

Effect of heat treatment on the properties of surimi gel from black mouth croaker (*Atrobuca nibe*)

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Abstract

The aim of this study was to evaluate the physicochemical characteristics of surimi gel-forming ability due to different heating conditions. Textural properties, whiteness, expressible moisture, microstructure and protein pattern of the gel specimens were determined. All gel samples were incubated for 18 hours at 4°C before applying for analyses. Kamaboko gel (KK) by setting sol at 40°C prior to cooking at 90°C for 20 min showed the highest breaking force (623.20±21.74 g), followed by directly heated (DH) and modori (MD) gels, respectively (p<0.05). The highest expressible moisture (5.269±0.09%) and whiteness (73.91±0.02%) were found in DH gel (p<0.05). Conversely, two-steps heated gels (i.e. KK and MD) showed lowest expressible drip and whiteness (p<0.05). KK had the higher interconnected three-dimensional protein networks than other gels. High Hardness (1.6-3.8 Kg force) and deformation (10.73 to 12.06 mm) of black mouth croaker surimi gels, introduce that this low fat and white-fleshed fish species could be used for producing high quality surimi-based products. Analysis of SDS- PAGE indicated that myosin heavy chain (MHC) as a major protein with a high density band in surimi, decreased in all gels. However, MHC was more retained in DH gel. In conclusion, preparation of KK enhanced the gel-forming properties of surimi.

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Introduction

Black mouth croaker (*Atrobuca nibe*) is the most abundant aquatic species by-catch content of lanternfishes commercial mid-water trawl exploitation of the Iranian site of Oman Sea and catch rate of this species reach at 863 tons in the recent years (Iranian Fisheries Organization Statistical Yearbook, 2012). Also, the presence of this deep sea fish species in the Indian Ocean has been reported earlier (Rajab, 1988; Johannesson and Valinassab, 1994). Since, croaker species are classified into the light muscle fish; they are the preferred raw material of the traditional kamaboko industry (Nopianti *et al.*, 2010) and commonly used as a raw white fish species for the surimi manufacturers in Aisa (Morrissey and Tan, 2000).

Myofibrillar proteins (i.e. myosin, actin, tropomyosin and troponin) are made up largest proportion of surimi through their gel-forming capacity when heated (Hall, 2011). These proteins are responsible for the formation of gel (Zhou *et al.*, 2006) and their properties are affected by the species,

fish freshness, pH, ionic strength and processing procedure parameters (Niwa, 1992; Shimizu *et al.*, 1992). It is inferred that surimi gel is a framework consists of a continuous macroscopic myofibrillar protein suspended in a semi-solid medium without showing the steady state flow.

Application of setting in the surimi industry has been used to improved gel properties (Kimura *et al.*, 1991; An *et al.*, 1996). Setting the surimi sol at different temperature and time may lead to different gel characteristics (Benjakul and Visessanguan, 2003; Benjakul *et al.*, 2003a; Arfat and Benjakul, 2012). It is believed that types of fish species according to their temperature habitat can be highly caused the different setting response (Shimizu *et al.*, 1981; Tsukamasa and Simizu, 1989; Tsukamasa and Shimizu, 1991; Morales *et al.*, 2001). The setting phenomenon closely related with polymerization of myosin heavy chain (MHC) induced by endogenous transglutaminase (TGas) and sulfhydryl enzyme (Kimura *et al.*, 1991; Wan *et al.*, 1994; Kumazawa *et al.*, 1995). Arfat and Benjakul (2012) expressed that gelling properties of yellow stripe trevally (*Selaroides*

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leptolepis) surimi were improved during setting the sol at 40°C followed by another kamaboko gel having setting temperature of 25°C, directly heated gel (90°C) and modori gel (60°C), respectively. Setting of surimi sol from *Priacanthus tayenus* at 40°C for 2 h and *P. macracanthus* at 25°C for 3 h showed the optimum gel-forming ability conditions, respectively (Benjakul and Visessanguan, 2003). Generally, the quality of gels those with prior setting are mostly wealthier than directly heated gels (Niwa, 1985; Van Phu *et al.*, 2010). However, occurring of modori phenomenon at temperature close to 60°C, resulting in brittle gel due to destroy the gel structure by activating proteases (Alvarez *et al.*, 1999). Benjakul *et al.* (2003a) reported that the application of medium setting temperature (25°C) on gel properties and cross-linking of myofibrillar protein in the Thailand surimi industry to obtain a better quality. Effective use of this new source of surimi is based on modifying of gel-forming ability characteristics. Hence, the present study determines the effect of setting on physical (i.e. textural properties, expressible drip, whiteness and microstructure) and chemical (i.e. SDS-Page) gelling properties of black mouth croaker surimi.

