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Preparation of surimi gels from striped mullet (*Mugil cephalus*) using an optimal level of calcium chloride

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Abstract

Optimal conditions for the setting phenomenon in surimi, prepared from striped mullet (*Mugil cephalus*) by adding calcium chloride, were determined. Concentrations of calcium chloride (0–0.4%), temperature (25–45 °C), and time (30–90 min) were optimised to improve the shear stress and shear strain properties of fish gels. After incubating under selected conditions for setting, all gels were cooked at 90 °C for 15 min. Shear stress was mainly affected by the calcium concentration, while shear strain was moderately affected. Maximum shear stress (89.6 kPa) was obtained by employing a concentration of calcium of 0.4%, a temperature of 39.3 °C and a time of 1 h. Under these conditions, a shear strain of 1.47 was obtained. The results suggest that the mechanical properties of surimi gels can be improved, simply, by adjusting the level of calcium to induce activity of the endogenous transglutaminase.

Keywords: Surimi; Calcium; Shear stress; Shear strain; *Mugil cephalus*; Striped mullet

1. Introduction

Mugil cephalus (striped mullet or sea mullet) is an extremely widespread fish species. This species is found in temperate and tropical waters throughout the world. Sea mullet is an object of both commercial fishery and game angling and it is not considered as a threatened or endangered fish species. The mullet caught on ocean beaches are mostly spawning run fish and their catches have increased as a result of a growing market for sea mullet roe, considered as a highly popular delicatessen fish product. Because of this, mullet is successfully cultivated in several countries. It is a popular food fish in the southeastern United States and Gulf coast, where they are sought after for both meat and roe. This fish feeds on floor silt and scales, scraping them off various underwater objects with its spade-like lower jaw. They feed by sucking in bottom sediments that contain decaying plant material, algae, and inorganic particles. They may also extract algae and microorganisms from scum that accumulates on the water surface or from the surface of submerged vegetation or other substrates (Collins, 1985; Murdy, Birdsong, & Musick, 1997).

The mullet has tasty fat flesh which can absorb odorous compounds from its diet. For this reason, in many countries, such as Mexico, it is only caught to export its roe and meat is considered as a by-product. The increasing demand for striped mullet roe requires the development of technologies to process the meat, which has low commercial value. Several works dealing with the feasibility of using the mullet flesh in commercial process or products have been reported. Wootton and Chuah (1981) found that mullet fillets can be used to produce cold marinades of the roll-mop type with attributes similar to commercial products obtained from herring. Fish protein isolates and hydrolysates obtained from *Mugil cephalus* showed high protein level (90%),

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good solubility and emulsifying capacity and were appropriate to fortify (with protein, 20–35%) common Mexican dishes (Morales de Leónic Gálvez-Mariszal, & Téllez-Sill, 1990). The flesh of sea mullet can be used to obtain salted fish products (El-Sahn, El-Sharnouby, & Moharram, 1997) and to obtain better cold-smoked fillets from fresh mullet, combining sodium tripolyphosphate and salt (Antoine, Marshall, Sims, O'Keefe, & Wei, 2000). Optimal conditions for producing surimi from striped mullet, employing microbial transglutaminase (MTGase) to induce the setting phenomena have also been studied (Ramírez, Rodriguez-Sosa, Morales, & Vázquez, 2000).

A more economical way to improve the mechanical properties could be the optimisation of processing conditions to reach maximum activity of the endogenous transglutaminase (TGase). The presence of an endogenous TGase in striped mullet with optimal activity at 30–35 °C and pH 7.5–7.7 has been reported (Lee, Chiang, & Pan, 1998). Previously, it was 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 20 °C (Nowsad, Katoh, Kanoh, & Niwa, 1996). Recently, it was found that endogenous fish and pig transglutaminases have higher deamidation activities than MTGase (Ohtsuka, Umezawa, Nio, & Kubota, 2001). However, there is not yet available any commercial TGase from any animal.

