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Peptides from white and red meat of yellowfin tuna (*Thunnus albacares*): A comparative evaluation

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ABSTRACT

Dark muscle from yellowfin tuna is rich in proteins and is an important byproduct from tuna cannery. However, it has a low market realisation and is currently utilised for preparation of fertilisers and animal feeds. Recovery and utilisation of this biomass to bioactive protein hydrolysate is a promising alternative as it facilitates food and pharmaceutical applications. However, the extent to which the properties of the hydrolysate vary in red meat derived-hydrolysate is less investigated. Hence the current study was aimed at exploring the characteristics of protein hydrolysates derived from red meat of yellowfin tuna (*Thunnus albacares*) in comparison to its white meat. Protein hydrolysate was prepared using 1% (w/w) papain for one hour at optimised temperature of 60°C and pH of 6.5 from white and red meat of yellowfin tuna to derive tuna white meat protein hydrolysate (TWPH) and tuna red meat protein hydrolysate (TRPH), respectively. Nutritional evaluation of tuna meat indicated comparable protein content in both white and red portions with a value of 25.99±0.24% and 24.03±0.11%, respectively and a recovery of 50.34% from white meat and 44.67% from red meat protein to their hydrolysate forms was observed. Comparative evaluation of the functional as well as bioactive properties of hydrolysates from white and red meat of tuna indicated better antioxidative activity for TWPH. However, except oil absorption capacity (OAC), functional properties *viz*., protein solubility, foaming capacity and emulsifying properties were higher for TRPH. Present study explores the application potential of tuna red meat to its bioactive peptides for food and pharmaceutical sectors.

Keywords: Antioxidative properties, Functional properties, Papain, Tuna protein hydrolysate, Yellowfin tuna

Introduction

Tuna resources are considered to be significant sources of seafood and are largely exploited on account of their increased global demand for thermally processed delicacies. However, tuna market mainly utilises the white meat thus resulting in the underutilisation of protein rich byproducts *viz*., red meat, head, skin, trimmings and viscera, that are discarded without recovery attempts and accounts for more than 60% of biomass (Chalamaiah *et al*., 2012). Of these, 10-12% is the dark meat portion which has nutrients especially proteins of high quality comparable to that of the white meat (Nishioka *et al*., 2007). Hence utilisation of these dark meat proteins is a serious matter to be addressed on account of the limited food resources for meeting nutritional security as well as increasing environmental pollution issues.

Proper exploitation of these nutrient rich fish processing discards could be achieved by enzymatic conversion of these protein sources into its hydrolysates, facilitating its effective utilisation. Protein hydrolysates are the breakdown products of proteins *viz*., smaller peptide chains with 2-20 amino acids obtained by hydrolysis either chemically or enzymatically. This process facilitates recovery of essential nutrients *viz*., amino acids and has immense scope in food, nutraceutical and pharmaceutical industry on account of the excellent physicochemical, functional as well as bioactive properties (He *et al*., 2013; Halim *et al*., 2016). Hence with increasing knowledge of these advantages, more research is being focused on the development of fish-derived functional and nutraceutical foods. The present study focused on deriving protein hydrolysates from white and red meat of yellowfin tuna (*Thunnus albacares*) under similar hydrolytic conditions using papain, to comparatively evaluate the characteristics of the derived hydrolysate for their further application potentials.

Materials and methods

Fish, enzyme and chemicals

Fresh yellowfin tuna (*T. albacares*) was purchased from fish landing centre at Mumbai and brought to the laboratory in iced condition. The white and red meat of tuna was separated and used as raw material for the

preparation of tuna white meat protein hydrolysate (TWPH) and tuna red meat protein hydrolysate (TRPH), respectively. Enzymatic hydrolysis was carried out using papain (Hi Media, India) obtained from papaya latex (proteolytic activity ≥ 4.5 ml of 0.1M NaOH). All the reagents used for the study were of analytical grade.

