



July – September 2018

Special Issue: International Conference on Probiotics and Food Sustainability 2018
23-24th September 2018, Johor Bahru, Malaysia

Theme: Food Security

Research paper

Investigation on Rheological and Thermodynamic Changes of Fish Processing Waste Hydrolysates from Atlantic Mackerel (*Scomber scombrus*) and Mixed Fish (MF) by Differential Scanning Calorimetry (DSC) and Small Deformation Rheology

Nor Raihana Mohamed Zam^{1,*} and Nazlin K Howell²

¹ Section of Food Engineering Technology, Universiti Kuala Lumpur, Malaysian Institute of Chemical & Bio-Engineering Technology (UniKL-MICET), Lot 1988, Kawasan Perindustrian, Bandar Vendor, Taboh Naning, 78000 Alor Gajah, Melaka, Malaysia.

² School of Biosciences and Medicine, Faculty of Health and Medical Sciences, University of Surrey, GU2 7XH Guildford, Surrey, United Kingdom.

Corresponding author's email address:

noraihana@unikl.edu.my



Received: September 25, 2018/ Accepted: November 10, 2018/ Published online: March 15, 2019

Academic Editor: Zanariah Hashim and Ida Idayu Muhammad

To cite this article:

Mohamed Zam, N.R. and Howell, N.K (2018). Investigation on Rheological and Thermodynamic Changes of Fish Processing Waste Hydrolysates from Atlantic Mackerel (*Scomber scombrus*) and Mixed Fish (MF) by Differential Scanning Calorimetry (DSC) and Small Deformation Rheology. *Asia-Pacific Journal of Food Safety and Security*, 4(3), 22-34.

Highlights

- Fish waste samples showed different protein denaturation transition peaks, indicating that enzymatic hydrolysis can affect the thermodynamic and functional properties of protein samples.
- The rheological properties were different for different Atlantic mackerel (AM) and mixed fish samples.
- Changes in myofibrillar as well as sarcoplasmic proteins of fish waste can be exploited in the future to monitor the effect of freezing and heating during processing and in storage.

About Author

Nor Raihana Mohamed Zam (PhD) is a Senior Lecturer in the Food Engineering section, Universiti Kuala Lumpur, MICET. Her research interests are in food science, food security, seafood protein, bioactive peptides and nutrition.

Abstract

Up to 50 % of fish is lost during filleting, including significant levels of high-quality protein (10-23 % [w/w]), which may be a source for bio-functional peptides. The fish processing waste protein hydrolysate can be a potential solution for minimizing the environmental issues related to marine processing products and act as an alternative to producing value-added fish processing by-products. The main aim of this study was to investigate the structural and thermodynamic changes in proteins from Atlantic Mackerel (*Scomber scombrus*) (AM) and mixed fish (MF) processing waste streams by differential scanning calorimetry (DSC) and small deformation rheology, respectively. The DSC thermograms from the samples indicated that the fish waste samples may be comparable. Moreover, in fish waste sample hydrolysates, the same trends were obtained in 10 kDa AM and MF fractions. Fish waste samples, especially hydrolysed fish waste samples, showed different protein denaturation transition peaks, indicating that enzymatic hydrolysis can affect the thermodynamic and functional properties of protein samples. Similarly, the rheological properties were different for fish samples (AM and MF) with AM showing higher G' or elastic modulus values ($p < 0.05$). This study will contribute to food security by reducing post-harvest losses in sustainable food product chains.

Keywords: fish waste protein, fish processing waste, food security, rheological properties, Atlantic Mackerel (*Scomber scombrus*), differential scanning calorimetry (DSC)

1- Introduction

In a living system, proteins are found in cell membranes, aqueous solutions, and in lipid-protein complexes. Protein denaturation is usually associated with conformational changes in the molecular structure. The denaturation process may include protein unfolding or association due to covalent and non-covalent bonds that occur at specific sites of interaction. Extensive protein-protein interactions, can lead to aggregation or polymerization, precipitation, coagulation and flocculation, which results in a higher molecular weight, with larger complexes. Gelation is a process involving native or partially denatured proteins. The exposed reactive groups in the unfolded protein molecules interact systematically to form networks via balanced protein-protein and protein-solvent interactions. This process can result in a three-dimensional protein matrix with water holding capabilities (Messens et al., 1997 and Howell, 1992).