The endogenous TGase is calcium-dependent and its activity can be optimised during the setting phenomenon by adding calcium to fish paste before incubation at 25–40 °C. It was found that the shear stress property of surimi gels from cold water fish species, such as Pacific whiting and Alaska Pollock, was increased by adding 0.2% calcium chloride (Lee & Park, 1998). The shear stress of surimi gels from warm-water fish species, such as, Mexican flounder (*Cyclopsetta chittendeni*) and Northern kingfish (*Menticirrhus saxatilis*) was also improved by adding 0.2% calcium chloride (Morales, Ramirez, Vivanco, & Vázquez, 2001). This author found that although endogenous TGase is present in surimi obtained from most fish species, the endogenous calcium level was a limitation in obtaining maximum shear stress in warm-water fish species studied. The objective of this work was to determine optimal conditions of time, temperature and $CaCl_2$ level in the surimi production from striped mullet.

2. Materials and methods

2.1. Frozen surimi

Fresh striped mullet (*Mugil cephalus*) were obtained from a fish market in Tampico, Tamaulipas, Mexico. The 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 wash of the mince was performed in wash tanks in <10 °C water, using a ratio of fish/water of 1/3 (w/w). The wash was followed by manually dewatering with cheesecloth. Surimi was mixed with 8% sucrose as cryoprotectant, using a Hobart mixer (model VCM. Troy, Ohio). 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

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, Ohio) for 3 min with 2.5% salt. Calcium chloride 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.

2.3. 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 centre. 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).

2.4. Statistical analysis

A second-order multiple regression analysis, using least squares regression methodology, was performed using Microsoft Excel 7.0 (Microsoft Corporation, Redmond, WA, USA, 1995) software. Microsoft PowerPoint 7.0 (Microsoft Corporation, Redmond, WA, USA, 1995) was used to plot the experimental data and models. All data presented are mean values of three determinations.

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
Calcium concentration	Ca	%	(0, 0.4)
Temperature	T	°C	(25, 45)
Incubation time	T	min	(30, 90)
(c) Dimensionless, normalised independent variables	Nomenclature	Definition	Variation range
Dimensionless calcium concentration	x_1	(Ca−0.2)/0.2	(−1, 1)
Dimensionless temperature	x_2	(T−35)/10	(−1, 1)
Dimensionless time	x_3	(t−60)/30	(−1, 1)
(d) Dependent variables	Nomenclature	Units	
Shear stress	y_1	Pa	
Shear strain	y_2	Dimensionless	

3. Results and discussion

To determine the optimal level of calcium addition to improve the mechanical properties of *Mugil cephalus* surimi gels, a set of experiments was conducted. The setting phenomenon was induced by adding different levels of calcium chloride (0–0.4%), at different combinations of temperature (25–45 °C), and time (30–90 min). The concentration of calcium chloride, temperature and time were considered as operational variables (denoted Ca, T and t, respectively) and their effects on selected dependent variables (shear stress, y_1 and shear strain, y_2) were measured.

The range of study for temperature (25–45 °C) and time (30–90 min) variables were selected because settings perform well in such ranges for warm-water fish species, according to previous reports (Ramirez, Rodriduez-Sosa et al., 2000; Ramirez, Santos, Morales, Morrissey, & Vázquez, 2000; Ramirez, Garcia-Carreño, Morales, & Sánchez, 2002). Calcium chloride was selected between 0 and 0.4% because 0.2% has been reported as adequate for several fish species (Lee & Park, 1998; Morales et al., 2001).

Table 1 summarises the variables involved in the optimisation of the setting process during the surimi production using calcium chloride as additive. For computation purposes, the normalized, dimensionless variables x_1, x_2 and x_3 were defined as:

$$x_1 = (\mathrm{Ca} - 0.2)/0.2;\quad x_2 = (\mathrm{T} - 35)/10;\quad x_3 = (\mathrm{t} - 60)/30 \qquad (1)$$

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:

Table 2
Operational conditions assayed and experimental results achieved

Experiment	Independent variables						Dependent variables	
	Dimensional			Dimensionless				
	Ca	T	t	x_1	x_2	x_3	y_1	y_2
1	0	25	30	−1	−1	−1	59 858	1.327707
2	0	25	60	−1	−1	0	60 482	1.316911
3	0	25	90	−1	−1	1	64 148	1.163566
4	0	35	30	−1	0	−1	64 595	1.347504
5	0	35	60	−1	0	0	70 757	1.499789
6	0	35	90	−1	0	1	66 423	1.339993
7	0	45	30	−1	1	−1	67 560	1.160542
8	0	45	60	−1	1	0	59 882	1.093104
9	0	45	90	−1	1	1	56 195	1.112842
10	0.2	25	30	0	−1	−1	52 087	1.174652
11	0.2	25	60	0	−1	0	54 407	1.288812
12	0.2	25	90	0	−1	1	67 150	1.162409
13	0.2	35	30	0	0	−1	66 834	1.277091
14	0.2	35	60	0	0	0	68 098	1.568659
15	0.2	35	60	0	0	0	69 046	1.449229
16	0.2	35	60	0	0	0	65 254	1.580168
17	0.2	35	60	0	0	0	66 834	1.573779
18	0.2	35	90	0	0	1	66 597	1.278137
19	0.2	45	30	0	1	−1	66 939	1.193385
20	0.2	45	60	0	1	0	63 700	1.035671
21	0.2	45	90	0	1	1	55 563	0.91847
22	0.4	25	30	1	−1	−1	85 414	0.91847
23	0.4	25	60	1	−1	0	81 870	1.103507
24	0.4	25	90	1	−1	1	86 373	1.249889
25	0.4	35	30	1	0	−1	83 951	1.221315
26	0.4	35	60	1	0	0	86 141	1.185062
27	0.4	35	90	1	0	1	96 380	1.152284
28	0.4	45	30	1	1	−1	89 546	1.270122
29	0.4	45	60	1	1	0	75 708	1.11036
30	0.4	45	90	1	1	1	74 892	1.110361

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

where y_j (j:1−2) and x_i or x_k (i or k:1–3, I⩾k) are the dependent or independent, normalised variables and b_{0j}...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.

The experimental values for shear stress (y_1) varied over a wide range (52–96 kPa). The analysis of the main experimental trends and the values of coefficients are listed in Table 3. The calcium chloride concentration (dimensional variable Ca or dimensionless variable x_1) had the most influence on shear stress, followed by the interactions of temperature and time. No influence was found for the second order term of time. This result is in accord with previously reported data (Ramirez, Santos et al., 2000) where no influence was found for the second order term of time on the shear stress of surimi gels from Silver carp (*Hypophthalmichthys molitrix*) obtained by different levels of MTGase, temperature and time. A similar study, using MTGase, conducted on striped mullet (*Mugyl cephalus*), also showed a low influence on shear stress ($P<0.05$) for the second order term of time (Ramirez, Rodriguez-Sosa et al., 2000).

Table 4 shows the statistical parameters R^2, F_{exp}, and *F*-test probability, measuring the correlation and significance of models. R^2 showed a good agreement between experimental and predicted data for both regressions. The statistical value of *F*-test probability showed that the model for shear stress (y_1) was accurate in describing the experimental data.

The resulting variation pattern is shown in Fig. 1. It describes the dependence of shear stress on calcium concentration and temperature at three representative values of time. Calcium concentration was the variable most influential for the setting of surimi from striped mullet. The maximum shear stress values were obtained at the highest level of calcium (0.4%). At this level of

Table 3
Regression coefficients and statistical significance

Coefficients	y_1 (j=1)	y_2 (j=2)
b_{0j}	66279[a]	1.4368[a]
b_{1j}	10576[a]	−0.0578[b]
b_{2j}	−100	−0.0389
b_{3j}	−170	−0.0223
b_{12j}	−1055	0.0550
b_{13j}	331	0.0268
b_{23j}	−4808[a]	−0.0531
b_{11j}	11268[a]	−0.0386
b_{22j}	−6798[a]	−0.2007[a]
b_{33j}	1323	−0.0894[c]

[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]	*F*-text probability
y_1	0.9199	25.53	0.8238
y_2	0.7009	5.20	0.3439

[a] F_{exp} defined as the ratio between the mean squares of model and error.