Materials and Methods

Fish samples

Sampling of black mouth croaker (*A. nibe*) was conducted at different depths, from surface to 400 m depth in the Iranian site of Oman Sea by R/V “Ferdows-1” that was equipped with a mid-water trawl net. Collected fish (0.631 ± 0.074 g mean weight) were iced with a fish/ice ratio of 1:2 (w/w) on board immediately after capturing and transported to the Fisheries Science laboratory, Islamic Azad University, Science and research branch, Tehran (less than 16 hours).

Preparation of surimi and gel

Preparation of surimi was based on the method reported by Lee (1984), with slight modifications. Briefly, fish were gutted, beheaded and washed with chilled fresh water manually. To obtain mince, skin and bones of iced fillets were first removed, and then minced by a bone separator machine with a 2 mm drum (SEPAmatic, Bergisch Gladbach, Germany), in a temperature-controlled room ($15 \pm 2^\circ\text{C}$). The minced meat was leached twice using 1 part fish minced to 3 parts cold distilled water (w/v). For the third washing, cold 0.3% NaCl solution was used. Each washing cycle was stirred gently and took place for 5 min at water temperature 2–4°C. At least, washed minced was wrapped in a folded silk cloth and squeezed

manually. Cryoprotectant agents (sucrose 4%, sorbitol 4%, and sodium tripolyphosphate 0.3%) were finally incorporated to the prepared dewatered mince with a mixer (FP 6001 Moulinex Food Processing, France) for a further 60s. To maintain the temperature of surimi paste below 10°C, before mixing processes, the bowl and blade of food processor were kept at -20°C about 1 h. Surimi was packed in airtight zip-lock polyethylene bags (500 ± 0.1 g). Each block was frozen individually using a spiral freezer at -35°C for 30 min (air flow of 5 m/s) and then kept at -20°C. Frozen surimi was stored not longer than 1 month.

To prepare the gels, frozen surimi was left in a refrigerator ($4 \pm 1^\circ\text{C}$) for 4–5 h until the core reach in zero temperature. The thawed surimi was cut into small pieces (about $2 \times 2 \times 2$ cm³), added with 2.5% salt (w/w) and chopped for 3 min to obtain the homogenous sol. During homogenization, iced-water was sprinkled over the mixture to adjust the moisture content to 80 ml/100 g. The resultant sol was stuffed into polyvinylidene chloride casing with a diameter of 2 cm, and both ends of the casing were sealed (Lanier 2000; Benjakul *et al.* 2003a). A directly heated gel (DH) was prepared by heating the sol in a water bath (Mettler, Germany) at 90°C for 20 min. The kamaboko gel (KK) was prepared by setting the sol at 40°C for 30 min, followed by heating at 90°C for 20 min. Setting the sol at 60°C for 30 min, followed by heating at 90°C for 20 min was referred as Modori gel (MD) (Benjakul *et al.* 2010). All heated gels were cooled immediately in iced-water for 30 min to stop any further effect of heat on the texture and stored at 4°C over night (18 h) prior to analysis.

Textural properties

Puncture test

Puncture test was carried out on the cylinder-shaped gel samples (20 mm diameter and 25 mm height) using a model CT3-4500 texture analyzer (Brookfield Engineering Laboratories, USA). Gels left to equilibrate at ambient temperature (27–29°C) for 30 min. Breaking force (g) and deformation (mm) were measured using the texture analyzer equipped with a stainless steel spherical plunger (diameter 5 mm, depression speed of 60 mm/min). The load cell capacity and trigger force used were 25 kg and 5 g.

Texture profile analysis (TPA)

Tempered gels were placed on a flat platform and were double compressed from 50% of original height by an acrylic cylindrical plunger (50 mm diameter) adapted to a 25 Kg load cell at a deformation rate of 60 mm/min. Textural parameters like hardness-1,

hardness-2, springiness and cohesiveness were calculated from force by time curve plot generated for each sample (Hayes *et al.*, 2005; Dey and Dora, 2011). Shear test was done according to the Dey and Dora (2011) with slight modification. Briefly, the cylindrical gel sample (20 mm diameter and 15 mm height) was placed horizontally on a platform and was cut into 2 pieces with a shearing speed of 50 mm/min (Warner-Bratzler shear probe; 4500 kg load cell).

