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Functional peptides from yellowfin tuna (*Thunnus albacares*): Characterisation and storage stability assessment

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ABSTRACT

Present study attempted the effective utilisation of tuna red meat which is a major cannery waste from tuna industry, by recovery in the form of bioactive peptides. Protein hydrolysate from yellowfin tuna red meat was, characterised for functional properties. Molecular weight profile of the derived hydrolysate revealed its heterogeneity in peptide pattern with a major distribution above 10 kDa (60%). A protein recovery of 39.64% was obtained from the raw material with a protein content of 88.57±0.66% in the derived tuna protein hydrolysate (TPH). Present study revealed TPH to have rich levels of amino acids like glutamic acid, aspartic acid, lysine and leucine while phenyl alanine, tyrosine, methionine and cysteine were found in lower amounts. Variations in foaming properties at different pH levels ranging from 2-10 indicated these properties to be maximum at pH 6.0. Similarly, emulsion stability index was highest (48.09±2.69 min) at pH of 6.0. However, emulsifying activity index increased with increase in pH. The storage stability studies carried out for TPH at ambient (28°C) and chilled storage conditions (4°C) for upto six months indicated an uptake of moisture, increase in oxidative indices as well as changes in functionality which was more prominent under ambient conditions. Results suggested protein hydrolysate from tuna red meat to be a promising source of bioactive peptides, finding suitability in formulation of functional foods as well as nutraceutical products.

Keywords: Functional properties, Papain, Storage stability, Tuna protein hydrolysate, Yellowfin tuna

Introduction

Tuna is one of the widely distributed fishery resources having high economic value on account of its demand for fresh as well as thermally processed delicacies. Simultaneously, the waste generation on account of the conversion of these resources to high value commodities is huge, to the tune of upto 70% which needs to be effectively addressed (Herpandi *et al*., 2011). Among the byproducts generated, protein rich dark meat from tuna has a share of about 10-12% and has immense recovery and utilisation options. Effective recovery by conversion of this biomass to bioactive protein hydrolysate is a potential alternative that facilitates various applications. Enzymatic hydrolysis enables conversion of native proteins into amino acids and peptides with high digestibility and excellent functionality (Wangkheirakpam *et al.,* 2019). Based on the extent of hydrolysis that the parent protein undergoes, the properties exhibited by the hydrolysates vary considerably.

Previous studies reported optimisation and characterisation of protein hydrolysates from various sources (Wangtueai *et al*., 2016; Awuor *et al*., 2017; Wang *et al*., 2017). Protein hydrolysates with various bioactive properties derived from tuna have been reported by Saidi *et al.* (2014); Parvathy *et al.* (2018a,,b) and Zhang *et al*. (2019). Exploration of application potentials of protein hydrolysate for the effective development of new food products or dietary supplements requires its prior characterisation. However, there are no comprehensive studies reported on characterisation of tuna red meat hydrolysate optimised for functional attributes with thrust on protein recovery as well as sensory parameters. Hence, novelty of the current investigation lies with regard to characterisation of functional protein hydrolysate derived from tuna red meat (a tuna cannery byproduct), under optimised set of process conditions with emphasis on maximum protein recovery and minimum bitterness. Although the specific health benefits from different hydrolysates are mostly supportable scientifically, the consistency of these benefits is debatable on account of the quality changes during storage. Considering this aspect, the optimised hydrolysates were also subjected to storage stability studies under chilled (4°C) as well as ambient conditions (28°C).

Materials and methods

Raw materials and chemicals

Tuna red meat from the cannery waste of Forstar Canning Company, Mumbai which had undergone a

precooking process was collected and subjected to boiled water wash (1:4 (w/v), pressed and further subjected to treatment with 0.2% cold sodium bicarbonate solution (1:4 (w/v) and then pressed to remove excess moisture. This washed meat was used as the starting material for deriving functional tuna protein hydrolysate using RSM (Response Surface Methodology) optimised hydrolytic conditions (data not shown) employing papain enzyme (HiMedia, India). All the reagents used for the study were of analytical grade.

Preparation of protein hydrolysate

Treated tuna red meat was well comminuted and mixed with twice the amount of water, cooked for 15-20 min at 85-90°C to inactivate endogenous enzymes. The protein solutions were allowed to equilibrate for 30 min before hydrolysis was initiated. Further hydrolysis was done using papain under RSM optimised conditions *viz.,* enzyme-substrate ratio - 0.34%, hydrolysis time - 30 min, temperature - 60°C and pH - 6.5 in a shaking water bath (Neolab Instruments, Mumbai, India) with continuous agitation. Hydrolysis was terminated by heating the solution to 85-90°C for 15-20 min. The resultant solution was cooled and centrifuged (K-24A, Remi Instruments, Mumbai) at 8000 *g* and 10°C for 20 min and the supernatant obtained was further spray dried (Lab 2 Advanced Laboratory type Spray Dryer, Hemraj, Mumbai) and used for analysis.

