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Extraction and Characterization of Myofibrillar Proteins from Different Meat Sources: A Comparative Study

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ABSTRACT

In the present study, myofibrillar proteins were extracted from the meat proteins of beef, lamb, chicken, tuna and emperor fish using non-denaturation method, and their physico-chemical and rheological properties were assessed. The myofibrillar proteins of beef, emperor and lamb samples had higher percentage of protein extractability than tuna and chicken samples. The tuna sample showed significantly higher bound bromophenol blue (BPB) value while lamb samples showed lower value ($P < 0.05$). The myofibrillar protein of chicken sample was found to have more ionic and hydrogen bonds than all other myofibrillar samples. The disulphide bonds in tuna and lamb myofibrillar protein samples were significantly higher than other three samples ($P < 0.05$). The myofibrillar protein samples showed major bands myosin heavy chain, α-actinin, desimin, actin, troponin, tropomyosin and myosin light chain with wider molecular weight distribution in the range of 20–200 ku. The myofibrillar proteins exhibited Newtonian and shear thickening nature behaviour at lower protein concentration (1 mg/mL) as revealed by flow profile and visco-elastic analysis using rheometer.

1. Introduction

Usually, muscle proteins can be categorised into three groups, sarcoplasmic, myofibrillar and stroma proteins/connective tissue proteins (Xiong, 1994). Myofibrillar proteins are the long fibril proteins, considered as major component of the skeletal muscle which accounts for 60%–70% of total muscle proteins. It mainly comprises of myosin (thick) and actin (thin) components and, mainly involves in muscle contraction. Myofibrillar proteins play an important role in producing meat products with required functional characteristic properties (Sun and Holley, 2011). Myosin is the primary component of myofibrillar proteins which has impacts in maintaining three-dimensional structure in meat proteins and, also is responsible for gelation in meat proteins. Myosin is the most abundant and asymmetric molecule of myofibrillar protein, with two heavy polypeptide chains and four light polypeptide chains. Myosin molecule has two proteolytic fragments namely heavy meromyosin (HMM) and light meromyosin (LMM). The LMM comprises of two parts of heavy chains, while the HMM contains two heavy chains and four light chains. Actin is another major component of myofibrillar protein which plays critical role in reinforcing the gel structure of myosin. The actin molecule exists in two forms G-actin and F-actin. The polymerization of G-actin into F-actin occurs in the presence of salt (López-Bote, 2017). However, the other

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components of myofibrillar proteins like tropomyosin and troponins have no effect on the gelation of myosin. The interaction between myosin and actin in actomyosin complex, reflects the degree of meat tenderness (Wang et al., 2020).

During the extraction process, the quality of myofibrillar proteins is considered to be the most important aspect, because it affects the protein intermolecular interactions, as well as the physical stability of formulated functional foods. Moreover, the extractability or solubility is another key factor that influences the functional and surface-active properties of food muscle proteins (Chen et al., 2017). Myofibrillar proteins have been extracted from different types of meat/muscle proteins of pork, beef, poultry and fish (Hopkins and Thompson, 2002; Mehta et al., 2017; Guo and Wang, 2018; Zhou et al., 2019). Different types of salts such as sodium chloride, potassium chloride and calcium chloride (NaCl, KCl, and $CaCl_2$) have been commonly used for the extraction of myofibrillar proteins. Further, the myofibrillar proteins have been subjected to different treatments like high pressure, ultrasonication, heat and thermal induction for the betterment of physico-biochemical and functional properties (Malva et al., 2018; Wang et al., 2018). Malva et al. (2018) compared the denaturation and non-denaturation methods of extraction of myofibrillar proteins from various types of meat proteins.

Protein gelation, functional, physico-chemical, structural and rheological properties are answerable for texture development in formulating functional foods, and therefore, consumer acceptability depends mainly on the quality of myofibrillar proteins. With this rationale, we have made an attempt to compare the myofibrillar proteins from different meat/muscle protein of beef, lamb, chicken and fish (tuna and emperor) and characterizing the physico-chemical and structural properties of obtained myofibrillar proteins.

2. Material and methods

Meat samples from *longissimus thoracis* muscle of beef and lamb; *pectoralis major* muscle of chicken; and dorsal white muscle of fish Bigeye tuna (*Thunnus obesus*) and Emperor (*Lethrinus nebulosus*) was procured from local market (Kochi, Kerala, India). Meat samples were packed and brought to laboratory in iced and frozen state condition. The meat samples were stored at –80 °C till myofibrillar proteins were extracted. All other chemicals and reagents used in the present study were either of analytical grade (AR) or guaranteed grade (GR).

2.1. Sample preparation

Connective and adipose tissues were removed from meat samples of beef, lamb and chicken, whereas the processing waste like bones, scales, and visceral organs were discarded from fish samples. All the fresh meat samples were finely minced using mortar and pestle at 4 °C, prior to myofibrillar protein extraction.

2.2. Extraction

The myofibrillar proteins were extracted according to the following method as described by Wang et al. (2020), with slight modifications. Meat muscles was finely chopped into small pieces, and was minced using mortar and pestle at 4 °C. The minced meat samples were homogenized with phosphate buffer (0.02 mmol/L, pH 7.0) (1:10 w/V), for 2 min using homogeniser Ultra Turrax homogenizer (Ultra Turrax, T 25, KG Staufen, Germany), followed by centrifugation at 8000 r/min at for 20 min at 4 °C using refrigerated centrifuge (Sorvall Legand XTR, Thermo Fisher Scientific, New Hampshere, USA), to separate sarcoplasmic proteins. The resultant pellet was washed twice with phosphate buffer (0.02 mmol/L PB, pH 7 with 0.6 mol/L KCl) and centrifuged as mentioned above. After second wash, the supernatant was filtered using Whatmann No. 1 filter paper and the resultant filtrate is designated as myofibrillar proteins. The obtained myofibrillar proteins were stored at –80 °C, till further analysis. The protein content of myofibrillar proteins was estimated by the method as described by Lowry et al. (1951), using bovine serum albumin (BSA, Sigma) as standard.

