

Physico-chemical properties of red tilapia (*Oreochromis* spp.) during surimi and kamaboko gel preparation

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Abstract

Physico-chemical properties of red tilapia mince during the preparation of surimi and kamaboko gel were evaluated to determine the potential of red tilapia as a source for surimi. Processing of red tilapia for surimi and kamaboko gel resulted in a significant lower Ca^{2+} ATPase activity, protein and fat than mincemeat. Thermograms of Differential Scanning Calorimetry (DSC) showed three peaks at 31.0, 54.5 and 72.0°C for the mince and only two peaks for surimi and kamaboko gel respectively. Enthalpies of myosin peaks in surimi and kamaboko gel were lower compared to the mincemeat, but there were no significant differences among the enthalpies (ΔH) of their actin peaks. The highest maximum storage modulus (G'_{max}) was obtained at 78.5°C for kamaboko gel which corresponded to 2420 Pa. The sodium dodecyl sulfate (SDS-PAGE) gel showed apparent intensity different in myosin (205 kDa) and no obvious differences for actin (43 kDa).

Keywords

Red tilapia
Thermal Property
Storage Modulus
Textural properties
 Ca^{2+} ATPase activity
SDS-PAGE

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Introduction

Surimi-based products originated from Japan, but they are now known in many parts of the world through products like crab meat analogues and products that require special textural properties. Gel-forming ability as one of the main indicators in the determination of surimi quality depends on several factors such as fish species (Benjakul *et al.*, 2001), freshness (Choi *et al.*, 2005), preparation method and the type of additives used in the product formulation (Mao and Wu, 2007), protein concentration, heating temperature and duration (Luo *et al.*, 2001). The gelation is due to the dissociation of actin-myosin complex and followed by interaction between myosin molecules to form a three-dimensional network; however, the cross-linking characteristics of myosin heavy chain (MHC) vary among fish species (Benjakul *et al.*, 2001). During the 2 stage-heating at 40-50°C and at 80-90°C, the cross-linking of mainly myosin by endogenous transglutaminase produced an elastic texture in the surimi gel (Lanier *et al.*, 2005). Luo *et al.* (2001) concluded that the protein concentration was the major factor affecting the gel strength.

Alaska Pollock (*Theragra chalcogramma*), the traditional raw material for surimi is not available in tropical warm waters. It is known that fish species

differ in muscle and protein composition. Red tilapia (*Oreochromis* spp.) is an abundant freshwater fish with a white flesh meat, but has not been fully explored for surimi processing. Klesk *et al.* (2000) reported that tilapia gels could have comparable or superior gel quality compared to pollock gels when set at setting temperatures of 60°C and 40°C, respectively. Among cultivated freshwater fish, red tilapia is popularly cultivated due to its rapid growth; however, tilapia consumption is still limited because of the characteristic muddy odor and intramuscular bones (Yarnpakdee *et al.*, 2004). Thus, processing of red tilapia into value-added seafood products such as surimi could overcome this constrain. Nonetheless, relatively few studies (Duangmal and Taluengphol, 2010; Mahawanich *et al.*, 2010; Tongnuanchan *et al.*, 2011) have been reported on tilapia surimi properties, in particular about its thermal stability and elasticity, which are important factors in determining quality of products formulated from tilapia surimi. Therefore, this study was conducted to determine the potential of red tilapia mince as a good source of surimi by evaluating physico-chemical properties of surimi and kamaboko gel prepared from the mince.

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Material and Methods

Raw material

Fresh red tilapia (*Oreochromis* spp.) weighing approximately 0.5 kg per head was purchased from a wholesale fish market and transported in ice to the laboratory. The fish was gutted, headed, washed and deboned in a deboner for surimi and surimi gel (kamaboko) preparation.