The term functional properties of proteins, which comprises gelation, foaming and emulsification that occur during food processing, storage and consumption relates to physicochemical properties. In native proteins, any changes in physicochemical properties are associated with changes in the primary structure, comprising a linear chain of amino acids, secondary structure (alpha helix, beta-sheet and random coil) as well as the tertiary structure. Furthermore, free amino acids and peptides which are the products resulting mainly from enzymatic hydrolysis (Chalamiah et al., 2012) or chemical modification may also contribute to enhancement of functional properties. Smaller polypeptides can improve the functional properties, particularly foaming and emulsification that can be used in different applications.

Therefore, enzymatic hydrolysis has been used successfully to obtain useful peptides from fish and fish by-products (Howell and Kasase, 2010, Halim et al., 2016).

According to Badii and Howell (2002), fish proteins are known to be exposed to aggregation and denaturation during storage as well as temperature, which affects fish texture and nutritional value. Protein denaturation can be measured by the reduction in extractable myofibrillar proteins; several studies have reported that during frozen storage the myosin heavy chain fraction of the myofibrillar proteins is sensitive to freeze denaturation (Del Mazo et al., 1999, Badii and Howell, 2002).

To date, whilst there are several papers published on the physicochemical properties of proteins in different types of fish under several conditions (freezing, drying and heating), only a few reports exist on fish waste protein fractions (Filho et al., 2011). Most papers on waste recovery from fish processing report functional properties related to fish oil. There are several studies that investigate the functionality (solubility, emulsion and foam capacity and stability, as well as water and fat binding) of fish protein hydrolysates, especially from the muscle, viscera, skin and roe (Halim et al., 2016).

According to Shahidi (1994), fish processing of by-products using appropriate enzymatic hydrolysis is known to enhance the quality as well as functional properties of the fish products. Physicochemical, functional, as well as nutritional and sensory properties of proteins in their original state can be improved by enzymatic hydrolysis. Thus, enzyme hydrolysis is often used to enhance the applications of low-value food proteins (Althouse et al, 1995; and Kuipers et al., 2005).

Very few papers on hydrolysates from fish processing by-products have been published; these include, for example, fish processing of viscera (Ovissipour et al., 2009), fish skin (Mendis et al., 2005), waste from cod (*Gadus morhua*), shrimp (*Pandalus borealis*) and sardines (*Sardina pilchardus*) and processing industry by-products (Bordenave et al., 2002 and Yin et al., 2010). Hydrolysates obtained from fish proteins are highly affected by the source of the protein, enzymes used, degree of hydrolysis, buffer pH and the reaction temperature and time. Examples of fish hydrolysates with useful functional properties include those from the skin of Nile Perch (*Lates niloticus*), Grass carp (*Ctenopharyngodon idella*) and Nile tilapia (*Oreochromis niloticus*) (Wasswa et al., 2008).

Protein denaturation can be measured thermodynamically and biochemically. The thermodynamic measurement can be performed by Differential Scanning Calorimetry (DSC analysis), which describes the changes of free energy enthalpy (ΔH) in folded and unfolded states after heating.

Molecules in solution exist in equilibrium as native (folded) and denatured (unfolded) conformations. On heating, the term thermal transition midpoint (T_m) is used when 50% of the molecules are unfolded; a high T_m denotes a more stable molecule. In addition, the change in heat capacity (ΔC_p), primarily due to changes in hydration of side chains that were buried in the native state but become solvent when exposed in the denatured state (Badii and Howell, 2006) can also be measured by DSC.

Denaturation changes affect the functional properties like gelation. Gelation properties of proteins are generally measured by rheological methods. Rheology is a term used to determine the flow and

deformation of materials that affects the texture and consumer acceptance of products, especially in food products. Rheology is also regarded as a key method for monitoring texture in food processing, quality control, and the shelf life of food materials (Ötles, 2011). Food materials display elastic and viscous behaviour, which is measured by viscosity and oscillatory shear, as well as a wide range of small oscillatory and large deformation rheological tests that are commonly used to characterize food gels.

2- Materials and Methods

2.1 Sample preparation and preservation

Fish waste (FW) from Atlantic Mackerel (AM) and mixed fish (MF) samples from fish industry processing, were bought from The Fish Society, Wormley, Surrey GU8 5TG, UK. The fish samples were usually dispatched in ice directly to the Food Science laboratory. The fish waste samples were processed upon arrival to the lab, weighed and homogenized in Omni Mixer-CAMLAB) at 6000 rpm for 30 seconds in ice. All chemicals used were mostly bought from Fisher Scientific, Loughborough, UK and Sigma Aldrich, Poole, UK.

