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Surimi gels from striped mullet (*Mugil cephalus*) employing microbial transglutaminase

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Abstract

Striped mullet (*Mugil cephalus*) is an abundant marine coastal fish of tropical waters and has a low commercial value. This work deals with the assessment of striped mullet as a resource for surimi gels. To improve the gels obtained, the addition of microbial transglutaminase was evaluated. Optimal conditions for setting were determined using mathematical models. Concentration of microbial transglutaminase (MTGase), temperature and time were studied to improve the mechanical properties of surimi gels from striped mullet. Shear stress was strongly affected by the variables studied while shear strain was moderately affected. Maximum shear stress (156 kPa) was obtained by employing the following setting conditions: a concentration of MTGase of 9.3 g/kg of surimi, a temperature of 37°C and a time of 3.9 h. Under these conditions the shear strain was 1.34. Maximum shear strain (1.57) was obtained by employing the following conditions: a concentration of MTGase of 5 g/kg of surimi, a temperature of 34.5°C and a time of 1 h. Under these conditions the shear stress was 123 kPa. © 2000 Published by Elsevier Science Ltd.

1. Introduction

Striped mullet (*Mugil cephalus*) is one of the few species of marine shore fish with a worldwide circumtropical distribution. This fish can live in coastal waters at a salinity as high as 45 ppt (Hotos & Vlahos, 1998). Striped mullet obtains feed from both the benthos and plankton. This species efficiently exploits certain other resources, such as fine detritus and small zooplankton (Cardona, Torras, Gisbert & Costello, 1996). The smell of this fish is not acceptable to consumers because it easily absorbs odorous compounds from the environment. Therefore, the economic value of striped mullet is small. The processing of striped mullet to obtain surimi gels can be a good alternative use for this abundant resource. To our knowledge, there are no reports about producing surimi gels from striped mullet.

To obtain a good surimi gel from striped mullet it is important to establish the optimum conditions for setting. Setting is a phenomenon of protein aggregation that it is shown by surimi gels when the mass is made soluble with salt and it is heated below 40°C. The time needed for setting can be as long as 24 h (Chan, Gill, Thompson & Singer, 1995) or as short as 15 min (Montejano, Morales & Díaz, 1994). Setting is considered to be a sole characteristic of fish protein (Matsumoto & Noguchi, 1992). Nowadays the setting has been associated with the presence and activity of an endogenous and calcium-dependent transglutaminase (TGase). This enzyme catalyzes the cross-linking of myosin. For this reason microbial transglutaminase (MTGase) has been studied as a means of improving the textural characteristics and mechanical properties of fish and meat products (Anon., 1998). Shear stress at failure and shear strain can be used to study the mechanical properties of surimi gels. Shear stress at failure determines the firmness of the gel and shear strain determines the fragility of the gel.

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A relationship between habitat temperature and optimal conditions for setting has been established by several authors (An, Peters & Seymour, 1996; Lee, Lanier, Hamann & Knopp, 1997; Lee & Park, 1998). Therefore, the setting of cold water species is at temperatures lower than the setting of warm water species. In Alaska pollock, a species from cold water, the setting can occur at 0°C/24 h (Kamath, Lanier, Foegeding & Hamman, 1992). On the other hand, Pacific whiting, a species from warm water, does not present setting at 0°C/20 h while at 25°C/3 h the setting occurs. The correlation between optimal conditions for setting and fish habitat temperature can be due to the following factors (Lanier, 1994): (i) the endogenous TGase has an optimal temperature for each species; (ii) the denaturation of myofibrillar protein is at different temperatures for each species; (iii) both phenomena. Several studies based on differential scanning calorimetry (DSC), show that the myofibrillar proteins from warm water species are more thermostable than the proteins from cold water species (Osawa, Kanamaru, Miyashita, Tamiya & Tsuchiya, 1995; Wu, Akahane, Lanier & Hamann, 1985; Yamamoto, 1990). This behavior is detected mainly in myosin, the protein responsible for mechanical and textural properties of fish gels (Davies, Ledward, Bardsley & Poulter, 1994; Hastings, Rodger, Park, Matthews & Anderson, 1985; Poulter, Ledward, Godber, Hall & Rowlands, 1985; Howell, Matthews & Donnelly, 1991).