Color

Surimi gel samples (50 mm thickness and 20 mm diameter) were measured for the degree of lightness (L^*), redness/greenness (a^*) and yellowness/blueness (b^*) using a colorimeter (Hunterlab Colorflex, USA). Whiteness index was calculated by the following formula of Park (1994):

$$\text{Whiteness} = [(100 - L^*)^2 + a^{*2} + b^{*2}]^{\frac{1}{2}}$$

Expressible moisture

Expressible moisture was measured according to the modified method of Benjakul *et al.* (2003b) by Arfat and Benjakul (2012). A gel sample with a thickness of 50 ± 0.1 mm was weighed (X in grams) and placed between two pieces of Whatman No. 1 filter paper at the top and three pieces of the filter paper at the bottom. The standard weight (5 kg) was placed on the top of the sample and maintained for 2 min. The sample was then removed from the filter paper and weighed again (Y in grams). Expressible moisture content was calculated and expressed as percentage of sample weight as follows:

$$\text{Expressible moisture (\%)} = \left[\frac{(x - y)}{x} \right] \times 100$$

Scanning electron microscopy

Method for scanning electron microscopy (SEM) of surimi gels was carried out according to Nurkhoeriyati *et al.* (2011) with slight modification. Gel samples with a thickness of 2 mm were freeze-dried at -56 °C for 28 h using a model UF40-350T freeze-dryer (Colora, Germany). Dried samples were mounted on a bronze stub and sputter-coated with gold layer. The specimens were visualized with a scanning electron microscope (LEO 440i, Oxford, UK) at an acceleration voltage of 15 kV and 5-10 Pa pressure.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

A discontinuous sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with 4% stacking gel and 10% running gel was used

to determine the protein pattern of surimi and surimi gels according to the method of Laemmli (1970). To prepare the protein sample, 3 g of sample was homogenized with 5% (w/v) SDS solution in a final volume of 30 ml using a homogenizer (Heidolph, type D-91126, Germany) at 1100 rpm for 3 min. The homogenate was incubated at 85°C for 1 h to dissolve total proteins. The samples were centrifuged at $4500 \times g$ for 30 min to sink undissolved debris and then supernatants solution mixed with sample buffer (0.5 M Tris-HCl, pH 6.8, containing 4% SDS, 20% glycerol and 10% 2- β -mercaptoethanol) at ratio of 1:1, the mixture was boiled for 3 min. An aliquot of 15 μL of samples was loaded into the stacking gel subjected to electrophoresis at a constant current of 15 mA/gel using a Mini Protein tetra cell (Bio-rad, Laboratories, Inc., USA). After separation, the proteins were stained with Coomassie Blue R-250 and then destained with 5% methanol and 7.5% acetic acid on a rotary rocker over night. A prestained protein ladder used as the marker (Fermentas, Product No. 26619), ranged from 10-250 kDa.

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) at the significance level of 5%, using statistical software (SPSS 15.0, package program for windows, Chicago, IL, USA). All graphs were drawn in Excel 2007 software.

Results and Discussion

Gel penetration property

Breaking force (gel strength) and deformation (elasticity/deformability) of black mouth croaker surimi gels at different heating conditions are shown in Figure 1. Setting the surimi sol at various heating conditions is one of the parameters that can be affecting the gel textural characteristics (Benjakul *et al.*, 2003a; Arfat and Benjakul, 2012). From the results, Kamaboko gel with setting at 40°C had the highest breaking force (623.20 ± 21.74 g) among other two gels ($p < 0.05$). Breaking force of KK was 1.46 and 1.53 times higher than DH gel and MD gel, respectively. This could be due to stabilize protein aggregation by various bands (i.e. disulfide bridges and hydrophobic interactions) in KK gel (Samejima *et al.*, 1981; Benjakul *et al.*, 2001). Kamaboko gel had lower deformation than both directly heated gel and modori gel ($p < 0.05$). It can be concluded that KK gel had the strongest and rigid protein network structure. This correlated well with lower elasticity (Park *et al.*, 2005) and lower water holding capacity of protein (Tanaka, 1981) resulting in lower deformation value.