2.3. Ultraviolet (UV) absorption spectra

Myofibrillar protein samples of 1 mg/mL concentration were prepared in 50 mmol/L phosphate buffer (0.6 mol/L KCl, pH 7.0) and filtered using a Whatman filter paper No. 1. The Ultraviolet (UV) absorption spectra were recorded in the wavelength range of 230–350 nm using double beam spectrophotometer (UV-VIS spectrophotometer, Shimadzu, Tokyo, Japan).

2.4. Protein extractability

The solubility of myofibrillar proteins was determined according to the following method of Robinson and Hogden (1940). The myofibrillar proteins were homogenised with 1:9 (w/V) phosphate buffer (0.02 mmol/L PB, pH 7 with 0.6 mol/L KCl), followed by centrifugation at 5000 r/min for 15 min at 4 °C. The protein extractability of myofibrillar protein was determined and expressed in percentage.

$$\text{Protein extractability (\%)} = \frac{\text{Protein after centrifugation}}{\text{Protein before centrifugation}} \times 100\% \quad (1)$$

2.5. Turbidity

The turbidity of myofibrillar protein samples was determined according to the following method as described by Wang et al. (2020). The 0.5 mg/mL concentration of myofibrillar proteins was prepared in 50 mmol/L phosphate buffer (0.6 mol/L KCl, pH 7.0), and the turbidity of myofibrillar proteins was monitored at 360 nm using double beam spectrophotometer.

2.6. Particle size distribution and zeta potential

The particle size distribution (PSD) and Zeta potential of myofibrillar protein samples (0.5 mg/mL) were determined by dynamic light scattering analyses (DLS) using a Zeta Sizer Nano Series (Malvern, Worcestershire, UK). For PSD analysis, the protein samples were loaded into a quartz cuvette with 1 cm path-length and were subjected to DLS measurement with a detection angle of 90° at (25 ± 0.1) °C. The hydrodynamic diameters of the particles and PSDs of myofibrillar proteins were determined and recorded based on the scattering intensity. For analysing zeta potential, the protein samples were filtered using 0.45 μm syringe filter membrane to remove large particles and measured with an angle of 15° at 25 °C.

2.7. Surface hydrophobicity

The surface hydrophobicity of the myofibrillar protein solutions was determined according to the following method as described by (Chelh et al., 2006). Myofibrillar protein samples (1 mL) was added to 200 μL bromophenol blue (BPB) (0.1% (w/V) in distilled water). Samples without myofibrils were treated as control, consisted only of 200 μL BPB and 1 mL of 50 mmol/L phosphate buffer (pH 7). The samples were agitated at 37 °C for 10 min prior to centrifugation for 15 min at 2000 r/min. The absorbance of the supernatant (diluted to 1/10-fold) was read at 595 nm against a phosphate buffer as blank. The amount of BPB bound was calculated using Eq. (2):

$$\text{Amount of BPB bound } (\mu g) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 200\ \mu g \tag{2}$$

2.8. Determination of chemical bonds

Chemical bonds present in myofibrillar protein samples were determined by the following method as described by Li et al. (2019). The myofibrillar protein samples were treated with different buffer solutions for their capacity to disrupt different kinds of specific bonds. The buffers used were namely (A) 0.05 mol/L NaCl, (B) 0.6 mol/L NaCl, (C) 0.6 mol/L NaCl + 1.5 mol/L urea, (D) 0.6 mol/L NaCl + 8 mol/L urea, and (E) 0.6 mol/L NaCl + 8 mol/L urea + β-mercaptoethanol. Briefly, 1 g of myofibrillar protein sample was added to 9 mL of each buffer solution and homogenized for 2 min using homogenizer. The resultant solutions were stirred for 2 min followed by centrifugation at 4 °C, 8000 r/min for 10 min. Protein concentration in supernatants was estimated by the method as described by Lowry et al. (1951). The chemical bonds namely ionic bonds, hydrogen bonds and disulfide bonds were calculated by the protein content differences between buffer solution A and B (ionic bonds), C and B (hydrogen bonds), E and D (disulfide bonds), respectively.

2.9. Molecular weight determination

The molecular weight determination of myofibrillar proteins was determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis through the following method as described by Laemmli (1970). Electrophoresis was carried out using polyacrylamide gel slabs of 10 cm × 8 cm (length × width) in a vertical slab electrophoresis apparatus (Mini Protean II tetra cell Bio-Rad Laboratories, CA, USA). An aliquot 20 μL of protein samples at 0.5 mg/mL were loaded onto a polyacrylamide gel (14% running gel and 4% stacking gel). The molecular weight of the proteins was approximated using standard wide range-molecular-weight protein markers (Sigma, St. Louis, MO, USA).

2.10. Flow behaviour study

The rheological flow properties (shear stress and viscosity vs. shear rate) of myofibrillar protein solutions were measured using Brookfield DV-III Ultra TM Programmable Rheometer (Brookfield Engineering Laboratories INC, Middleboro, USA). The myofibrillar samples at 2 mg/mL concentration and temperature of 37 °C were used for measuring flow properties. The cone and plate spindle (CP- 41 model) with measuring geometry of 5 cm was used with a gap of 0.05 mm and a shear rate ranging from 10 to 400 s^{-1}. A flow curve was obtained by plotting shear stress and viscosity vs. shear rate values using a steady state flow program. The Herschel-Bulkley model was selected as best-fit model based on standard error of shear stress-shear rate and shear stress-viscosity data. The Herschel-Bulkley model equation is as follow:

$$T = T_o + k D^{\eta} \tag{3}$$

where, T is the shear stress (Pa), T_o is the yield stress (Pa), D is the shear rate (s^{-1}), k is the consistency coefficient and η is the flow behaviour index (dimensionless). The consistency coefficient (k) and flow behaviour index (η) of myofibrillar protein solutions were determined by the steady state flow program software.