Preparation of tuna protein hydrolysate

The separated white and red meat of tuna were comminuted thoroughly using a kitchen blender and added with twice the amount water to get fine slurry of meat which was further subjected to 80-90°C for 30 min for complete endogenous enzyme inactivation. Further enzymatic hydrolysis was performed in a shaking water bath (Neolab Instruments, Mumbai, India) maintained at 60°C, employing papain at physiological pH (6.5). Enzyme:substrate (E/S) ratio and duration of hydrolysis were maintained at 1.0% and 60 min, respectively. Termination of hydrolysis was brought out by raising the process temperature to 80-90°C for 15-20 min and the resultant solution was course filtered and centrifuged (K-24A, Remi Instruments, Mumbai) at 8000 *g* at 10°C for 20 min to obtain protein hydrolysate solution which was further spray dried (Hemaraj Enterprises, Mumbai) and subjected to quality analysis.

Protein content and protein recovery

Analysis of the protein content of tuna meat and hydrolysates were done as per AOAC (2012) adopting micro-Kjeldahl method. The protein recovery in hydrolysate was estimated as the percentage of protein recovered in hydrolysate to the total amount of protein in raw material.

Degree of hydrolysis and proteolytic activity

Degree of hydrolysis (DH) was determined as the percentage of α-amino nitrogen in hydrolysates, determined by formol titration method (Taylor, 1957) to the total nitrogen content in the raw material (AOAC, 2012).

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

Yield

Yield of fish protein hydrolysate (%) was calculated as the quantity of hydrolysate powder obtained after spray drying from the amount of raw material used for hydrolysis.

Colour and browning intensity

Hunter Lab colourimeter (Colorflex EZ 45/0, Hunter Associates Lab inc., Reston, Virginia, USA) was employed to analyse the colour of the sample (1% hydrolysate solution) *viz*., L^* (the degree of lightness), a^* [redness(+)/ greenness (-)] and b^* [yellowness (+) or blueness (-)].

The browning intensity of hydrolysate samples were determined by measuring the absorbance of filtered samples (80 mg ml^{-1}) spectrophotometrically (Lambda 25 UV/Vis, Perkin Elmer Life and Analytical Sciences, Singapore) at 420 nm.

Ultraviolet absorption spectra

UV absorption spectra of the samples (2 mg ml^{-1}), in the wavelength range of 200-330 nm and scan speed of 2 nm sec^{-1} were determined using UV-VIS spectrophotometer (Lambda 25 UV/Vis, Perkin Elmer Life and Analytical Sciences, Singapore) (Elavarasan and Shamasundar, 2016).

Functional properties

Protein solubility

Hydrolysate samples (10 mg ml^{-1}, 20 ml) was well dispersed in distilled water by stirring at room temperature for 30 min followed by centrifuging at 7500 *g* for 15 min to collect the supernatant. Protein solubility was calculated as percentage of total protein in supernatant to the total protein in sample (Morr *et al*., 1985).

Foaming properties

Foaming properties of the protein solution were determined by the methodology of Sathe and Salunkhe (1981). A known volume of protein solution (1%) was homogenised (230 VAC T-25 digital Ultra-turrax, IKA, India) at 16,000 rpm for 2 min at ambient temperature and the whipped sample was immediately transferred to a measuring cylinder and the volume read immediately and after 3 min to calculate the properties as:

$$\text{Foaming capacity/Stability \%} = \frac{(A-B)}{B} \times 100$$

where, A = foam volume after whipping to determine foaming capacity; foam volume after standing for 3 min (foam stability) and B = volume before whipping.

Emulsifying properties

A mixture containing oil (10 ml) and hydrolysate solution (1%, 30 ml) were thoroughly homogenised (230 VAC T-25 digital Ultra-turrax, IKA, India) at 20,000 rpm for 1 min. An aliquot of the emulsion (50 µl) pipetted from

the bottom of the container at 0 and 10 min were mixed with 0.1% sodium dodecyl sulphate solution (5 ml) to read the absorbance at 500 nm (Lambda 25 UV/Vis, Perkin Elmer Life and Analytical Sciences, Singapore) immediately (A_0) and 10 min (A_{10}) after emulsion formation (Pearce and Kinsella, 1978):

$$\text{EAI (Emulsion Activity Index) } (m^2\ g^{-1}) = \frac{2 \times 2.303 \times A0}{0.25 \times \text{wt of protein}}$$

$$\text{ESI (Emulsion stability index) (min)} = \frac{A_{10} \times \Delta t}{\Delta A}$$

Oil absorption capacity (OAC)

Hydrolysate sample and sunflower oil [1: 20 (w/v)] taken in a pre-weighed centrifuge tube was vortexed (Expo Hitech, India) for 30 s, centrifuged (K-24A, Remi Instruments, Mumbai) at 2800 *g* for 25 min and the supernatant was drained off (Shahidi *et al*., 1995). OAC was calculated as weight of oil held per gram of the sample (g oil g^{-1} sample).