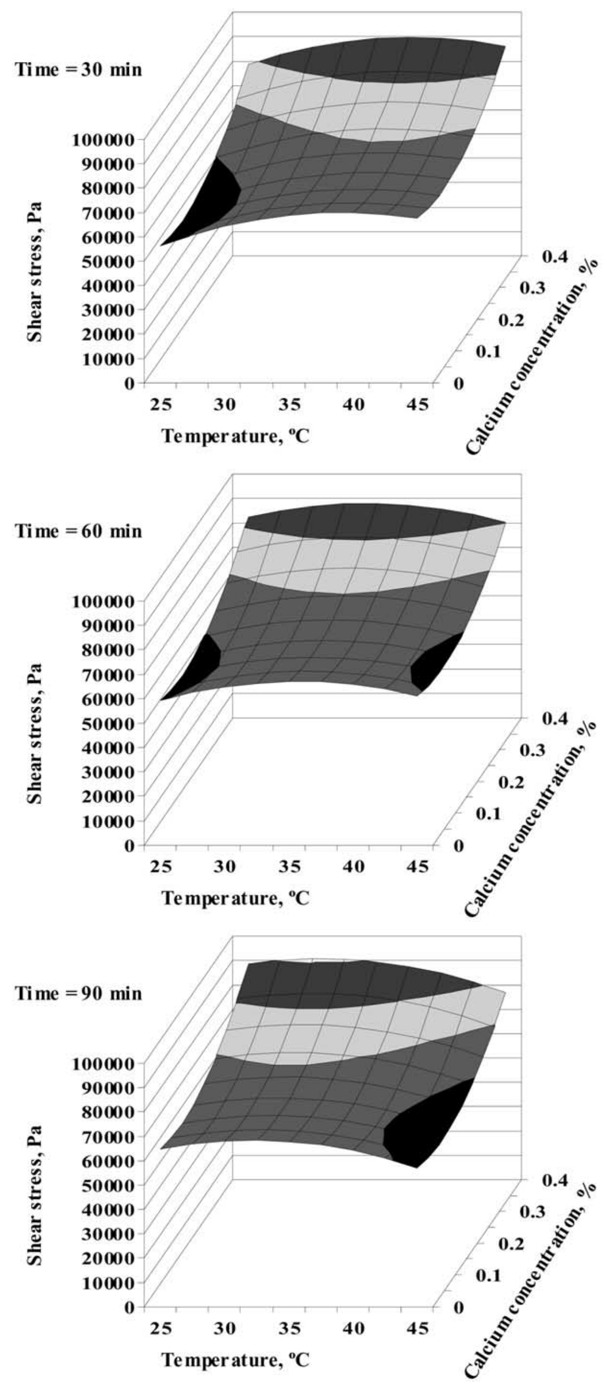

Fig. 1. Calculated dependence of shear stress (y_1) on temperature and calcium chloride concentration for several incubation times.