2.11. Functional properties of myofibrillar proteins

2.11.1. Foaming properties

The foaming properties (foaming capacity and foam stability) were determined according to the method as described by Sathe and Salunkhe (1981). Myofibrillar protein sample (2%) solution (20 mL) was whipped at a speed of 13 500 r/min using homogenizer to incorporate the air for 2 min at room temperature. The whipped sample was immediately transferred into a 100 mL measuring cylinder and volume was recorded. The foaming capacity was calculated using the following equation:

$$\text{Foaming capacity} = \frac{(\text{Volume after whipping}) - (\text{Volume before whipping})}{\text{Volume before whipping}} \times 100\% \quad (4)$$

The whipped sample was allowed to stand at 20 °C for 30 min and the volume of whipped sample was then recorded. Foam stability was calculated as follows:

$$\text{Foaming stability} = \frac{(\text{Volume after standing}) - (\text{Volume before whipping})}{\text{Volume before whipping}} \times 100\% \quad (5)$$

2.11.2. Emulsion property

The emulsion properties (emulsion activity index (EAI) and emulsion stability index (ESI)) were determined according to the method as described by Pearce and Kinsella (1978). Myofibrillar protein sample (2%) (30 mL) and refined sunflower oil (10 mL) were mixed and homogenized at 20500 r/min for 1 min. A 50 μL of emulsion was taken from the bottom of the container at 0 and after 10 min and mixed with 5 mL of 0.1% sodium dodecyl sulphate (SDS) solution. The absorbance of the solution was recorded at 500 nm using double beam spectrophotometer. The absorbance was measured immediately 0 min (A_0) and after 10 min (A_{10}) of emulsion formation. The readings were used to calculate the EAI and ESI as follows:

$$\text{EAI} = \frac{2 \times 2.303 \times \text{Absorbance}_{500}}{0.25 \times \text{Protein concentration}} \quad (6)$$

$$\text{ESI} = \frac{A_0 \times \Delta t}{\Delta A} \quad (7)$$

where ΔA, absorbance at 0 min (A_0) – absorbance after 10 min (A_{10}); $\Delta t = 10$ min.

2.11.3. Water holding capacity

Water holding capacity (WHC) was determined according to the following method as described by Diniz and Martin (1997); Kumar et al. (2017). The WHC was calculated and expressed as mL of water absorbed per g of myofibrillar protein sample.

$$\text{WHC (mL/g)} = \frac{(\text{Initial volume of distilled water} - \text{Volume of supernatant})}{\text{Weight of myofibrillar protein}} \quad (8)$$

2.12. Statistical analysis

One-way ANOVA was used to analyse the data. The experiments were carried out in triplicates independently. Significant difference between the means of triplicates were determined by Duncans multiple comparison test using statistical software IBM SPSS.2 (SPSS Inc, Illinois, USA).

3. Results and discussion

3.1. The UV absorption spectra

The UV absorption spectra analysis is an effective analytical tool used to detect the conformational changes that occur in protein structure. The UV absorption spectra of myofibrillar protein samples showed absorption maxima in the wavelength region of 240–300 nm (Fig. 1). The humps at this region (240–300 nm) indicates the presence of aromatic amino acid residues like tyrosine, phenylalanine and tryptophan (Donovan, 1969). Wang et al. (2020) demonstrated that the peak at 285–290 nm region can be attributed to aromatic amino acids (tyrosine and tryptophan), while the peak at 296 nm region can be assigned to tryptophan, respectively.

3.2. Protein extractability

Solubility of protein is defined as the amount of total muscle proteins that dissolve in buffer solution and at saturation point it represents an equilibrium between the protein and water. The solubility is a significant index of protein functionality that directly reflects denaturation and aggregation of protein (Sun and Holley, 2011). The myofibrillar proteins of beef, emperor and lamb samples have higher solubility than other two samples ($P < 0.05$) (Fig. 2A). High solubility of proteins might be due to cleavage of proteins into smaller peptide molecules, and simultaneously the number of polar groups will be increased to form hydrogen bonding with water molecules. At certain iso-electric point, interaction between salt used for the extraction process may induce the intermolecular forces such as attraction or repulsion between the protein molecules, which leads to variations in solubility. During extraction process, the disruption and dispersion of filaments that caused by the swelling of myofibrils helps in promoting the solubility of myofibrillar

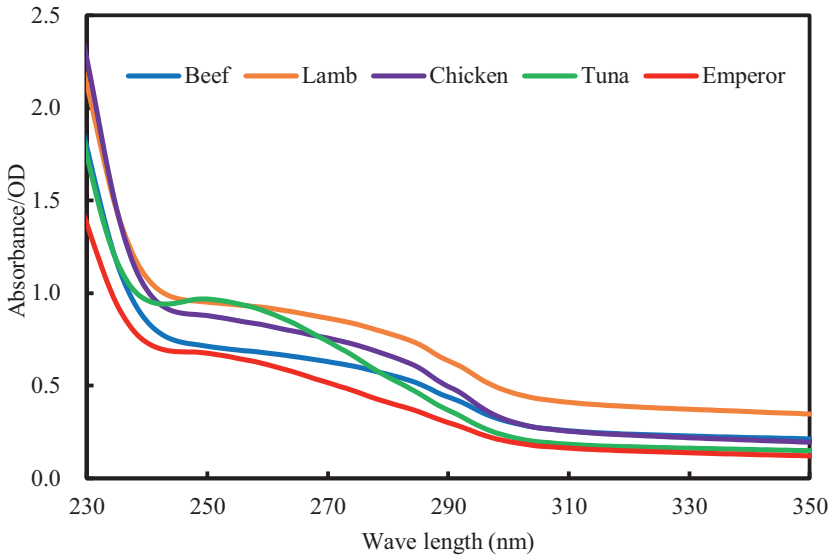

Fig. 1. Ultraviolet (UV) absorption spectra of myofibrillar proteins

Table 1
Particle size and Zeta potential value of myofibrillar proteins.