Moreover, the deformation of KK gel from black mouth croaker was higher than the results obtained for white mouth croaker (8.86 ± 0.8 mm) (Cortez-Vega *et al.*, 2012) and also slightly similar to kamabako gel prepared from big eye croaker (by setting at 25°C for 30 min) (Benjakul *et al.*, 2003a). Breaking force of directly heated gel was lower than that of kamaboko gel but was slightly higher than that of modori gel ($p < 0.05$) in agreement with results reported from yellow stripe trevally surimi (Arfat and Benjakul, 2012).

The Ca^{2+} and endogenous transglutaminase (TGas) could enhance the cross-linking of myofibrillar proteins, especially myosin molecular as a substrate for endogenous TGas (Lee and Park, 1998). Although, endogenous TGas activity depends on the fish species (Lanier, 2000) but differences in setting condition can affect cross-linking of gel network by endogenous TGase stability (Seki *et al.*, 1990; Kumazawa *et al.*, 1995). Setting surimi sol at high temperature is exposing hydrophobic amino acids, leading to hydrophobic interactions due to instability of hydrogen bonds during high heat (Niwa, 1992). However, setting at low temperature cause non-covalent bonding (Nowsad *et al.*, 1996). In MD gel the lowest breaking force was measured ($p < 0.05$). It was caused by hydrolysis of the protein molecules due to fish muscle proteinases activation at temperature range from 60 - 65°C (Takagi, 1973; An *et al.*, 1996; Benjakul *et al.*, 1997). Several investigators reported the poor textural characteristics from modori gel (e.g. sardine surimi, Alvarez *et al.*, 1999; Indian mackerel surimi, Chaijan *et al.*, 2010). Generally, enzymatic proteolytic degradation at modori temperature (Niwa, 1992), thermal coagulation of protein molecular and non-enzymatic interaction between proteins (Iwata *et al.*, 1977) might responsible for weak modori gel. Directly heated gel had lower breaking force in comparison with kamaboko gel ($p < 0.05$). It was hypothesized to be due to rapid formation of disulfide and hydrophobic protein-protein bonds in the absence of prior setting required for the proteins to orient to form a gel protein network in order to occur some weak protein coagulation (Niwa, 1985). Deformation did not significantly differ among MD gel and DH gel ($p < 0.05$).

From the results, the gel deformation of surimi from black mouth croaker (10.73 to 12.06 mm) is superior compared to those found in surimi from bigeye snapper (8 to 10 mm) (Benjakul *et al.*, 2002), frigate mackerel (6 to 10 mm), Indian mackerel (9 to 10 mm) (Chaijan *et al.*, 2010) and significantly inferior to that reported for yellow stripe trevally surimi (16.33 to 19.10 mm) (Arfat and Benjakul, 2012).

Table 1. Texture profile analysis of black mouth croaker surimi gels with different heating conditions

| Sample | Hardness 1 (Kg f.) | Hardness 2 (Kg f.) | Cohesiveness | Springiness (mm) | Gumminess (Kg f.) | Shear force (Kg/g) |
|--------|----------------------|---------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| KK | 3.8 ± 0.09 a* | 3.6 ± 0.05 a | 0.8 ± 0.04 ^a | 1.3 ± 0.11 ^a | 2.9 ± 0.12 ^a | 1.8 ± 0.11 ^a |
| DH | 2.4 ± 0.01 b | 2.2 ± 0.09 b | 0.8 ± 0.01 ^a | 1.0 ± 0.06 ^b | 1.3 ± 0.04 ^b | 1.2 ± 0.08 ^b |
| MD | 1.6 ± 0.06 c | 1.5 ± 0.07 c | 0.7 ± 0.01 ^a | 1.1 ± 0.14 ^b | 1.8 ± 0.06 ^c | 0.78 ± 0.04 c |

*Mean \pm SD (n = 3). Different superscripts in the same column indicated a significant difference ($P < 0.05$).

