Enhancement of the Gelling Properties of Sardine Surimi with Transglutaminase and Optimization of its Activity Using Response Surface Methodology

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Abstract

In order to improve the gelling properties of sardine surimi gel and to determine the maximum activity range of microbial transglutaminase (MTGase), we investigated the characteristics of surimi gel as a function of MTGase concentration, as well as temperature and reaction time, using the response surface methodology. Specifically, we assessed the following mechanical and physicochemical characteristics of the gel: rheological properties, disulphide bond and total sulfhydryl group content, and water-holding capacity. Our results demonstrated that temperature and enzyme concentration had more influence than reaction time on all dependent variables, incorporation of MTGase markedly ameliorated all the responses. The optimal properties were predicted to be obtained by employing the optimised setting conditions as follows: a concentration of MTGase of 10 g/kg of surimi at 45°C for 1 h. All the mathematical models derived for the various responses were found to be a good fit to predict the data.

Keywords: Microbial transglutaminase; Setting; Sardine surimi; Gelling; Response surface methodology

Introduction

In recent years, the increasing demand for "fresh food products" worldwide has prompted attempts to better understand and study restructured product. The addition of ingredients or additives can lead to improve these products in order to make them with new textural properties and/or a new appearance. Most of these products, particularly seafood analogues, are made from surimi of different sources of protein. Surimi is a structure of myofibrillar protein concentrates obtained from fish muscle, with a high commercial value and a wide range of applications in seafood production [1]. Given the limited availability of white muscle fish due to their overexploitation, it would be desirable to use lower-quality surimi from other species and to improve it by further processing [2]. Therefore, more attention has been paid to dark muscle fish species as an alternative raw material [3-5].

Among all dark-fleshed fish species, sardine was one of the most abundant species caught in Tunisia. The use of this small pelagic fish for surimi production is a promising way and an alternative to revalue and convert underutilized fish protein resources into foods of premium quality, particularly protein gel-based products. However, the characteristics of surimi gel depend on the properties of the myofibrillar proteins, and these are determined by the species and freshness of the fish, as well as the processing parameters [6].

Small pelagic fish species, such as sardine, produce surimi with poor gel characteristics due to the high content of dark muscle, comprising a considerable amount of lipids and sarcoplasmic proteins [7,8]. The presence of sarcoplasmic proteins of dark muscle has often been cited as one of the reasons for the poorer gelation properties of dark muscle fish compared to light muscle fish [9]. In this context, microbial transglutaminase (MTGase) has been studied as a means of improving the textural characteristics and mechanical properties of fish as well as meat products [10]. MTGase induces the formation of ε-(γ-glutamyl) lysine cross-link in the proteins via acyl transfer between the ε-amino groups of a lysine residue and γ-amide group of a glutamine residue [11]. The reactions promoted by the enzyme create profound changes

in the proteins in food matrices, which results in the improvement of textural properties and stability in terms of temperature, syneresis, emulsifying properties, gelation and water-holding capacity (WHC), without changing the pH, colour, flavour or nutritional quality of food, and may even render it more nutritious due to the possibility of adding essential amino acids [12]. However, the efficiency of MTGase in improving gelation properties of proteins depends on many factors, including the amount of MTGase, type of fish, and fat content [13-15].

In addition to the gel enhancer used, setting response is an important step in the formation of surimi gel that contributes to the different gelling properties. Setting or suwari is a treatment prior to cooking, which involves pre-incubation of salted surimi paste, generally at temperatures between 0°C and 40°C [16]. This results in the formation of a myosin network through cross-linking induced by endogenous transglutaminase (TGase) [17,18]. Considering this, a study to determine the optimal conditions for setting, using MTGase, can be based on analysing the relationship between enzyme concentration, time and temperature.

To determine the best conditions for TGase activity in foods, several authors have used the response surface methodology (RSM) [19-21]. This methodology develops a suitable experimental design by collecting statistical and mathematical tools that are useful for the modelling and analysis of problems in which a response of interest is influenced by several variables, with the objective of optimizing the response [22]. The RSM involves full factorial research to explore

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simultaneous, systematic and efficient variations in the important components, identifying potential interactions and higher-order effects, and thereby determining the optimal operational conditions [23]. Although MTGase has been successfully used for improving the gelation properties of surimi, no information has been reported regarding the use of MTGase in surimi gel from dark-fleshed fish species, such as sardine. The aim of this study was to use sardine (*Sardina pilchardus*) resource and transform it from low-valued fish to high-value-added product "surimi gel", to evaluate the effects of MTGase in sardine surimi gel and to optimize the conditions for enzyme activity during setting (concentration, temperature and time) using the RSM.