Characterisation studies

Degree of hydrolysis and proteolytic activity

The methodology described by Hoyle and Merritt (1994) was followed to evaluate the degree of hydrolysis. One volume of 20% trichloroacetic acid (TCA) was added to the protein hydrolysate supernatant, followed by centrifugation at 2560 *g* for 15 min to collect the 10% TCA soluble fraction and the degree of hydrolysis (DH) was computed as:

$$\% \text{ DH} = \frac{\text{10\% TCA soluble } N_2 \text{ in the sample}}{\text{Total } N_2 \text{ in the sample}} \times 100$$

Proteolytic activity of the sample was estimated with respect to its tyrosine content (Gajanan, 2014). Absorbance of a known quantity of diluted liquid hydrolysate solution was measured at 280 nm (Lambda 25 UV/Vis, Perkin Elmer Life and Analytical Sciences, Singapore). Standard curve of L-tyrosine in 0.2 M HCl (0.025 - 0.2 mg ml^{-1}) was used to determine the tyrosine content of sample, expressed in µ mole of tyrosine liberated per mg of protein.

Protein recovery and yield

Protein recovery in hydrolysate was defined as the amount of protein obtained from the raw material upon hydrolysis to the total amount of protein in raw material. Yield of the sample was derived from the amount of spray dried hydrolysate powder obtained from the initial amount of raw material.

Molecular weight

Molecular weight of the tuna protein hydrolysate sample was determined by employing molecular weight cutoff centrifugal filters (Amicon®Ultracel®, Merck Millipore Ltd., Ireland) *viz.,* 100kDa, 50kDa, 30kDa, 10kDa and 3kDa under a processing time ranging from 15 to 40 min (depending on the filter size). A known volume and concentration of the protein solution was taken in centrifuge tubes with cut-off filters and subjected to pre-set centrifuging conditions. The device was spun in a fixed-angle rotor centrifuge (K-24A, Remi Instruments, Mumbai). The concentrate was collected from the filter device sample reservoir using a pipettor, while the ultra filtrate was collected from the centrifuge tube. Further, the volume and protein concentration of the filtrate as well as the concentrate was analysed for determining the molecular weight distribution pattern of peptides.

Nutritional profile

Proximate composition of tuna protein hydrolysates *viz.,* moisture, protein, fat and ash were estimated as per AOAC (2012). Precolumn derivatisation method was adopted for determination of amino acids using o-phthalaldehyde (OPA) and 9-fluorenylmethyl-chloroformate (FMOC) using HPLC (Shimadzu Prominence, Japan) equipped with UV detector (Shimadzu SPD-20A, Japan) and Poroshell HPH-C_{18} column (4.6 mm dia, 100 mm length and 2.7 µ particle size). Gradient mobile phase having phosphate and borate buffer at pH of 8.2 was used. A wavelength of 338 nm was employed for detecting aminoacids except for proline which was detected at 262 nm. The oven temperature was maintained at 40°C and the run time was 30 min.

Functional properties

Foaming properties

Foaming properties *viz.,* foaming capacity and stability of fish protein hydrolysate were determined at different conditions of pH as well as protein concentration (Sathe and Salunkhe, 1981). Protein solution (10 mg ml^{-1}) was prepared under different pH *viz.,* 2, 4, 6, 8 and 10. Further the solution was whipped for 2 min at a speed of 16000 rpm using a homogeniser (230 VAC T-25 digital Ultra-turrax, IKA, India) and poured into a 100 ml graduated cylinder. The foaming capacity was calculated as:

$$\text{FC } \% = \frac{V_2 - V_1}{V_1} \times 100$$

where V_2 is the volume immediately after whipping (ml) (0 min) and V_1 is the volume before whipping (ml). The whipped sample was further allowed to stand at room temperature for 3 min to determine the foam stability as:

$$FS\% = \frac{V_2 - V_1}{V_1} \times 100$$

where V_2 is the volume after standing (ml) and V_1 is the volume before whipping (ml). Similarly, the foaming capacity and foam stability of protein solution at different concentrations *viz.,* 0.1, 0.5, 1.0, 2.0 and 3.0%, under neutral pH were determined.

Emulsifying properties

Vegetable oil and protein solution (10 mg ml^{-1}) adjusted to a pH of 2, 4, 6, 8 and 10 were mixed in the ratio of 1:3 (v/v) and homogenised using a digital homogeniser (230 VAC T-25 digital Ultra-turrax, IKA, India) at a speed of 20000 rpm for 1 min. An aliquot of the emulsion (50 µl) was pipetted from the bottom of the container at 0 and 10 min after homogenisation and mixed with 5 ml of 0.1% sodium dodecyl sulphate (SDS) solution. The absorbance of the diluted solution was measured at 500 nm using a spectrophotometer (Lambda 25 UV/Vis, Perkin Elmer Life and Analytical Sciences, Singapore). The absorbance was measured immediately (A_0) and 10 min (A_{10}) after emulsion formation to calculate the emulsifying activity index (EAI) and the emulsion stability index (ESI) (Pearce and Kinsella, 1978):

$$EAI\ (mg^{-2}) = \frac{2 \times 2.303 \times A_0}{0.25 \times \text{Wt of protein}}$$

$$ESI\ (min) = A_{10} \times \Delta t \Delta A$$

where Δt =Time and $\Delta A = A_0 - A_{10}$

Similarly, the emulsifying properties of protein solution at different concentrations *viz.,* 0.1, 0.5, 1.0, 2.0 and 3.0% at neutral pH were determined.

