Original paper

Textural Properties of Heat-induced Gels Prepared Using Different Grades of Alaska Pollock Surimi under Ohmic Heating

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Frozen Alaska pollock surimi (FA, A, and RA grades) was used to clarify how the heating method (ohmic heating and water bath heating) and heating rate affect the physical properties of heat-induced gels. Textural properties were significantly influenced by the heating method and heating rate. In 1-step heating, slow heating enhanced the gel strength of high-grade surimi more effectively than that of low-grade surimi. The quality of gels prepared by water bath and ohmic heating differed even if the heating time to the final temperature was the same, probably due to differences in the linear and non-linear temperature patterns of the two heating methods. The results of gels formed by 2-step heating and those with suppressed setting by EDTA confirmed that a slow heating rate enhanced gel strength by altering the setting phenomenon, but was also influenced by the modori effect, and the degree of enhancement differed depending on the surimi grade.

Keywords: ohmic heating, surimi, setting, modori, EDTA, heating rate

Introduction

Ohmic heating (OH) is a process in which alternating current passes through conductive food products and generates heat (De Alwis and Fryer, 1990). According to Sastry and Li (1996), OH differs from other rapid heating methods by its use of food contact electrodes. The voltage difference between two electrodes creates an electric field, which forces electrons to move, causing an electric current. OH systems have been applied on a commercial scale to process a wide range of food products over the past few decades (Sastry and Salengke, 1998).

The advantage of ohmic treatment is that both solid and liquid phases display identical and simultaneous conductivities, thereby distributing temperature uniformly within the food product (Sastry *et al.*, 2014). Furthermore, ohmic cooking displays some outstanding advantages over traditional heating,

such as reduced processing time and increased productivity, as well as retained nutritional value and food color (Yildiz-Turp *et al.*, 2013; Castro *et al.*, 2004; Icier and Ilicali, 2005; Park and Reed, 2014; Vasanthi *et al.*, 2007). For the above stated reasons, OH is widely applied in food technology for preheating, cooking, blanching, pasteurization, sterilization, and extraction (Lima and Sastry, 1999; Icier and Ilicali, 2005; Knirsch *et al.*, 2010).

Currently, OH technology is being increasingly applied to the production of surimi-based foods (Shiba, 1992; Pongviratchai and Park, 2007; Tadpitchayangkoon *et al.*, 2012; Moon *et al.*, 2017). Surimi (refined and stabilized fish protein with cryoprotectant) is a transitional raw material used for processing “kamaboko,” a traditional Japanese seafood, and various seafood analogs (i.e., crabstick). When surimi is ground with salt, its salt-soluble myofibrillar protein is solubilized,

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forming a protein network structure, which forms a gel with unique elasticity when heated.

OH is considered more advantageous than external heating, such as water-bath heating (WB), because the heating rate in OH can be regulated by adjusting the current, allowing uniform heat distribution via electrical resistance within the surimi paste regardless of the heating rate (Yongsawatdigul *et al.*, 1995; Park and Yongsawatdigul, 1999). However, in surimi ground with salt, gel strengthening by the cross-linking enzyme transglutaminase (setting) and gel degradation by the proteolytic enzyme (modori) are both enhanced during the heating process. Furthermore, several types of chemical interactions including non-covalent cross-linkages such as hydrophobic interactions, hydrogen bonds, and ionic bonds, and covalent interactions such as disulfide bonds are also involved in the formation of network structures of surimi gels, (Gilleland, 1997), indicating that the effect of heating on the texture of heat-induced gels is highly complicated.

Yongsawatdigul *et al.* (1995) reported that since products heated by OH may reach 90 °C within a minute, the process may minimize proteolysis or inactivate protease enzymes in Pacific whiting (PW) surimi, since proteolytic enzymes are considered to be most active at 60-70 °C in temperate water fish species and 50-60 °C in cold-water fish species. It was also reported that rapid heating was superior for the production of PW surimi, in which proteolysis is predominant (Yongsawatdigul and Park, 1996; Zhu *et al.*, 2011).