2.2 Heat Treatment

FW samples were treated with heat at 65° C in a water bath for 20 minutes to achieve microbiological quality and avoid the existence of any endogenous enzymes.

2.3 Fish Protein Hydrolysate

The method used was that of Megias et al., (2004), the powder form of the water-soluble fish waste protein was mixed with milli-Q water in the ratio (1:10 w/v) and homogenized (Omni mixer homogenizer, USA). The pH was adjusted to pH 2.0 using 1M HCl. Then, pepsin was added to the suspension with a substrate/enzyme ratio of 20:1. After 3 hours, the pH of the mixture was adjusted again to pH 7.5 using 1M NaOH. Subsequently, pancreatin was added using the same ratio and time of digestion as described for pepsin. In order to deactivate the enzyme reaction, the mixture was immersed in boiling water for 20 minutes. Then, after cooling, the sample was filtered and lyophilized for further study.

2.4 Differential Scanning calorimetry (DSC)

Protein Denaturation was measured by using a Setaram Micro DSC VII differential scanning calorimeter (Setaram, Lyons, France)

The fish sample (0.8-1 g wet weight) and reference (distilled water) were scanned at 1° C min⁻¹ over the range 10-90° C and cooled to 10° C (first scan). The sample was rescanned (second scan); this was subtracted from the first scan to obtain a heat flow-corrected signal as described in the Setaram DSC handbook and Setsoft software. For each peak, a linear baseline was constructed by joining the first point at the peak start and the last selected point at the end of the peak, according to the Setsoft software. The total energy required to denature the protein, i.e. the change in enthalpy (ΔH), was estimated

by integrating the area under each peak. The temperature reached when half of the protein is denatured is the transition temperature (T_m), which was measured at the tip of the peak (Setaram DSC handbook and Setsoft software). Three replicates of each sample were tested.

2.5 Small Deformation Rheology Test

Small deformation rheological analysis of fish water soluble and salt soluble protein concentrations of Atlantic Mackerel and mixed fish were determined on a Rheometrics (Leatherhead, Surrey, UK) controlled stress 200 rheometer using a 40 mm parallel plate geometry and a 0.3 mm gap. A temperature sweep, with a stress of 0.1 Pa and a frequency of 1rad/s was used. Evaporation during heating was minimized by using silicone oil.

The temperature of the Peltier plate was programmed to ramp, at a rate of $2^{\circ}\text{C min}^{-1}$, from 20 to 90°C , was then cooled to 20°C at the rate of $2^{\circ}\text{C min}^{-1}$. Changes in the elastic or storage modulus (G') and loss modulus (G'') were recorded and G'/G'' crossover point were recorded.

2.6 Statistical Analysis

Statistical analysis by t-test was performed using Microsoft Excel. All values represent means of triplicate analysis and are given with standard deviations and those at $p < 0.05$ were considered significant.

3- Results and discussion

3.1 Differential Scanning Calorimetry (DSC)

3.1.1 Water soluble fish protein (Atlantic Mackerel)

The DSC thermogram analysis parameters include; heat energy uptake (C_p endotherm), transition peak (T_m) as well as calorimetric enthalpy change (ΔH_{cal}) (**Figure 1**)

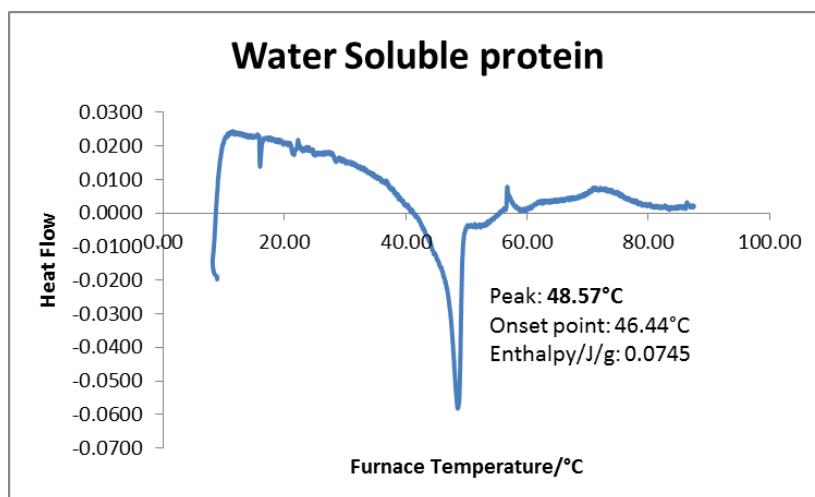


Figure 1. DSC thermogram of Atlantic Mackerel water-soluble fish protein

On heating the sample at a constant rate, heat change was detected due to thermal denaturation. The main transition temperature (T_m) was 48.57° C, which is the point where water soluble proteins started to denature.