Sensory properties

The sample acceptability was assessed using nine point hedonic scale as per the methodology by Normah *et al.* (2013). Rawa porridge, used as carrier for the study, was mixed with 1% [5:1 (w/v)] hydrolysate solutions and evaluated for attributes *viz*., colour, odour, taste and overall acceptability. The sample bitterness was scored using 10 point scale designed with 01 as 'no bitterness' to 10 indicating 'extreme bitterness' (Nilsang *et al*., 2005). The scale of bitterness was anchored using standard caffeine solution as the reference.

Antioxidative properties

DPPH radical scavenging activity

Equi-quantity of 0.1 mm DPPH in 95 % ethanol and sample solution (2 mg protein ml^{-1}) was vortexed for 10 min and incubated in the dark for 30 min. Absorbance of the resultant solution was recorded at 517 nm (Lambda 25 UV/Vis, Perkin Elmer Life and Analytical Sciences, Singapore). Sample solution and ethanol (1:1 v/v) served as sample blank and DPPH with distilled water (1:1v/v) was used as control (Shimada *et al*., 1992):

$$\text{DPPH (\%)} = \frac{[\text{Absorbance control} - (\text{Absorbance of sample} - \text{Absorbance of sample blank})]}{\text{Absorbance of control}} \times 100$$

Reducing power

A premix containing known concentration of hydrolysate sample (10 mg ml^{-1}), 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide (1:5:5) was incubated at 50°C for 20 min. Further, an aliquot (2.5 ml) of 10% trichloroacetic acid was added to the mixture, centrifuged at 3000 rpm (K-24A, Remi Instruments, Mumbai) for 10 min and 2.5 ml of supernatant mixed with equal volume of distilled water and 0.1% ferric chloride to read the absorbance at 700 nm (Lambda 25 UV/Vis, Perkin Elmer Life and Analytical Sciences, Singapore) (Oyaiza, 1986).

Ferric reducing antioxidant power (FRAP)

FRAP solution was prepared freshly by incubating a premix containing 300 mm acetate buffer (pH 3.6) (25 ml), 10 mm TPTZ (2,4,6-tripyridyl-s-triazine) solution (2.5 ml) in 40 mm HCl and 2.5 ml of 20 mm $FeCl_3$. $6H_2O$ solution at 37°C for 30 min and 2850 µl of this FRAP solution was mixed with 150 µl of 10 mg ml^{-1} sample and incubated in dark for 30 min to form ferrous tripyridyltriazine complex which was measured for absorbance at 593 nm (Lambda 25 UV/Vis, Perkin Elmer Life and Analytical Sciences, Singapore) (Benzie and Strain, 1996). Ascorbic acid standard curve for FRAP was derived to measure the FRAP activity in terms of mM ascorbic acid (mM AA) equivalents.

Metal chelating activity

One ml of known concentration of sample solution was mixed with 3.7 ml of distilled water and to this diluted solution, 2 mm $FeCl_2$ (0.1 ml) and 5 mm 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine) (0.2 ml) was added, incubated at ambient conditions for 20 min and the absorbance was read at 562 nm (Lambda 25 UV/Vis, Perkin Elmer Life and Analytical Sciences, Singapore). Control was prepared in the same manner with distilled water instead of sample (Decker and Welch, 1990) and metal chelating activity was calculated as:

$$\text{MC (\%)} = \frac{1 - (\text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

ABTS radical [2,20 -azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] scavenging activity

The modified method of Re *et al*. (1999) was followed to determine ABTS radical scavenging activity of the sample. The stock solution of ABTS radical stock solution consisting of 7 mm ABTS in 2.45 mm potassium persulfate, was pre-incubated in dark for 16 h at ambient temperature. The working solution of ABTS radical with absorbance of 0.70±0.02 at 734 nm was prepared and mixed with 20 µl of sample (10 mg ml^{-1}) or distilled water (in control) and the reduction in absorbance was noted (Lambda 25 UV/Vis, Perkin Elmer Life and Analytical Sciences, Singapore) after incubation at 37°C for 10 min in dark.

$$\text{ABTS radical scavenging activity} = \frac{(1\text{-Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Statistical interpretation

All experiments done in triplicate were subjected to analysis of variance (ANOVA) and the differences between means were evaluated by Duncan's multiple range test. SPSS programme (SPSS 16.0 for Windows, SPSS Inc., Chicago, IL,) was used for data analysis.