Surimi preparation

Surimi was prepared according to the method of Benjakul *et al.* (2005). The fish was deboned in a deboner machine (Baader 694, Taiwan) with a drum of an aperture size of 4.5 mm for the mince. The mince was collected and washed for 5 minutes with cold water (5°C) at a mince to water ratio of 1:3 (w:v). The water was decanted and the slurry was placed into two layers of cheese cloth and manually squeezed. Washing and dewatering process were repeated three times. Finally, water was decanted in a surimi decanter (model BAN 153, Japan) for 5 minutes. The mince was then mixed with 4% sucrose and 4% sorbitol in the domestic blender (Kitchen Aid, Model 5K5SS, USA) for 5 minutes, packed in 1 kg pack and kept at -18°C until use.

Gel preparation

The frozen surimi was thawed in running tap water for about 15 minutes to reach a temperature of 5°C. It was chopped into small pieces and blended with 2.5% sodium chloride for 4 minutes (4°C) in a super blender (Pensonic, Model PB-326, Malaysia) to obtain a homogenous surimi paste. The moisture content of paste was adjusted to 80% by adding ice during the blending. The paste (500 g) was stuffed into a cylindrical low density polyethylene tubing measuring 3 × 15 cm (diameter × length) and both ends were then sealed. The cylinders were placed in a water bath for the 2 step heating process at 45°C for 30 minutes and then at 90°C for 20 minutes. The heated surimi gel was cooled in the ice water. The samples were equilibrated at room temperature and kept for 24 h at 4°C prior to analyses according to the procedure of Benjakul *et al.* (2005).

Preparation of natural actomyosin (NAM)

Actomyosin was extracted from fish mince, surimi and kamaboko gel according to the procedure of Benjakul *et al.* (2005). Samples (3 g) were homogenized in 30 ml of chilled (4°C) 0.6 M potassium chloride solution (pH 7.0) for 4 minutes (for each 20 seconds homogenization, a 20 seconds resting was allowed). The extract was centrifuged at

8730 × g for 30 minutes at 4°C. Pellets were collected and dissolved in 3 volumes of chilled deionized water and centrifuged at 8730 × g for another 20 minutes at 4°C. The collected actomyosin pellets were dissolved by blending for 30 minutes in an equal volume of the 0.6 M potassium chloride solution. Undissolved debris was removed from the preparation by further centrifugation at 8730×g for another 20 minutes at 4°C. The retained supernatant was the NAM used for Ca²⁺ ATPase analysis.

pH determination

All pH values of samples were determined in triplicates by homogenizing 3 g of samples in 30 ml of deionized water (w/v). pH was determined using a calibrated digital pH meter (Mettler Toledo, Model DELTA 320).

Proximate composition

Proximate analyses of all the three samples were calculated on dry weight basis according to the method of AOAC (2002). The crude protein was calculated based on the conversion factor of 6.25.

Ca²⁺ ATPase activity determination

Ca²⁺ ATPase activity was determined according to Zhou *et al.* (2006) and expressed as microgram of inorganic phosphate (Pi)/mg protein/min at 27°C. The NAM of respective sample was diluted to 2.5-4 mg/ml with 0.6 M potassium chloride (pH 7.0) and 1 ml of the diluted solution was added to 0.6 ml of 0.5 M Tris-maleate (pH 7.0), followed by the addition of 10 ml 10 mM CaCl₂ and 7 ml of distilled water. To the mixture, 0.5 ml of 20 mM adenosine 5'-triphosphate was added to initiate the reaction. The reaction was then conducted at 25°C for 10 minutes and terminated by adding 5 ml of chilled 15% (w/v) trichloroacetic acid. The mixture was centrifuged at 3500×g for minutes and the inorganic phosphate released in the supernatant was estimated by ammonium molybdate according to the procedure described by Fiske and Subbarow (1925).

