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Surimi of fish species from the Gulf of Mexico: evaluation of the setting phenomenon

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Abstract

Atlantic croaker (*Micropogon undulatus*), Mexican flounder (*Cyclopsetta chittendeni*) and Northern kingfish (*Menticirrhus saxatilis*) are warm water species abundant in the Gulf of Mexico, usually obtained as shrimp by-catch. Gels from these species were obtained by several treatments: (1) setting at 25°C/3 h followed by cooking at 90°C/15 min; (2) setting at 40°C/30 min and 90°C/15 min; (3) 90°C/15 min (control). Three different additives were studied: 0.66% ammonium chloride, 0.2% EDTA and 0.2% calcium chloride. The setting phenomenon was induced at 40°C in the three species. 0.2% calcium chloride improved shear stress and shear strain in surimi gels from Atlantic croaker and Northern kingfish. 87.98 and 98.49 kPa for shear stress, and 2.23 and 2.15 for shear strain were achieved, respectively.

1. Introduction

Atlantic croaker (*Micropogon undulatus*), Mexican flounder (*Cyclopsetta chittendeni*) and Northern kingfish (*Menticirrhus saxatilis*) are warm water fish species present in the shrimp by-catch. They are abundant species in the Gulf of Mexico and they have low commercial value. The processing of these fish species to obtain surimi can be a good alternative use for this abundant resource. A considerable biomass with appropriate mechanical properties for surimi gel (strength and elasticity) are present in these species. Several food ingredients from surimi are known, such as emulsifiers (Ramírez, Díaz-Sobac, Morales, & Vázquez, 1999).

After being mixed with salt, surimi sols prepared below 40°C from most fish species have a unique ability to form a translucent, highly deformable gel. This process is called setting. A stronger gel results by heating at higher temperatures than by cooking directly (Lanier, 1986).

An endogenous transglutaminase (TGase) has been found to be responsible for the setting of surimi (An, Peters, & Seymour, 1996). TGase is a calcium-dependent enzyme that catalyzes an acyl transfer reaction between γ-carboxyamide groups of glutamyl residues in proteins and primary amines. When the primary amine is an ϵ-amino group of lysine or lysyl residues, the ϵ-(γ-glutamyl)lysine cross-linking occurs (Kumazawa, Seguro, Takamura, & Motoki, 1993; Seki, Nakahara, Takeda, Maruyama, & Nozawa, 1998). Although low temperature setting has been related to TGase activity, high temperature setting has been associated with the unfolding of proteins at 30–40°C (An et al., 1996).

A difference in setting response, depending upon the fish species, has been reported (Shimizu, Machida, & Takanemi, 1981). In addition, a relationship between the habitat temperature of fish species and optimal conditions for setting has been observed. Thus, surimi gels from cold water species present better mechanical properties with low temperature setting, while gels from warm water species present better attributes with high temperature setting (Kamath, Lanier, Foegeding, & Hamman, 1992; Lee & Park, 1998; Ramirez, Rodríguez-Sosa, Morales, & Vázquez, 2000). Nowsad, Katuh, Kanch, and Niwa (1996) found that endogenous TGase has an optimal temperature in the range 25–30°C for several cold water fish species, with a minimum effect at

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20°C. The strongest gels were obtained at 35–40°C without calcium in the medium. The effect of calcium in the range 0–0.2%, on setting at 25°C/3 h or 5°C/20 h, in surimi from Pacific whiting and Alaska pollock, has been reported by Lee and Park (1998). These results indicate that the presence of calcium increases the values of shear stress at failure, in both species. The effect on shear strain was negligible. These studies suggest that, although the endogenous enzyme is present and the concentration of calcium is high, the variable temperature and time are important to allow the protein cross-linking.

Kumazawa, Seguro, and Motoki (1995) found that increasing of gel strength obtained in surimi grade FA was associated with polymerisation of the myosin heavy chain, mediated by the presence of an endogenous TGase.

Ammonium chloride is a specific inhibitor for transglutaminase and ethylenediaminetetraacetate (EDTA) is a calcium-chelating agent (Lee, Lanier, & Hamann, 1997b). The employing of both ammonium chloride and EDTA could suppress the setting phenomenon, decreasing the gel strength, myosin heavy chain polymerization and formation of the ϵ-(γ-glutamyl) lysine bonds.

Calcium could be a limiting factor during the setting of warm water fish species; therefore, the use of calcium chloride could improve the mechanical properties of the surimi obtained.