Sample	Average particle size (nm)	Polydispersity index (PDI)	Zeta potential
Beef	314.67±6.91	0.492±0.065	−14.15±2.19
Lamb	131.07±2.66	0.405±0.015	−38.9±8.57
Chicken	122.37±2.46	0.518±0.054	−0.018±0.01
Tuna	105.20±0.87	0.290±0.064	−0.072±0.01
Emperor	80.87±1.44	0.427±0.017	−0.024±0.004

proteins (Wang et al., 2018). It was stated that the protein extractability from skeletal muscles is a complex phenomenon which depends on several parameters such as method of extraction, solubilization process, type of muscle tissue and its structure, pH, post-mortem changes that occur during the transformation of muscle (Zayas, 1997). The solubility of myofibrillar proteins from white muscle is usually reported to have higher percentage than red muscle. This disparity probably could be due to different degrees of structural hindrance of the myofibrils, specifically Z-disc and M-protein. Nevertheless, the extraction of myofibrillar proteins depends on the isoform of myosin and polymorphism of structural proteins like M-protein and α-actinin which mostly intervene in sarcomere organisation (Xiong, 1994). It is important to note that the hydrophobic interactions are greatly implicated in protein-protein associations, and in this point of view the protein surface hydrophobicity might influence the protein extractability (Chen et al., 2017).

3.3. Turbidity

The tuna myofibrillar protein has more turbidity value which is significantly higher than other myofibrillar proteins ($P > 0.05$) (Fig. 2C). No significant difference was found in the turbidity values of beef, lamb and chicken myofibrillar proteins. The higher turbidity might be due to the continuous formation of large protein aggregates that causes light scattering and impede light transmission (Jia et al., 2015). The disparity of turbidity probably might be due to the difference in particle size and/or rate of protein aggregation (Chan and Gill, 1994). The results suggested that the tuna myofibrillar proteins could undergo more aggregation than other myofibrillar protein samples. The presence of large-aggregates formation is apparently a criterion for the formulation of good elastic gel (Chan et al., 1992). The differences in aggregation behaviour of myofibrillar proteins were assumed to result in the different gelling properties between species. It has been reported that the differences in amount of hydrophobic and disulfide bonds might also be one of the possible reasons for difference in aggregation and certainly, lead to changes in turbidity (Yarnpakdee et al., 2009).

3.4. Particle size distribution and Zeta potential

Beef myofibrillar proteins have significantly higher size than all other proteins ($P < 0.05$) (Table 1). Tuna myofibrillar proteins have higher particle size than emperor myofibrillar proteins which is indicated by higher turbidity. The salt concentration used for the extraction of myofibrillar proteins influences the particle size (Jia et al., 2015), however, 0.6 mol/L KCl was used in the present study. Chen et al. (2018) demonstrated the effect of temperature on chicken myofibrillar protein and it can be seen that the particle size

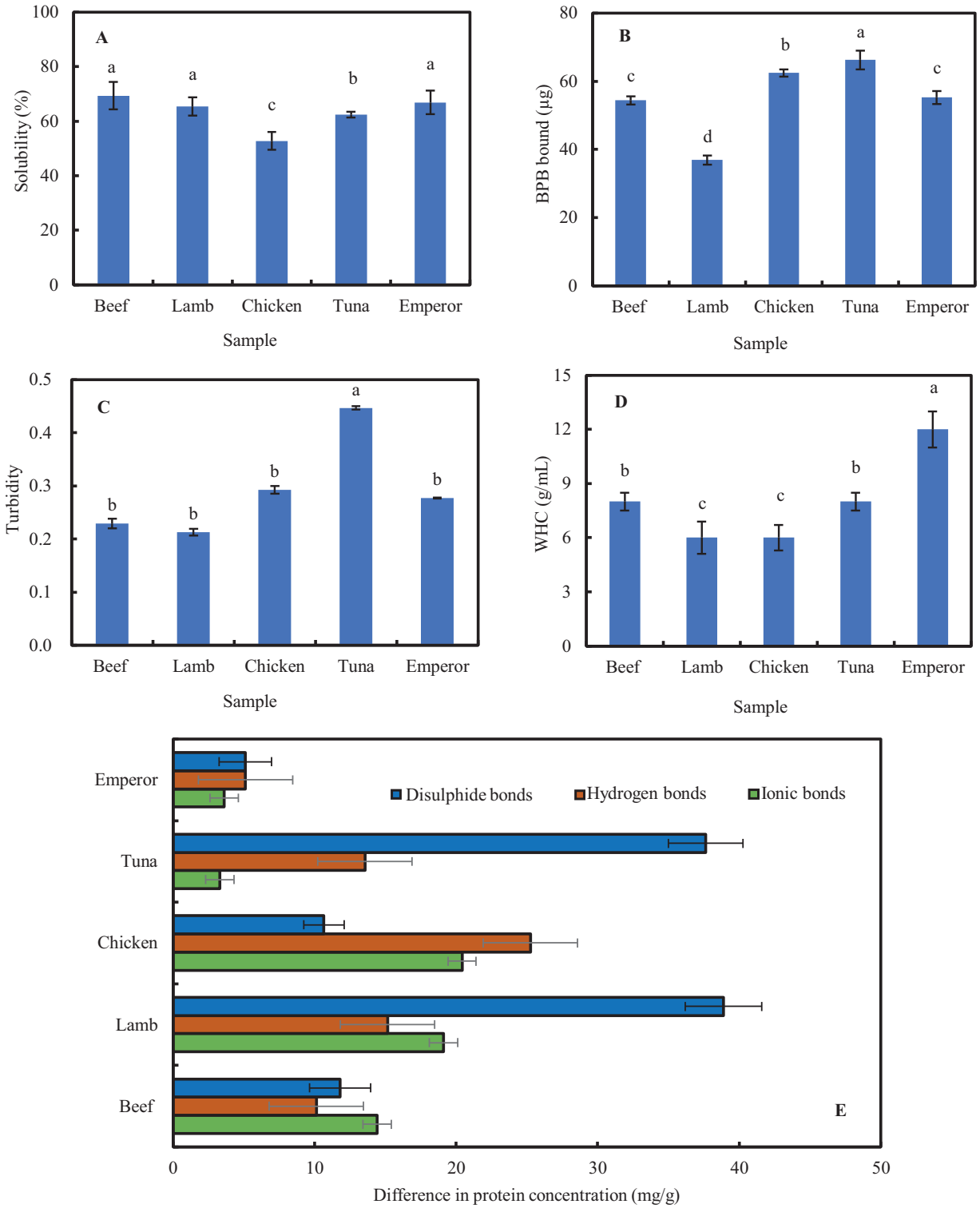

Fig. 2. (A) Solubility; (B) Surface hydrophobicity; (C) Turbidity; (D) Water holding capacity (WHC); (E) Chemical bonds of myofibrillar proteins. Different lowercase letters on the error bars indicate that the results are significantly different ($P < 0.05$).