Gel electrophoresis by sodium dodecyl sulfate polyacrylamide

Laemmli (1970) method was used for SDS-PAGE run. The samples for the SDS-PAGE runs were prepared according to Benjakul *et al.* (2006). About 6 g of the sample was homogenized for 2 minutes in 54 ml of 5% (w/v) SDS solution which was pre-heated to 85°C for 1 hr. The homogenate was then incubated at 85°C for 1 h to dissolve all proteins. Any undissolved residues were removed by centrifugation at 5000×g for 15 minutes. Sample aliquots (~10µl)

containing approximately 20 µg protein and prepared molecular weight marker (SIGMA, Co, Ltd, USA) were loaded into the gel (12.5% separation gel and 4% stacking polyacrylamide gel). Electrophoresis was carried out at 20 mA constant current in 1xTAE (Tris-acetate-EDTA) buffer using a Mini Protein II unit (BioRad Laboratories, CA, USA). The gels were then stained with Coomassie brilliant blue R-250 overnight and destained in a solution containing 15 % methanol (v/v) and 10% (v/v) acetic acid. The apparent molecular weight was calculated using the relative migration distance (Rf) of the peptides.

Texture profile analysis (TPA)

The texture analysis was performed on fish meat cut into 3 × 3 cm from the fillet, surimi and kamaboko gels (heated surimi paste) which were also cut into 3 × 3 cm from the gel prepared in the gel preparation step mentioned earlier for texture analysis using texture analyzer (Stable Micro System, Surry, England) by a double compression test using cylindrical plunger (diameter 50 mm) and the gels were prepared for the test as described under the gel preparation method. The samples were compressed to 60% of the original height at a speed of 60 mm/min using a 5 N load cell according to Martinez *et al.* (2004). Samples with apparent air bubbles were discarded to prevent inaccurate readings. Samples hardness, springiness, cohesiveness, chewiness and adhesiveness index were recorded. Each sample was run in triplicate.

Viscoelastic Measurement The storage modulus of samples during heating in the temperature range of 20°C to 90°C were measured using a Thermo Electro Corporation Rheostress (HAAKE, RT 20, ROTOVISCO, Germany), which was equipped with C35/2°Ti cone and plate geometry. An oscillation of 0.1 Hz with a resistance stress of 3 Pa was used for testing (Rawdkuen *et al.*, 2008). Samples were covered with a thin layer of paraffin oil to avoid evaporation during the analysis. Reading reported were the average from at least three runs.

Thermal properties

A differential scanning calorimeter (DSC7, Perkin–Elmer) was used to determine maximum transition temperature (T_m) and protein denaturation enthalpy of samples according to the method of Karayannakidis *et al.* (2008) with slight modifications. An amount of 10 ± 0.01 mg of sample was placed into a 50µl Perkin–Elmer aluminum pan and sealed using a sample sealer (Perkin–Elmer – 0219 – 0061). The sealed sample was scanned at a heating rate of 5°C/min from 27°C to 100°C. An empty××pan was used as a reference. Maximum temperatures and changes

in enthalpy were determined from the thermogram.

Statistical analysis

Data collected were analyzed statistically by a 1-way ANOVA using a Minitab software (Version 16.0 software, Minitab Inc., PA, USA) followed by Tukey's multiple range test to determine significance between treatments ($P < 0.05$).

Results and Discussion

Changes in pH

pH values of all samples are in the vicinity of 6.6 (Table 1). Slight differences were the result of washing where water-soluble acid elements such as free amino acids, lactic acid and free fatty acid were partially removed. The denaturation of nitrogenous compounds in proteins could also lead to the observed increase in pH during surimi preparation (Benjakul *et al.*, 2002).

Changes in proximate composition

Changes in proximate composition are shown in Table 1. Significantly lower protein and lipid contents were observed in surimi and kamaboko gel compared to the fish mince ($P < 0.05$) which was due to removal of sarcoplasmic proteins during the washing process and also could be due to the increase in the moisture content of the samples. Significant increase in moisture contents of surimi and kamaboko gel could probably be due to the absorption of water by hydrophilic remains of myofibrillar proteins (Karthikeyan *et al.*, 2006). The higher ash and carbohydrate contents in surimi and kamaboko were expected due to the addition of additives such as sucrose and sorbitol. The increase in the ash content of kamaboko gel compared to surimi was due to the addition of salt during kamaboko gel preparation. While the addition of sorbitol and sugar contributed to the increase of carbohydrate content.

