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

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Abstract

Heat-induced gelling abilities 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 improved CW gels most, but seemed to adversely affect MTGase activity in AC and AL surimi. AC and AL surimi gels were of lower whiteness than were CW surimi gels.

Keywords: Transglutaminase; Surimi; Acid; Alkaline; Solubilization; Menhaden

1. 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 under-utilized or under-valued, 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 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, chapter 3). 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

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crosslinking and other protein–protein interactions (Kristinsson & Hultin, 2003a, 2003b; Pérez-Mateos, Amato, & Lanier, 2004).

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 as to determine their suitability as substrates for a microbially-derived TGase (MTGase) additive recently approved for food use (Ashie & Lanier, 2000, chapter 6; Motoki & Seguro, 1998).

2. Materials and methods

2.1. 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% total lipid 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, SC), then frozen and stored at −20 °C until needed (within 2 months).

2.2. Processing into surimi

Frozen stabilized mince (1200 g) was briefly tempered at room temperature before cubing with a knife while still frozen, then homogenized with eight 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, CT). 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 4000*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 four 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 about 10 min per batch. The resulting precipitate (surimi) was collected by centrifugation at 4000*g* for 5 min at 4 °C and excess moisture was removed by squeezing manually between "chiffon" mesh fabrics. Conventionally water-leached (CW) surimi was also prepared as described by Pérez-Mateos et al. (2004) by three water washings.

Cryoprotectants, in proportions previously described, were added to all surimis before freezing. Antioxidants were added, based on an estimated 3% of remaining lipid content, in the same proportion as 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.

2.3. Gel preparation

Frozen surimi was briefly tempered at room temperature 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, NJ) 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 *Streptoverticilium* (Activa™ TI, containing 100 U of MTGase/g dispersed in maltodextrin; Ajinomoto USA, Inc., Teaneck, NJ) was added, yielding 20 U 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 at 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.

2.4. 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 centre of gels at a penetration speed of 1.1 mm/s. Breaking force (g) and deformation (penetration depth, mm) were determined on at least six specimens per treatment.

2.5. Gel whiteness

Colour was measured on six specimens per treatment with a Chroma Meter CR 300 (Minolta Camera Co., Ltd., Osaka, Japan), using the CIE Lab scale (D65/10°),

with 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: Whiteness $= 100 - [(100 - L)^{*2} + a^{*2} + b^{*2}]^{1/2}$.

2.6. 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, CA) loaded with 6 μg protein per lane. Densitometer scans were made on gels using an AlphaImager™ (Alpha Innotech Corporation, San Leandro, CA). 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.

2.7. 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.

3. Results and discussion

3.1. 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 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 about 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 3–5 times higher punch force values when incubated at 30 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.