The fish proteins in aqueous solution were in equilibrium between the native and denatured (unfolded) conformation. The native state remains stable depending on the size of the Gibbs free energy (ΔG) and the thermodynamic interplay between enthalpy (ΔH) and entropy (ΔS) changes. Therefore, a positive ΔG is characteristic of a stable the native state rather than the denatured state. Proteins denature and unfold when covalent and non-covalent bonds are broken. When conformational entropy overcomes the bonding forces, the protein is able to unfold on heating to temperatures where entropy becomes the main factor (Johnson, 1995).

3.1.2 Fish waste samples (Atlantic Mackerel and Mixed Fish)

The DSC analysis was undertaken on waste obtained from Atlantic Mackerel and mixed fish as well as hydrolysates samples prepared from fish waste (10 kDa of Atlantic Mackerel and 10 kDa mixed fish). The DSC thermograms from the samples indicated that they may be comparable. By investigating the denaturation peaks enthalpies (J/g) and denaturation temperatures (°C) (**Table 1**), the t-test of significance showed that there were no significant differences between the two types of fish waste samples ($p > 0.05$).

The denaturation enthalpy change for myosin in Atlantic mackerel (0.349 J/g) was higher at the transition temperature (T_m) of 49.3° C than mixed fish (0.286 J/g at 47.4° C). For actin denaturation, mixed fish had the highest enthalpy change values (0.12 J/g) compared to Atlantic Mackerel (0.055 J/g), and there was no significant difference between samples ($p > 0.05$). Sarcoplasmic proteins in mixed fish waste sample denatured more readily with a lower enthalpy change value than in Atlantic Mackerel fish waste. Different habitats and type of fish have different muscle fibre composition, i.e. mixed fish had more white muscle fibre than Atlantic mackerel. White muscle fibres comprise myofibrils, and a few lipid droplets. The fish fat content may contribute to different heat conductivity, which is also different among the species.

For the DSC thermograms of fish waste sample hydrolysates, the same trends were obtained in 10 kDa AM and MF. Atlantic Mackerel showed higher enthalpy change values for myosin (0.69 J/g) and lower enthalpy change values for actin (0.024 J/g) than mixed fish (0.202 J/g for myosin and 0.12J/g in actin), showing that there were significant differences ($p < 0.05$) among the samples.

The controlled enzymatic hydrolysis of fish waste is known to affect the functional characteristics, such as foaming and emulsification, due to the production of smaller polypeptides (Chalamiah et al., 2012 in Halim et al., 2016). In addition, the small peptides are reported to have several therapeutic properties, including anti-hypertensive and anti-cancer properties (Yousr and Howell, 2015).

Table 1. Transition temperature and enthalpy change (J/g) for fish waste (FW) samples in Atlantic Mackerel (AM), mixed fish (MF), 10 kDa AM and 10 kDa MF

FW Sample	AM	MF	10 kDa AM	10 kDa MF
Peak 1 (°C)	49.3 ± 0.2 _a	47.4 ± 0.5 _a	48.1 ± 0.5 _a	47.1 ± 0.1 _a
Enthalpy change (J/g)	0.349 ± 0.06 _a	0.286 ± 0.050 _a	0.69 ± 0.005 _a	0.202 ± 0.0080 _a
Peak 2 (°C)	60.1 ± 0.3 _a	--	59.7 ± 0.7 _a	--
Enthalpy change (J/g)	0.001 ± 0.001 _a	--	--	--
Peak 3 (°C)	71.2 ± 0.2 _a	--	70.1 ± 0.6 _a	--
Enthalpy change (J/g)	0.007 ± 0.002 _a	--	0.007 ± 0.001 _a	--
Peak 4 (°C)	80 ± 0.3 _a	76.7 ± 0.1 _a	79.4 ± 0.003 _a	75.2 ± 0.001 _a
Enthalpy change (J/g)	0.055 ± 0.002 _a	0.120 ± 0.030 _a	0.024 ± 0.002 _b	0.120 ± 0.020 _b

Values expressed as mean ± SD (n=3) of triplicate measurements.

a-b Means within a column with different letters are significantly different (P<0.05)

ANOVA single factor was carried out by Least Significant Difference (LSD) test, followed by the T-test.