Changes in Ca^{2+} ATPase activity

Ca^{2+} ATPase activity of NAM extracted from all the samples are as shown in Table 1. Ca^{2+} ATPase activity of tilapia mince was 0.17 ± 0.04 (µmol Pi/mg protein/min) and a significant increase of Ca^{2+} ATPase activity in surimi and kamaboko gel of red tilapia was observed ($P < 0.05$) compared to that of fish mince. These values are comparable to the values of Ca^{2+} ATPase activities of four marine species (threadfin bream, bigeye snapper, lizard fish and croaker) by Benjakul *et al.* (2005). Although heating would decrease the Ca^{2+} ATPase activity, the higher Ca^{2+} ATPase activity in surimi and kamaboko

Table 1. pH, Ca²⁺ ATPase activity ($\mu\text{mol Pi/mg protein/min}$) and proximate composition (%) of fish flesh, surimi and kamaboko gel from red tilapia

Sample	pH	Ca ²⁺ ATPase activity	Protein	Fat	Moisture	Ash	Carbohydrate
Fish mince	6.60 \pm 0.03 ^a	0.17 \pm 0.04 ^a	18.15 \pm 0.38 ^a	3.10 \pm 0.41 ^a	75.42 \pm 0.48 ^a	2.32 \pm 0.12 ^a	1.01 \pm 0.08 ^a
Surimi	6.70 \pm 0.02 ^a	0.27 \pm 0.01 ^b	14.83 \pm 0.95 ^b	0.95 \pm 0.18 ^b	79.23 \pm 0.62 ^b	2.02 \pm 0.22 ^b	2.97 \pm 0.05 ^b
Kamaboko gel	6.67 \pm 0.04 ^a	0.20 \pm 0.05 ^c	14.13 \pm 0.23 ^b	0.91 \pm 0.23 ^b	78.30 \pm 0.52 ^b	3.65 \pm 0.26 ^c	4.01 \pm 0.06 ^c

Values were means \pm SD after three replications. Different letters in the same column indicated significant difference $p < 0.05$

Table 2. Texture attributes of red tilapia fish mince, surimi and kamaboko gel

Sample	Hardness (g)	Springiness (cm)	Cohesiveness (dimensionless)	Chewiness (g.cm)
Fish mince	267.25 \pm 66.5 ^a	0.80 \pm 0.03 ^a	0.63 \pm 0.03 ^a	135.40 \pm 44.47 ^a
Surimi	443.50 \pm 24.04 ^b	0.80 \pm 0.04 ^a	0.68 \pm 0.02 ^b	282.20 \pm 23.5 ^b
Kamaboko gel	663.06 \pm 37.2 ^c	0.81 \pm 0.03 ^a	0.71 \pm 0.04 ^c	486.66 \pm 5.51 ^c

Values were means \pm SD after three replications. Different superscript in the same column indicated significant difference at $p < 0.05$

gel was due to the washing process, which removed sarcoplasmic proteins, lipid, and other undesirable compounds thus concentrating myofibrillar proteins and consequently a higher initial Ca²⁺ ATPase activity relative to the fish mince (Chaijan *et al.*, 2004) and subsequently higher content of myosin heavy chain. Since, the globular heads of myosin are responsible for Ca²⁺ ATPase activity, higher Ca²⁺ ATPase activity would be detected. The increase in Ca²⁺ ATPase activity from 0.17 \pm 0.04 in mince fish to 0.27 \pm 0.01 in surimi corresponded to the increase of pH (from 6.60 \pm 0.03 to 6.70 \pm 0.02) after the washing process. Denaturation of protein or decreasing in Ca²⁺ ATPase activity affected the textural and functional properties of protein by making the texture more fibrous and tough due to the lower protein solubility and water holding capacity. A regular decrease in ATPase activity with the increase in temperature was the result of unfolding of actomyosin molecules and the surfacing of the SH groups in the molecules (Sano *et al.*, 1994); however, we observed an insignificant decrease of Ca²⁺ ATPase activity in kamaboko gel compared to surimi.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE)

The SDS-PAGE profiles of myofibrillar proteins of the fish mince, surimi and kamaboko gel are as shown in Figure 1. The bands at \sim 205 kDa and \sim 43 kDa were that of myosin heavy chain and actin, respectively. The intensity of myofibrillar proteins, particularly MHC increased after washing compared to initial unwashed fresh minced fish. Similar observations were reported for threadfin bream