The setting can be considered to be due to chemical reactions. From this point of view, a study to determine the optimum conditions for setting, using MTGase, can be based on the interrelationship among the variables, enzyme concentration, time and temperature. Microbial TGase performs at 50°C (Anon., 1998). It can be expected that an increase of the temperature up to 50°C leads to a major extension of the reaction and consequently to an increase in the values of shear stress and shear strain. On the other hand, an increase in the temperature leads to the denaturation and the irreversible aggregation of proteins. Moreover, it is known that endogenous proteases perform above 50°C. Therefore, faulty gels are obtained using setting temperatures around 50°C.

This work deals with the assessment of striped mullet (*Mugil cephalus*) as a resource to obtain surimi gels that can give added value to this species. The setting with MTGase has been studied. Mathematical models, that allow the determination of optimum conditions for setting of striped mullet, have been developed.

2. Materials and methods

2.1. Microbial transglutaminase

Microbial transglutaminase, Active TG-TI, was supplied by Ajinomoto USA, INC. (Teaneck, NJ). Composition of product is: maltodextrin 99%, transglutaminase 1%. Transglutaminase activity: approx. 100 units/g. In this work the enzyme concentration is reported as commercial concentration.

2.2. Frozen surimi

Fresh striped mullet (*Mugil cephalus*) was obtained from a fish market in Tampico, Tamaulipas, Mexico. Fish were headed, gutted and washed. Skin and bones were removed with a Bibun deboning machine (Model NF2DX, Fukuvama, Miroshima, Japan) with a drum having 5 mm diameter perforations. The washing of the mince was performed in wash tanks in < 10°C water using a ratio of fish/water of 1/3 (w/w). Washings were followed by manually dewatering with cheesecloth. Surimi was mixed with 8% sucrose as a cryoprotectant, using a Hobart mixer (model VCM, Troy, OH). 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.

2.3. Surimi gel preparation

Samples (250 g) of surimi were selected from a 2 kg bag, partially thawed at room temperature, cut into small pieces and chopped in a 5 qt capacity Hobart cutter (Model 84145, Troy, OH) for 3 min with 2.5% salt. Microbial TGase was dispersed with salt and added to the surimi paste. The final chopping temperature was maintained below 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 treatments for setting followed by cooking at 90°C for 20 min. After cooking, the tubes were immediately removed, placed in a cold 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.

2.4. Torsion test

Gels were kept at room temperature prior to the torsion test. Gels were cut into 3.0 cm lengths and milled into an hourglass shape with a minimum diameter of 1 cm in the center. Each gel was placed in a modified torsion apparatus composed of a Brookfield digital viscometer (Model 5XHBTD, Brookfield Engineering Laboratories, Inc., Stoughton, MA). The texture of each gel was then measured by twisting the sample at 2.5 rpm until structural failure occurred. Shear stress and true shear strain at failure were calculated as described by Hamann, Amato, Wu and Foegeding (1990).

2.5. Statistical analysis

A second-order multiple regression analysis using least squares regression methodology was performed

using Microsoft Excel 7.0 (Microsoft Corporation, Redmont, WA, USA, 1995) software. Microsoft PowerPoint 7.0 (Microsoft Corporation, Redmont, WA, USA, 1995) was used to plot the experimental data and models. All data presented are mean values of three determinations.

3. Results and discussion

The influence of MTGase concentration, temperature and time during setting on the striped mullet surimi production was assessed using the response surface methodology which has been successfully used to optimize biochemistry and biotechnology processes related with food systems (Roberto, Sato, Mancilha & Tacheda, 1995; Saval, Pablos & Sánchez, 1993; Vázquez & Martin, 1998). The set of experiments followed a second-order, factorial structure (Box, Hunter & Hunter, 1988).

The concentration of enzyme, temperature and time were considered as operational variables (denoted E, T and t, respectively) and their effects on selected dependent variables (shear stress y_1 and shear strain y_2) were calculated. For computation purposes, the normalized, dimensionless variables x_1, x_2 and x_3 were defined as:

$$x_1 = (E\text{-}5)/5; \quad x_2 = (T\text{-}35)/10; \quad x_3 = (t\text{-}3)/2 \qquad (1)$$

Table 1 summarizes the variables involved in the optimization of the setting process during the surimi production using MTGase as additive.