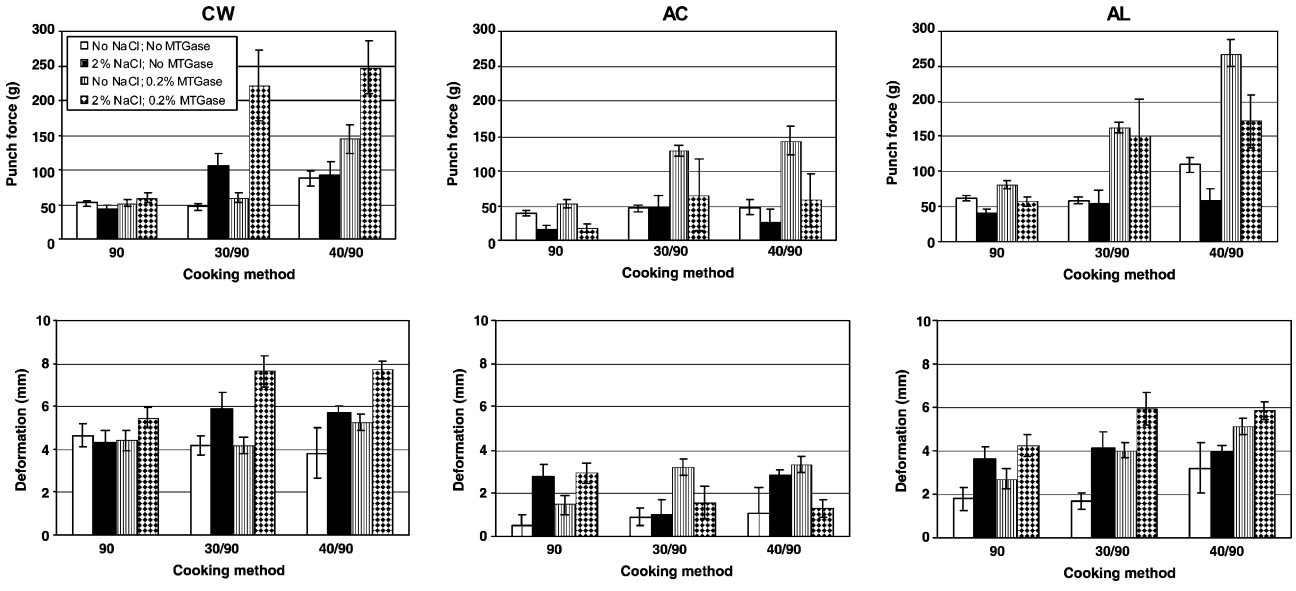

Fig. 1. Comparison of puncture test values for surimi gels made with three surimi types: conventionally washed (CW), acid-solubilized (AC) and alkaline-solubilized (AL). Gels were made with or without 2% NaCl, and with or without 0.2% added microbial transglutaminase (TGase). 90 indicates that gels were cooked at 90 °C for 20 min, 30/90 and 40/90 indicate gels incubated at 30 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.

The punch force and deformation for gels made from alkaline-aided (AL) surimi were the same as 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/reprecipitation 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 were 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 (Kim, Park, & Choi, 2003; Kristinsson & Hultin, 2003a; Pérez-Mateos et al., 2004; Undeland, Kelleher, & Hultin, 2002; Yongsawatdigul & Park, 2004).

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, 2003b). 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.

3.2. SDS–PAGE

In general, for surimi prepared by all three methods, with and without addition of 2% NaCl (Figs. 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 lower intensities 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 move 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

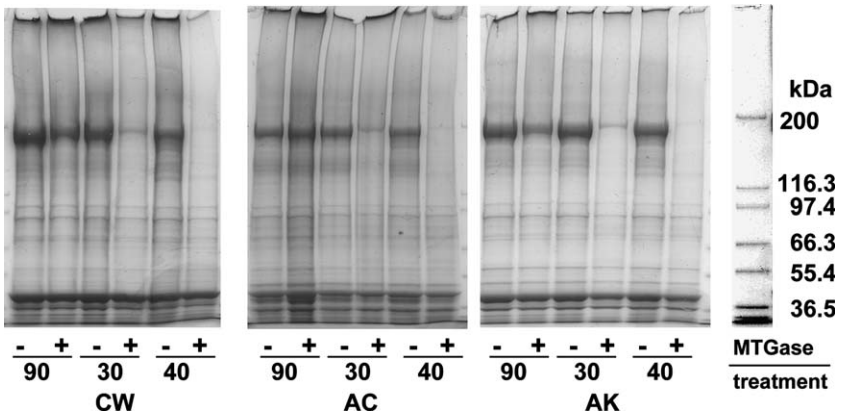

Fig. 2. Comparison of the effect of MTGase and 2% NaCl on muscle proteins in surimi gels made with three surimi types: conventionally washed (CW), acid-solubilized (AC) and alkaline-solubilized (AK), where MTGase "−" and "+" indicate results for surimi gels made with no or with added MTGase, respectively, and, for 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.

