Comparison of Atlantic menhaden gels from surimi processed by acid or alkaline solubilization

Pérez-Mateos, M. ^(a) and Lanier, T.C. ^(b)

(a) Instituto del Frío (CSIC), Dpto. Ciencia y Tecnología de Carnes y Pescados

José Antonio Nováis, 10, E28040 Madrid, Spain

(b) North Carolina State University, Dept. of Food Science

Raleigh, NC 27695-7624, USA

(*) Corresponding author Pérez-Mateos (E-mail : miriam@if.csic.es Fax: 34915493627)

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Abstract

Heat-induced gelling ability of surimis prepared by pH shifting (isoelectric precipitation following acid (AC) or alkaline (AL) solubilization) were compared to that of conventionally washed (CW) surimi. Greater endogenous transglutaminase activity (evidenced as enhanced strength of cooked gels subjected to 30-40 °C preincubation) was measured for CW and AL surimi than for AC surimi (all at pH 7). Upon addition of microbial transglutaminase (MTGase), increased crosslinking of myosin heavy chain and gel strengthening during 30-40 °C preincubation were apparent for all three types of surimi, most markedly in CW and AL surimi. Salt addition most improved CW gels, but seemed to adversely affect MTGase activity in AC and AL surimi. AC and AL surimi gels were of lower whiteness than CW surimi gels.

Key words: transglutaminase, surimi, acid, alkaline, solubilization, menhaden

INTRODUCTION

Despite a continuing strong market for surimi-based foods, there is increasing demand for fillets from whitefish species that have traditionally been used for surimi production. This portends a future time when surimi production may increasingly depend upon abundant pelagic species that are presently underutilized or undervalued, such as mackerel or menhaden (Davis, 1988). Menhaden surimi has previously been shown to have excellent gelling properties (Boye & Lanier 1988), but poor lipid stability in frozen storage.

Hultin and coworkers (Hultin & Kelleher, 1999; 2000a) proposed a new process for surimi production based on solubilization at high or low pH followed by isoelectric precipitation. Besides yielding a higher recovery of the proteins, this pH-shifting approach is particularly promising for dark fleshed fish since centrifugation of solubilized proteins during these processes was found to facilitate removal of membranous material high in content of unstable phospholipids leading to a more stable product in frozen storage (Hultin & Kelleher, 2000b). Recently it has been found that the alkaline pH-shifting process further contributes to lipid stability by stabilizing residual heme groups (from myoglobin or hemoglobin), reducing its reactivity with lipids (Kristinsson & Hultin, 2004).

Conventionally produced menhaden surimi will exhibit a strong “setting” ability induced by low temperature preincubation (optimum at 40°C for menhaden) (Boye & Lanier, 1988). This setting phenomenon has been attributed to endogenous transglutaminase (TGase) activity that induces protein crosslinking and gel strengthening. Since sarcoplasmic proteins are retained in both the acid and alkaline pH-shifting processes, endogenous transglutaminase activity may be even greater in such surimi than in conventionally washed surimi if the extremes in pH do not inactivate the TGase. Conformational changes in the myofibrillar proteins during acid and alkaline processing may also expose more

functional groups for TGase-induced crosslinking and other protein-protein interactions (Pérez-Mateos, Amato & Lanier, 2004; Kristinsson & Hultin, 2003a,b).

This work was conducted to compare the effects of the acid or alkaline pH-shifting methods of surimi preparation to that of the conventional washing process for surimi manufacture on the gel forming properties of surimis made from Atlantic menhaden. A particular interest was to measure the endogenous setting ability (TGase activity induced by low temperature preincubation) of surimi prepared in these ways, as well to determine their suitability as substrates for a microbially derived TGase (MTGase) additive recently approved for food use (Motoki & Seguro, 1998; Ashie & Lanier, 2000).