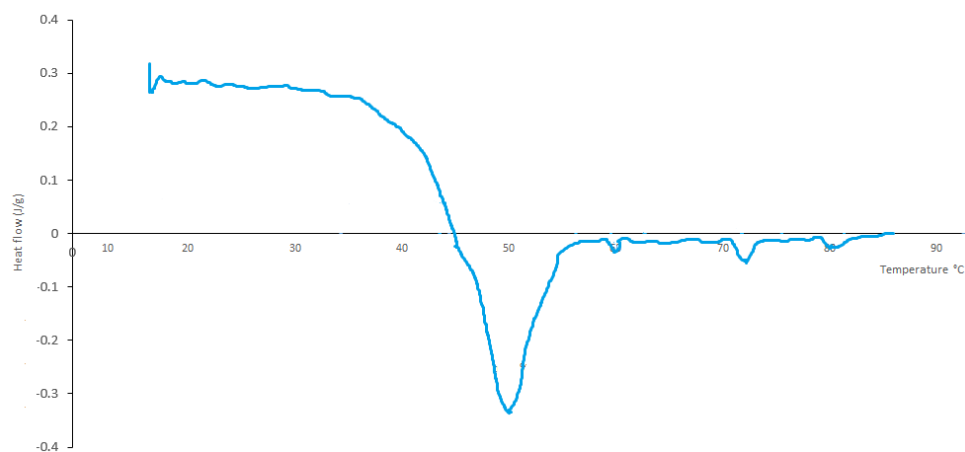


Figure 2. DSC thermogram of Atlantic Mackerel (AM) fish waste sample

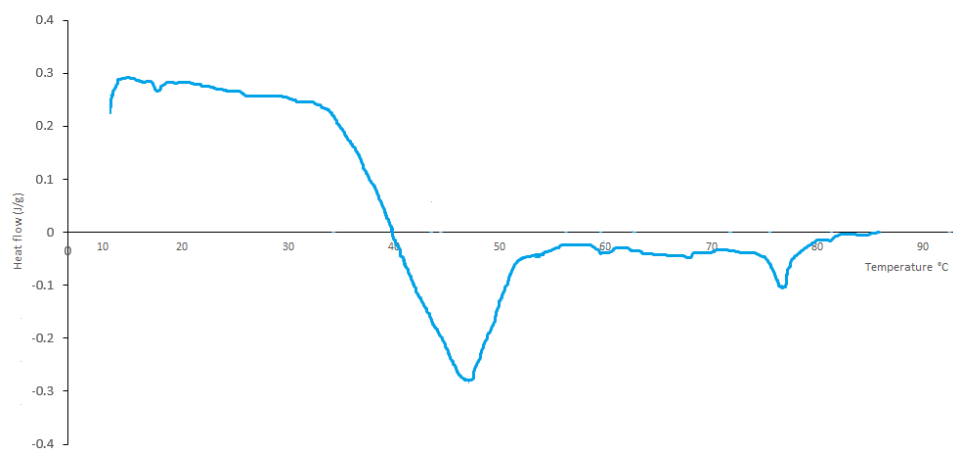


Figure 3. DSC thermogram of mixed fish (MF) waste sample

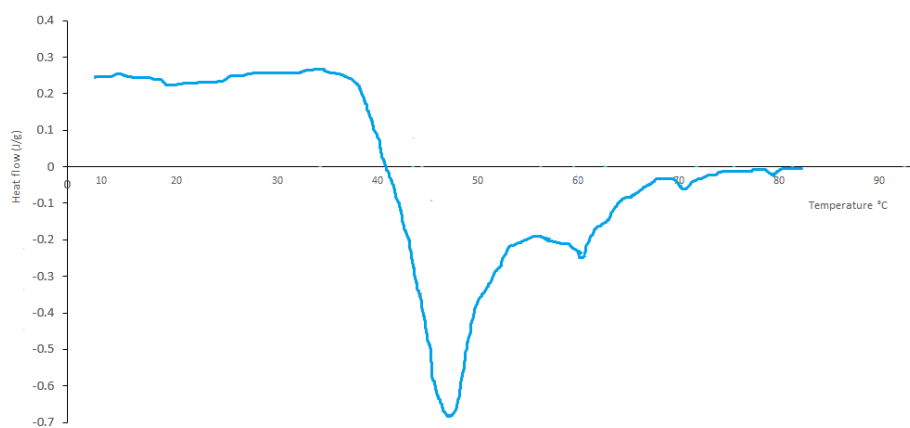


Figure 4. DSC thermogram of 10 kDa Atlantic Mackerel (AM) fish waste hydrolysate sample