increases with temperature. Zeta potential is one of the significant parameters that reflects the stability of nanoparticles in aqueous environment. The Zeta potential of a myofibrillar protein particle is pH-independent, whereas the isoelectric point (pI) resembles the net zero potential charge (Malhotra and Coupland, 2004). The Zeta potential values of all myofibrillar proteins were observed to be negative. At neutral pH, the acidic amino acids such as glutamic acid and aspartic acid signifies the negative charge of the protein molecule. A higher drop of negative charge was observed which indicated the formation of cross-linkage bridge between the positively charged ions of KCl and negatively charged residues of protein (Jia et al., 2015). In the present study, the chicken, tuna and emperor were found to have higher Zeta potential which indicates stability in aqueous solution. The size distribution (polydispersity index, PDI) profile data of the myofibrillar proteins was in a narrow size range of distribution (PDI < 1).

3.5. Surface hydrophobicity

The hydrophobic groups of protein molecules characterize the tertiary structure of the protein. The hydrophobic interaction forces help in maintaining protein structure, also influence the stability and functional properties of myofibrillar proteins (Chelh et al., 2006). Fig. 2b demonstrates the surface hydrophobicity of myofibrillar proteins. The tuna sample showed significantly higher bound BPB value than all other samples, while lamb samples showed lower value of 36.8 μg ($P < 0.05$). This increase in bound BPB value indicates the unfolding of myofibrillar proteins and exposure of non-polar amino acid residues to their surface (Chan and Gill, 1994). Nevertheless, the degree of this unfolding of myofibrillar proteins depends on factors such as ionic environment, extraction condition, and intrinsic factors of the protein (Xiong, 1994). Hayakawa et al. (1996) stated that difference in hydrophobicity was related to the cleavage of hydrogen bonds between the water that present on the surface globular protein molecules and hydrophilic regions. Conformational changes in the α-helix structure of protein tend to increase surface hydrophobicity, thereby promoting intermolecular interactions (Stone and Stanley, 1992). In another study by Chapleau and de Lamballerie-Anton (2003), it was observed that the secondary structure modification, destruction of α-helices present in heavy chains of myosin and conformational changes in actomyosin leads to change in surface hydrophobicity of myofibrillar proteins.

3.6. Determination of chemical bonds

Fig. 2e illustrates the chemical bonds of myofibrillar proteins. The myofibrillar protein of chicken sample was found to have more ionic and hydrogen bonds than other myofibrillar samples. The ionic bonds in fish myofibrillar proteins were significantly lower than beef, lamb and chicken samples ($P < 0.05$). The disruption of hydrogen bonds between water and protein molecules leads to unfolding of protein molecules, and these results to a gradual decrease in apparent viscosity (Liu et al., 1982). The presence of more hydrogens bonds is possibly to be the reason for more bound surface hydrophobicity in chicken samples. Ionic and hydrogen bonds play important roles in gel formation and stabilizing protein conformation. At low temperature (< 40 °C), ionic and hydrogen bonds contribute higher stability to the mammalian protein compared to that of fish protein (Liu et al., 2011). The disulphide bonds in tuna and lamb myofibrillar protein samples were significantly higher than other three samples ($P < 0.05$). It is stated that the disulphide bonds involve in cross-linking of denatured protein molecules as well as aggregation (Choi et al., 2006). It is usually accepted that inherent disulphide bonds stabilize the properly folded conformation of proteins and/or destabilize denatured conformations by decreasing conformational entropy (Bulaj, 2005).

3.7. Molecular weight determination

The molecular weight distribution of myofibrillar proteins was characterized by SDS-PAGE analysis (Fig. 3). The myofibrillar protein samples showed major bands myosin heavy chain, α-actinin, desimin, actin, troponin, tropomyosin and myosin light chain with wider molecular weight distribution in the range of 200 – 20 ku. The 200 ku molecular weight band can be designated as myosin heavy chain component, whereas the other three bands of 55 ku, 45 ku and 36–20 ku are actin, tropomyosin and myosin light chain components. Myosin mainly contributes tensile strength to muscle, whereas α-actinin and desmin are cytoskeletal proteins accountable for the maintenance of structural and mechanical integrity of actin filaments in the Z-disc (Lana and Zolla, 2016). The electrophoretic pattern of myofibrillar protein revealed that fish samples are devoid of troponin complex and, lamb and beef protein samples contain desimin molecules. The reduced quantity of myofibrillar proteins in beef has been observed, this might be due to proteolysis or denaturation (Malva et al., 2018). Furthermore, the polypeptides residues formed during proteolysis could not be able to bind to the staining dye, which leads to less intensity of molecular pattern bands (Tejada et al., 2003). It has been noticed that the type of extraction method implied and ionic strength of the extraction buffer used for the extraction have impact on proteins with high molecular weight (Chen et al., 2017). In the present study, the extraction with low ionic strength and nondenaturing method of extraction was used for the extraction of myofibrillar proteins.