Texture profile analysis

Results of the texture profile analysis of different gels made from black mouth croaker are shown in Table 1. In all samples, the hardness 1 value was always higher than hardness 2 value due to firm texture of compressed sample (Dey and Dora, 2011). In kamabako gel, the highest hardness value coincided with highest breaking force was obtained ($p < 0.05$). Textural properties of kamabako gel prepared from black mouth croaker were markedly higher than kamabako gel from other scianids fish, *Johnius gangeticus* (Dey and Dora, 2011). There was a decrease in hardness when setting sol was prolonged at 60°C . When cohesiveness value reached closed to 1, it is indicating that the intactness of sample is high after first compressing cycle of the TPA (Munizaga and Canovas, 2004). From the results, lower cohesiveness values was obtain from modori gel, suggesting that the gel has a lower tendency of recovery to its original structure after first compressing, compared to KK and DH gels ($p < 0.05$). Cohesiveness values were within the same range for KK and DH, while it was slightly decreased in MD gel which indicated lower MD gel property than other two gels ($p < 0.05$). Maximum gumminess value was recorded in kamabako gel ($p < 0.05$). The shear force values decreased in DH and MD gels, respectively ($p < 0.05$), which showed MD gel had more soft tissue. Decreasing of shear force values in the samples was in agreement with the hardness values. In general, the texture profile analysis of modori gel decreased. This finding is similar to sardine surimi gel (Alvarez *et al.*, 1999). Generally, reliable assessment of the textural characteristics of surimi productions are obtained from the results of breaking force and gel strength (Park and lin, 2005; Ramadhan *et al.*, 2014).

Whiteness

Slight differences in whiteness were observed in different gels (Table 2). The whiteness of DH was

Table 2. Whiteness and expressible drip of surimi gels from black mouth croaker with various heating conditions

| Samples | Whiteness | Expressible drip (%) |
|---------------------|--------------|----------------------|
| Kamaboko gel | 73.30±0.01c* | 4.39±0.180c |
| Directly heated gel | 73.91±0.02b | 5.27±0.099b |
| Modori gel | 73.31±0.06c | 4.86±0.076a |

*Mean ± SD (n = 3). Different superscripts in the same column indicated a significant difference (P<0.05).

better than both two-step heated gel samples ($p<0.05$; Table 2). Since, preparing of KK and MD gels carried out in higher extent with a longer exposure heating time, hence the Maillard browning reaction takes place more in two-step heated gels (Whistler and Daniel, 1985; Arfat and Benjakul, 2012). Lipid oxidation products (aldehydes or carbonyl compounds) which interact with protein amino groups resulting in nonenzymatic browning (Panpipat *et al.*, 2010) and also increasing metmyoglobin formation (Chaijan *et al.*, 2004) may cause a negative effect on gel color. Black mouth croaker is a white-fleshed mesopelagic fish and has low lipid content. Consequently, the lipid oxidation of this species could occur to a lesser extent during heat-induced gelation. Nevertheless, the lipid oxidation impact cannot be ignored. Similar to results from this study, Arfat and Benjakul (2010) observed the highest whiteness index of directly heated gel ($74.41\pm 0.08\%$) from yellow stripe trevally surimi compared to modori gel ($73.18\pm 0.14\%$) and kamaboko gels (by setting at 40°C and 25°C). The whiteness of KK gel prepared from black mouth croaker surimi was inferior to that obtained from white mouth croaker (*Micropogonias furnieri*) surimi ($77.3\pm 0.9\%$) (Cortez-Vega *et al.*, 2012) and superior to that reported for Tigertooth croaker (*Otolithes ruber*) surimi (Panpipat *et al.*, 2010). Whiteness is an index for determining the quality and general appearance acceptability of surimi gel (Park, 2005; Yoon *et al.*, 1997). Differences of whiteness index in fish species may be related to the sarcoplasmic protein removal efficiency (Kang *et al.*, 2008), metmyoglobin content (Panpipat *et al.*, 2010) and level of muscle lipid composition (Chaijan *et al.*, 2004), which mostly depend on raw material and processing parameters (Cortez-Vega *et al.*, 2012).