Results and discussion

Protein content and protein recovery

Comprehending the nutritional composition of food is useful in streamlining the processing possibilities as well as for enhancing their utility for various food and pharmaceutical applications. Fish byproducts like tuna red meat are rich in nutrients like protein which can be diverted to high value products, thus enhancing their economic value (Herpandi *et al*., 2011). Analysis of protein content in tuna white meat and red meat revealed a value of 25.99±0.24 and 24.03±0.11%, respectively which got concentrated to 84.4±2.35 and 85.87±0.91%, on conversion to their respective protein hydrolysates *viz*., TWPH and TRPH. Enzymatic hydrolysis of parent protein facilitates their selective extraction by proper solubilisation yielding higher protein content in the derived hydrolysate. Several reports suggest a protein content range of 60-90% of total composition in hydrolysates derived from different seafood substrates (Choi *et al.,* 2009; Khantaphant *et al.,* 2011). Recovery of protein is a major determinant factor that indicates the efficiency of a hydrolysis process and the current study indicated a recovery of 50.34 and 44.67%, respectively in TWPH and TRPH from their parent substrates. Higher protein recovery was observed from tuna white meat than their counterparts, to their respective hydrolysates. He *et al.* (2013) reported that the extent of hydrolysis, influenced by factors like protein substrate used, type and amount of enzymes used and hydrolysis period, to have a major influence in the recovery of protein. More breakage of peptide bonds occur during hydrolysis thus releasing low molecular weight protein hydrolysates which are more water soluble, thereby increasing the protein content in the resultant hydrolysate solution. Similarly serial enzymatic hydrolysis of parent protein can also facilitate higher protein extraction from the substrate (Binsi *et al.,* 2016).

Degree of hydrolysis (DH) and proteolytic activity

The extent of protein hydrolysis can be determined by measuring the degree of hydrolysis (DH) which in turn is clearly one of the most important variables that influences the attributes exhibited by the protein hydrolysates (Himonides *et al*., 2011). Under similar hydrolysis condition, except for variations in substrate used, a DH of 15.48±0.17 and 15.69±0.21% was observed for TWPH and TRPH, respectively after 60 min hydrolysis time, which was not significantly different ($p>0.05$).The amount of tyrosine liberated during hydrolysis on account of the peptide breakage is indicative of the extent of hydrolysis and it was observed that the proteolytic activity was in well proportion with the DH values of 0.274±0.011 and 0.274±0.007 for TWPH and TRPH, respectively. Gajanan (2014) in her studies reported the proteolyic activity to be increasing with hydrolysis in threadfin bream frame waste hydrolysate.

Yield

Product yield, a determinant variable for economic and sustainable process depends on various factors like DH and drying method adopted. The present study revealed a comparable yield of 6.1 and 6.0%, for spray-dried TWPH and TRPH, respectively. Lower yields are generally reported for spray-dried hydrolysates as only the soluble fractions are dried as well as on account of the solid losses occurring in the instrument during operation. Liceaga-Gesualdo and Li-Chan (1999) reported a yield of about 6.6% in freeze dried herring fish protein hydrolysate. Gajanan *et al*. (2016) reported a hydrolysate yield ranging from 4.6 to 9.7% from threadfin bream frame waste for DH ranging from 5-15%.