On the other hand, it has been reported that slow heating is more suitable for Alaska pollock (AP) surimi, as proteolysis is almost absent (Yongsawatdigul and Park, 1996). These findings indicated that the possibility of myosin heavy chain (MHC) polymerization, catalyzed by the endogenous transglutaminase enzyme, was accelerated during slow heating. This cross-linking process, which is termed setting (suwari in Japanese), varies according to the species (Nishimoto *et al.*, 1987; Seki *et al.*, 1990; Kimura *et al.*, 1991).

The ability of salted pastes to form cohesive gels at temperatures below 40 °C, depending on the species, is confirmed as an exclusive property of surimi that is commonly obtained via setting. In general, due to differences in the habitat temperatures of the fish studied, the optimal setting temperature may vary (Kato *et al.*, 1984). Cold-water species show optimal setting at 5-20 °C, temperate water species set at 20-30 °C, and tropical species set at 30-40 °C (Park *et al.*, 2014). As described above, since the temperature dependence of gelation differs depending on the species, the optimum heating method also varies depending on the species. Therefore, in order to obtain a gel with enhanced properties, it is necessary to apply a suitable heating condition (such as heating rate) when applying electric heating.

On the other hand, for surimi produced from one type of fish, the setting and modori effects may vary depending on the grade of surimi, attributable to differences in intrinsic factors

associated with gel setting (suwari) and gel softening (modori), respectively. In the case of industrially important AP surimi, various grades are manufactured, wherein low-grade surimi (such as RA grade) is comprised of surimi that may have been damaged during harvesting from by-products or during post-harvest storage, whereby myofibrillar proteins may be denatured or surimi protease levels may be increased. Further, the cost of surimi continues to increase, resulting in a demand for reduced production costs. Therefore, the development of efficient processing methods, which use different grades of surimi as raw materials to manufacture surimi-based products, is required. However, there is insufficient information on the effect of OH conditions governing gel quality, such as texture and water holding capacity, which may enable OH to be used effectively. Besides, slight quality differences seen between industrial OH-based products and conventionally heated products remain to be clarified.

Thus, the objectives of the current study were to clarify the effects of the 2 heating methods (OH and WB) and heating rates on the physical properties of heat-induced gels using different grades of AP surimi, and to assess the differences in effects among various surimi grades.

Materials and Methods

(1) Materials Commercial frozen Alaska pollock (AP) (*Theragra chalcogramma*) surimi (FA, A, and RA grades) containing 4 % sucrose, 5 % sorbitol, and 0.25 % sodium polyphosphate were provided by Glacier Fish Company (Seattle, WA, USA). Immediately following delivery to the laboratory, a large, 10 kg block of surimi was cut into small pieces (~500 g), vacuum packaged, and stored in a freezer (-30 °C) until use. The proximate composition of each surimi is shown (Table 1). The protein and moisture contents were different among the 3 grades of surimi and corresponded to the surimi quality grades. The gel forming ability corresponded to the surimi grade and was classified as FA > A > RA, as shown in Fig.2 (WB heating).

Ethylenediaminetetraacetic acid (EDTA), obtained from Wako Pure Chemical Industry, Ltd. (Osaka, Japan), was used as a calcium chelating agent (Saeki *et al.*, 1988). All chemicals and reagents were of the highest purity available.

(2) Preparation of surimi paste In order to prepare surimi paste, frozen surimi was thawed at -3 °C overnight before being cut into small cubes (1.0-1.5 cm³). Surimi cubes were chopped at 1 500 rpm for 1.5 min under vacuum of 0.9 bar, using a vacuum cutter (UMC-5, Stephan Machinery Corp, Hameln, Germany) equipped with a chilling system. Sodium chloride (1.5 % w/w) was added according to the results of a preliminary experiment (data not shown). At the same time, 0 or 5 mmol EDTA/kg was added (Kumazawa *et al.*, 1995), and this mixture was chopped for 1.5 min at 1 500 rpm. Cold water was added to a moisture content of 81.0 % and the mixture was chopped continuously at 1 500 rpm for 5 min while maintaining a

Table 1. Proximate compositions of Alaska pollock surimi (FA, A and RA grades) (%)

Components	FA grade	A grade	RA grade
Moisture	73.9	74.6	75.5
Crude protein	17.0	16.9	15.6
Crude lipid	0.1	0.1	0.1
Carbohydrate	8.4	7.8	8.3
Crude ash	0.7	0.7	0.6

temperature below 5 °C. To control for the negative effect of air bubbles on the measurement of physical properties, surimi paste was prepared under vacuum conditions and stored in ice until gel preparation.