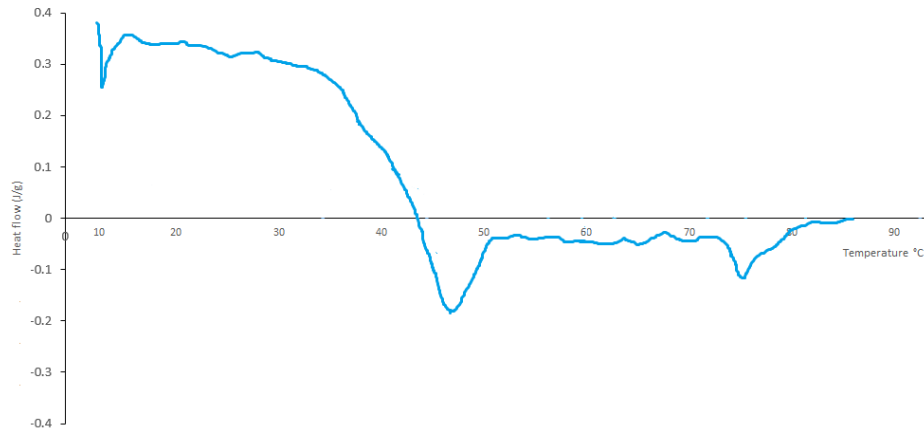
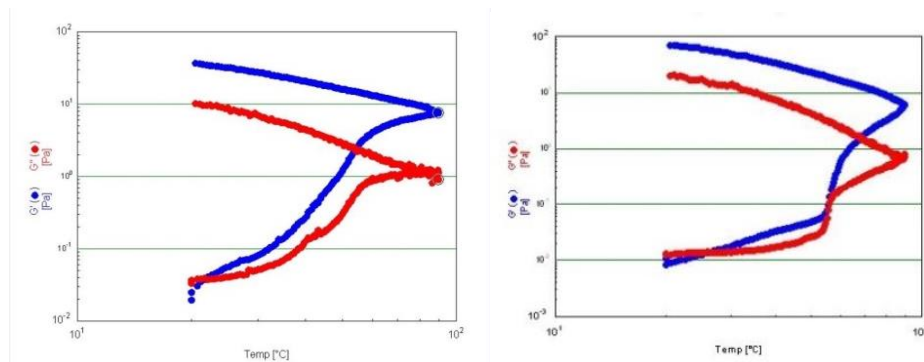


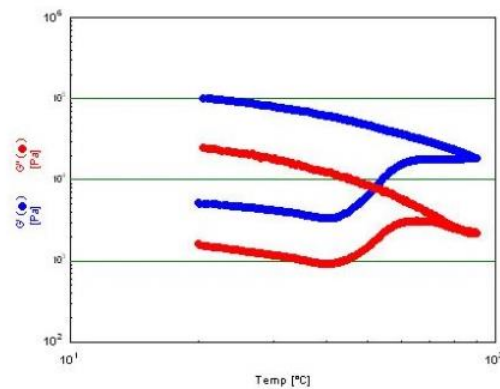
Figure 5. DSC thermogram of 10 kDa mixed fish (MF) waste hydrolysate sample

3.2 Small Deformation Rheology test



(a) Water soluble fish protein

(b) Salt soluble actomyosin fish protein



(c) Fresh Atlantic Mackerel fish

Figure 6. (a) Temperature sweep and rheological properties (G' and G'') of water-soluble fish protein from processing waste sample; (b) Temperature sweep and rheological properties (G' and G'') of salt soluble actomyosin from fish processing waste sample and (c) Temperature sweep and rheological properties (G' and G'') of fresh Atlantic Mackerel fish processing waste sample.