Expressible drip

Kamaboko gel set at 40°C rendered the lowest expressible moisture content compared with other gels, suggesting that the protein network of the gel was the highest in water holding capacity ($p<0.05$; Table 2). The maximum expressible drip was recorded in DH and MD gel ($p<0.05$), indicating the

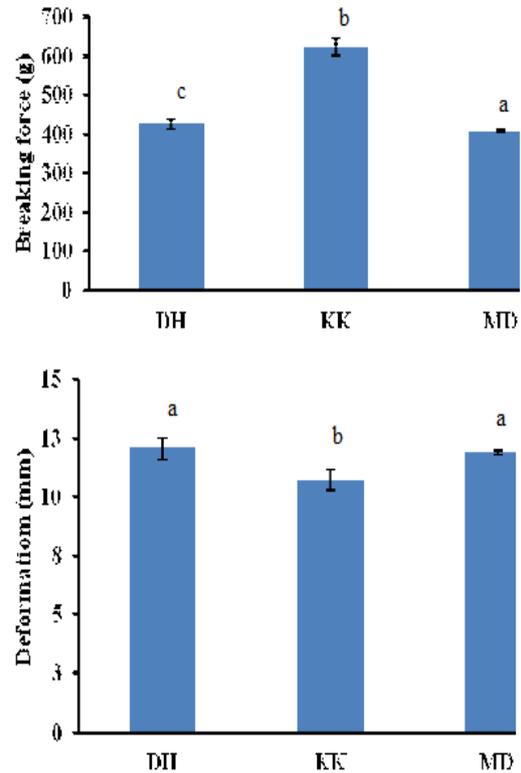


Figure 1. Force and deformation of different gels from black mouth croaker. Directly heated gel (DH); Kamaboko gel (KK) and Modori gel (MD). Bars indicate the standard deviations and different letters on top of the bars indicate significant differences (n=3, $p<0.05$)

lowest protein-protein bonds water binding capacity of these gels (Niwa, 1992; Alvarez *et al.*, 1999). From the result, marked decrease of expressible drip was in association with increased breaking force (Figure 1). Niwa (1992) reported that releasing more water from directly heated gel network without prior setting is in consequence of rapid unfolding of proteins and therefore irregular dispersion of proteins. In Sardine surimi, kamaboko gel showed higher water holding capacity than modori gel (Alvarez *et al.*, 1999). Similar results obtain from goatfish surimi (Benjakul *et al.*, 2010). Arfat and Benjakul (2012) reported that kamaboko gel (set at 40°C prior to heat at 90°C) from yellow stripe trevally surimi has the lowest expressible drip ($4.43\pm 0.01\%$) in comparison to directly heated gel ($5.77\pm 0.02\%$) and modori gel ($5.30\pm 0.01\%$), respectively.

Microstructure

The internal network and porous structures of black mouth croaker surimi gel with different heating conditions are illustrated in Figure 2. The fine gel network was obtained in the kamaboko gel, suggested higher interconnected three-dimensional gel network. Thus kamaboko gel exhibited a finer strand, regular

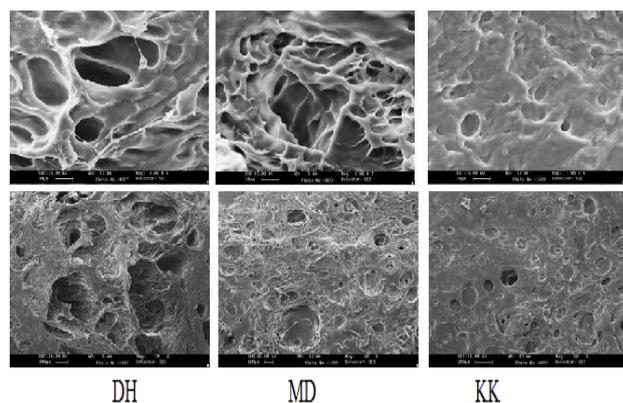


Figure 2. Microstructure of black mouth croaker gels set at different conditions, showing regular and denser protein network in KK gel than others. Directly heated gel (DH); Kamabako gel (KK) and Modori gel (MD) (Magnification 50 \times and 1000 \times)

structure and denser protein network than directly heated gel and modori gels. This was in agreement with the higher breaking force (Figure 1) and higher water holding capacity (Table 2). However, the gel network of directly heated and modori gels were ragged and three-dimensional protein networks were destroyed. This was coincidental related to the lower breaking force with less water holding capacity. Large scale protein-protein interactions and disulfide bonds occurred in modori gel due to high temperature during setting (Careche *et al.*, 1995; Alvarez *et al.*, 1999). The weak gel three-dimensional network might be related to the degradation of MHC by proteases activity around 60 $^{\circ}$ C (Makinodan *et al.*, 1987; Saeki *et al.*, 1995; Cao *et al.*, 1999). In directly heated gel, although the orientation of protein-protein bonds in the networks were restricted to prevent breakdown of the gel, but the largest voids were observed.