The objective of this work was to evaluate the effect of different heat treatments in the surimi production using, as raw material, fish species from the shrimp by-catch of the Gulf of Mexico. The effect of ammonium chloride and EDTA on the presence and activity of the endogenous TGase, as well as the effect of $CaCl_2$ on the setting process, were studied.

2. Materials and methods

2.1. Frozen surimi

Atlantic croaker (*Micropogon undulatus*), Mexican flounder (*Cyclopsetta chittendeni*) and Northern kingfish (*Menticirrhus saxatilis*) were obtained as part of the shrimp by-catch of a commercial vessel, which was fishing in the Gulf of Mexico on the coast of Tamaulipas, Mexico. In general, all fishes weighed an average of 150 g and their length was near 15 cm. Whole fishes were washed and kept in ice until processing. Fish was processed into surimi about 12 h after being caught. Fish were headed, gutted and washed by hand. Skin and bones were removed with a Bibun deboning machine (Model NF2DX, Fujiyama, Japan) with a drum having 5 mm diameter perforations. Fresh mince was washed-dewatered three times, with a ratio of 3:1 (water/mince). Each wash consisted in stirring the mince with water for 7 min. The temperature was maintained below 10°C by the addition of ice during the washing process. The last wash included 0.25% NaCl to facilitate dewatering. Surimi was packed into polyethylene bags (2 kg), frozen within 5 h at −30°C in a Crepaco plate freezer (Model B-5854-AM12, Crepaco, Inc., Chicago, IL) and stored at −20°C until needed.

2.2. Surimi gel preparation

A 250-g surimi sample was selected from a 2 kg bag, partially thawed at room temperature, cut into small pieces and chopped in a Hobart cutter (Model 84145, Troy, Ohio) for 3 min with 2.5% salt. Calcium chloride (0.2%), ammonium chloride (0.66%) or EDTA (0.2%) were dispersed with salt and added to surimi paste. The moisture content of each formulation was adjusted to 78%. Final chopping temperature was maintained at <15°C. The paste was stuffed into stainless tubes (diameter = 1.87 cm; length = 17.75 cm), and sprayed with commercial vegetable oil to prevent sticking. Tubes were capped before thermal treatment. After cooking, tubes were immediately removed, placed in a water bath and cooled at 4–5°C for 30 min. All gels were removed from the tubes and stored overnight at 4°C in polystyrene bags, prior to testing.

2.3. Torsion test

Gels were kept at room temperature (approx. 20°C) before the torsion test. Gels were cut into 3.0 cm length and milled into an hourglass shape with a minimum diameter of 1 cm in the centre. Each milled sample was then mounted and fixed in a special fixture of a Brookfield digital viscometer (Model 5XHBTD, Brookfield Engineering Laboratories, Inc., Stoughton, MA). The sample was twisted at 2.5 rpm to the point of failure while a computer recorded the plot of torque versus angular rotation (deformation) produced. From such a plot, the peak torque and angular deformation at the point of sample failure (breakage) was directly obtained. Under these conditions, the shear stress and shear strain at the failure were calculated as described by Hamann, Amato, Wu, and Foegeding (1990). All analyses were performed with six replicates for each treatment.

2.4. Chemical analysis

Total solids and moisture were determined according to AOAC standard methods (AOAC, 1984). All analyses were performed in triplicate for each surimi.

2.5. Protein solubility

Surimi (50 g) was solubilised in 300 ml phosphate buffer, 50 mM, pH 7.0, 0.6 M KCl at 4°C employing a

homogenizer, avoiding foaming formation. After solubilisation, samples were divided in aliquots of 5 ml and incubated at 25°C for 3 h or at 40°C for 15 min, with a subsequent cooling at 5°C by immersion in an ice-water bath before centrifugation. Protein solubility was determined as the protein failing to precipitate after centrifugation at 1800 ***g*** for 10 min at room temperature (25±2°C). Protein concentration in the supernatant was determined using the Lowry technique described by Robyt & White (1990). All analyses were performed in triplicate for each surimi.

2.6. Statistical analysis

Statistical analysis was performed using Statgraphics 5.0 (Software Publishing Corporation, Bitstream Inc.). LSD multiple range tests were used to determine significant differences ($P<0.05$) among treatments after initial demonstration of a treatment related effect by analysis of variance.