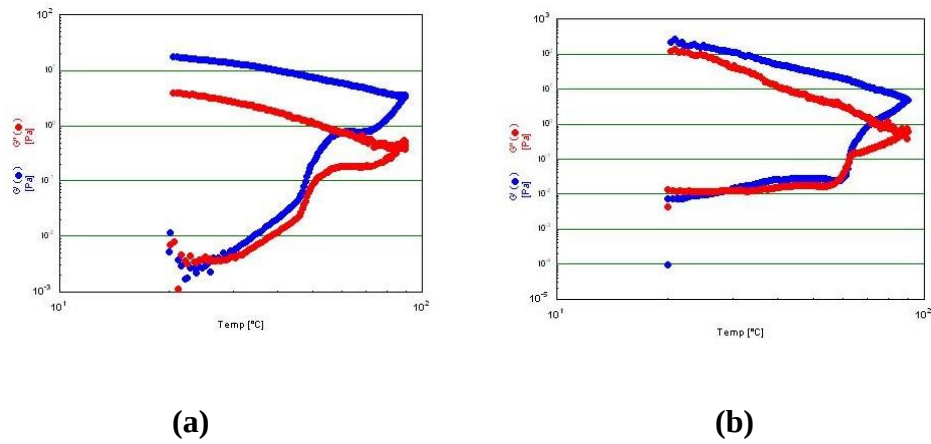


Figure 7. Temperature sweep and rheological properties (G' and G'') of **(a)** water-soluble, and **(b)** actomyosin proteins from fish processing waste stream sample of mixed fish

The results obtained for both water-soluble fish protein fractions showed that prior to heating, the storage modulus (G') was greater than the loss modulus (G''), demonstrating that the samples had a gel-like structure as shown in **Figure 6** and **Figure 7**. The viscous and loss modulus of both Atlantic Mackerel and mixed fish increased with time and temperature after storage at -80°C .

The G' (storage modulus) of Atlantic Mackerel fish sample were considerably higher at 90°C (18399 Pa) and cooled to 20°C ($G'=1.02\text{E}+05$ Pa) compared with water and salt soluble sample at 90°C ($G'=7.62$ and 6.15 Pa respectively) and 36.82 , 70.93 Pa respectively when cooled at 20°C ($p<0.005$).

Meanwhile, the G' (storage modulus) of salt soluble mixed fish processing waste streams protein was higher at $90^{\circ}\text{C}=5.0268$ Pa and cooled to 20°C ($G'=215.97$ Pa) compared to the water-soluble sample at 90°C ($G'=3.6774$ Pa) and 17.629 when cooled at 20°C ($p<0.05$).

As G' is the energy stored elastically in the structure, this shows that Atlantic Mackerel sample was tougher than the mixed fish sample. The difference between the G' values of the water-soluble (small proteins, peptides) and salt soluble proteins (high MW myosin and actin) indicates that the latter can form stronger 3D gel networks and influence the rheological and texture properties.

4- Conclusions

Fish waste samples, especially hydrolysed fish waste samples, showed different protein denaturation transition peaks, indicating that enzymatic hydrolysis can affect the thermodynamic and functional properties of protein samples. Similarly, the rheological properties were different for different fish samples (AM and MF) with AM showing higher G' or elastic modulus values. The salt soluble actomyosin fraction had higher G' values than the water-soluble fraction ($p<0.05$).

Changes in myofibrillar as well as sarcoplasmic proteins of fish waste can be exploited in the future to monitor the effect of freezing and heating during processing and in storage as well as to study the effect of antioxidants added to preserve the fish waste samples.

Further work to be taken into consideration includes measurement of the amino acid composition by HPLC in order to see if the different amino acid content in both Atlantic Mackerel and mixed fish processing waste protein contributed to the gel strength.

Few papers report changes in water holding capacity of fish due to surface dehydration of proteins as well as the relationship between protein solubility of muscle and toughening of tissue during frozen storage fish (Badii and Howell, 2002). Thus, further work can be done on moisture content and protein solubility of fish proteins (myofibrillar protein and other mixture of protein components) alongside the small deformation testing.