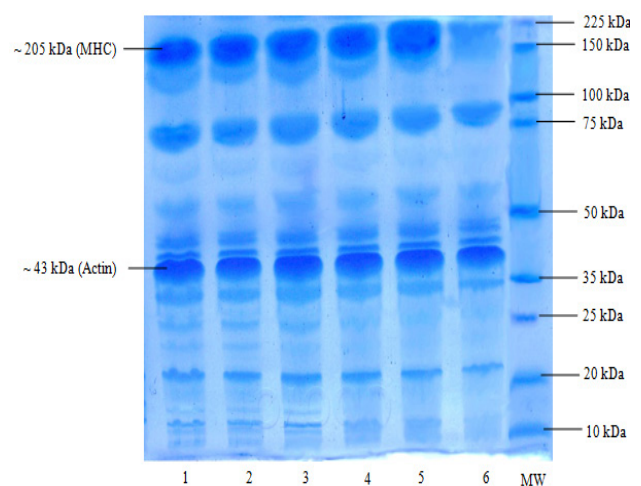


Figure 1. SDS-PAGE profile of myofibrillar protein of 1) unwashed fish mince 2) fish mince after first washing 3) fish mince after second washing 4) fish mince after third washing; 5) surimi 6) kamaboko gel, MW- molecular weight of protein markers

(Karthikeyan *et al.*, 2006). Some peptide bands disappeared in surimi and kamaboko gel. The lowest intensity of MHC was observed in the kamaboko gel, which could be due to the formation of cross-linking between myosin heavy chains. Moreover, gelation of fish paste during setting temperature have a close relationship to the formation of cross-linking between myosin heavy chain and also the thermal formation of non-covalent bonds and disulfide bonds of the myofibrillar proteins (Benjakul *et al.*, 2003).

Texture profile analysis (TPA)

Significant differences ($P < 0.05$) in hardness, chewiness and cohesiveness among the samples were

Table 3. G'_{max} (Pa) and T_{max} ($^{\circ}$ C) of three clear peaks observed in the rheometer measurement of the fish mince, surimi and kamaboko gel

Sample	Peak 1		Peak 2		Peak 3	
	G'_{max}	T_{max}	G'_{max}	T_{max}	G'_{max}	T_{max}
Fish mince	1130 \pm 5.0 ^a	44.3 \pm 0.01 ^a	1710 \pm 4.0 ^a	64.1 \pm 0.02 ^a	5120 \pm 6.0 ^a	76.0 \pm 0.03 ^a
Surimi	1930 \pm 6.0 ^b	41.2 \pm 0.02 ^b	2890 \pm 5.0 ^b	68.3 \pm 0.03 ^b	3342 \pm 4.0 ^b	76.5 \pm 0.01 ^a
Kamaboko gel	2445 \pm 5.0 ^c	40.2 \pm 0.05 ^c	2950 \pm 3.0 ^c	66.5 \pm 0.06 ^c	3420 \pm 3.5 ^b	78.5 \pm 0.04 ^c

Values were means \pm SD after three replications. Different superscript in the same column indicated significant difference at $p < 0.05$

Table 4. Maximum transition temperature (T_m $^{\circ}$ C) and enthalpy (ΔH J/g) of fish mince and kamaboko gel from red tilapia

Sample	myosin peak		sarcoplasmic peak		actin peak	
	ΔH	T_m	ΔH	T_m	ΔH	T_m
Fish mince	0.40 \pm 0.04 ^a	31.0 \pm 0.3 ^a	0.58 \pm 0.03	54.5 \pm 0.5	0.45 \pm 0.02 ^a	72.0 \pm 0.2 ^a
Surimi	0.35 \pm 0.01 ^b	30.2 \pm 0.3 ^a	ND ¹	ND	0.43 \pm 0.03 ^b	71.0 \pm 0.3 ^a
Kamaboko gel	0.35 \pm 0.02 ^b	31.5 \pm 0.5 ^a	ND	ND	0.43 \pm 0.01 ^b	72.5 \pm 0.3 ^b