(3) *Preparation of heat-induced surimi gels* Surimi gels were prepared using two different heating methods: 1-step (Fig. 2D) and 2-step (Fig. 3D) heating.

(a) *1-step heating* Surimi paste was packed into plastic tubes (diameter, 2.5 cm; length, 2.5 cm) and heated in a water bath (WB) at 90 °C for 30 min or heated via ohmic heating (OH) using an ohmic heating machine (Frontier Engineering, Co., Ltd., Tokyo, Japan). For OH, samples were placed between two movable electrodes attached to the holding chamber, and then the upper electrode was pressed down to form a tight attachment to both sides of the tubes in order to secure a sample length of 2.5 cm. The samples were heated from approximately 0 to 90 °C using 4 different heating rates: 3, 10, 40, and 80 °C/min. Following heat treatment, samples were cooled down by being placed in a plastic bag and immersed immediately in ice/water for 30 min, and then stored in a refrigerator (4 °C) overnight.

(b) *2-step heating* 2-step heating was conducted using WB and OH. Surimi paste was packed into plastic tubes, which were placed in a plastic bag and then submerged in a water bath (30 °C) for 30 min to induce setting. Subsequently, the preset samples were removed from the water bath and subjected to WB (90 °C, 30 min) or OH at different heating rates (3, 10, 40, and 80 °C/min). The heating conditions are summarized in Table 2.

(4) *Temperature profile of surimi paste heated by WB and OH* The temperature patterns of WB and OH gels were recorded using a compact thermo logger (TR-50U, T and D Co., Ltd., Tokyo, Japan). A thermo sensor was inserted into the geometric center of the samples to measure temperature changes during heating via the two heating methods. The data thus obtained were compared.

(5) *Puncture test* In order to evaluate deformability, breaking strength (g) and breaking distance (mm) were determined using a texture analyzer (RE-3350B, Yamaden Ltd., Tokyo, Japan) according to the method described by Benjakul and Visessanguan (2003), with modifications. Six cylindrical-shaped samples (2.5 cm long) were prepared by keeping them at room temperature (25 °C) for 1 h prior to

analysis. A spherical probe (diameter 5 mm) was used to penetrate the center of the gel sample perpendicularly at a constant crosshead speed (60 mm/min) until the sample was punctured. The force to puncture the gel was recorded as breaking strength, and the distance from the point where the probe touched the gel to the point where the gel was punctured was recorded as breaking distance (mm).

(6) *Expressible moisture* Expressible moisture was measured according to the method described by Rawdkuen *et al.* (2004), with modifications. Gel samples were cut into thin slices (approximately 1.0 g) (W1) and placed between 4 layers of filter paper (No. 4A paper on the inner side and No. 2 paper on the outer side). The filter papers were purchased from Advantec, Inc., Tokyo, Japan. Samples were then placed between 2 plastic plates and a constant force (10 kg/cm²) was applied to the top of samples for 20 s using a rheometer (RE-3305, Yamaden Ltd., Tokyo, Japan) with 2 plastic plates attached to the crosshead (top) and the metal plate (bottom), and then the sample was weighed again (W2). Expressible drip content was calculated using the following equation and expressed as a percentage of sample weight:

$$\text{Expressible drip (\%)} = [(W1 - W2) / W1] \times 100 \quad \dots\dots\text{Eq. 1}$$