MATERIALS AND METHODS

Stabilized mince preparation

Atlantic menhaden (*Brevoortia tyrannus*) was harvested off the North Carolina coast. Fresh, net-caught fish were headed, eviscerated, and thoroughly washed. Skinless, boneless mince was obtained by passing these through a Yanagiya mini belt deboner, having drum perforations of 5 mm diameter. As cryoprotectants, 10% sucrose and 0.3% sodium tripolyphosphate w/w were added. As antioxidants, 0.2% sodium ascorbate w/w on total weight and 0.02 % propyl gallate based on an estimate of 10% lipid total were added; and all mixed for 2 min in a large dough mixer. The stabilized mince (MacDonald, Wilson & Lanier, 1990) was portioned (1 kg) and vacuum-packed in oxygen-impermeable bags (Cryovac[®] CN-590 cook-in material bags; Cryovac Division of W. R. Grace and Co., Duncan, S.C.), then frozen and stored at -20 °C until needed (within 2 mo).

Processing into surimi

Frozen stabilized mince (1200 g) was tempered at room temperature briefly before cubing with a knife while still frozen, then homogenized with 8 parts deionized cold water (0-6 °C) for 1 min in a blender at maximum speed (Model CB-6/34BL22, Waring Products Division, Dynamics Corporation of America, New Hartford, Conn.). To solubilize proteins, the pH was adjusted from about 5.0 (native) to 2.5 using 1 N cold HCl for the acid-aided process (AC), or to pH 10 with 1 N cold NaOH for alkaline-aided surimi (AL). Homogenates were centrifuged at 4,000 x g for 5 min at 4 °C. The top layer (containing neutral lipid) and bottom layer (containing insoluble lipid membranes and connective tissue) were discarded. The middle layer containing dilute solubilized protein, of low viscosity, was filtered through 4 layers of cheesecloth, then adjusted to pH 5.5 using 1 N cold NaOH or 1 N cold HCl with slow stirring. The average time for pH adjustment was ca. 10 min per batch. The resulting precipitate (surimi) was collected by centrifugation at 4,000 x g for 5 min at 4 °C and excess moisture was removed by squeezing manually between "chiffon"

mesh fabric. Conventionally water-leached (CW) surimi was also prepared as described by Pérez-Mateos et al. (2004) by 3 water washings.

Cryoprotectants, in proportions previously described, were added to all surimis before freezing. Antioxidants were added based on an estimated 3% remaining lipid content, in the same proportion of the stabilized mince. Samples were packed flat in sealed plastic bags and stored in a freezer (-20 °C) overnight. For AC and AL surimi the pH during storage was 5.5, while the pH of CW during storage was near 7.

Gel preparation

Frozen surimi was tempered at room temperature briefly before cubing with a knife while still frozen. Sodium chloride (0% or 2% w/w), ice sufficient to achieve 78% (w/w) final moisture content, as well as 1 N cold NaOH or 1 N HCl (between 5 and 10 mL) for pH adjustment to 7.0 were added together and the mixture was chopped in a food processor (Model LPP; Cuisinart, East Windsor, N.J.) to a final temperature of 5 °C (about 6 min). To some pastes 0.2% of a commercial preparation of microbial transglutaminase (MTGase) derived from *Streptoverticillium* (Activa™ TI, containing 100 units of MTGase/g dispersed in maltodextrin; Ajinomoto USA, Inc., Teaneck, N.J.) was added, yielding 20 units of MTGase activity per 100 g surimi paste (Lee, Lanier, Hamann & Knopp, 1997).

The pastes were extruded into open-ended (ends cut off) polypropylene centrifuge tubes (9 cm length, 1.4 cm i.d.), previously sprayed inside with a lecithin-based release agent to prevent gel adhesion. The tubes were capped on both ends with serum sleeve stoppers clamped with metal thumbscrew clamps. They were then heated by one of three water bath treatments: (1) 90 °C for 20 min; (2) 30 °C for 2 h followed by 90 °C for 20 min; (3) 40 °C for 2 h followed by 90 °C for 20 min. Immediately after heating, the

tubes were cooled in ice water. Gels were then removed from the tubes and held at 4 °C overnight in sealed plastic bags.

Gel Strength and Deformability

Gel samples (1.4 cm long, 1.4 cm i.d.) were tested as described by Pérez-Mateos et al. (2004) by axial puncture with a 3.175 mm dia ball in the center of gels at a penetration speed of 1.1 mm/s. Breaking force (g) and deformation (penetration depth, mm) were determined on at least 6 specimens per treatment.