Storage stability

Tuna protein hydrolysate samples were kept in air tight plastic containers and stored under chilled (4°C) and ambient conditions (25°C) for a period of six months. Samples were drawn monthly and analysed for quality parameters *viz.,* moisture (AOAC, 2012), pH using pH meter (ECPH S1042S, Eutech Instruments, Singapore), colour *viz.,* L^*, a^* and b^* using Lab colorimeter (Colorflex EZ 45/0, Hunter Associates Lab inc., USA), protein solubility (Morr *et al.,* 1985), thiobarbituric acid reactive substances, TBARS (Tarladgis *et al*., 1960), trimethylamine-nitroen (TMA-N) by Conway microdiffusion assay (Conway, 1950), sensory indices (carried out with ten trained panelists for indices *viz.,* appearance, odour, flavour, colour, texture and overall acceptability using a 9 point hedonic scale as per Meilgaard *et al*., 2006) and aerobic plate count (APC) by pour plate technique (USFDA, 2001).

Statistical analysis

All analyses were carried out in triplicate, except for sensory attributes (n = 10). Analysis of variance (ANOVA) was performed for the data collected and comparison of mean was done by Duncan's multiple range test considering level of significance at 5%. SPSS software (SPSS 16.0 for Windows, SPSS Inc., Chicago, USA) was used for all statistical analyses.

Results and discussion

Characteristics of tuna protein hydrolysate

Degree of hydrolysis (DH) and proteolytic activity

DH is an important parameter that facilitates understanding of the extent of protein hydrolysis to liberate a mixture of high and low molecular weight peptides and free amino acids. It is constructive in establishing the relationships between proteolysis and their associated functional, bioactive as well as sensory properties (Cheison *et al.,* 2009). DH of the optimised functional hydrolysate was observed to be 14.35±0.15% and the corresponding proteolytic activity, in terms of amount of tyrosine liberated was 0.329±0.003 µ moles of tyrosine per mg protein. The physicochemical conditions of the reaction determine the degree of hydrolysis as well as the molecular weight of the peptides which are contributors to the properties exhibited by the peptides (Ren *et al*., 2008). Haldar *et al*. (2018) reported that functional properties like foaming and emulsifying capacities are governed by their DH with better properties exhibited by larger peptides. In the current investigation too, major contribution was made by larger peptides (above 10 kDa) which can be related to superior functional characteristics of the tuna protein hydrolysate derived.

Protein recovery and yield

Recovery of protein, the compound of interest in hydrolytic process, determines the process efficacy. This is governed by various other factors including the physico-chemical conditions of the process. Protein recovery reported for the optimised hydrolysate sample was 39.64%. Parvathy *et al.* (2018b) also observed a protein recovery of 36.87% from tuna (*Euthynnus affinis*) red meat in its hydrolysate form. Adoption of optimised hydrolytic conditions followed by subsequent filtration and centrifugation promoted the concentration of desirable protein in the derived sample excluding

undesirable components like fat, minerals and pigments. Correspondingly a yield of 5.9% (w/v) was observed for tuna protein hydrolysate (TPH) from their respective solutions while it was about 6.9% (w/w) from the substrate. The yield obtained was also subjective to the amount of protein recovered into the solution which was further spray dried to powder. Generally, a yield of 3-15% (Gajanan *et al.,* 2016; Parvathy *et al.,* 2018a) is observed during hydrolysis from different substrates based on conditions adopted. In general, these lower yields are due to selective extraction followed by dehydration of the protein soluble fraction and further losses during spray drying operations.

Molecular weight

The size of the peptides derived upon hydrolysis determines its functional and bioactive properties which in turn is crucial for its effective utilisation (Li *et al*., 2013; Taheri *et al*., 2014). Analysis of the molecular weight profile of the derived hydrolysate revealed the heterogeneity of peptides in the sample. Furthermore, the pattern of molecular weight peptides positively correlated with the degree of hydrolysis. A distribution of 60% peptides above 10 kDa (>30 kDa - 10%; 10-30 kDa - 50%; 3-10 kDa - 35% and < 3 kDa - 5%) were observed upon fractionation. Studies conducted by Samsudin *et al*. (2018) reported eel protein hydrolysate to exhibit yields of 59.0%, 24.5% and 16.5% for 10 kDa, 5 kDa and 3 kDa fractions, respectively indicating higher amount of larger molecular weight peptides than lower molecular weight peptides. These differences in observations reported could be on account of the varying hydrolysis conditions adopted. Chi *et al*. (2014) in their studies reported a positive correlation between the peptide molecular weight and functional properties. Van der Ven *et al*. (2002) correlated the foam stability of casein hydrolysates to their molecular weight distribution, where a high proportion of peptides of molecular weight >7 kDa, was found to be positively related to foam stability.