(7) *Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE)* To investigate MHC polymerization in surimi gels heated by WB or OH, SDS-PAGE experiments were conducted according to the method described by Laemmli (1970). Samples were prepared for SDS-PAGE according to Kamath *et al.* (1992). The surimi gel (0.5 g) was cut and placed in 20 mL of buffer solution (pH 8.0) containing 2 % SDS, 8 M urea, 20 mL Tris-HCl, and 2 % (v/v) β-mercaptoethanol (pH 8.8), namely SDS-solution. The samples were boiled at 100 °C and shaken continuously at room temperature for 24 h to solubilize the gel. The homogenized solutions were centrifuged at 10 000×g (Sorvall, DuPont Co., Newton, CT, USA) for 10 min at room temperature. The protein concentration of supernatants was measured using Lowry's method with bovine serum albumin as a standard. The SDS-solubility was then calculated by the protein concentration ratio in the SDS-solution before and after centrifugation. Samples (12 µg protein) were loaded onto polyacrylamide gels comprised of a 7.5 % stacking gel and 10 % running gel

Table 2. Heating conditions of heat-induced surimi gels

Heating condition			Voltage (V)	Voltage gradient (V/cm)	Heating time (s)	Initial temperature (°C)	Target temperature (°C)
1-step	WB	WB heating			1 800	0	90
	OH	OH - 3 °C/min	11.5	4.6	1 800	0	90
		OH - 10 °C/min	16.5	6.6	540	0	90
		OH - 40 °C/min	30.0	12.0	135	0	90
		OH - 80 °C/min	46.0	18.4	70	0	90
2-step	WB-WB	WB Setting* → WB heating (90 °C)			1 800	30	90
	WB-OH	WB Setting* → OH - 3 °C/min	11.5	4.6	1 200	30	90
		WB Setting* → OH - 10 °C/min	16.5	6.6	360	30	90
		WB Setting* → OH - 40 °C/min	30.0	12.0	90	30	90
		WB Setting* → OH - 80 °C/min	46.0	18.4	45	30	90

*Setting: Heated by WB at 30°C for 30 min

(e-PAGEL, Atto Co., Ltd., Tokyo, Japan) and subjected to electrophoresis using PAGE equipment (Atto Co., Ltd., Tokyo, Japan) at a constant current of 20 mA. Polyacrylamide gels were stained with 0.05 % Coomassie Brilliant Blue R-250 and de-stained with 30 % methanol/10 % acetic acid/60 % water (v/v). Gel images were acquired using an Atto SDS-PAGE analysis system.

The quantitative analysis of MHC as relative staining intensity to actin (MHC/Ac) from the results of SDS-PAGE was conducted by analyzing the one-dimensional electrophoretic gels using the ImageJ software (NIH, Bethesda, MD, USA).

(8) *Proximate compositions of surimi* The proximate analyses of 3 grades of AP surimi were performed according to the methods proposed by the Association of Official Analytical Chemists (AOAC, 2000). Briefly, moisture (method 925.09) was determined by drying the sample in a convection oven at 105 °C for 5 h. Crude protein content (method 954.01) was estimated using the Kjeldahl method. Crude lipid was extracted via the Soxhlet method (method 920.39), and crude ash (method 923.03) was quantified by burning the sample at 550 °C for 5 to 6 h in a muffle oven. Finally, the carbohydrate content was calculated following the equation:

$$\text{Carbohydrate (\%)} = 100 - (\text{moisture} + \text{crude protein} + \text{crude lipid} + \text{crude ash}) \quad \dots \text{Eq. 2}$$

(9) *Statistical analysis* For each measurement, 6 samples were used to obtain the mean and standard deviation (SD) of breaking strength and distance, respectively. Next, 4-5 samples were used to obtain the mean and SD of expressible moisture. Data were subjected to one-way analysis of variance using MS-Excel 2010 (Microsoft, Redmond, WA, USA). Differences between the samples were compared statistically using Tukey's method at a significance level of $p < 0.05$.