Gel Whiteness. Color was measured on 6 specimens per treatment with a Chroma Meter CR 300 (Minolta Camera Co., Ltd., Osaka, Japan) using the CIE Lab scale (D65/10°) where L* (black 0 to light 100), a* (red 60 to green -60) and b* (yellow 60 to blue -60) to measure lightness, redness and yellowness. Whiteness was calculated according to the following formula: $\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

The activities of either endogenous TGase or MTGase, resulting in covalent crosslinking of proteins, or (endogenous) proteolytic enzymes, resulting in small molecular weight breakdown products, were monitored by SDS-PAGE of the solubilized gels according to the method of Pérez-Mateos et al. (2004) using NuPAGE® Tris-acetate buffered (pH=7) polyacrylamide gradient gels (3-8 %) (Invitrogen, Carlsbad, Calif.) loaded with 6 µg protein per lane. Densitometer scans were made on gels using an Alphamager™ (Alpha Innotech Corporation, San Leandro, Calif.). Protein was quantified as percentages of the total protein in each lane. Three regions were measured for each lane: the sum of bands above myosin heavy chain (MHC), sum of bands below MHC, and the MHC band itself.

These data were used to determine statistical differences between treatments and are not included in the results.

Statistical analysis

Data were subjected to analysis of variance and planned, paired comparisons were made using the Student's t-test with significance of difference set at $p < 0.01$ unless otherwise indicated in the text.

RESULTS AND DISCUSSION

Rheological properties

As expected (Boye & Lanier 1988), in the presence of added NaCl conventionally water-leached (CW) menhaden surimi evidenced setting when incubated at 30°C or 40°C prior to cooking (Fig. 1) as an approximate doubling of punch force after cooking at 90 °C. Without added NaCl, however, this surimi evidenced setting only at 40°C, with a punch force only ca. 1.5 times that of the 90 °C control. Similarly, when MTGase was added to induce/enhance setting, the sample incubated at 30 °C did not exhibit a setting effect unless 2% NaCl was included in the formulation. Addition of MTGase to all other CW surimi gels resulted in 3 to 5 times higher punch force values when incubated at 30 °C or 40 °C prior to cooking (90 °C). Gel deformability (fracture deformation) was increased with the addition of MTGase to CW surimi gels preincubated at lower temperatures before the final cook. CW gels containing NaCl had higher deformability than their counterpart treatments without added NaCl except for the controls cooked at 90 °C only.

Gels made from acid aided (AC) surimi were generally of poorer quality than those made with CW surimi, with lower punch force and deformation values for comparable treatments (Fig. 1). No endogenous setting was apparent in AC surimi gels, but with addition of

MTGase, a setting effect was exhibited at both preincubation temperatures. Unlike the CW surimi gels, punch force was much greater when salt was not added to the MTGase-containing formulations.

The punch force and deformation for gels made from alkaline aided (AL) surimi were the same or lower than those made with the CW surimi except for MTGase-containing gels, made with no added NaCl and incubated at 30 °C. Similar to CW surimi gels, AL gels made with the addition of salt were more deformable (all but gels containing MTGase and incubated at 40 °C). Endogenous TGase activity was only evident in AL surimi gels made without added salt and incubated at 40 °C (Fig.1). When MTGase was added, gel enhancement occurred at both setting temperatures, with highest activity of MTGase evident for gels formulated without salt and incubated at 40 °C.

These results suggest that in AL surimi the presence of a higher relative content of sarcoplasmic proteins neither impeded nor enhanced the (M)TGase mediated protein crosslinking process. Endogenous TGase activity in AL surimi was apparently lower than in CW surimi, but nonetheless evident. There did not appear to be any enhancement of substrate response to (M)TGase activity due to the acid or alkaline solubilization/precipitation processes used to make AC or AL surimi as compared to that of CW surimi.

NaCl addition clearly improved gelation of CW surimi and especially TGase-linked setting. NaCl addition had variable effects on AC and AL surimi, seeming to interfere with MTGase activity in both. Pérez-Mateos et al. (2004) found that the addition of MTGase and NaCl enhanced the gel strength (punch force) of CW and AL gels made from Atlantic croaker, but had the opposite effect on croaker AC surimi.