3.8. Flow behaviour study

Flow behaviour is usually used to specify protein-protein interactions and it represents the functionality of food formulations in terms of viscosity (Chen et al., 2018). Different sort of physico-dynamic or computer rheological models such as Herschel-Bulkley, Newtonian, Bingham, Casson, Power law were usually used to present the flow profile of fluids, and their measurement in suspensions, rheological fluids, food hydrocolloids and muscle proteins to describe shear stress and shear behaviour, among which the Herschel-Bulkley equation is mostly preferred, because it results in more accurate models of rheological behaviour when adequate experimental

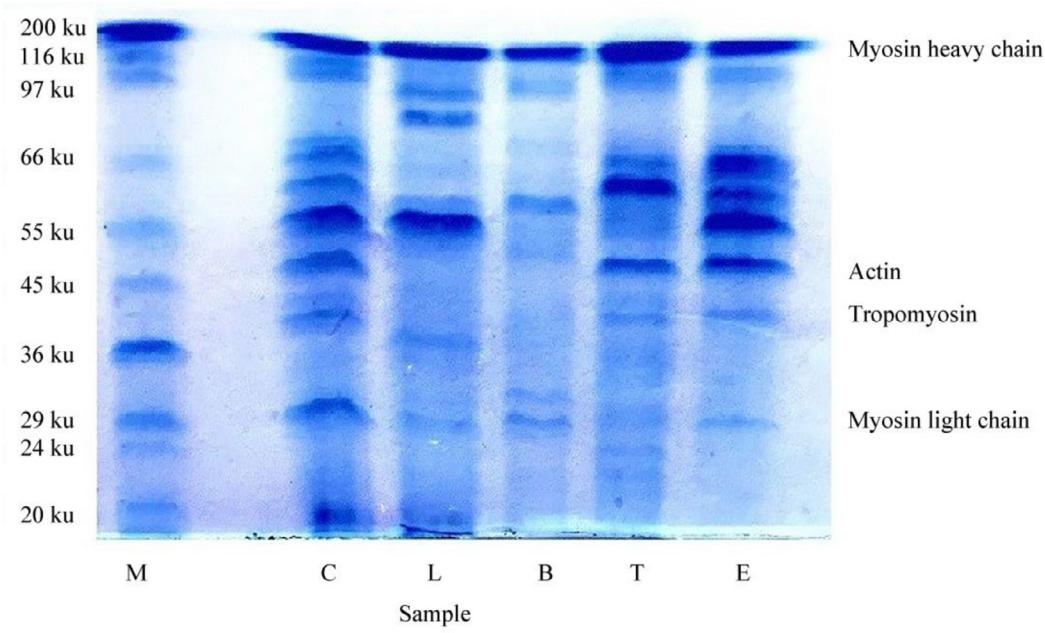

Fig. 3. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern of myofibrillar proteins

Table 2
Herschel-Bulkley parameters for myofibrillar protein.

Sample	Concentration (mg/mL)	T_o	k	η	R^2
Beef	1	0.89	5.5	1.38	0.99
	3	1.16	14.1	0.72	
	5	1.96	24.0	0.86	
Lamb	1	0.52	3.69	1.32	0.99
	3	0.69	20.0	0.64	
	5	1.30	39.5	0.91	
Chicken	1	1.71	58.2	0.40	0.99
	3	2.35	66.1	0.47	
	5	3.37	73.9	0.51	
Tuna	1	1.32	12.3	1.29	0.99
	3	1.67	15.1	0.72	
	5	1.72	22.0	0.76	
Emperor	1	1.28	15.4	1.22	0.99
	3	1.91	33.6	0.60	
	5	2.63	44.3	0.72	

Notes: T_o, yield stress; k, consistency coefficient; η, flow behavior index; R^2, regression coefficient.

data are available (Lee et al., 2009). In the present study, the rheological Herschel-Bulkley model was used to analyse the experimental result data (shear stress and viscosity-shear rate) of myofibrillar protein solutions. The R^2 values of Herschel-Bulkley model were found to be 0.99 which is a high-level indicative of linearity relation between the measuring points (Table 2). The yield stress (T_o), flow behaviour index (η) and consistency coefficient (k) were also calculated using the software provided with the rheometer.

Yield stress (T_o) is a finite stress to be exceeded for a material to flow (Macosko and Larson, 1994). The rheological Herschel-Bulkley model exhibited yield stress values for all myofibrillar protein samples at all concentrations (1, 3 and 5 mg/mL) studied (Table 2). At all concentrations, the yield stress values of chicken myofibrillar protein solutions were found to be higher than all other myofibrillar proteins. The higher yield stress might be due to higher intermolecular attraction. It has been stated that the method of analysis and experimental condition influences the yield stress (Christianson and Bagley, 1984).

The myofibrillar protein solutions at concentration 3 and 5 mg/mL, exhibited shear thinning and pseudoplastic behaviour, which represents an irreversible structural break down (Fig. 4). The decline in viscosity at this region might be due to polymer deformation and molecular alignment that occur within the substance (Glicksman, 1969). The viscosity of myofibrillar protein solutions at concentration 3 and 5 mg/mL was found to be increased with shear rate till 100 (s^{-1}), and then decreased with increasing shear rate. The viscosity of a shear thinning liquid has a tendency to decrease when the shear rate increases, while a shear thickening liquid has a linearity between viscosity and shear rate (Liu et al., 2018). The flow behaviour of these myofibrillar proteins at concentration 3 and