Colour and browning intensity

Colour and appearance of the product are aesthetic attributes which influence the consumer acceptability and they are in turn influenced by other factors like substrate type, hydrolysis conditions and the drying methods adopted. The current work indicated that TRPH was darker in comparison to its counterparts mainly on account of the raw material compositional variations. The L^*, a^* and b^* value of tuna white and red meat were 37.54±0.11, 9.71±0.16, 16.85±0.37 and 22.46±0.25, 10.37±0.05, 14.82±0.09, respectively indicating significant difference ($p<0.05$). These variations were noticeable in the derived hydrolysate solutions too with L^*, a^* and b^* values of 2.58±0.05, -0.1±0.04, -0.03±0.01, respectively for TWPH and 2.69±0.06, 0.83±0.03, 2.14±0.15, respectively for TRPH. Parvathy *et al*. (2016) have also reported these colour variations in hydrolysate due to the compositional variation of the raw material used. Similar to the colour values which indicated a higher redness and yellowness in TRPH solutions, browning intensity values also showed a significantly higher ($p<0.05$) value of 0.35±0.12 for hydrolysate derived from tuna red meat than their counterpart which exhibited a value of 0.115±0.001. The presence of pigments like myoglobin and melanin in the red meat in comparison to white meat and their oxidation must have resulted in this variation in the derived hydrolysates (Jemil *et al.,* 2014).

UV absorption spectra

The absorption spectra of tuna protein hydrolysates in the UV region (Fig. 1) indicated nearly identical absorption pattern with maximum absorbance at 200-230 nm. A rapid decrease in the absorption was observed from 230 to 240 nm which was further followed by a gradual decrease in wavelength ranging from 240-300 nm. Reports suggest peptides to absorb wavelength in spectra ranging from 180-230 nm and aromatic side chains of tyrosine, tryptophan and phenyl alanine to have absorption affinity in range of 270-280 nm (Elavarasan and Shamasundar, 2016). The present study indicated absence of aromatic side chains in the derived peptides. Further there were only slight variations in the spectral properties of the hydrolysate with tuna white meat hydrolysate indicating a comparatively higher UV absorption pattern in the wave length range of 230-280 nm than red meat hydrolysate which must be on account of the raw material compositional variations.

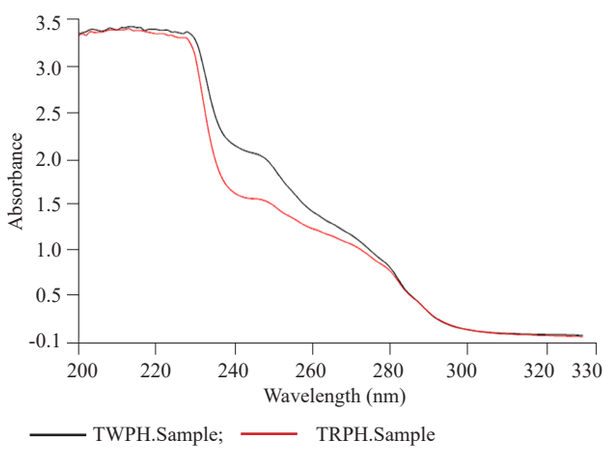

Fig. 1. UV absorption spectra of protein hydrolysate from white and red meat of yellowfin tuna

Protein solubility

Protein solubility is one of the main physico-chemical property of protein hydrolysate as it influences the other functional properties exhibited by the hydrolysate (Jemil *et al*., 2014). The present study revealed a fairly high protein solubility of 86.53±0.73 and 88.74±0.53%, respectively for TWPH and TRPH (Fig. 2a). Geirsdottir *et al.* (2011) observed a drastic increase in the solubility pattern, indicating a solubility of 15% in unhydrolysed to 70% in hydrolysed fish protein from blue whiting. Enzymatic hydrolysis of proteins facilitate an increased exposure of hydrophilic polar groups thereby releasing more water soluble peptides into the solution which facilitate increased solubility in comparison to the intact protein.