Table 1
Variables used in the study

a. Fixed variables			
NaCl concentration	25 g/kg		
Final heating	90°C		
Time for final heating	20 min		
b. Dimensional independent variables	**Nomenclature**	**Units**	**Variation range**
Enzyme concentration	E	g/kg	(0,10)
Temperature	T	°C	(25,45)
Incubation time	t	h	(1,5)
c. Dimensionless, normalized independent variables	**Nomenclature**	**Definition**	**Variation range**
Dimensionless enzyme concentration	x_1	(E−5)/5	(−1,1)
Dimensionless temperature	x_2	(T−35)/10	(−1,1)
Dimensionless time	x_3	(t−3)/2	(−1,1)
d. Dependent variables	**Nomenclature**	**Units**	
Shear stress	y_1	Pa	
Shear strain	y_2	Dimensionless	

The operational conditions assayed (in terms of dimensional and dimensionless operational variables) as well as the experimental results determined for y_1 and y_2 are shown in Table 2. The interrelationship between operational and dependent variables was established through an equation including linear, interaction and second-order terms:

$$y_j = b_{0j} + \Sigma_i b_{ij} x_i + \Sigma_i \Sigma_k b_{ikj} x_i x_k \qquad (2)$$

where y_j (j: 1–2) and x_i or x_k (i or k 1–3, $i \geqslant k$) are the dependent or independent, normalized variables and $b_{0j} \ldots b_{ikj}$ are regression coefficients calculated from the experimental data by multiple linear regression. Table 3 shows the values of coefficients from the mathematical models and their statistical significance, and Table 4 shows the statistical parameters R^2 and F_{exp}, measuring the correlation and significance of models, respectively.

The range of study for the variable temperature was selected between 25 and 45°C because 25°C is the temperature commonly recommended for setting in cold water

Table 2
Operational conditions assayed and experimental results achieved

Experiment	Independent variables						Dependent variables	
	Dimensional			Dimensionless				
	E	T	t	x_1	x_2	x_3	y_1	y_2
1	0	25	1	−1	−1	−1	59.540	1.2466
2	0	25	3	−1	−1	0	66.149	1.2385
3	0	25	5	−1	−1	1	62.673	1.2544
4	0	35	1	−1	0	−1	60.382	1.2428
5	0	35	3	−1	0	0	80.159	1.3415
6	0	35	5	−1	0	1	100.330	1.3550
7	0	45	1	−1	1	−1	58.302	1.2500
8	0	45	3	−1	1	0	50.507	1.0319
9	0	45	5	−1	1	1	26.439	0.6700
10	5	25	1	0	−1	−1	81.686	1.3918
11	5	25	3	0	−1	0	105.570	1.4124
12	5	25	5	0	−1	1	86.900	1.3324
13	5	35	1	0	0	−1	99.040	1.5930
14	5	35	3	0	0	0	144.254	1.3668
15	5	35	3	0	0	0	154.000	1.6716
16	5	35	3	0	0	0	148.520	1.6932
17	5	35	3	0	0	0	145.597	1.5347
18	5	35	5	0	0	1	147.993	1.3294
19	5	45	1	0	1	−1	125.952	1.4444
20	5	45	3	0	1	0	110.758	1.1410
21	5	45	5	0	1	1	108.388	1.1742
22	10	25	1	1	−1	−1	91.798	1.3919
23	10	25	3	1	−1	0	89.165	1.2404
24	10	25	5	1	−1	1	135.801	1.3223
25	10	35	1	1	0	−1	104.017	1.3383
26	10	35	3	1	0	0	154.024	1.2415
27	10	35	5	1	0	1	154.735	1.1743
28	10	45	1	1	1	−1	129.086	1.2484
29	10	45	3	1	1	0	152.365	1.1848
30	10	45	5	1	1	1	116.683	1.1145