Results and Discussion

(1) *Temperature profiles of conventional WB and OH* The temperature profile of surimi paste during heating via conventional WB or OH using different heating rates is shown in Fig. 1. OH showed a linear heating pattern, while WB showed a non-linear heating pattern (Fig. 1). OH at different heating rates of 3, 10, 40, and 80 °C/min took approximately 30, 9, 2, and 1 min for the center temperature of the gel to reach 90 °C, respectively, while for WB, it took approximately 30 min (Table 2). Thus, although WB and OH (3 °C/min) took the same time to reach 90 °C, the temperature histories were quite different.

In general, the setting temperature of AP is considered to be approximately 25-35 °C (Kim *et al.*, 1993), and the time taken to pass through this temperature zone was approximately 250, 60, 15-20, and 5-10 s for 3, 10, 40, and 80 °C/min OH, respectively, while it was about 60 s for WB.

On the other hand, the modori temperature of AP is considered to be approximately 50-60 °C (Klesk *et al.*, 2000), and the time taken to pass through this temperature zone was approximately 200, 60, 10-15, and 5-7 s for 3, 10, 40, and 80 °C/min of OH, respectively, while it was about 120 s for WB.

(2) *Texture properties of surimi gels prepared by 1-step heating*

(a) *Comparison of the effect of heating rate* Breaking strength, breaking distance, and expressible moisture of FA, A, and RA grade AP surimi gels at various heating rates by 1-step heating via WB and OH are shown (Figs. 2 A, B, and C, respectively). Under all heating conditions, the breaking strength and breaking distance of FA grade gels were the highest, followed by grades A and RA ($p < 0.05$; Fig. 2 A).

Firstly, when OH gels were compared at different heating rates (3, 10, 40, 80 °C/min), the lower the heating rate, the higher the breaking strength and breaking distance for any

Fig. 1. Temperature profile of surimi paste heated by water bath (WB) and ohmic heating (OH) at different heating rate. (A), (B), (C) = the time that samples exposed to setting temperature under OH-3 °C/min, WB, and OH-10 °C/min, respectively; (a), (b), (c) = the time that samples exposed to modori temperature under OH-3 °C/min, WB, and OH-10 °C/min, respectively.

surimi grade. This tendency was notable in FA and A. As shown in Fig. 1, the time taken to pass through the setting temperature zone was 4-5 min, 1 min, and 5-10 s at 3, 10, and 80 °C/min, respectively. Therefore, in the case of slow heating, it was suggested that a higher breaking strength was associated with a longer heating time at the setting temperature.

The results stated above indicate that slow heating by OH remarkably improved the textural properties of AP surimi gel by promoting setting (suwari). This result corresponded with the results of Park and Yongsawatdigul (1999), and Park and Reed (2014). Furthermore, higher grade surimi is considered to display a higher setting in general, and the results were consistent with this tendency. This suggested that the effect of different heating rates on breaking strength differed depending on the surimi grade, which was attributed to differences in the ability to set among the surimi grades.

On the other hand, the change in the heating rate also affected the heating period in the modori temperature zone. In AP surimi, modori occurs due to a protease enzyme that degrades MHC at around 50-60 °C (Park *et al.*, 2014), and the slow heating rate increased the time taken to pass through this temperature zone. However, no reduction in gel strength was observed in any of the surimi grades. It was speculated that breaking strength and distance did not decrease with a slow heating rate because the enhancing effect of setting on gel strength was greater than the reducing effect of modori on gel strength. On the other hand, in Fig. 1, a comparison of OH (10 °C/min) and WB shows that samples heated by the former were exposed to the modori temperature zone for a significantly shorter time than the latter; meanwhile, both showed a similar

duration in the setting temperature zone. Correspondingly, the breaking strength and breaking distance of the gel by the former showed a significantly higher value than the latter (Fig. 2). This result suggests the merit of fast heating by OH to pass through the modori temperature zone.