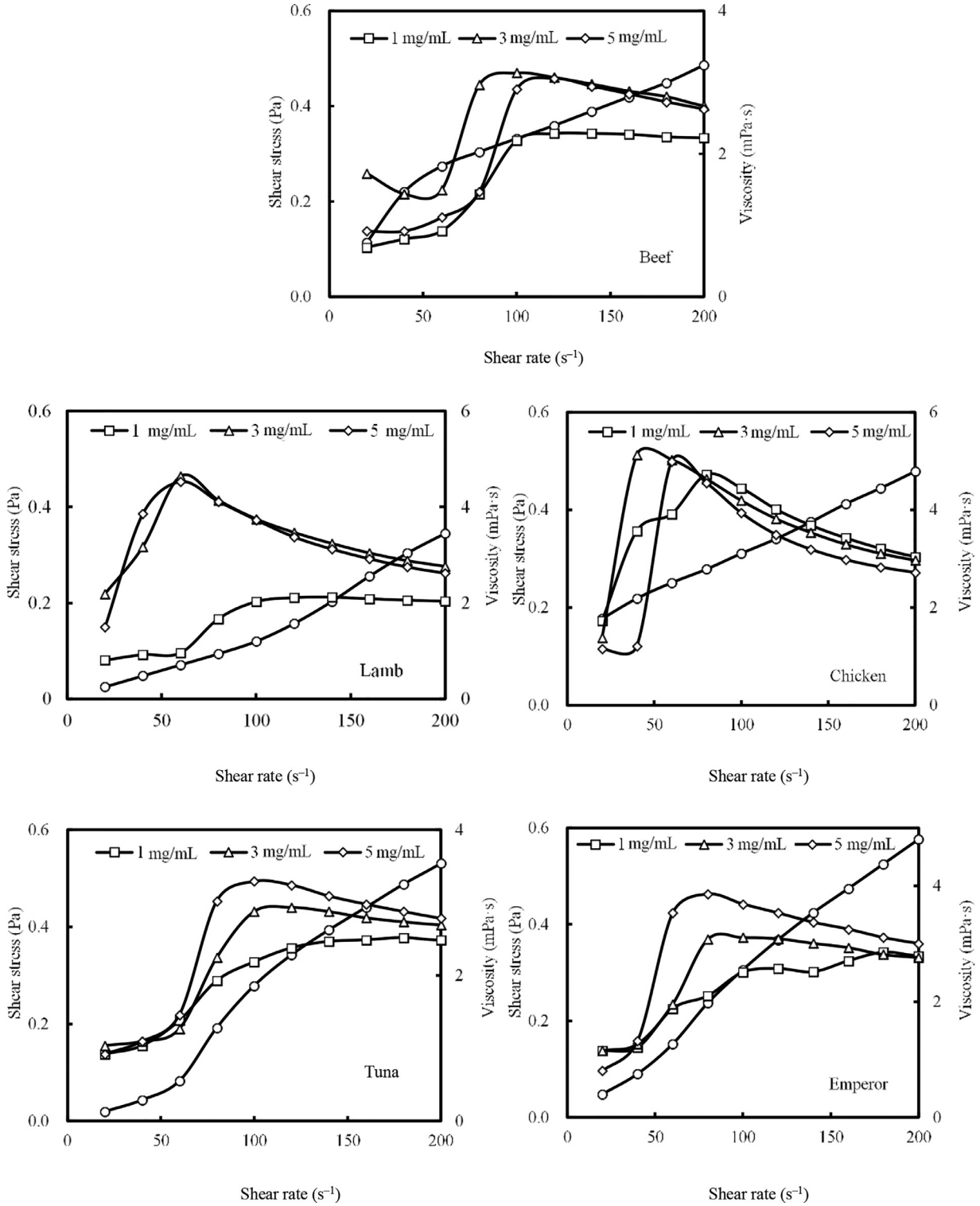

Fig. 4. Flow properties (shear stress and viscosity vs. shear rate) of myofibrillar proteins

5 mg/mL shows the transition of Newtonian to non-Newtonian. The intrinsic factors between white and red fibre of muscular proteins lead to rheological variations in myofibrillar proteins. Further the polymorphism phenomenon of myosin molecule and the ability of white myosin molecule possibly will explain the changes in viscosity between the white and red myofibrillar proteins (Xiong, 1994). The viscosity values of beef and chicken myofibrillar proteins solutions at higher concentrations were found to be upswing at low shear rates which is an indicative of an apparent yield stress (Rao and Tattiyakul, 1999). The viscosity of the fluid varies with the shear stress and the consistency depends upon the duration and shear rate (Lee et al., 2009). At a specific shear rate, the myofibrillar proteins seems to have Newtonian or shear thickening behaviour with a rather low consistency. The higher shear rate induces a modification of myosin molecule and consequently the dissociation of the actomyosin complex via non-covalent bonds which leads to less viscosity of fluids (Liu et al., 1982).

The consistency coefficient (k) value of myofibrillar protein solutions are presented in Table 2. The k values were increased with the increase in concentration of myofibrillar proteins. The consistency coefficient (k) values of chicken myofibrillar proteins were comparably higher than other myofibrillar proteins. The conversion of polypeptide chain to random chain molecules is the most likely reason for the increase in k values (Flory and Weaver, 1960). Besides protein concentration and physico-chemical characteristic properties, the rheological behaviour of myofibrillar proteins in solution depends on the size and shape of the molecules (Chapleau and de Lamballerie-Anton, 2003).

The flow behaviour index, $\eta = 1$ for a Newtonian fluid, $\eta < 1$ for a shear thinning fluid or rheofluidifying, whereas $\eta > 1$ for shear thickening or rheothickening fluid. Liu et al. (2018) demonstrated about two systems namely, liquid-dominated system (LDS) and a solid-dominated system (SDS) corresponding viscosity vs. shear rate. It was stated that the flow indices in the LDS were found to be 1 ($\eta = 1$), and in the SDS, the flow indices were lower than 1 ($\eta < 1$), which indicates that the two systems were Newtonian and non-Newtonian, respectively. In the present study, η values of beef, lamb, tuna and emperor at 1 mg/mL concentration indicate a Newtonian and shear thickening nature. The value of flow behaviour index and its change are highly dependent on molecular size (Rao and Kenny, 1975).

3.9. Functional properties of myofibrillar proteins

Foaming capacity and stability of myofibrillar protein samples at 2% concentration are given in Fig. 5A. The foaming capacity of beef and tuna myofibrillar protein samples was found to be higher as compared to other myofibrillar protein samples ($P < 0.05$). The foaming stability of beef and chicken myofibrillar protein samples was significantly higher than other myofibrillar protein samples studied ($P < 0.05$). The foaming properties of proteins are influenced by the source of the protein, methods and thermal parameters of processing, protein extraction, temperature, pH, protein concentration (Zayas, 1997). The protein solubility, the ratio of hydrophobic and hydrophilic groups and viscosity of protein solution determine the foaming properties of proteins (Sathe and Salunkhe, 1981). In the present study, tuna myofibrillar proteins were found to have more hydrophobicity and disulphide bonds than other proteins, and this might be the possible reason for more foaming capacity. It indicates the direct linear correlation between the surface hydrophobicity and its ability to lower surface and interfacial tension. At gas-liquid interface, the absorption of partially denatured protein molecules increases due to changes in molecular arrangement (Chen et al., 2017).