Foaming properties

Proteins in dispersions cause a lowering of the surface tension at the water/air interface, thus creating foam. Protein hydrolysis results in the exposure of more of hydrophobic residues facilitating enhanced foaming properties. Foaming properties *viz*., foaming capacity and foam stability of tuna protein hydrolysates were determined (Fig. 2b). Foaming capacity of TWPH and TRPH were 126.7±5.8 and 150±10%, respectively indicating significant difference ($p<0.05$) whereas comparable foaming stability was observed after 3 min, being 40±10 and 36.7±5.8%, respectively. The properties exhibited by the hydrolysate cannot be explained with respect to the extent of hydrolysis as the source of raw material used were different which also have influence on the resultant hydrolysate functionalities. Sanchez-Zapata *et al.* (2011) have reported variations to occur in

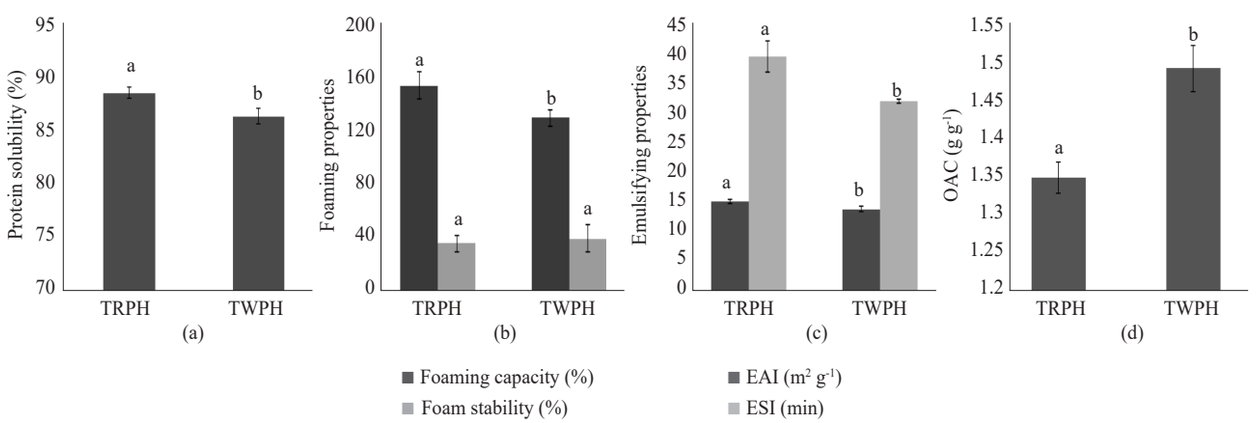

Fig. 2. Functional properties of white meat and red meat hydrolysates from yellowfin tuna a) Protein solubility; b) Foaming properties; c) Emulsifying properties and d) Oil absorption capacity

functionality on account of variations in the raw material composition, especially the type of protein used for hydrolysate preparation.

Emulsifying properties

Emulsifying properties of hydrolysed proteins are directly related to surface properties with influence the degree of hydrolysis which effectively reduces interfacial tension between hydrophilic as well as hydrophobic components in food system (dos Santos *et al*., 2011). Emulsifying properties (Fig. 2c) *viz*., emulsifying activity index (EAI) and emulsion stability index (ESI) were observed to be 13.85±0.36 m^2g^{-1} and 31.39±0.32 min, respectively for TWPH and 15.04±0.36 m^2g^{-1} and 38.71±2.51 min, respectively for TRPH. Results indicated significantly higher ($p<0.05$) emulsifying properties for TRPH compared to TWPH. Taheri *et al.* (2013) in their study reported the peptide sequence as well as its amphiphilic nature to be important factors influencing emulsion properties than peptide length or extent of hydrolysis. Hence, though similar range of DH was observed for the hydrolysate samples in the present study, the properties exhibited by them varied. Jemil *et al.* (2014) reported an EAI in the range of 21.31-47.58 m^2 g^{-1} and an ESI ranging from 22.64-47.75 min in hydrolysates derived from different sources *viz*., *Sardinella*, zebra, blenny, goby and ray muscle.

Oil absorption capacity

The ability of peptides to bind fat, influences the palatability of food products and thereby its applicability in the food industry (Tanuja *et al*., 2012). The present study indicated an OAC of 1.49±0.03 g g^{-1} and 1.35±0.02 g g^{-1}, respectively for TWPH and TRPH which were significantly different ($p<0.05$) (Fig. 2d). Oil absorption mechanism is a combined attribute of physical entrapment of oil together with sample hydrophobicity and reports suggest excessive hydrolysation to compromise the molecule's structural integrity resulting in its degradation and resultant capacity to entrap oil (He *et al*., 2013). Present study revealed fairly good OAC for the hydrolysates on account of the limited hydrolysis. Foh *et al.* (2011) and Geirsdottir *et al.* (2011) reported comparatively superior oil binding capacity for hydrolysates from species like tilapia and blue whiting, to that of commercial food-grade oil binders proving their potential to be utilised as commercial oil binders in food industry.