The expressible moisture of gels changed with the grade of surimi and heating conditions ($p < 0.05$; Fig. 2 C). Rawdkuen *et al.* (2004) stated that expressible moisture content could be considered as an indicator of the water holding capacity of surimi gels. Increased expressible moisture content of gels indicated that less water was retained or bound in the gel network (Chanarat and Benjakul, 2013). Low expressible moisture (high water holding capacity) may be the result of protein network structures, formed via various bonds between protein molecules, which are more stable in high-grade surimi gels than low-grade surimi gels (Mahawanich *et al.*, 2010).

High-quality surimi (FA) gel exhibited the lowest expressible moisture of all grades, and the amount of water released from the FA grade surimi gel was highly similar regardless of the heating rate ($p < 0.05$). For RA grade surimi gels, high expressible moisture was observed at all heating rates.

As described above, in 1-step heating of AP surimi, the effect of setting was more significant when the heating rate was slower; however, the effect of heating rate differed depending on the grade of surimi.

(b) *Comparison of gels heated by WB and slow OH (3 °C/min)* Gels produced by WB and OH (3 °C/min), in which the same duration was required to complete the heating process, were analyzed.

Fig. 2. Breaking strength (A), breaking distance (B), and expressible moisture (C) of AP surimi gels (FA, A, and RA grade) by 1-step heating (WB and OH) at different heating rates. Bars represent the standard deviation ($n = 6$). Different letters on the bar, including the control, indicate a significant difference ($p < 0.05$); (D) The flowchart of preparation of heat-induced surimi gels by 1-step heating by WB and OH

OH (3 °C/min) gels exhibited higher breaking strength and distance than WB gels for all 3 grades of surimi ($p < 0.05$). The breaking strength of RA and A grade OH (3 °C/min) surimi gels was increased by approximately 1.2 and 1.6 times compared to WB heating, respectively, while the enhancement was more noticeable in FA grade surimi. FA grade gels heated ohmically showed approximately 2.0- and 1.3-fold increases in breaking strength and distance, respectively, as compared to WB gels (Fig. 2 A, B).

Although the heating time of WB and OH (3 °C/min) was almost the same (approximately 30 min), the temperature history including setting time of the 2 heating methods was markedly different (Fig. 1). For OH (3 °C/min), samples were exposed to the setting temperature (25–35 °C) for about 4–5 min; however, just over 1 min of exposure occurred in the case of conventional WB. Also, the duration in which the samples were subjected to the modori temperature (50–60 °C) was different for both OH and WB, 3.5 and 2 min, respectively. This indicated that the level of setting and modori in WB and OH (3 °C/min) differed due to differences in temperature history, which was attributed to differences in linear and non-linear heating.

The above findings suggested that the quality of the gels prepared using WB and OH may differ due to linear and non-linear differences in the temperature patterns of the two methods, even though the heating time taken to reach the final temperature was similar.

(3) Texture properties of surimi gels by 2-step heating

(WB-WB and WB-OH) Based on the above results (Fig. 2), it was hypothesized that slow ohmic heating would significantly improve the textural properties of AP surimi gels by promoting setting at around 30 °C. However, it was still unclear if the difference in OH heating rates at temperatures above the setting temperature would affect the texture of the surimi gel. Therefore, the second step in the traditional 2-step WB heating was conducted using OH, while the first heating step was conducted under the setting temperature using WB (Fig. 3).

In this experiment, samples prepared from the 3 grades of surimi underwent the setting process in a 30 °C water bath for 30 min, and were then subjected to OH under the conditions described in Table 2. Results indicated that 2-step (WB-OH) heating significantly improved the breaking strength, breaking distance and expressible moisture of gels of all 3 grades, compared with direct heated gels prepared using only WB. Furthermore, there were no significant differences in breaking strength, breaking distance or expressible moisture among the 3 surimi grades when gels were prepared via 2-step heating, regardless of the heating method or heating rate used in the second step (30–90 °C; $p < 0.05$). Heat treatment at 30 °C (setting) was performed under the same condition for all samples, and the results demonstrated that gel quality was hardly affected even when the heating rate of 30–90 °C was changed. This suggests that the reason for the difference in gel quality with the various heating rates shown in the previous section [section (2a)] was mainly due to the difference in temperature history during the setting process, but not during

Fig. 3. Breaking strength (A), breaking distance (B), and expressible moisture (C) of AP surimi gels set in a water bath at 30 °C for 30 min before being heated ohmically at different heating rates. Bars represent the standard deviation ($n = 6$). Different letters on the bar, including the control, indicate a significant difference ($p < 0.05$). (D) The flowchart of preparation of heat-induced surimi gels by 1-step heating by WB and two-step WB-WB and WB-OH

modori.