Nutritional profile

Proximate composition

Hydrolysis facilitates selective extraction of proteins from the substrate by proper solubilisation yielding higher protein content in the derived hydrolysate. As the other superfluous components are removed during this process as well as subsequent centrifugation process, the final product will be concentrated form of protein ranging between 60-90% based on the process conditions adopted (Choi *et al*., 2009; Khantaphant *et al*., 2011). A protein content of 88.57±0.66% was observed in the tuna protein hydrolysate which demonstrated its potential use as protein supplement for human nutrition. A lower fat content of 0.49±0.09% was present, favouring desirable range for stable keeping quality. This low fat content of the resultant fish protein hydrolysates must be on account of the pre-treatment adopted for removal of fat from the parent source as well as removal of lipids with insoluble protein fractions during subsequent hydrolysis. As the hydrolysate was dehydrated by spray drying, the moisture content could be reduced to 7.59±0.18%. Previous studies also demonstrated that protein hydrolysates from various seafood sources contain moisture below 10% (Chalamaiah *et al*., 2010; Foh *et al*., 2011; Parvathy *et al*., 2016) which facilitates better handling as well as storage stability. The ash content of the protein hydrolysate was 2.42±0.08% indicating a lower value in comparison to the range *viz.,* 0.45 to 27% previously reported (Yin *et al*., 2010; Mazorra-Manzano *et al*., 2012).

Amino acid profile

Hydrolysates, composed of a mixture of long and short chain peptides as well as free amino acids exhibit many advantages as nutraceuticals or functional foods on account of their amino acid profile (Wiriyaphan *et al*., 2015). Present study revealed TPH to be rich in amino acids like glutamic acid, aspartic acid, lysine and leucine while amino acids *viz.,* phenyl alanine, tyrosine, methionine and cysteine were found to be in lower amounts (Table 1). Under the conditions of acid hydrolysis, tryptophan was destroyed and thus was not detected. Similar to the present results, aspartic acid and glutamic acid were the major amino acids in most of the reported studies on fish protein hydrolysates (Klompong *et al*., 2009; Yin *et al*., 2010; Ghassem *et al*., 2014). Sathivel *et al.* (2003) reported higher levels of glutamic acid, aspartic acid, lysine and leucine in hydrolysates prepared from whole herring as well as herring body. Reports by Gamarro *et al.* (2013) suggested that tuna red meat contained all the essential amino acids (EAA) contributing to about 49-52% of the total amino acids. The EAA value of 49.96% in the functional hydrolysate was well above the reference value of 40% recommended by WHO/FAO/UNU (2007). The present study also indicated a high essential amino acid/ non-essential amino acid ratio of 1.01 strengthening its suitability as a dietary protein supplement. Further, the hydrolysate had an extremely high content of flavour enhancers *viz.,* glutamic acid, aspartic acid, glycine and alanine (38.31% of the total amino acids) which recommends its role in a variety of food applications.

Functional properties

Effect of pH

Food and nutraceutical applications of protein hydrolysates in different forms such as liquid beverages and protein powder, demand the product to undergo

Table 1. Amino acid profile of tuna protein hydrolysate

Amino acids	Percentage of total amino acids
Essential amino acids	
Arginine	6.36
Histidine	4.88
Isoleucine	3.77
Leucine	8.23
Phenyl alanine	2.76
Threonine	4.40
Valine	5.73
Methionine	2.34
Lysine	9.42
Tyrosine	2.07
Total	49.96
Non-essential amino acids	
Alanine	6.86
Aspartic acid	9.70
Glycine	4.90
Glutamic acid	16.85
Proline	6.38
Serine	3.67
Cysteine	0.90
Total	49.26

varying pH conditions. Hence its stability under different pH settings needs to be comprehended for its effective incorporation. The foaming properties *viz.*, foaming capacity and foam stability at different pH *viz.,* 2, 4, 6, 8 and 10 indicated neutral pH (6.0) to be ideal for preserving the functional properties (Fig. 1). Deviation from the neutral to acidic and alkaline pH range exhibited lowering of these properties signifying the hydrolysate to have a well-ordered orientation under neutral conditions. Similar to the present study, Parvathy *et al.* (2016) reported foaming properties to be maximum at pH 6.0 in protein hydrolysate from yellowfin tuna waste and the properties were adversely affected by deviations in pH. Studies by Samsudin *et al.* (2018) also reported the foaming stability of fractionated eel protein hydrolysate to be highest at pH 6. Klompong *et al.* (2007) reported maximum foaming capacity for yellow stripe trevally hydrolysate at pH 6 with a slight decrease at alkaline pH using alcalase. The lowering of foaming properties of proteins at acidic conditions can be coincided with the lowest solubility at or near their isoelectric pH of 4.0.