Sensory properties

The degree of bitterness that develops during hydrolysis is associated with the level of hydrophobic amino acids and the release of bitter tasting peptides (Nilsang *et al.,* 2005). Factors *viz*., type of substrate, nature of enzymes as well as the hydrolysis conditions play effective roles in determining the final physico-chemical properties of hydrolysate especially sensory acceptability with regard to the bitterness generated (Normah *et al.,* 2013). Effective application of a product in food system demands a combination of enhanced functionality along with sensory acceptability. In the current study, the samples exhibited very slight bitterness (2.3±0.5) in TRPH while hardly any bitterness (1.5±0.7) was observed in TWPH when incorporated in porridge at 0.2%. Similarly the acceptability studies indicated a sensory score of 6.7±0.5 for TRPH whereas it was 7.4±0.5 for TWPH ($p<0.05$). This variation in the sensory properties must be on account of the variation in nature of substrate used for the study. However the observations indicated its suitability to be incorporated in food system.

Antioxidative properties

DPPH radical scavenging activity

Hydrolysis of protein results in its structure unfolding to facilitate the exposure of more of hydrophobic amino acids which in turn leads to improved antioxidative activity compared to the intact protein (Sarmadi and Ismail, 2010). Depending on the assay system, an antioxidant may exhibit variations in its potential, based on the antioxidative mechanism being measured as well as the reaction conditions used in the various assays (Najafian and Babji, 2012). Hence in the present study, different antioxidant assays were carried out to comparatively analyse the properties exhibited by the tuna hydrolysates. DPPH radical-scavenging activity determines the hydrogen-donating ability of protein hydrolysates which assists in breaking of the radical chain reaction (Yarnpakdee *et al.,* 2015). The comparison of this antioxidant assay in 0.2 % solutions of TWPH and TRPH indicated a significant difference ($p<0.05$) revealing higher potential for TWPH than TRPH (Fig. 3a). Yarnpakdee *et al.* (2015) observed differences in the DPPH radical scavenging activity in Nile tilapia hydrolysate to be associated with the extent of hydrolysis with an increase upto 30% beyond which it decreased. However, the better antioxidant potential of TWPH in comparison to TRPH in the present study cannot be related to the extent of hydrolysis undergone by the sample. Similar observations were made by Slizyte *et al.* (2016) mentioning that DH and size of peptides produced cannot alone predict the DPPH scavenging ability.

Reducing power and FRAP

Protein hydrolysates possess the ability to donate electron/hydrogen and free radicals facilitating oxidation stable substances by interrupting the free radical chain reactions or prevent their formation (You *et al.,* 2010). The