3. Results and discussion

Atlantic croaker, Mexican flounder and Northern kingfish were processed to obtain surimi. It has been reported that optimal setting temperature for surimi from cold water fish species is 25°C and for warm water fish species is 40°C (Kamath et al., 1992; Lee and Park, 1998). Therefore, to establish the better setting temperature, three thermal treatments were studied: (1) setting at 25°C/3 h followed by cooking at 90°C/15 min; (2) setting at 40°C/30 min and cooking at 90°C/15 min; (3) only cooking 90°C/15 min. Mechanical properties (shear stress and shear strain) and protein solubility were measured in the surimi obtained. Table 1 summarises the results obtained. These showed that the surimi produced from Atlantic croaker, incubated at 40°C/30 min before cooking, showed the highest shear stress value (95.22 kPa), according with that expected for a warm water fish species.

Also, the surimi from Mexican flounder improved its shear stress due to the setting at 40°C/30 min, but with a slight effect (63.43 kPa). But Northern kingfish showed different behaviour. No setting was observed at 40°C as could be expected from a warm water fish. The shear stress decreased slightly when the gel was exposed to setting temperatures (Table 1).

The second mechanical parameter (shear strain) did not show any significant difference ($P<0.05$) compared with the control (90°C/15 min) in all three species.

It is known that, during the setting phenomenon, fish protein aggregates, inducing the gel formation (Niwa, Ueno, & Kanoh, 1992). Table 1 shows that the protein solubility is lower when the setting temperature is 40°C than when it is 25°C. This means that proteins, from the fish species studied, aggregated when incubated at 40°C/30 min, but remained soluble when incubated at 25°C/3 h.

Northern kingfish showed the lowest protein solubility (5.97 g/l). Although a protein aggregation was observed in this species, the protein aggregation was not directly associated with mechanical properties, because the shear stress was higher in the control (42.24 and 45.12 kPa, respectively).

Setting phenomenon has been associated with an endogenous calcium-dependent TGase. The addition of a TGase inhibitor was evaluated to determine the participation of the endogenous TGase. Ammonium chloride (0.66%) was selected as TGase inhibitor (Kumazawa et al., 1995). A new set of experiments adding 0.66% ammonium chloride was performed and surimi gels were produced under different thermal conditions. Fig. 1 shows the results obtained. In the surimi from Atlantic croaker and Mexican flounder, the addition of ammonium chloride decreased the shear stress values of surimi compared with the control (Table 1). In the surimi from Northern kingfish, ammonium chloride confers no advantage, because the enzyme was not active, perhaps due to a deficient concentration of the cofactor calcium in the medium. In Fig. 1b it can be observed that ammonium chloride also decreased shear strain values for the three species.

Table 1
Results achieved from the study of thermal conditions of surimi processing

Species	Conditions	Shear stress (kPa)	Shear strain(dimensionless)	Protein solubility (g/l)
Atlantic croaker (*Micropogon undulatus*)	90°C/ 15 min	36.12	2.06	13.6
	25°C /3 h + 90°C/ 15 min	49.62	1.81	16.1
	40°C /30 min + 90°C/ 15 min	95.22	2.19	8.72
Mexican flounder (*Cyclopsetta chittendeni*)	90°C/ 15 min	46.02	1.62	10.4
	25°C /3 h + 90°C/ 15 min	56.02	1.55	12.0
	40°C /30 min + 90°C/ 15 min	63.43	1.91	6.36
Northern kingfish (*Menticirrhus saxatilis*)	90°C/ 15 min	45.12	1.99	14.2
	25°C /3 h + 90°C/ 15 min	40.24	1.70	15.7
	40°C /30 min + 90°C/ 15 min	42.24	1.93	5.97

The addition of a calcium-chelating agent was evaluated to determine the participation of the calcium as cofactor of the endogenous TGase. A concentration of 0.2% EDTA was selected (Kumazawa et al., 1995). A new set of experiments with 0.2% EDTA was performed and surimi gels were produced under different thermal conditions. Fig. 2 shows the results obtained. Addition of EDTA decreased the shear stress values of surimi gels from Atlantic croaker and Mexican flounder.

EDTA had no significant effect on surimi gels from Northern kingfish which gels only with cooking (90°C/15 min). Moreover, an increase in shear stress was observed in the surimi gels treated at setting temperatures (25 or 40°C). This positive effect of EDTA in surimi preparation from Northern kingfish could not be explained.

EDTA also decreased the shear strain values for the three species. So we can conclude that the endogenous TGase of these three species is calcium dependent but the behaviour of the enzyme from Northern kingfish indicates that different endogenous TGases are present in warm water fish species. Further studies are needed to identify these TGases.