Table 3
Regression coefficients and statistical significance

Coefficients	y_1 ($j=1$)	y_2 ($j=2$)
b_{0j}	140 764	1.5053
b_{1j}	31 288[a]	0.0348
b_{2j}	5511	−0.0873[a]
b_{3j}	7230[c]	−0.0789[a]
b_{12j}	11 207[b]	0.0317
b_{13j}	5925	0.0077
b_{23j}	−9515[b]	−0.0719[b]
b_{11j}	−21 143[a]	−0.1806[a]
b_{22j}	−26 875[a]	−0.1468[a]
b_{33j}	−11 544[c]	−0.0162

[a] Coefficients significant at 99% confidence level.
[b] Coefficients significant at 95% confidence level.
[c] Coefficients significant at 90% confidence level.

Table 4
Statistical parameters measuring the correlation and significance of models

Variable	R^2	F_{exp}[a]	Prob
			$F_{exp} > F_{st}$[b]
y_1	0.8711	15.02	<0.01
y_2	0.7439	6.46	<0.01

[a] F_{exp} defined as the ratio between the mean squares of model and error.
[b] F_{st} defined as the statistical value of F for the degrees of freedom of the model and error.

species such as Alaska pollock (Lee et al., 1997) while 45°C is the maximum due to the Modori phenomenon in the range 50–70°C (An et al., 1996). The importance and origin of the Modori in fish species from the Gulf of Mexico has been previously reported (Ramos-Martínez, Morales, Ramírez, García-Carreño & Montejano-Gaitán, 1999; Sánchez, Ramírez, Morales & Montejano, 1998). The range for the variable time was selected at 1–5 h because MTGase performs in the range 2–5 h on Alaska pollock at 25°C (Lee & Park, 1998).

The experimental values for shear stress (y_1) varied over a wide range (26–155 kPa). The analysis of the main experimental trends and the values of coefficients listed in Table 3 suggest that the interrelationship of y_1 with the independent variables was characterized by a complex influence of the effects considered significant being three terms of the model at 99% confidence level, two terms significant at 95% confidence and two additional terms significant at 90% confidence. The resulting variation pattern is shown in Fig. 1. It describes the dependence of shear stress on enzyme concentration and temperature at three representative values of time. MTGase concentration was the variable most influencing the setting of striped mullet.

Shear strain (y_2) showed behavior different from y_1. The experimental results varied within the narrow range 0.67–1.69. The most favorable conditions were defined