Values were means \pm SD after three replications. Different superscript in the same column indicated significant difference at $p < 0.05$.

observed (Table 2). Kamaboko gel had significant increase in hardness and chewiness compared to surimi. However, no significant difference was observed for springiness among the samples. Klesk *et al.* (2000) did not use the same method for comparison. The slight shift to higher pH in surimi and kamaboko gel obtained by washing may also contributed to the increase of the textural properties as suggested by Jin *et al.* (2007) where a slight pH different of 0.1 unit resulted in a change of textural properties since this relate to the shear force of the gel. The formation of disulfide bonds was favorable at neutral and alkaline pH values (6.5 to 9.5) were also noted by Shimada and Cheftel (1988) to contribute to the elasticity of whey protein isolate gels which could be due to the increase exposure of the reactive SH groups, which presumably promoted myosin head-to-head aggregation during gelation (Kristinsson and Hultin, 2003). Fish myofibrillar proteins has their isoelectric point in the vicinity of 4 to 6 (Yongsawatdigul and Park, 2004).

Viscoelastic measurement

The storage modulus (G') of fish mince, surimi and kamaboko gel of red tilapia surimi are as shown in Table 3. All samples showed an increasing trend in their G' when the temperature was increased from 20 $^{\circ}$ C to 90 $^{\circ}$ C. An increase in G' of raw material such as fish flesh is correlated to the protein-protein and protein-lipid interactions (Dileep *et al.*, 2005). The three peaks were related to three thermodynamic stages for the formation of meat gel, namely suwari (up to 50 $^{\circ}$ C), modori and kamaboko which develop as

heating proceeds (Jafarpour *et al.*, 2009). The suwari peak (G') for kamaboko gel was obtained at 42.0 $^{\circ}$ C, which was similar to Alaska Pollock surimi 42 $^{\circ}$ C (Noguchi, 1986). The suwari stage is correlated with the cross-linking of actomyosin and myosin. Saroat *et al.* (2008) reported that unfolded actomyosin entangled and formed gel networks as shown by an increase of G' at temperature greater than 55 $^{\circ}$ C. Maximum storage modulus values of third peaks were obtained at temperatures greater than 76 $^{\circ}$ C, which is higher than that of Alaskan Pollock and Pacific whiting surimi (74 $^{\circ}$ C) as reported by Yoon *et al.* (2004).

Thermal Properties Thermal stability of myosin differ from species to species of fish; some had three endotherm peaks, while others had two (Ogawa *et al.*, 1994). Enthalpy and transition temperature of the endothermic peaks are as listed in Table 4. Three clearly endothermic peaks at 31.0, 54.5 and 72.0 $^{\circ}$ C were observed in the thermogram of the fish mince, which were related to the denaturation of myosin, sarcoplasmic proteins and actin, respectively. Only myosin and actin peaks were recorded for surimi and kamaboko gel. The T_m of both myosin and actin shifted to lower temperature in surimi and kamaboko gel compared to fish mince. Sarcoplasmic proteins was removed during washing process of surimi preparation, hence the absence of the peak. The myosin transition temperature of fish mince and kamaboko gel was similar but the enthalpy of myosin peak for kamaboko gel was lower as noted by Park and Lanier (1989). A shift in the thermal

transition of which could be due to the NaCl addition to surimi that caused the unfolding of actin to higher temperature (72 to 72.5°C) in surimi and kamaboko gel thus indicated an increase in thermal stability of protein.

Conclusion

The DSC and storage modulus results indicated that the tilapia surimi forms a strong gel structure at temperatures greater than 72°C. The significant higher Ca²⁺ ATPase activities in surimi and kamaboko gel supported the results from the texture profile analysis on the increase hardness of the kamaboko gel. The present study contributes useful information for the seafood industries on physicochemical properties of red tilapia mince when converted to surimi and kamaboko gel preparation, which takes place during surimi-based products formulations. As a conclusion, the red tilapia exhibited acceptable properties as a source for surimi.

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