(4) *Effect of heating conditions under suppression of setting by EDTA* The results discussed in (2) and (3) suggested that differences in the quality of AP surimi gels generated by WB and OH using different heating rates were mainly due to variability in the degree of setting, which were caused by differences in temperature histories. On the other hand, the effect of the modori enzyme, which was suspected of showing activity around 50–60 °C, could not be confirmed. Therefore, in the next step, the surimi gel was prepared under conditions that suppressed setting, and the effect of the temperature history on the modori temperature zone was examined.

Kimura *et al.* (1991) reported on the mechanism underlying the cross-linking of myosin in relation to setting and suggested that the effect of transglutaminase was activated by calcium ions. This has been supported by the observation that transglutaminase, a calcium-dependent acyl-transfer enzyme, catalyzed MHC cross-linking during the setting of surimi paste (Kumazawa *et al.*, 1995; Kimura *et al.*, 1991). Therefore, EDTA (a calcium chelating agent), which binds calcium and prevents transglutaminase from cross-linking proteins, was added to surimi, and heat-induced surimi gels were prepared using different heating methods and heating rates.

It is reported that 1 kg of AP surimi contains approximately 0.6 mmol of calcium ions (Saeki *et al.*, 1988). Therefore, the addition of 5 mmol EDTA per kg surimi was reported to chelate almost all calcium ions in the surimi paste sample (Kumazawa *et al.*, 1995).

The results of breaking strength, breaking distance, and expressible moisture of surimi gels prepared with EDTA under

the above condition are shown in Fig. 4. The breaking strength of gels without EDTA by 1-step WB was somewhat higher than those with EDTA generated by OH and 2-step WB heating. This result can be explained by the complete suppression (mainly by EDTA) of the setting effect in OH and 2-step WB gels, while setting was obtained in 1-step WB heating despite the short duration (Fig. 1). Breaking strength and distance of gels without EDTA were in the order of 3 °C/min > 10 °C/min > 80 °C/min (Fig. 2), whereas breaking strength and distance with EDTA were in the order of 3 °C/min $\approx$ 10 °C/min $\approx$ 80 °C/min and were not affected by the heating rate of OH. Therefore, the increase in gel strength caused by lowering the heating rate was properly inhibited by EDTA addition (Fig. 4 A).

On the other hand, the breaking distance of EDTA-added gels increased as the heating rate increased (3 °C/min < 10 °C/min < 80 °C/min) (Fig. 4 B). Expressible moisture became lower as the heating rate increased, indicating the increased water holding capacity of the gels (Fig. 4 C).

As mentioned above, once EDTA was shown to eliminate the gel strengthening effect during heating at the setting temperature zone, the suppressive effect of fast heating on gel degradation by the modori phenomenon was more apparent. Expressible moisture of RA grade surimi gels was decreased by about 10 % by fast heating (80 °C/min), attributed to its ability to inhibit the modori effect. This also suggested that the modori effect was more sensitively reflected by breaking distance and water holding capacity compared to breaking strength.

(5) *Protein patterns* The SDS-PAGE results of 3 different

Fig. 4. Breaking strength (A), breaking distance (B), and expressible moisture (C) of AP surimi gels prepared with EDTA before being heated ohmically at different heating rates. Bars represent the standard deviation ($n = 6$). Different letters on the bar, including the control, indicate a significant difference ($p < 0.05$). (D) The flowchart of preparation of heat-induced surimi gels with or without EDTA

AP surimi gel grades, subjected to various OH heating rates with or without EDTA as well as 1-step and 2-step WB heating, are shown in Fig. 5 A. Fig. 5 B shows the results of the quantitative analysis of MHC by SDS-PAGE as relative staining intensity to actin.