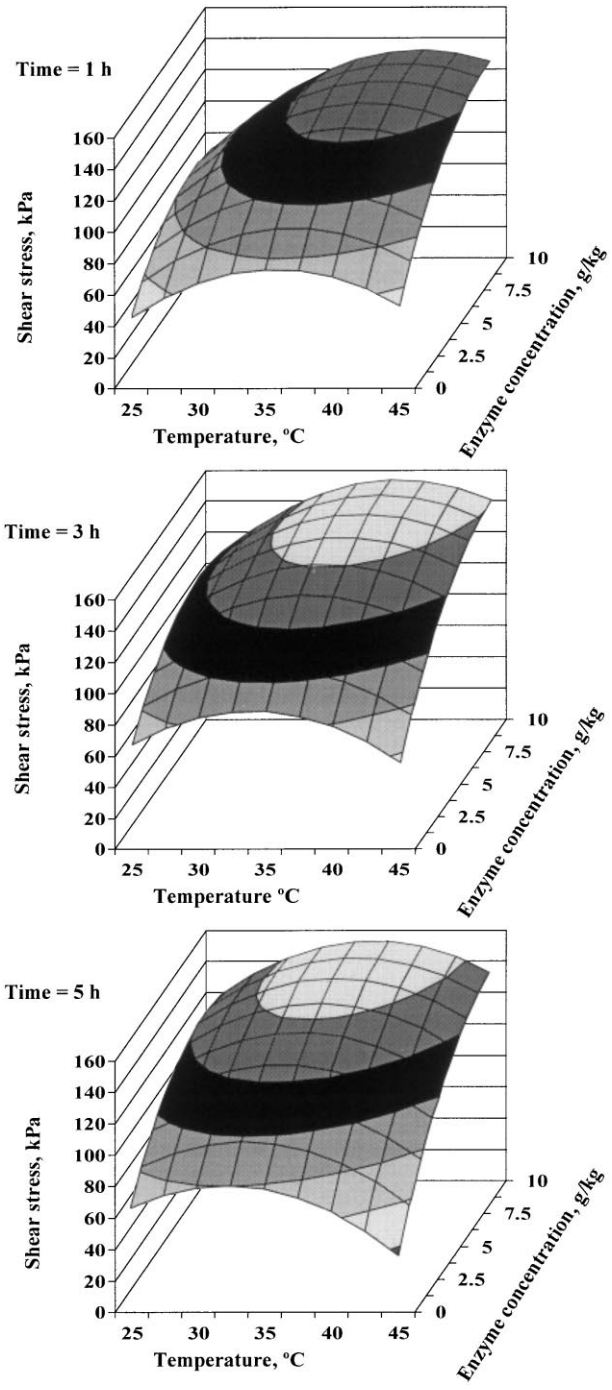

Fig. 1. Calculated dependence of shear stress (y_1) on temperature and commercial enzyme concentration for several incubation times.

by intermediate values of enzyme concentration and temperature and the lowest values of time (experiment 13). The significance analysis of the regression coefficients for y_2 showed that the shear strain was not significantly affected by the variable enzyme concentration but was significantly affected by the second-order term of this variable. The predictions of the empirical model for the dependence of shear strain on the enzyme concentration and temperature at several incubation times

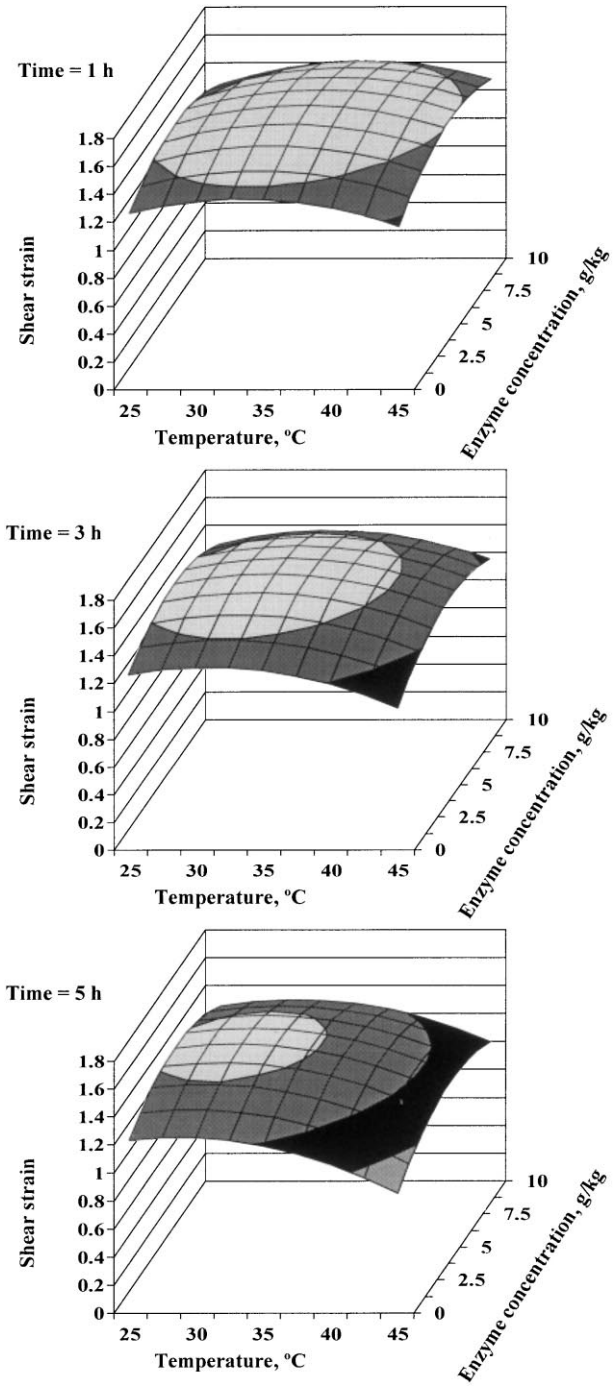

Fig. 2. Calculated dependence of shear strain (y_2) on temperature and commercial enzyme concentration for several incubation times.

is shown in Fig. 2. High values of time and temperature produce a surimi gel with low shear strain.