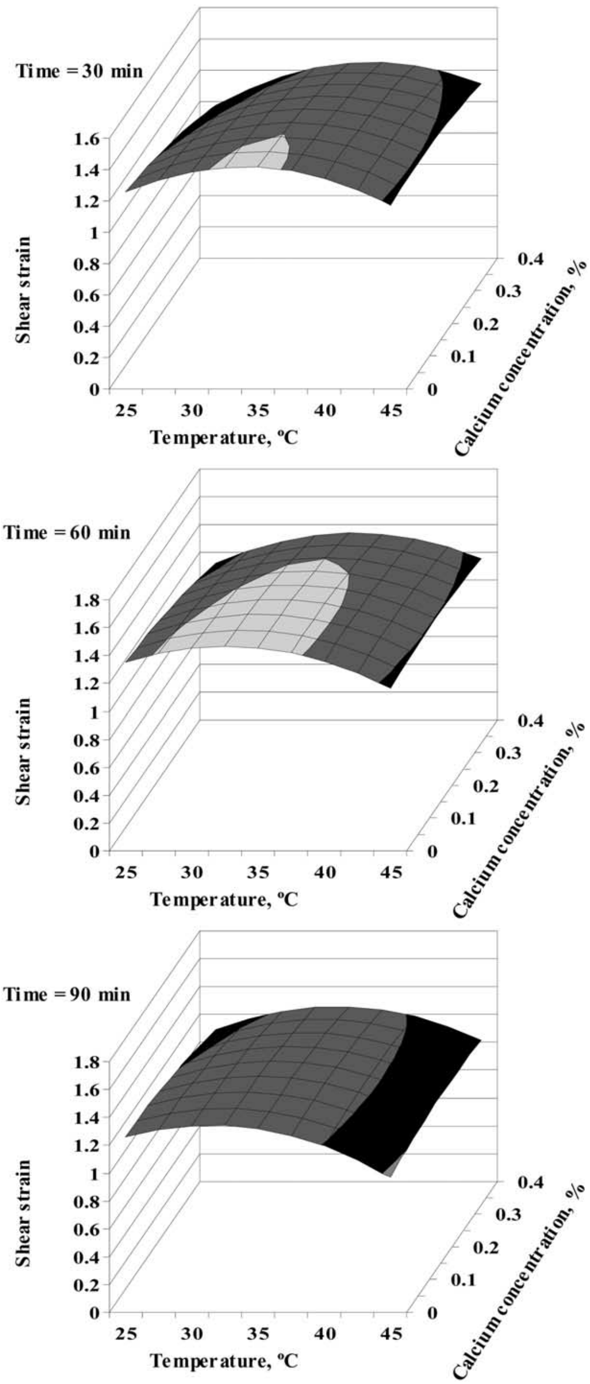

Fig. 2. Calculated dependence of shear strain (y_2) on temperature and calcium chloride concentration for several incubation times.

calcium addition, a small but significant interactive effect was observed between time of incubation and temperature. The gels incubated for 30 min showed maximum shear stress at 40 °C, while gels incubated for 90 min showed maximum shear stress at temperature near to 30 °C. This behaviour could be associated with the heat induced protein denaturation. Gels obtained with short times of incubation could show higher protein denaturation at higher temperatures. On the other hand, a long period of incubation induces a higher level of protein denaturation and lets the endogenous TGase catalyse protein crosslinking between adjacent polymeric chains. The decrease in shear stress of gels incubated at 45 °C for 90 min has been associated with endogenous proteases (An, Peters, & Seymour, 1996; Sánchez-Varela, Ramirez, Morales, & Montejano, 1998; Ramos-Martínez, Morales, Ramirez, Garcia-Carreño, & Montejano-Gaitán, 1999; Ramírez et al., 2002).

Shear strain (y_2) showed different behaviour from y_1. The experimental results varied within the narrow range 0.92–1.58. The significance analysis of the regression coefficients for y_2 showed that the shear strain was significantly affected by the variable calcium chloride concentration and by the second-order term of the variables temperature and time. The predictions of the empirical model for the dependence of shear strain on the enzyme concentration and temperature at several incubation times are shown in Fig. 2. The most favourable conditions were defined by intermediate values of temperature. A low significant effect was obtained by increasing calcium chloride level. High values of time and temperature produced a surimi gel with low shear strain. As discussed previously, this phenomenon has been associated with the activity of endogenous proteases.

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 (89.6 kPa) and a maximum shear strain (1.47). Table 5 shows that the operational conditions for maximum shear stress and maximum shear strain are different.

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 significantly most affected by the effect of endogenous TGase.

Table 5
Canonical analysis of response surface: optimum values predicted in this study with addition of calcium chloride

Dependent variables	Coded			Uncoded			Predicted value	Stationary point
	x_1	x_2	x_3	Ca	T	t		
Y_1	1	0.428	−1	0.4	39.3	30	89605	Maximum
Y_2	−0.9627	−0.201	−0.210	0.01	33.0	53.7	1.4709	Maximum

Table 6
Experimental and predicted results of verification experiment under selected operational conditions (calcium, 0.4%; temperature, 39.3 °C; time 30 min)

Variable	Experimental value	Predicted value
y_1	88785	89605
y_2	1.4586	1.4709

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 (89.6 kPa) produce a surimi gel with a shear strain too small (1.47). 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 obtained as needed in a wide range, using the mathematical model to select the adequate operational conditions.

