PPHMG ($p < 0.05$). Similar results have been reported by Samaranayaka and Li-Chan (2008) for Pacific hake hydrolysates (metal chelating activity in the range of 7–46%). The formation of peptide and free amino acids upon enzymatic hydrolysis directly impacted the chelating ability. Metal chelating may be affected by size and amino acid composition (Kumar et al. 2011). Reduction in metal chelating activity may be attributed to differences in the composition of PPH and PPHMG, wherein PPHMG could have been diluted due to the addition of maltodextrin and gum arabic in its composition.

DSC analysis

Fishes are poikilothermic in nature, with heat-sensitive muscle proteins, which have the tendency to denature upon heating (Sikorski et al., 1994). Denaturation temperatures are important when evaluating the thermal stability of spray-dried protein hydrolysates. DSC analysis showed that the PPH sample had two degradation curves, whereas a single degradation curve was observed in the PPHMG sample. The first denaturation peak maximum was recorded at 108.9°C for PPH (Figure 6A) and at 96.16°C for PPHMG (Figure 6B). The denaturation temperatures are influenced by various factors such as concentration of the sample, heating rate, etc. Total heat content (enthalpy) of PPH and PPHMG was higher, 8.45 J/g and 15.84 J/g, respectively, indicating its vulnerability during processing, handling, and storage.

Antimicrobial properties

PPH or PPHMG alone did not exhibit antimicrobial activity, but in combination with chitosan, they exhibited antimicrobial activity against Gram-positive (*B. cereus*) and Gram-negative (*E. coli*)

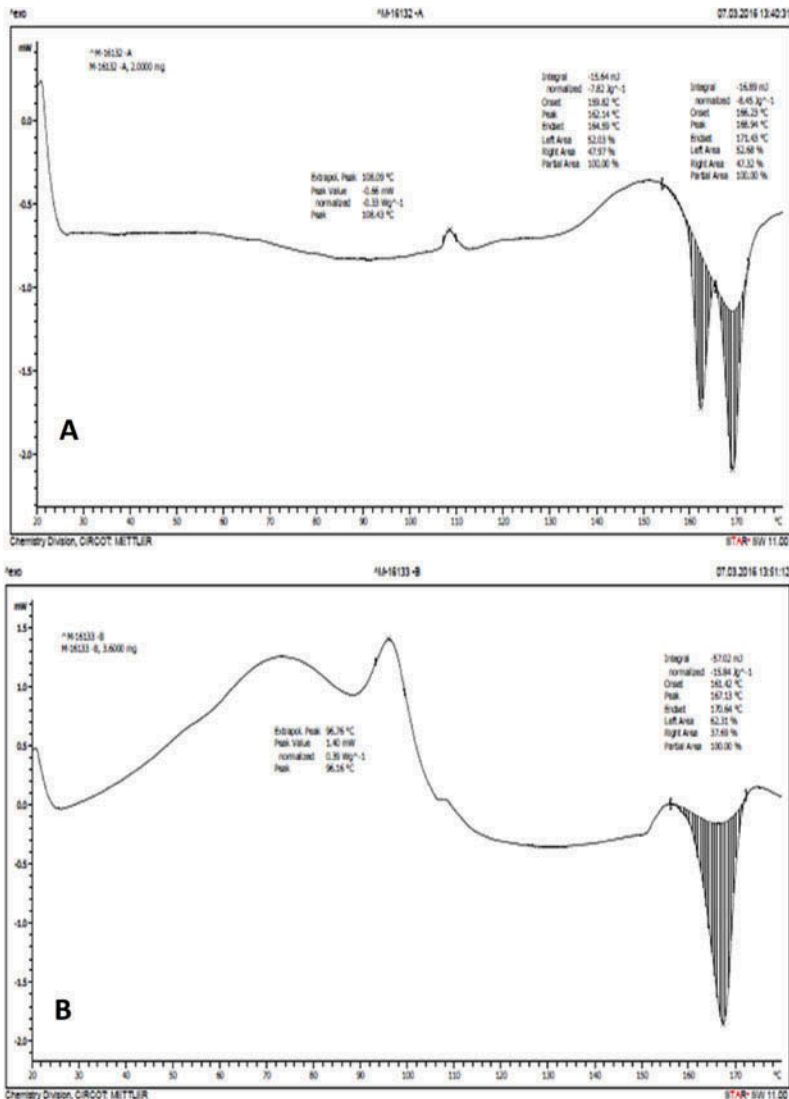


Figure 6. DSC analysis of A. PPH and B. PPHMG.

pathogens. Chitosan alone against *E. coli* showed an inhibition zone with a diameter of 17 mm, and PPH in combination with chitosan enhanced the antimicrobial activity further to the 24-mm inhibition zone against *E. coli*. Further increase in inhibition zone diameter was observed for the PPHMG samples in combination with chitosan against *E. coli* (Figure 7). A similar trend was observed against *B. cereus*. The results for the antimicrobial activity of PPH and PPHMG in combination with chitosan are given in Table 4.