Araft and Benjakul (2012) reported that higher breaking force and water holding capacity were obtained in kamabako gel (set at 40 $^{\circ}$ C) with finer and ordered gel network in comparison to directly heated and modori gels. Similar results were obtained from bigeye snapper gels (Rawdkuen and Benjakul, 2008). It is noticeable that the formations of protein-protein bonds were occurred strongly in kamabako gel, producing a very compact structure. Thus finer gel network with smaller voids was observed in gel with the highest breaking force and water holding capacity as compared with the poor gel structure with an amorphous gel network structure due to lower breaking force and water holding capacity. It is concluded that the internal structure of surimi gel can be expressed the gel quality.

Protein pattern

To illustrate the behavior of the polymerization and degradation of proteins in the surimi gels

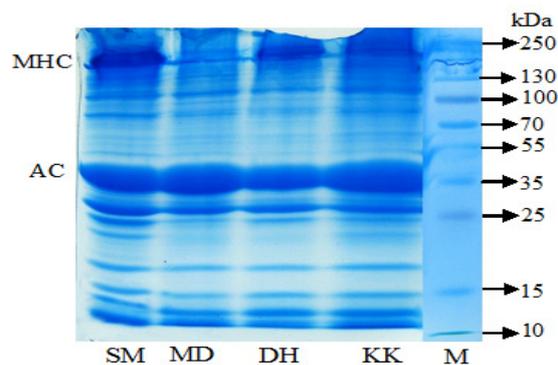


Figure 3. SDS-PAGE patterns of surimi and surimi gel from black mouth croaker with different setting conditions. M, marker; SM, surimi; KK, MD, modori gel; kamabako gel; DH, directly heated gel; MHC, myosin heavy chain and AC, actin

with different heating conditions under reducing conditions, SDS-PAGE patterns of surimi and surimi gels was carried out (Figure 3). The myosin heavy chain (MHC) showed the highest band intensity in surimi. The MHC band markedly decreased in all gels. This was attributed to the presence of TGase in the surimi gel network that promotes the cross-linking of MHC (Niwa *et al.*, 1985; Seki *et al.*, 1990; Araki and Seki, 1993; Nowsad *et al.*, 1995; Nowsad *et al.*, 1996). Although the role of alkaline proteases to contribute to the protein degradation in surimi gels is non-negligible (Toyohara *et al.*, 1987; Boye and Lanier 1988). However, MHC was more appear on directly heated gel SDS-PAGE, all though it was faint. The results suggested that MHC becomes more undergo either polymerization or degradation in two-steps heated gels than those without prior setting (directly heated gel). Furthermore, it was found that maximum polymerisation or degradation occurred in kamabako gel (by setting at 40 $^{\circ}$ C). These results are in agreement with Benjakul *et al.* (2003a) and Arfat and Benjakul (2012). Generally, decreasing of MHC after heating was presumed to be due to the polymerisation or degradation (Benjakul and Visessanguan, 2003). The substances between MHC and actin bands were slightly produced with setting time. This was suggested the degradation of MHC (Van Phu *et al.*, 2010). Some investigators reported that MHC of surimi gel was more susceptible to cross-linking (Benjakul and Visessanguan, 2003) and more prone to proteolytic degradation (Benjakul *et al.*, 1997) than other muscle proteins, including actin, troponin and tropomyosin during setting. Degradation of MHC band and occurrence of new protein bands was reported in modori and kamabako gels from yellow stripe trevally surimi (Arfat and Benjakul, 2012).

Conclusions

High hardness (1.6-3.8 Kg force) and deformation (10.73 to 12.06 mm) of black mouth croaker surimi gels, introduce that this low fat and white-fleshed fish species can be used for producing high quality surimi-based products in large scale. Setting the surimi sol at different heat treatment may lead to different gel characteristics. Although, whiteness of directly heated gel was better than two-steps heated gel samples but two-steps heated gels showed lowest expressible drip and whiteness. Kamabako gel exhibited a finer strand, regular structure and denser protein network with highest breaking force than directly heated gel and modori gels. Therefore, preparation of KK is enhancing gel-forming ability of the surimi. Conversely, modori phenomenon heated at temperature close to 60°C, resulting in brittle surimi gel.

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