It can be observed from Figs. 1 and 2 that a maximum response can be obtained within the range of the study. Canonical analysis allows the prediction of a maximum shear stress (156 kPa) and a maximum shear strain (1.57). Table 5 shows that the operational conditions for maximum shear stress and maximum shear strain are different.

Table 5
Canonical analysis of response surface: optimum values obtained in this study

	Critical values							
	Coded			Uncoded				
Dependent variables	x_1	x_2	x_3	E	T	t	Predicted value	Stationary point
y_1	0.856	0.201	0.450	9.3	37.01	3.90	156 343	Maximum
y_2	0.071	−0.045	−1	5.0	34.55	1	1.57	Maximum

Table 6
Experimental and predicted results of verification experiment under selected operational conditions

Conditions selected			Experimental	Predicted	Experimental	Predicted
E	T	t	y_{11}	y_1	y_2	y_2
9.3	37.01	3.9	149 250	156 342	1.35	1.34
8.5	39.28	1	130 124	134 250	1.45	1.47

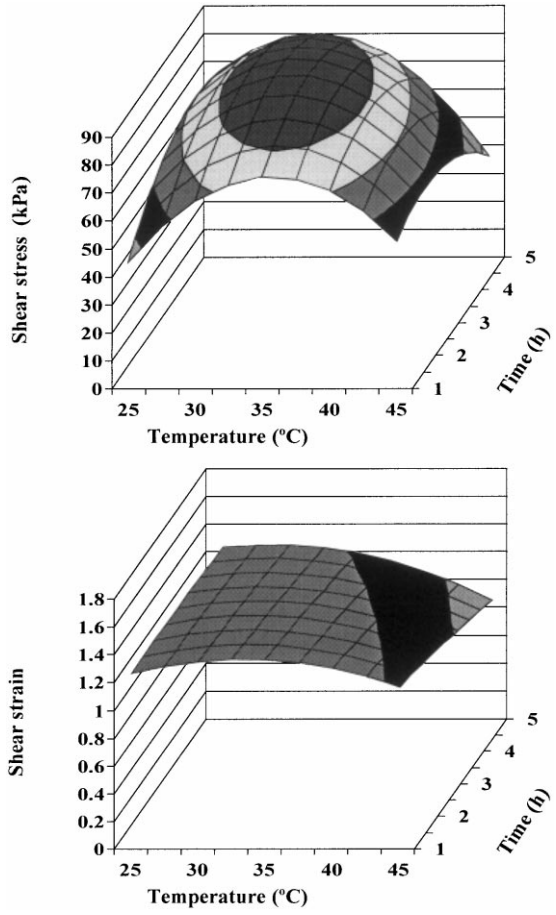

Fig. 3. Calculated dependence of shear stress (y_1) and shear strain (y_2) on temperature and time without addition of MTGase.

Verification experiments were performed with the purpose of confirming the models obtained. The conditions for maximum shear stress were selected because this parameter is the mechanical property most significantly most affected by the addition of MTGase. Also, a verification experiment was performed using the lower time studied (1 h) because this variable does not significantly affect the process and short times are preferred for hygienic and economical reasons.

Table 6 presents the experimental data obtained in the verification experiments under the selected conditions, as well as the values predicted by the models. The results confirm that the mathematical models obtained can be used to predict the mechanical properties of the surimi gels from striped mullet under different conditions of setting. The conditions selected to achieve the maximum shear stress (156 kPa) produce a surimi gel with a shear strain too small (1.34). This means that the surimi gel produced was brittle. Further studies are needed to increase the value of shear strain. The value of shear stress can be selected using the mathematical model in as wide a range as needed.