Among the tested organisms, PPHMG and chitosan in combination was more effective than the PPH and chitosan combination. These results are in agreement with the report given by Serra et al. (2008). The mechanism of antimicrobial activity is because the outer cell membrane or cytoplasmic membrane of the bacterium is essentially composed of phospholipid bilayer and proteins and is the major site of interaction with antimicrobial compounds.

Overall acceptability of vegetable soup fortified with fish protein hydrolysate

Peptide size and hydrophobic nature allow it to bind to bitter taste receptors (Ishibashi et al. 1988). In relation to the safety of fish protein hydrolysates, protein hydrolysates are considered to be safe when they are derived from proteins with a history of safe use and when they are hydrolyzed using food-grade proteases and commonly used methods of processing (Schaafsma 2009). In the present study, the raw material used was pink perch fish meat, which is consumed widely; the enzyme used for hydrolysis was papain, which was derived from a plant source. Furthermore, fish protein hydrolysates produced by enzymatic, microwave-intensified enzymatic, chemical, and microwave-intensified hydrolysis have been safely used in food formulations (deep-fried battered fish and fish cake) up to 10% concentration (He et al. 2015). Atlantic salmon hydrolysate was safely supplemented to malnourished children in a chocolate drink and was found to be well-tolerated (Nesse et al. 2014). The bitter and salty effect of FPH should be taken into account when adding FPH to food products.

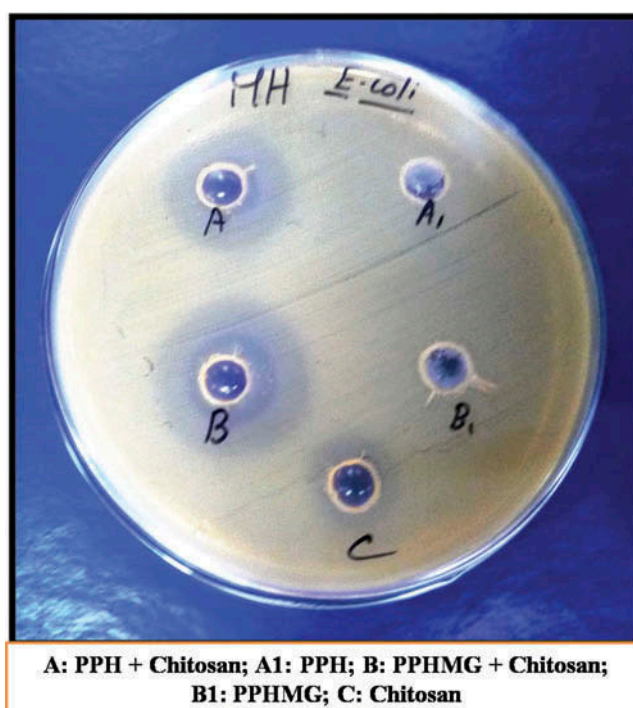


Figure 7. Antimicrobial activity of PPH and PPHMG in combination with chitosan against *E. coli*.

Table 4. Antimicrobial activity of PPH and PPHMG in combination with chitosan against Gram-positive and Gram-negative bacteria.

Incubation time (h)	Inhibition zone diameter (mm)				
	A	B	C	A ₁	B ₁
Gram -ve bacteria (<i>E. coli</i>)					
24	24	28	17	–	–
48	24	28	17	–	–
72	24	28	17	–	–
Gram +ve bacteria (<i>B. cereus</i>)					
24	17	21	14	–	–
48	17	21	14	–	–
72	Culture overgrowth, not observed proper inhibition zone				

A: PPH in combination with chitosan; B: PPHMG in combination with chitosan; C: Chitosan alone; A₁: PPH alone; B₁: PPHMG alone

Hence, based on the previous literature, PPH and PPHMG concentrations of 2, 3, and 4% were selected for fortification in sweet corn vegetable soup.

Sensory acceptability of the samples in soup revealed that up to 4% PPHMG was highly scored without any traces of bitterness; however, for PPH, a concentration of up to 3% was acceptable. No significant difference was observed in the taste of soup added with PPHMG at 4% concentration and control. Spray-dried casein hydrolysates containing gum arabic as the carrier agent also exhibited reduced bitterness (Subtil et al., 2014).

Fish protein-fortified soup powder could be considered part of a healthy diet (rich in bioactive and functional compounds) as it also masks the bitterness of FPH effectively.

Conclusion

PPH and PPHMG were found to be rich sources of protein (80–90%) and exhibited good antioxidant properties, antimicrobial properties (in combination with chitosan), and functional properties to incorporate in the food system. PPH or PPHMG-fortified soup powder can be considered as part of a healthy diet. The results of this study indicated that the spray-drying of hydrolysate with maltodextrin and gum arabic as the carrier agents (PPHMG) is efficient in attenuating the bitter taste of hydrolysate, which may also increase its storage stability.

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