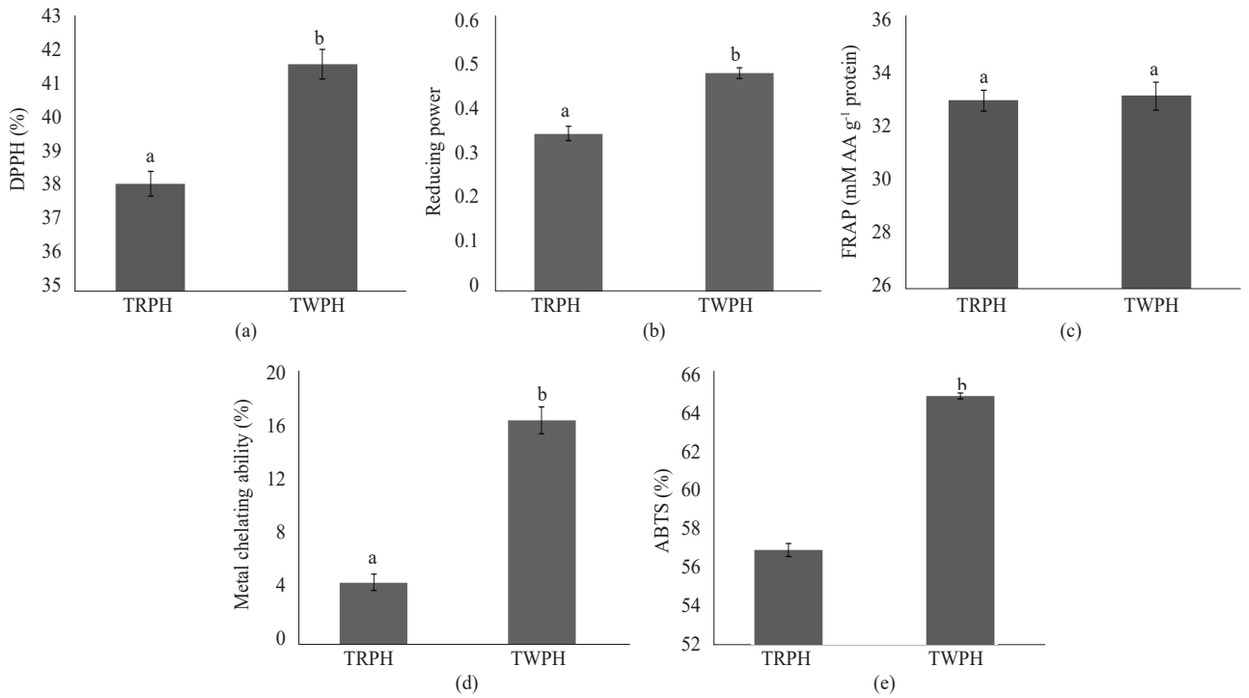

Fig. 3. Antioxidative properties of white meat and red meat hydrolysates from yellowfin tuna. a) DPPH radical scavenging activity; b) Reducing power; c) FRAP; d) Metal chelating ability and e) ABTS radical scavenging activity

present study reported a reducing power of 0.470±0.011 and 0.341±0.016, respectively for TWPH and TRPH with significant difference ($p<0.05$) (Fig. 3b). However the FRAP of the samples were comparable indicating a value of 33.09±0.49 for TWPH and 32.92±0.38 for TRPH (Fig. 3c). Choonpicharn *et al.* (2014) have reported good FRAP activity in hydrolysates from tilapia skin. Bougatef *et al.* (2010) suggested antioxidative properties of fish peptides to be related to their sequence, composition as well as hydrophobicity. Therefore, though the DH remained comparable between the hydrolysates, the different pattern of peptides derived from them must have resulted in diverse reducing activity.

Metal chelating ability

Different mechanisms of actions are adopted by antioxidant peptides to terminate free radical scavenging activity, of which sequestration of prooxidative metals facilitate effective retardation of oxidation (Yarnpakdee *et al*., 2015). The present study revealed a significantly ($p<0.05$) higher metal chelating activity of 16.53±0.96% for TWPH whereas it was 4.73±0.58% for TRPH (Fig. 3d). Similar to the present study, Tanuja *et al.* (2012) reported a lower metal chelating activity (<20%) for papain and bromelain derived hydrolysates from frame meat of striped cat fish.

ABTS radical scavenging activity

In the present study, TWPH and TRPH reported significantly different ($p<0.05$) ABTS values of 64.57±0.16 and 56.83±0.35%, respectively (Fig. 3e). Earlier studies conducted by Bernardi *et al.* (2016) in hydrolysates from Nile tilapia byproducts indicated superior ABTS activity. The amino acid constituents and the sequence of the peptides are determinant factors for their antioxidant activity which are dependent on substrate type, selection of appropriate proteolytic enzymes and the physico-chemical conditions of hydrolysis (Samaranayaka and Li-chan, 2011). Enzymes like papain exhibits specific substrate preferences, primarily for bulky hydrophobic or aromatic residues which must have resulted in variations in the nature of peptides formed as well as resultant properties (Tavano, 2013).

Tuna red meat, generally discarded as a byproduct in canning industry is a potential source of high quality proteins which can be effectively utilised for conversion to hydrolysate which has immense application potential in food and pharmaceutical industry. The present study revealed its application potential by comparison of the hydrolysates from red meat with a reference source *i.e*. tuna white meat. The extent of hydrolysis undergone was similar but the properties indicated variations as they were dependent mainly on the nature of peptides formed during hydrolysis rather than the peptide chain length. The current investigation paves possibilities for further exploration of this protein rich substrate for its commercial applications.

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