As reported by Yongsawatdigul and Piyadhamviboon (2007), almost all bonds in surimi gels except non-disulfide covalent bonds were dismantled following solubilization of gels in solutions containing SDS, urea, and β -mercaptoethanol. Therefore, the formation of greater intra- and intermolecular cross-linking of proteins via non-disulfide covalent bonds during heating probably resulted in the decreased intensity of MHC bands in SDS-PAGE.

As shown in Fig. 5 A, relatively reduced staining intensity of MHC was observed in the 2-step heated gel (WB-WB, S) without EDTA, especially for FA and A grades of surimi compared to the directly heated gel (WB, C), suggesting that cross-linking of MHC progressed. The low staining intensity of MHC/Ac in Fig. 5 B also confirmed this tendency of the 2-step heated gel.

Furthermore, it was confirmed that the staining intensity of MHC/Ac showed a decreasing tendency as the heating rate decreased, and this tendency was almost similar to the change in breaking strength of OH gels with different heating rates (Fig. 2). However, the decrease of SDS-solubility, indicating an increase in components that could not enter the gel due to the polymerization of MHC, was not observed so clearly, and the value was nearly 100% in all samples.

On the other hand, the intensity of MHC/Ac in the gels with EDTA did not show a clear difference at all heating rates,

and this tendency was almost the same as the results of gel properties shown in Fig. 3. These results suggest that the progress of the cross-linking reaction of MHC may be one of the reasons for the improvement in gel properties by the slow heating rate indicated in Fig. 2.

However, no clear differences among surimi grades were observed in the SDS-PAGE patterns of OH gels without EDTA at different heating rates (Fig. 5), notwithstanding the result showing considerable differences in gel strength among the surimi grades (Fig. 2). Furthermore, no apparent degradation of MHC was observed, notwithstanding the modori effect of the gels containing EDTA, by slowing the heating rate (Fig. 4). These results suggest that various bondings, which are not reflected in the SDS-PAGE patterns obtained in this study, other than MHC cross-linking by transglutaminase, are related to the differences in the gel property at various heating rates.

Conclusions

In 1-step heating, the textural properties of all grades of AP surimi were significantly influenced by the heating method and heating rate. The increase in breaking strength and breaking distance of AP gels was highest at slower heating rates. Slow heating enhanced the gel strength of high-grade surimi more effectively than that of low-grade surimi. Furthermore, the quality of gels prepared by WB and OH differed even when the time to heat the gels up to the final temperature was the same. This may be due to differences in the linear and non-linear temperature patterns of these two heating methods.

The results obtained using gels with progressed setting (2-step heating) and gels with suppressed setting (EDTA addition)

Fig. 5. SDS-PAGE pattern of surimi gels (FA, AA, and RA grades) prepared with and without 5 mmol EDTA/kg paste as affected by heating methods. A = SDS-PAGE pattern; B = the quantitative analysis of MHC as a relative staining intensity to actin on SDS-PAGE by using ImageJ. M = molecular weight marker, MHC = myosin heavy chain, C = control (WB heating), S = 2-step heating (WB-WB); 3, 10, 80 (°C/min) = ohmic heating, + = samples prepared with EDTA.

confirmed that the effect of slow heating on gel strength was due to the enhancement of setting. Furthermore, it was confirmed that the modori effect in AP surimi was not strong under conditions conducive to strong setting, whereas modori appeared more apparent under low setting conditions. The modori effect, which is subtle and requires sensitive detection, was largely reflected in breaking distance and water holding capacity compared to breaking strength.

The above results indicated the effectiveness of slow heating for the production of heat-induced surimi gel from high-grade surimi, and rapid heating for that from low-grade surimi, and the effect of OH heating rate on the gel properties differed depending on the grade of AP surimi.

The results of this study demonstrate the various merits of OH in the production of surimi gels. In order to achieve the desired OH gelation, it will be necessary to optimize the conditions according to the characteristics of each kind of material.

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