Variations in emulsifying properties in terms of, EAI indicated a linear increase in the values with pH from 46.43±1.74 $m^2 g^{-1}$ at pH 2.0 to 162.01± 2.43 $m^2 g^{-1}$ at pH 6.0;, thereafter showing a reduced rate of increase, reaching 192.78±7.56 $m^2 g^{-1}$ at pH 10.0 (Fig. 2). However, ESI was highest (48.09±2.69 min) at a pH of 6.0 and was lower towards the extremes of pH, more decrease noted at a pH of 2.0 (20.1±1.66 min). Samsudin *et al.* (2018) in their studies reported emulsifying activity index of fractionated eel protein hydrolysate to increase with increase in pH from 2 to 10. Similarly, Cho *et al.* (2014) reported a decrease in EAI of protein hydrolysate from egg white with decrease in pH. Solubility was lowest at pH 4 affecting the movement of peptides rapidly to the interface and their net charge was minimised. Reports by Taheri *et al.* (2013) indicated the presence of negatively charged peptides under highly alkaline conditions leading to more polypeptide cleavage resulting in more hydrophilic and hydrophobic peptide residues being exposed during emulsion, promoting significant interactions at the oil-water interface and stabilisation of the emulsion.

Effect of concentration

Functional properties of optimised tuna protein hydrolysate exhibited a proportional increase in foaming properties (foaming capacity and foam stability) as well as emulsifying properties (EAI and ESI) with protein concentration. Foaming capacity of the hydrolysate varied from 90±10% for the lowest concentration of 0.1% to 170±10% (3% protein concentration) (Fig. 3). The rate of increase in foaming capacity was higher initially following a linear pattern (from 0.1% to 0.5% protein

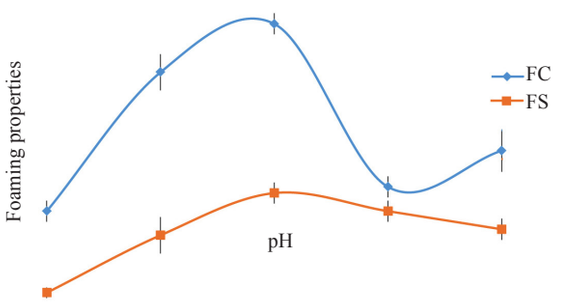

Fig. 1. Foaming properties of tuna protein hydrolysate (TPH) at varying pH conditions

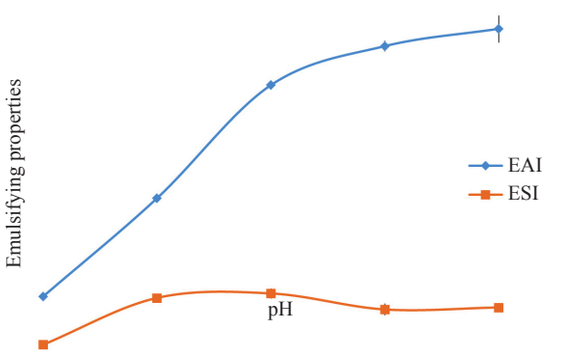

Fig. 2. Emulsifying properties of tuna protein hydrolysate (TPH) at varying pH levels

concentration). However, the rate of increase was affected with further increase in concentration. Similarly, a linear increase in foam stability with protein concentration from 0.1 to 1.0% was observed with further stagnation up to 3%. Foam stability varied from 16.7±2.9% for 0.1% protein concentration to 140±10% for 3% concentration (Fig. 3). Studies conducted by Salem *et al.* (2017) in octopus protein hydrolysate reported an increase in foaming properties with increase in protein concentration from 0.5 to 2%.

Emulsifying properties *viz.,* EAI exhibited direct and linear relation with protein concentration (R^2 = 0.960). It increased from 104.46±3.39 m^2 g^{-1} for 0.1% protein concentration to 200.76±8.43 m^2 g^{-1} for 3% protein concentration. Similarly, ESI ranged linearly from 28±1.63 min (0.1%) to 43.46±0.35 min (3%) with an R^2 of 0.916 (Fig. 4). The N-terminals of hydrophobic amino acids bind with oil and form emulsion at the air-water interface. As observed in the amino acid pattern, the relatively high amount of hydrophobic amino acids in the derived peptide resulted in a higher emulsifying activity index. The emulsifying properties of TPH could be explained by the fact that hydrolysis liberated high and medium molecular weight peptides from the native protein, which enhanced the flexibility of the peptides at the oil/water interface, resulting in a larger surface area and consequently, greater emulsion formation.