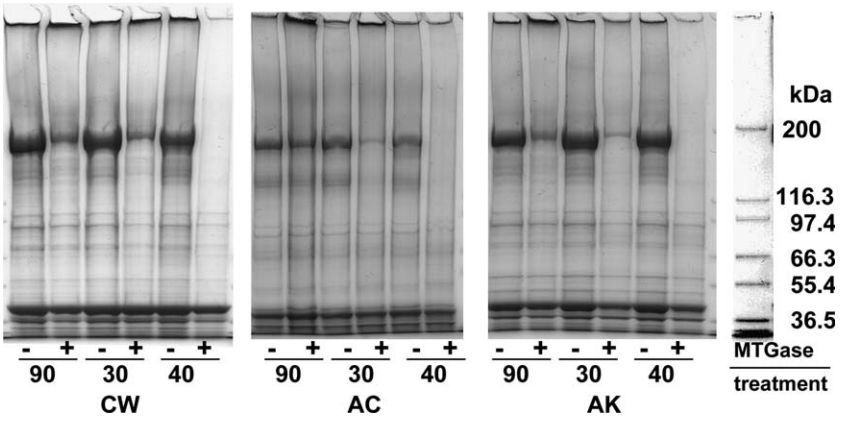

Fig. 3. Comparison of the effect of MTGase on muscle proteins in surimi gels made with the three types of surimi and without the addition of NaCl. Locations of treatment combinations are the same as those in Fig. 2.

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 previously in our laboratory when solubilizing cooked pollock (Lee et al., 1997) and croaker (Pérez-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 a 2 to 3 h 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 at low or 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 7.5–8.0; Choi, Lanier, Lee, and Cho (1999) reported maximum caseinolytic activity at pH 8 and 55 °C; Choi, Cho, and Lanier (1999) measured maximum Z-Phe-Arg-Nmec-hydrolyzing activity at pH 7.4 and 40–50 °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 would inactivate cathepsin L-like activities.

3.3. 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 did AC or AL surimi gels, due to

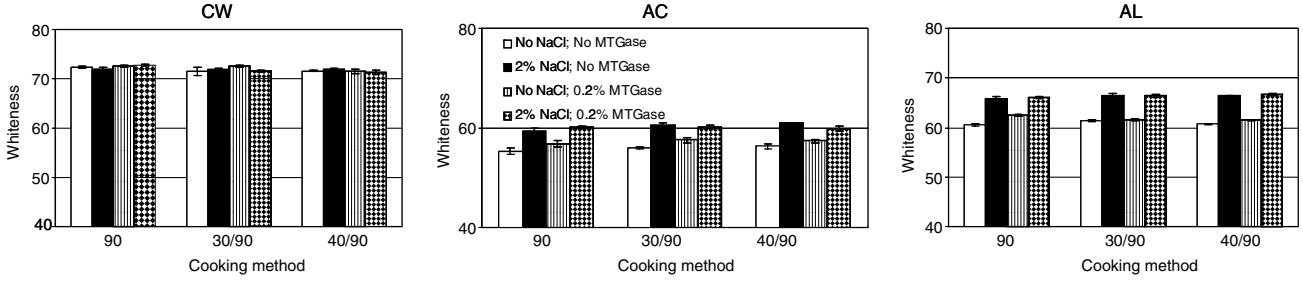

Fig. 4. Comparison of whiteness values for surimi gels made with three surimi types. Locations of treatment combinations are the same as those in Fig. 1.

the higher lightness (L^*) and lower yellowness (b^*) and redness (a^*) values. This can be explained since this surimi was obtained after three washing steps and more myoglobin was 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 other studies of surimi made by acid and/or alkali solubilization, followed by isoelectric precipitation, higher L^* values in CW gels were reported (Choi & Park, 2002; Undeland et al., 2002; Yongsawatdigul & Park, 2004), 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 this 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 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 or 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.

4. Conclusion

For processing menhaden into surimi, alkaline solubilization processing produced the highest gelling quality only in one washing step but gave poorer colour 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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