The EAI and ESI of myofibrillar protein samples at 2% concentration is shown in Fig. 5B. A higher EAI in beef and ESI in lamb myofibrillar protein samples were recorded. The EAI of beef, lamb, chicken, tuna and emperor were found to be 28.9, 23, 18.3, 2.1 and 12.9 m^2/g, respectively, showing significant differences ($P < 0.05$). The ESIs of beef, lamb, chicken, tuna and emperor were found to be 21.7, 31.4, 21.3, 3.7 and 16.8, respectively. The difference in emulsion properties might be due to several factors such as type of meat, variation of the protein fraction, protein conformation, temperature of oil, and protein solution, physicochemical properties, and functional groups of the proteins (Yapar et al., 2006). The fish proteins were found to have less connective tissue and more myofibrils than meat proteins of beef, lamb and chicken (Mehta et al., 2017). The protein solutions with high hydrophobicity and high solubility are usually reported to have good emulsifying functionalities. However, the proteins are highly sensitive to surface denaturation especially at oil-water and air-water interface, and this sensitivity may influence the surface-active properties (Zayas, 1997). Proteins with a greater number of hydrophobic amino acids interact more significantly with the oil surface. Nevertheless, the balance between hydrophilic and hydrophobic groups is required to lower the interfacial and surface tension (Sathe and Salunkhe, 1981).

The WHC of myofibrillar proteins are given in Fig. 2D. Emperor myofibrillar proteins have higher WHC than all other protein samples ($P < 0.05$). The WHC of beef and tuna protein samples is significantly higher than lamb and chicken protein samples. Liu et al. (2014) stated that the WHC holds a direct linear relationship with pH. In the present study, it can be seen the difference in WHC though the pH is the same in all the samples. The WHC of mammalian myofibrillar proteins are comparably lesser than fish myofibrillar proteins. This might be due to the changes in the protein-protein and protein-water interactions (Chen et al., 2017). The WHC of myofibrillar proteins is also governed by a large content of acidic amino acid such as aspartic and glutamic acid residues (Zayas, 1997).

4. Conclusion

The myofibrillar proteins of beef, lamb, chicken, tuna and emperor were extracted with a high percentage of protein extractability. Physico-chemical, functional and rheological properties of obtained myofibrillar proteins were characterized. The flow profile and visco-elastic analysis revealed the Newtonian and shear thickening nature behaviour at lower protein concentration. Further, the

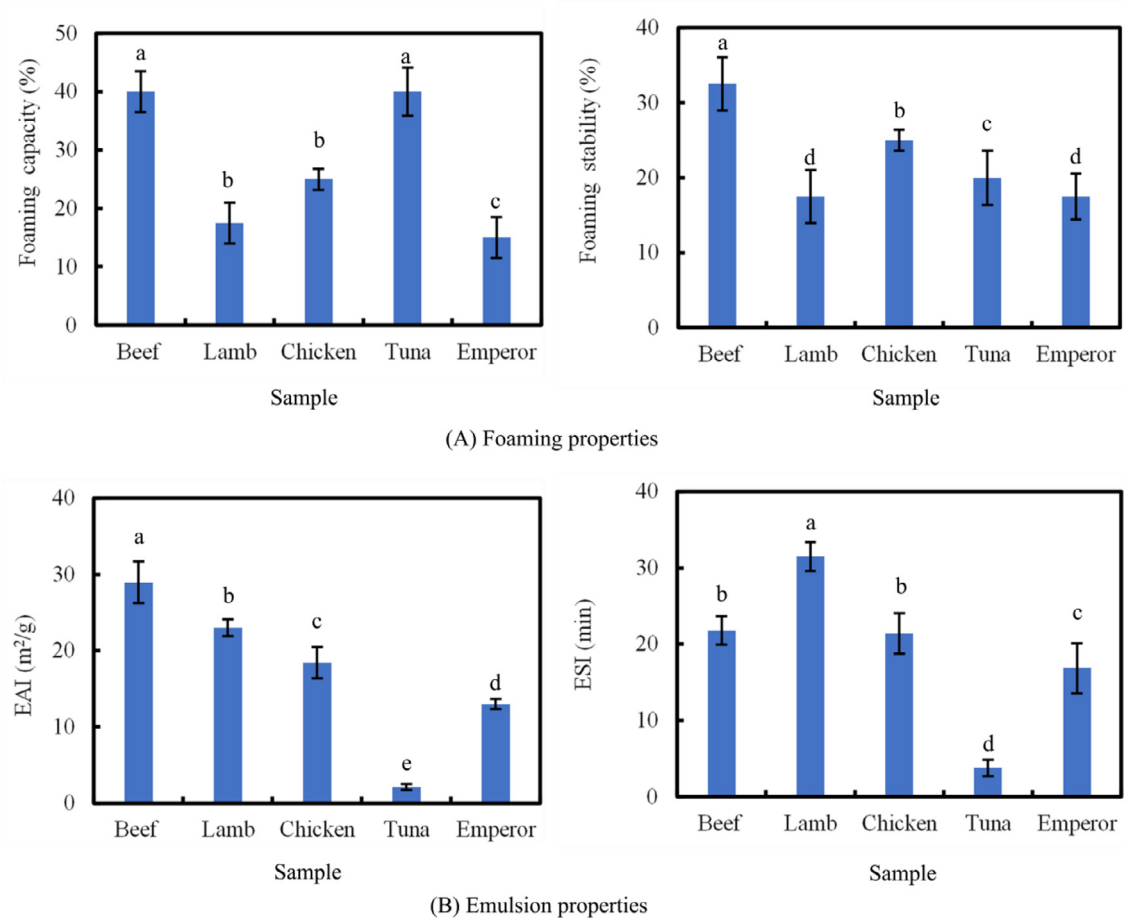

Fig. 5. Functional properties of myofibrillar proteins. Different lowercase letters on the error bars indicate that the results are significantly different ($P < 0.05$).

molecular interaction, surface hydrophobicity and molecular size of myofibrillar proteins were found to have a linear direct relationship with functional and physico-chemical properties. The underlying mechanisms and structural elucidation involved are not fully understood. Hence, further theoretical and experimental studies are needed to investigate the complex behaviour and interaction of meat proteins and with other polymers. And this will make more useful way to develop the formulations of functional foods and nutraceutical products.

Declaration of Competing Interests

All authors declare no competing financial interest.

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