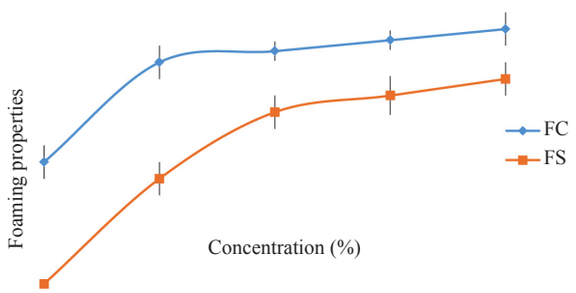

Fig. 3. Foaming properties of tuna protein hydrolysate (TPH) at varying concentrations

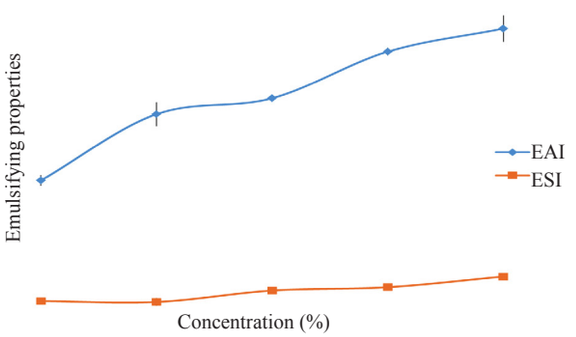

Fig. 4. Emulsifying properties of tuna protein hydrolysate (TPH) at varying concentrations

Storage stability studies

Moisture

Moisture content is a major parameter determining the product stability as it influences other physico-chemical attributes. The hygroscopic nature of protein hydrolysate leads to moisture absorption on exposure to unfavourable conditions resulting in rapid physical and chemical changes. In the present study, an increase in moisture content was observed in samples under both chilled and ambient conditions. It increased from an initial value of 5.82±0.42% to 7.35±0.08% (chilled storage) and 9.01±0.24% (ambient storage) during the six months storage period. During the initial period, the variations in moisture content were not significant but towards the fourth month, a significant difference was observed ($p<0.05$). Between the storage period also, there was no significant difference initially for three months of storage (Table 2). However, the increase was more prominent under ambient conditions suggesting further possibilities of related physico-chemical reactions to occur.

pH

In the present study, pH of the hydrolysate samples showed a slight increase ranging from 5.75-6.01 under ambient temperature while it ranged between 5.75 to 5.92 under chilled storage which were not significant (Table 2). pH variations during sample storage is widely assessed for determining its stability. Previously, studies conducted by Klompong *et al.* (2012) reported no marked changes in pH of yellow stripe trevally protein hydrolysate stored for a period of 12 weeks at room temperature.

Colour

Colour variations in the hydrolysate during two storage temperatures *viz.,* ambient as well as chilled storage indicated the changes to be more prominent during ambient conditions (Table 2). Lightness values decreased while a proportional increase in redness as well as yellowness was observed ($p<0.05$). Similar to the present observations, Hoyle and Merritt (1994) reported herring hydrolysates to exhibit decrease in lightness and increase in yellowness indicating sample darkening during storage. Reports by Klompong *et al.* (2012) also indicated trevally protein hydrolysate to exhibit a slight decrease in lightness whereas redness and yellowness gradually increased during storage. These colour variations must be associated with non-enzymatic browning reaction during storage and these changes are reported to be dependent on storage temperature as well as relative humidity (Rao and Labuza, 2012; Rao *et al*., 2012). In addition, the oxidation

Table 2. Variations in parameters of tuna protein hydrolysate (TPH) at ambient (28°C) and chilled conditions (4°C)