The addition of calcium chloride can determine whether the calcium is the limiting factor during the setting of warm water fish species. Surimi gels with 0.2% calcium chloride were produced and their mechanical properties were analysed. Fig. 3a shows the values of shear stress obtained in surimi supplemented with 0.2% calcium chloride produced under different thermal conditions. Comparing data from Table 1 (no calcium) and Fig. 3a (calcium added) we can conclude that the addition of calcium chloride has no effect on the shear stress of surimi gels from Atlantic croaker ($P<0.05$). Similar patterns were observed in the surimi obtained from Mexican flounder. No significant difference was observed between surimi processed with or without calcium added.

Different behaviour was observed in the surimi obtained from Northern kingfish. The addition of 0.2% calcium chloride improved the mechanical properties at 40°C but not at 25°C. The shear stress, incubating at 40°C, was 98.49 kPa, 233% higher than the value obtained in surimi without addition of calcium. These results suggest that TGase was presented in the surimi, but a low level of calcium in the paste repressed its activity.

Fig. 3b shows the values of shear strain measured in surimi gels with calcium added. Shear strain did not show any significant difference ($P<0.05$) from surimi gels without calcium added (Table 1). This fact accords with the fact that the setting phenomenon induces changes mainly in shear stress with little effect on shear

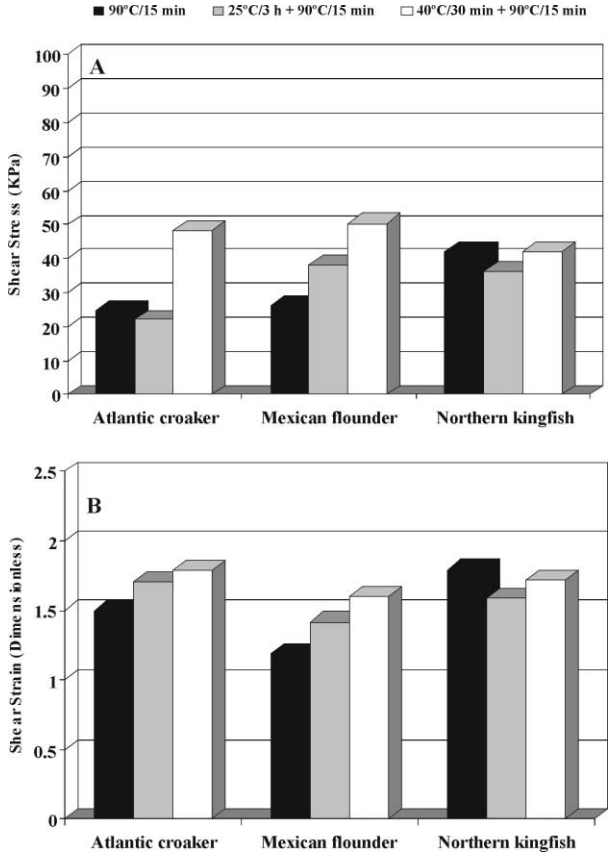

Fig. 1. Effect of 0.66% ammonium chloride on shear stress and shear strain of surimi gels from different warm water fish species.

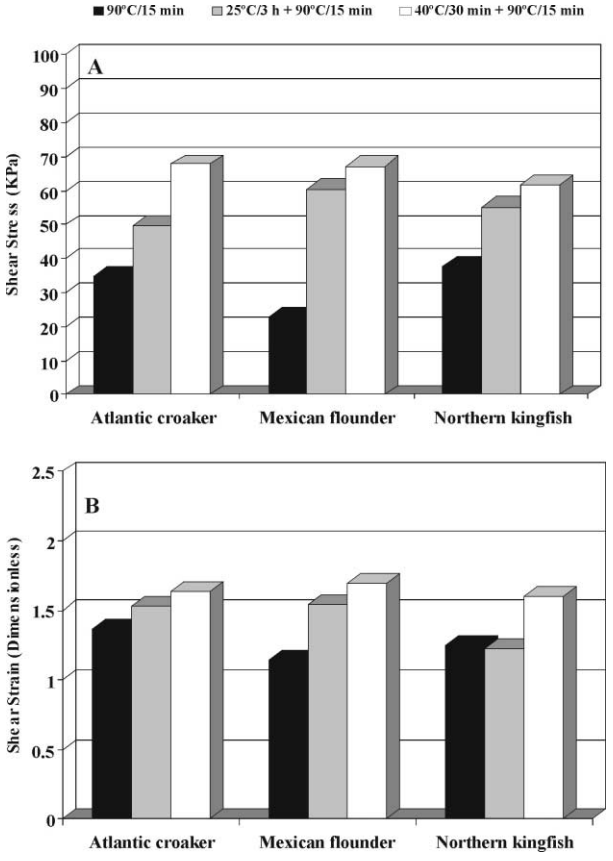

Fig. 2. Effect of 0.2% EDTA on shear stress and shear strain of surimi gels from different warm water fish species.