References

- Althouse, P. J., Dinakar, P. and Kilara, A. (1995). Screening of proteolytic enzymes to enhance foaming of whey protein isolates. *Journal of Food Science*, 60 (5), 1110-1112.
- Badii, F. and Howell, N.K. (2002). Changes in the texture and structure of cod and haddock fillets during frozen storage. *Food Hydrocolloids*, 16, 313-319.
- Badii, F. and Howell, N.K. (2006). Fish gelatin: Structure, gelling properties and interaction with egg albumin proteins. *Food Hydrocolloids*, 20, 630-640.
- Binsi, P.K., Shamasundar, B.A., Dileep, A.O., Badii, F. and Howell, N.K. (2009). Rheological and functional properties of gelatin from the skin of Bigeye snapper (*Priacanthus hamrur*) fish: Influence of gelatin on the gel-forming ability of fish mince. *Food Hydrocolloids*, 23, 132-145.
- Bordenave, S., Fruitier, I., Ballandier, I., Sannier, F., Gildberg, A., Batista, I. and Piot, J. M. (2002). HPLC preparation of fish waste hydrolysate fractions. Effect on guinea pig ileum and ACE activity. *Preparative Biochemistry and Biotechnology*, 32(1), 65-77.
- Chalamaiah, M., Hemalatha, R. and Jyothirmayi, T. (2012). Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. *Food Chemistry*, 135(4), 3020-3038.
- Del Mazo, M. L., Torrejón, P., Careche, M. and Tejada, M. (1999). Characteristics of the salt-soluble fraction of hake (*Merluccius merluccius*) fillets stored at -20 and -30° C. *Journal of Agricultural and Food Chemistry*, 47(4), 1372-1377.
- FILHO, P. O., Sobral, P. J. A., Trindade, M. A. and Viegas, E. M. M. (2011). Protein denaturation during the processing of sausage prepared with Nile tilapia fillet and minced fish. Volume XXIII Number 2 2011, 23, 194.

Halim, N. R. A., Yusof, H. M. and Sarbon, N. M. (2016). Functional and bioactive properties of fish protein hydrollysates and peptides: A comprehensive review. *Trends in Food Science & Technology*, 51, 24-33.

Howell, N. K. and Kasase, C. (2010). Bioactive peptides and proteins from fish muscle and collagen. *Biologically Active Food Proteins and Peptides in Health – Fundamental and Clinical Aspects*. Ch 14. Ed Yoshinori Mine, Eunice C.Y. Li-Chan, and Bo Jiang. Blackwell-Wiley Publ. London.203-224.

Howell, N.K. (1992). Protein-protein interactions. In *Biochemistry of Food Proteins*. Ed. B.J.F.Hudson. Elsevier Applied Science Publ. Ltd., Essex, pp 35-74.

Ishak, N.H. and Sarbon, N.M. (2018). A Review of Protein Hydrollysates and Bioactive Peptides Deriving from Wastes Generated by Fish Processing. *Food Bioprocess Technology*, 11, 2-16.

Johnson, C. M. and Fersht, A. R. (1995). Protein stability as a function of denaturant concentration: the thermal stability of barnase in the presence of urea. *Biochemistry*, 34(20), 6795-6804.

Kuipers, B. J., van Koningsveld, G. A., Alting, A. C., Driehuis, F., Gruppen, H. and Voragen, A. G. (2005). Enzymatic hydrolysis as a means of expanding the cold gelation conditions of soy proteins. *Journal of Agricultural and Food Chemistry*, 53(4), 1031-1038.

Mendis, E., Rajapakse, N., Byun, H.g. and Kim, S.K. (2005). Investigation of jumbo squid (*Dosidicus gigas*) skin gelatin peptides for their in vitro antioxidant effects. *Life Sciences*, 77, 2166-2178.

Messens, W., Van Camp, J. and Huyghebaert, A. (1997). The use of high pressure to modify the functionality of food proteins. *Trends in Food Science & Technology*, 8(4), 107-112.

Otles, S. (Ed.). (2011). Methods of analysis of food components and additives. CRC Press.

Shahidi, F. (1994). Seafood processing by-products. In *Seafoods: Chemistry, Processing Technology and Quality* (pp. 320-334). Springer US.

Wasswa, J., Tang, J. and Gu, X. (2008). Functional properties of Grass carp (*Ctenopharyngodon idella*), Nile perch (*Lates niloticus*) and Nile tilapia (*Oreochromis niloticus*) skin hydrollysates. *International Journal of Food Properties*, 11(2), 339-350.

Yin, H., Pu, J., Wan, Y., Xiang, B., Bechtel, P. J. and Sathivel, S. (2010). Rheological and functional properties of Catfish skin protein hydrollysates. *Journal of Food Science*, 75, E11-E17.

Yousr, M. and Howell, N. (2015). Antioxidant and ACE Inhibitory Bioactive Peptides Purified from Egg Yolk Proteins. *International Journal of Molecular Sciences*, 16(12), 29161–29178.

© 2018 by the authors; licensee 2050Science Publishers, Christchurch, New Zealand. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.