Breaking strength and deformation was noticeably lower in gels made from the AC surimi, and this surimi exhibited no apparent TGase/setting activity. Oddly this report did not find higher gelling properties for AC or AL surimi as compared to CW surimi, as have others, which have particularly shown the best gel-forming ability in AL surimi (Pérez-Mateos et al., 2004; Kim, Park & Choi, 2003; Yongsawatdigul & Park 2004; Undeland, Kelleher & Hultin, 2002; Kristinsson & Hultin, 2003a).

The ability to form strong, deformable gels in the absence of NaCl appears to be species dependent (Hennigar, Buck, Hultin, Peleg & Vareltizis, 1989) and is enhanced by the pH-shifting method of surimi preparation. Apparently acid- or base-induced solubilization leads to substantial changes in the conformation and structure of fish proteins, such that upon refolding these proteins have different properties (Kristinsson & Hultin 2003a,b). Related studies (Chang, Feng & Hultin, 2001; Wright & Lanier, 2005) suggest that a more thorough disruption of muscle structure by acid or alkaline processing may also play a key role in better distributing proteins for heat-induced gel formation and thus obviate the need for salt to accomplish the same effect.

SDS-PAGE

In general for surimi prepared by all 3 methods, with and without addition of 2% NaCl (Fig. 2 and 3, respectively), SDS-PAGE profiles appeared similar among treatments with and without added salt, though the rheological characteristics of these gels differed (Fig.1). The insoluble fraction and/or the size of polymers that were too large to enter the polyacrylamide stacking gel could be very different and also the overall spatial arrangement of the proteins in the gel could still differ as particularly in TGase activity. There was no change in the actin band in all the treatments; however Yongsawatdigul and Park (2004) found out lower intensive of actin bands in AC and AL surimi gels made from rockfish in comparison with the washed mince.

Pérez-Mateos et al. (2004) reported proteolysis by SDS-PAGE in croaker surimi gels when preincubated at 30 or 40 °C and higher in the presence of salt due to a stimulating effect of NaCl on autolysis of myosin. In our case, the addition of salt in AC and AL surimi seems to adversely affect MTGase activity (Fig. 1) rather than proteolysis breakdown (Fig.2).

SDS-PAGE revealed that myosin heavy chain concentration [MHC] of cooked gels remained relatively unaffected by a setting preincubation when MTGase was not added. The gel strength enhancement which we attributed to endogenous TGase activity in the previous discussion (Fig.1) apparently did not affect [MHC] enough to be detectable. Gels made with the addition of MTGase did not completely solubilize in the denaturing buffer used to prepare the samples for electrophoresis. This has been seen before in our laboratory when solubilizing cooked pollock (Lee et al. 1997) and croaker (Perez-Mateos et al., 2004) surimi gels which included MTGase in their formulations. Lee et al. (1997) further investigated the gels containing 0.2% MTGase for ϵ -(γ -glutamyl)lysine dipeptide content by HPLC analysis of protease-digested samples, which avoided the problems with the aforementioned solubilization process. They found that ϵ -(γ -glutamyl)lysine content increased linearly with setting time while [MHC] measured by SDS-PAGE decreased substantially with little to no [MHC] after 2 to 3 hr setting at 25 °C. They were then able to substantiate that a decrease in [MHC] was concomitant with increased ϵ -(γ -glutamyl)lysyl covalent crosslinking. In surimi gels without evidence of proteolytic activity, therefore, myosin polymerization can be reliably measured by disappearance of MHC.

In AC surimi gels [MHC] by SDS-PAGE was lower compared to other treatments, with appearance of smaller MW bands (about 150 KDa). This fact, together with the lower fracture force and deformation (Fig.1) for AC gels may indicate some degradation by proteolytic activity and/or irreversible damage of the protein at acidic pH (Yongsawatdigul

& Park, 2004). Salt, Leslie, Lillford and Dunnill (1982) noted that hydrochloric acid can cause substantial damage to soya proteins. Both Undeland et al. (2002), and Choi and Park (2002), also noted greater proteolysis in AC surimi. However Kristinsson, Theodore, Demir and Ingadottir (2005) did not find hydrolytic breakdown during low and high pH.