Table 2
Comparison of shear stress and shear strain values of surimi gels from different warm water fish species

Species	Conditions	Shear stress (kPa)	Shear strain (dimensionless)	Reference
Atlantic croaker	90°C/15 min	32	1.9	Sánchez, Ramírez, Morales, and Montejano (1998)
(*Micropogon undulatus*)	90°C/15 min	38	2.1	Ramos-Martínez, Morales-González, Ramírez, García-Carreño, and Montejano-Gaitán (1999)
	40°C/30 min + 90°C/15 min (2% $CaCl_2$)	87.98	2.23	This work
Mexican flounder	90°C/15 min	45	1.5	Sánchez et al. (1998)
(*Cyclopsetta chittendeni*)	90°C/15 min	46	1.7	Ramos-Martínez et al. (1999)
	40°C/30 min + 90°C/15 min (2% $CaCl_2$)	71.79	1.55	This work
Northern kingfish	90°C/15 min	43	1.9	Ramos-Martínez et al. (1999)
(*Menticirrhus saxatilis*)	40°C/30 min + 90°C/15 min (2% $CaCl_2$)	98.49	2.15	This work

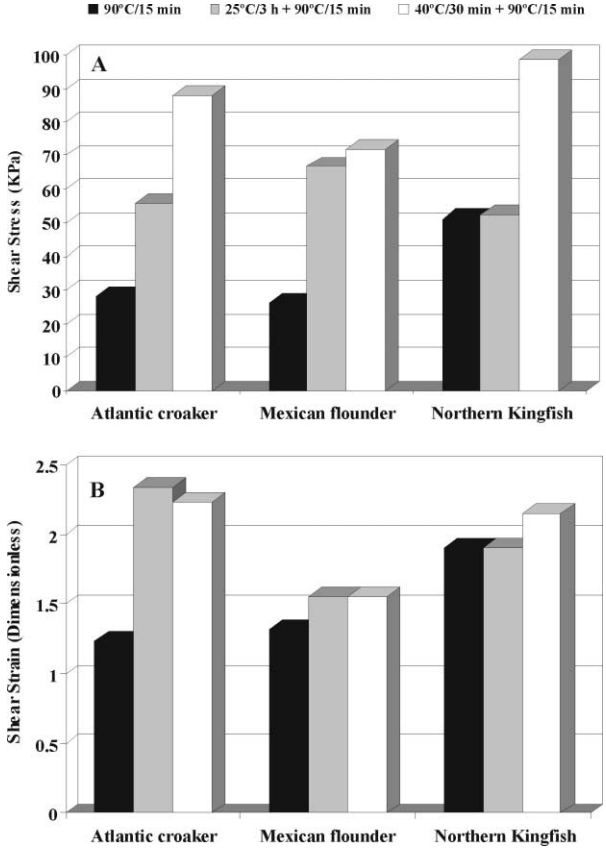

Fig. 3. Effect of 0.2% calcium chloride on shear stress and shear strain of surimi gels from different warm water fish species.

strain (Lee, Lanier, Hamann, & Knopp, 1997a; Lee et al., 1997b).

Shear strain values higher than 1.9 indicate elastic gels with AA value (Lanier, 1992). Surimi gels from Atlantic croaker and Northern kingfish, obtained at 40°C/30 min, followed by 90°C/15 min in the presence of 0.2% calcium chloride, presented values of shear strain higher than two and shear stress higher than 80 kPa. These results suggest that surimi gels from both fish species had appropriate mechanical properties for surimi production.

Finally, for comparative purposes, Table 2 shows the scarce data from previous studies. The values of shear stress and shear strain obtained in our work compare very well with those reported in the literature for this shrimp by-catch species.

4. Conclusion

Surimi gels from Atlantic croaker, Mexican flounder and Northern kingfish show different responses to thermal treatments and additives used in our study.

The results obtained from this study showed that surimi from Atlantic croaker and Northern kingfish presented the setting phenomenon at 40°C. Addition of 0.2% calcium chloride is recommended in these species.

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