Parameters	Storage period (months)	Ambient condition (25°C)	Chilled condition (4°C)
Moisture (%)	0	$5.82^{dA} \pm 0.42$	$5.82^{cA} \pm 0.42$
	1	$6.11^{cdA} \pm 0.20$	$5.79^{cA} \pm 0.21$
	2	$6.21^{cdA} \pm 0.26$	$5.98^{cA} \pm 0.18$
	3	$6.54^{cA} \pm 0.54$	$6.01^{cA} \pm 0.14$
	4	$7.89^{bA} \pm 0.32$	$6.33^{bcB} \pm 0.04$
	5	$7.72^{bA} \pm 0.49$	$6.65^{bB} \pm 0.14$
	6	$9.01^{aA} \pm 0.24$	$7.35^{aB} \pm 0.08$
pH	0	$5.75^{aA} \pm 0.02$	$5.75^{aA} \pm 0.02$
	1	$5.92^{aA} \pm 0.02$	$5.78^{aA} \pm 0.05$
	2	$5.89^{aA} \pm 0.03$	$5.81^{aA} \pm 0.02$
	3	$5.85^{aA} \pm 0.03$	$5.79^{aA} \pm 0.02$
	4	$5.90^{aA} \pm 0.04$	$5.84^{aA} \pm 0.01$
	5	$5.92^{aA} \pm 0.02$	$5.91^{aA} \pm 0.03$
	6	$6.01^{aA} \pm 0.04$	$5.92^{aA} \pm 0.03$
Colour L*	0	$89.12^{aA} \pm 0.30$	$89.12^{aA} \pm 0.30$
	1	$86.55^{bB} \pm 0.45$	$87.84^{bA} \pm 0.57$
	2	$84.54^{cB} \pm 0.13$	$85.41^{cA} \pm 0.19$
	3	$83.40^{dB} \pm 0.56$	$85.30^{cA} \pm 0.37$
	4	$82.51^{eB} \pm 0.40$	$84.82^{cdA} \pm 0.12$
	5	$81.03^{fB} \pm 0.50$	$84.12^{deA} \pm 0.12$
	6	$79.72^{gB} \pm 0.80$	$84.00^{eA} \pm 0.33$
a*	0	$0.14^{gA} \pm 0.05$	$0.14^{fA} \pm 0.05$
	1	$1.59^{fA} \pm 0.22$	$1.04^{eB} \pm 0.06$
	2	$1.93^{eA} \pm 0.06$	$1.59^{dB} \pm 0.08$
	3	$2.11^{dA} \pm 0.06$	$1.65^{dB} \pm 0.10$
	4	$2.57^{cA} \pm 0.05$	$1.81^{cB} \pm 0.03$
	5	$3.08^{bA} \pm 0.03$	$2.09^{bB} \pm 0.08$
	6	$3.31^{aA} \pm 0.06$	$2.26^{aB} \pm 0.04$
b*	0	$16.41^{gA} \pm 0.09$	$16.41^{eA} \pm 0.09$
	1	$20.73^{fA} \pm 0.10$	$18.67^{dB} \pm 0.11$
	2	$21.27^{eA} \pm 0.04$	$18.89^{dB} \pm 0.06$
	3	$22.63^{dA} \pm 0.26$	$19.95^{cB} \pm 0.05$
	4	$23.49^{cA} \pm 0.27$	$20.04^{cB} \pm 0.20$
	5	$24.83^{bA} \pm 0.09$	$21.24^{bB} \pm 0.30$
	6	$25.38^{aA} \pm 0.04$	$21.75^{aB} \pm 0.21$
Solubility (%)	0	$88.30^{aA} \pm 0.80$	$88.30^{aA} \pm 0.80$
	1	$85.97^{aA} \pm 1.33$	$87.03^{abA} \pm 1.16$
	2	$80.37^{bB} \pm 1.02$	$84.80^{bA} \pm 0.56$
	3	$76.30^{cB} \pm 1.90$	$81.97^{cA} \pm 2.04$
	4	$73.27^{dB} \pm 1.63$	$81.67^{cA} \pm 1.29$
	5	$67.90^{eB} \pm 2.57$	$76.33^{dA} \pm 1.16$
	6	$61.07^{fB} \pm 0.49$	$69.50^{eA} \pm 0.85$
TBARS (mg malonaldehyde kg^{-1})	0	$0.89^{dA} \pm 0.05$	$0.89^{bA} \pm 0.05$
	1	$1.77^{cdA} \pm 0.11$	$1.05^{bA} \pm 0.12$
	2	$2.19^{cA} \pm 0.07$	$1.73^{abA} \pm 0.06$
	3	$2.34^{bcA} \pm 0.06$	$2.23^{aA} \pm 0.07$
	4	$2.78^{abcA} \pm 0.06$	$1.89^{abA} \pm 0.07$
	5	$3.35^{abA} \pm 0.06$	$2.54^{aA} \pm 0.14$
	6	$3.63^{aA} \pm 0.08$	$2.25^{aB} \pm 0.09$

Contd.....

Parameters	Storage period (months)	Ambient condition (25°C)	Chilled condition (4°C)
TMA-N (mg%)	0	$7.00^{eA} \pm 0.00$	$7.00^{cA} \pm 0.00$
	1	$10.50^{eA} \pm 4.95$	$7.00^{cA} \pm 0.00$
	2	$14.00^{deA} \pm 0.00$	$7.00^{cA} \pm 0.00$
	3	$21.00^{cdA} \pm 9.90$	$10.50^{bcB} \pm 4.95$
	4	$28.00^{bcA} \pm 0.00$	$14.00^{abcB} \pm 0.00$
	5	$35.00^{bA} \pm 0.00$	$14.00^{abB} \pm 0.00$
	6	$49.00^{aA} \pm 0.00$	$21.00^{aB} \pm 0.00$
Sensory indices	0	$7.00^{aA} \pm 0.47$	$7.00^{aA} \pm 0.47$
	1	$6.00^{bB} \pm 0.47$	$7.00^{aA} \pm 0.67$
	2	$5.00^{cB} \pm 0.47$	$7.00^{aA} \pm 0.53$
	3	$4.00^{dB} \pm 0.24$	$6.00^{bA} \pm 0.82$
	4	$3.00^{eB} \pm 0.47$	$6.00^{bA} \pm 0.47$
	5	$3.00^{eB} \pm 0.82$	$5.00^{cA} \pm 0.67$
	6	$2.00^{fB} \pm 1.16$	$5.00^{cA} \pm 0.82$
TPC (log cfu g^{-1})	0	$4.40^{dA} \pm 0.20$	$4.40^{cdA} \pm 0.20$
	1	$4.50^{dA} \pm 0.30$	$4.40^{cdA} \pm 0.10$
	2	$4.70^{cdA} \pm 0.17$	$4.10^{dB} \pm 0.27$
	3	$5.00^{bcA} \pm 0.17$	$4.90^{aA} \pm 0.17$
	4	$5.00^{bcA} \pm 0.27$	$4.50^{bcB} \pm 0.17$
	5	$5.10^{abA} \pm 0.17$	$4.80^{abA} \pm 0.27$
	6	$5.40^{aA} \pm 0.17$	$5.10^{aA} \pm 0.17$