Menhaden has been reported to have high levels of endogenous proteases. Boye and Lanier (1988) reported maximum caseinolytic activity at 60 °C and pH 7.5 to 8.0; Choi, Lanier, Lee and Cho (1999b) reported maximum caseinolytic activity at pH 8 and 55 °C; Choi, Cho and Lanier (1999a) measured maximum Z-Phe-Arg-Nmec hydrolyzing activity at pH 7.4, 40 to 50 °C. Choi and Park (2002) attributed lower breaking force values for gels from Pacific whiting AC surimi to the activity of retained cathepsin B and L enzymes. Kim et al. (2003) reported that neither acid nor alkaline pH-shifting processes will inactivate cathepsin L-like activities.

Color

Comparing the whiteness of the three types of surimi gels (Fig.4), it is clear that CW surimi gels exhibited a higher whiteness than AC or AL surimi gels, due to the higher lightness (L^*) and lower yellowness (b^*) and redness (a^*) values. It can be explained since this surimi was obtained after three washing steps and more myoglobin likely removed from the CW surimi during washing, increasing its lightness and decreasing its redness and yellowness, whereas AC and AL surimi had only one “washing” step. In most others studies of surimi made by acid and/or alkali solubilization followed by isoelectric precipitation higher L^* values in CW gels are reported (Yongsawatdigul & Park, 2004, Choi & Park, 2002; Undeland et al., 2002); except in a recent work using catfish muscle (Kristinsson et al., 2005).

Higher whiteness was obtained in AL gels than in AC gels. This is in agreement with other studies in other species (Kim et al., 2003; Kristinsson et al., 2005). However Kim et al. (2003) pointed out that it was only found at alkaline pH (10.5-11) but not in gels made from surimi treated at pH 12.

Regarding the effect of heat treatment or by the addition of ingredients (salt, MGTase) no significant differences were found for CW surimi gels. For AC and AL surimi gels, however, NaCl addition increased the whiteness, while no significant differences due to heat treatment nor MTGase addition were seen.

Whiter gels could likely be obtained by these new processes by increasing the proportion of mince : water to about 1:20 (Hultin & Kelleher, 1999) or washing with ice water before mincing (Choi & Park, 2002) or by air-flotation (Choi, 2005) before adjusting to pH 11 though gel properties could be reduced.

CONCLUSION

For processing menhaden into surimi, alkaline solubilization processing produced the highest gelling quality only in one washing step but poorer color than CW surimi from Atlantic menhaden. Further experimentation is needed to improve whiteness of this surimi if it is to be competitive with surimi now available from whitefleshed species.

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Figure 1- Comparison of puncture test values for surimi gels made with 3 surimi types: conventionally washed (CW), acid-solubilized (AC) and alkaline-solubilized (AL). Gels were made with or without 2% NaCl, and with or without 0.2% added microbial transglutaminase (TGase). 90 indicates that gels were cooked at 90 °C for 20 min, 30/90 and 40/90 indicate gels incubated at 30 °C and 40 °C for 30 min, respectively, followed by cooking at 90 °C for 20 min. Error bars represent the standard deviation of 6-10 samples.

Figure 2- Comparison of the effect of MTGase and 2% NaCl on muscle proteins in surimi gels made with 3 surimi types: conventionally washed (CW), acid-solubilized (AC) and alkaline-solubilized (AK). Where MTGase “-” and “+” indicates results for surimi gels made with no or with added MTGase, respectively; and where cooking treatment: “90” = 90 °C for 20 min; “30” = 30 °C for 2 h followed by 90 °C for 20 min; “40” = 40 °C or 2 h followed by 90 °C for 20 min.

Figure 3- Comparison of the effect of MTGase on muscle proteins in surimi gels made with the 3 types of surimi and without the addition of NaCl. Locations of treatment combinations are the same as those in Figure 2.

Figure 4- Comparison of whiteness values for surimi gels made with 3 surimi types. Locations of treatment combinations are the same as those in Figure 1.