Additionally, the models can be used to predict the behaviour of the setting without the addition of calcium. Fig. 3 shows the dependence of shear stress (y_1) and shear strain (y_2) on temperature and time without the addition of calcium and Table 7 shows the conditions leading to maximum values. The most favourable conditions are defined by intermediate values of temperature and time. Operational conditions to obtain maximum mechanical properties (shear stress and shear strain) were the same with or without calcium. Maximum shear stress at temperatures near to 39 °C for 30–60 min has been previously reported for warm-water fish species (Ramírez, Rodriguez-Sosa et al., 2000, Ramírez, Santos et al., 2000).

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 calcium chloride have better mechanical properties, due to the higher values of shear stress and shear strain obtained. Hence the addition of 0.4% calcium can be considered useful to prepare surimi gels from striped mullet. Previously, a 0.2% calcium level has been reported as useful for Atlantic croaker, Mexican flounder and Northern kingfish (Morales et al., 2001) Pacific whiting and Alaska pollock (Lee & Park, 1998).

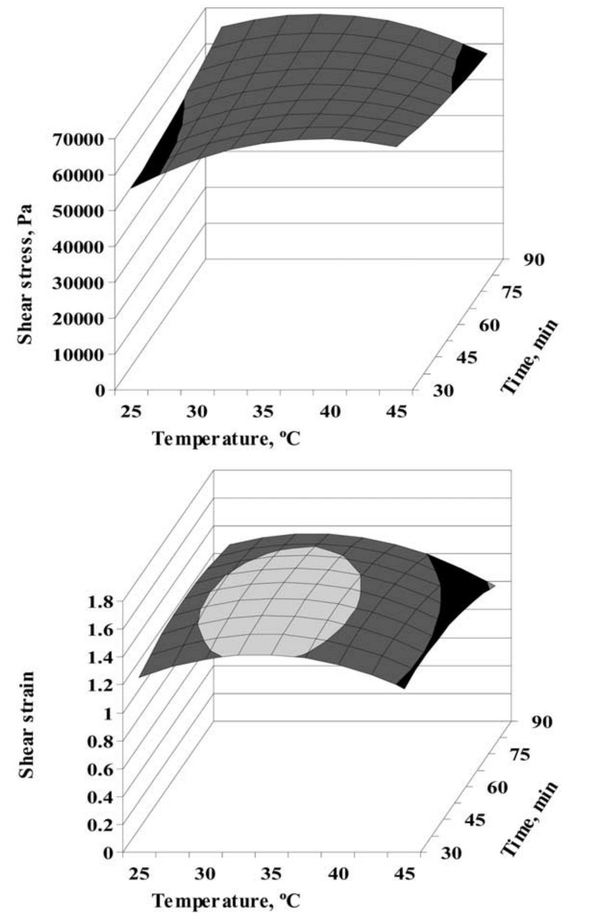

Fig. 3. Calculated dependence of shear stress (y_1) and shear strain (y_2) on temperature and time without addition of calcium chloride.

Without the addition of calcium chloride, the shear strain was considerably affected by temperature and time, while shear stress was moderately affected. The addition of calcium 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 calcium chloride needed for obtaining a surimi with the mechanical properties required by consumers. The

Table 7
Canonical analysis of response surface: optimum values predicted in this study without addition of calcium chloride

Dependent variables	Coded			Uncoded			Predicted value	Stationary point
	x_1	x_2	x_3	Ca	T	t		
y_1	−1	0.4239	−1	0	39.2	30	70019	Maximum
y_2	−1	−0.201	−0.210	0	33.0	53.7	1.4709	Maximum

results suggest that setting is a phenomenon dependent on both protein denaturation/aggregation and enzymatic activity induced by the endogenous transglutaminase, both processes occurring in the setting phenomenon.

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