Values are expressed as Mean ± SD; n = 3 and n = 10 (sensory score); Different superscripts in the same column indicate significant differences (a>b>c>d; $p<0.05$)
Different superscripts in the same row indicate significant differences (A>B; $p<0.05$)

of myoglobin and melanin pigment in the hydrolysate sample must have led to colour variations. Previously, Thiansilakul *et al.* (2007) observed intensification of yellowness in protein hydrolysates during storage, which was more pronounced at 25°C than at 4°C.

Solubility

Hydrolysate samples indicated a significant decrease ($p<0.05$) in protein solubility during storage, more distinct under ambient conditions in comparison to chilled storage. This resulted in significant variations between samples ($p<0.05$) during storage, from the second month of storage (Table 2). The present solubility results were in concurrence with the reports by Thiansilakul *et al.* (2007) who also observed decrease in solubility of round scad protein hydrolysates during storage. This decrease in solubility must be associated with aggregation of peptides which undergo irreversible covalent bonding affecting the other functional properties as well (Thiansilakul *et al*., 2007; Weiss *et al*., 2009).

TBARS

Variations in TBARS during storage of hydrolysate samples showed a rise in the values and oxidation was more pronounced and significant ($p<0.05$) at ambient temperature. The initial TBARS was 0.89 mg malonaldehyde kg^{-1} which crossed the acceptability limit of 2 mg malonaldehyde kg^{-1} (Connell, 1990) in the second month (2.19 mg malonaldehyde kg^{-1}) under ambient temperature, while it was extended to three months reaching a value of 2.23 mg malonaldehyde kg^{-1} when stored under chilled conditions (Table 2). In concurrence to these results, Klompong *et al*. (2012) observed a rise in TBARS values in yellow stripe trevally protein hydrolysate stored at room temperature during a storage period of 12 weeks. They further reported higher lipid oxidation possibilities in dried samples on account of the enhanced exposure of lipids present in the sample, to oxygen at a low water level. Previous studies have also reported that fish protein hydrolysates are prone to oxidation on account of high content of unsaturated fatty acids (Sohn *et al*., 2005; Yarnpakdee *et al*., 2012; Rao *et al*., 2016).

TMA-N

Similar to the trend observed in the other quality parameters during storage, variations in TMA-N were more prominent during ambient temperature in comparison to chilled conditions resulting in significant difference between the samples ($p<0.05$) from third month of storage. During chilled storage, the variations were limited and between the samples, a significant difference ($p<0.05$) was observed from third month onwards. The acceptability limit of 15 mg% (Lakshmanan, 2000) was crossed during

different storage periods. TMA-N increased prominently at ambient temperature, crossed the acceptability limit and attained a value of 21 mg% by third month while it was extended to 6 months (21 mg%) on storage under chilled conditions.

Sensory indices

Sensory evaluation is one of the significant quality attribute determining the storage stability of a commodity. There was a significant reduction in sensory score for TPH stored under ambient conditions ($p<0.05$) whereas the reduction was not significant for initial two months under chilled conditions (Table 2). On account of this, a significant difference in the sensory score was noticed between samples stored under the different storage conditions from first month onwards. Results indicated an acceptability period of two months and five months for the samples stored under ambient and chilled conditions, respectively.

Aerobic plate count

During storage, a significant difference ($p<0.05$) was observed in the microbial count of TPH. It increased from an initial value of 4.40±0.20 log cfu g^{-1} to 5.40±0.17 log cfu g^{-1} and 5.10±0.17 log cfu g^{-1} for samples stored under ambient and chilled conditions, respectively (Table 2). In general, an increase by one log cycle was observed for the samples during storage. However, the samples were within the microbial acceptable limit of 7 log cycle (ICMSF, 1998) throughout the period. The proportional increase in microbial index can be related to the role played by water activity (aw), which was evident from the variations in moisture upon storage, contributing to favourable conditions for the microbial activity.

Present study attempted to characterise the optimised functional hydrolysate from yellowfin tuna cannery waste. Molecular weight profile of the tuna protein hydrolysate derived in the present study indicated major contribution of peptides above 10 kDa (60%). Protein recovery of 39.64% was observed on hydrolysis and a protein content of 88.57±0.66% with balanced amino acid profile was obtained for the TPH. Storage studies carried out revealed a stability for up to two months under ambient conditions whereas under chilled conditions, it was stable for up to five months. Current study indicated the superior nutritional as well as functional attributes of the TPH clearly indicating its suitability for application in different foods.

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