Additionally, the models can be used to predict the behavior of the setting without addition of MTGase. Fig. 3 shows the dependence of shear stress (y_1) and shear strain (y_2) on temperature and time without addition of MTGase. The most favorable conditions are defined by intermediate values of temperature and time. In the absence of MTGase, the temperature needed to obtain a maximum shear stress is 34°C, 5° lower than using MTGase. This fact can be explained because the optimal temperature of the MTGase is higher than that of the endogenous TGase. From the models, it can be concluded that the optimal temperature of the MTGase is in the range 35–40°C while that of the endogenous TGase is in the range 32–36°C.

It can be observed from Fig. 3 that, although a maximum response for shear stress can be obtained within the range of the study, the gels achieved using MTGase have better mechanical properties due to the higher values of shear stress and shear strain obtained. Hence MTGase can be considered a useful additive to prepare surimi gels from striped mullet.

Finally, for comparative purposes, Table 7 shows the results of previous studies using other species. The values of shear stress obtained in this work compare very well with those reported in the literature. The optimal setting temperature for striped mullet was higher that those reported for cold water fishes which suggests an environmental role for setting.

Table 7
Comparison of shear stress and shear strain values of surimi gels from different species

Specie	Conditions	Shear stress	Shear strain	Reference
Pacific whiting	25°C/3 h + 90°C/15 min	62.40	2.57	Lee and Park, 1998
Merluccius productus	5°C/20 h + 90°C/15 min	48.97	2.54	Lee and Park, 1998
	25°C/3h + 90°C/15 min (0.2% calcium)	96.90	2.73	Lee and Park, 1998
	5°C/20 h + 90°C/15 min (0.2% calcium)	59.95	2.50	Lee and Park, 1998
	90°C/15 min	52.5	2.75	Yoon, Park and Kim, 1997
	90°C/40 min	60.71	2.71	Reppond and Babbit, 1997
Alaska pollock	25°C/3 h + 90°C/15 min	66.81	60.70	Lee and Park, 1998
Theragra chalcogramma	5°C/20 h + 90°C/15 min	79.10	2.89	Lee and Park, 1998
	25°C/3 h + 90°C/15 min (0.1% calcium)	86.82	2.94	Lee and Park, 1998
	5°C/20 h + 90°C/15 min (0.1% calcium)	78.03	2.96	Lee and Park, 1998
	90°C/15 min	64.5	2.90	Yoon et al., 1997
	90°C/20 min	21	2.65	Lee et al., 1997
	25°C/1 h + 90°C/20 min	39	2.5	Lee et al., 1997
	25°C/0 h + 90°C/20 min (2 g/kg MTGase)	42	3.1	Lee et al., 1997
	25°C/1 h + 90°C/20 min (2 g/kg MTGase)	70	3.1	Lee et al., 1997
	25°C/2 h + 90°C/20 min (2 g/kg MTGase)	80	3.0	Lee et al., 1997
	25°C/3 h + 90°C/20 min (2 g/kg MTGase)	85	2.9	Lee et al., 1997
	90°C/40 min	60.70	2.21	Reppond and Babbit, 1997
Pacific herring	90°C/40 min	67.85	2.21	Reppond and Babbit, 1997
Clupea harengus pallasi	30°C/40 min + 90°C/40 min	60.1	2.13	Reppond, Babbit, Bernsten and Tsuruta, 1995
	90°C/30 min	37	1.70	Chan et al., 1995
	10°C/24 h + 90°C/30 min	98	2.38	Chan et al., 1995
Sole	90°C/15 min	45	1.5	Sánchez et al., 1998
Cyclopsetta chittendini	90°C/15 min	45	1.7	Ramos-Martínez et al., 1999
Trout	40°C/15 min + 90°C/15 min	60	2.8	Montejano et al., 1994
Cyanoscion nothus				
Striped mullet	34.5°C/1 h + 90°C/20 min	123	1.57	This work
Mugil cephalus	(5 g/kg MTGase)			

Without the addition of MTGase, the shear stress was strongly affected by temperature and time, while shear strain was moderately affected. The addition of MTGase, for the setting in the surimi production of striped mullet, improves the mechanical properties of the surimi obtained. The mathematical model proposed can be used to determine the MTGase needed for obtaining a surimi with the mechanical properties required by consumers. The results suggest that setting is a phenomenon dependent on both protein denaturation/aggregation and enzymatic activity, both processes occurring in the setting phenomenon.

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