Materials and Methods

Microbial transglutaminase

MGTase from *Streptoverticillium mobaraense* was supplied by Ajinomoto USA, INC. (Teaneck, NJ, USA). Enzyme activity reported by the supplier was 100 units/g dry weights. The enzyme powder consisted of 99% maltodextrin and 1% enzyme by mass.

Surimi paste preparation

In order to prepare surimi by the conventional washing process [24], fresh sardine (*Sardina pilchardus*) was purchased from a fish market in Tunisia. Fish were headed, eviscerated and washed. Skin and bones were removed by hand in the laboratory. Fillets were minced in a meat mincer (TC-32 EL/80 Tre Spade, Torino, Italy). Fish mince was washed with cold water (4°C) using a water-to-mince ratio of 3:1 (v/w). The mixture was stirred gently for 10 min and the washed mince was filtered with a layer of nylon screen. This washing process was performed thrice. Finally, the washed mince was dewatered by centrifugation at a speed of 700×*g* for 15 min at 4°C (Model CE 21 K, Grandimpianti, Belluno, Italy). A cryoprotectant mixture was added to the washed mince (4% sorbitol and 4% sucrose w/w). Surimi was packed into polyethylene bags (250 g) and stored at -20°C until used for gel preparation. The storage time did not exceed 1 month.

Surimi gel preparation

Frozen surimi samples were partially thawed at 4°C, then cut into small pieces and chopped with 2.5% NaCl (w/w) for 3 min. MTGase was then added to the surimi paste and the mixture was chopped for another 5 min. Then moisture content of the surimi was adjusted to 80% with iced water and the chopping was continued for 15 min. For the entire chopping process, the temperature was kept below 10°C.

The paste was stuffed into stainless steel tubes (diameter=2.5 cm; length=15 cm), inner wall of which was coated with a film of vegetable oil to prevent gel adhesion. Both ends of the tubes were sealed tightly. The paste samples were allowed to set and then heated at 90°C for 20 min. The gel samples were cooled rapidly in iced water and kept at 4°C overnight until analysis.

Rheological measurements

Linear dynamic viscoelasticity measurements were determined using a controlled-stress rheometer (AR2000) from TA Instruments (New Castle, DE, USA) equipped with two parallel plates (40 mm diameter) with rough surfaces, setting a gap between plates of 0.5 mm [25]. Before measurement, the gels were tempered at room temperature and cut to size, namely, to the same diameter as that of the plate and at a thickness of 0.5 mm. Samples were covered with a thin film of petroleum jelly to avoid evaporation. All determinations were carried out at least in triplicate.

Stress sweep tests: In order to determine the linear viscoelastic (LVE) region, stress sweeps were conducted at 6.28 rad/s with a constant temperature at 25°C. The shear stress (σ) of the input signal was programmed from 1 to 1000 Pa.

Frequency sweep tests: Frequency sweep tests were carried out at different frequencies ranging from 10 to 0.1 Hz under a constant shear strain amplitude (γ=0.2%) within the LVE range. Storage modulus (G′), loss modulus (G′) and loss tangent (tan δ) were recorded at 1 Hz.

Disulphide bonds

The disulphide bond (DB) content was determined using a 2-nitro-5-thiosulphobenzoate assay method [26]. Freshly prepared 2-nitro-5-thiosulphobenzoate assay solution (3 ml) was added to the protein solution (0.5 ml) and the mixture was allowed to set in the dark at room temperature (26°C to 28°C) for 25 min. Absorbance was measured at 412 nm. DB content was calculated using the molar extinction coefficient of 13,900 M^{-1} cm^{-1} using a Genesys-20 spectrophotometer (Thermo Scientific, USA).

Total sulfhydryl group content

The total sulfhydryl (TSH) content was determined using 5,5'-dithio-bis (2-nitrobenzoic acid) method [27] with some modifications. Samples (70 μL of protein solution) were homogenised with 1 ml of solubilizing buffer (20 mMTris–HCl, 8 M urea, 10 mM EDTA, pH 8.0). An aliquot (100 μL) of Ellman's reagent was added. The mixture was incubated in the dark at room temperature for 30 min. As for DB content, the amount of TSH was measured at 412 nm with a molar extinction coefficient of 13,600 M^{-1} cm^{-1} using a Genesys-20 spectrophotometer (Thermo Scientific, USA).

Water-holding capacity

WHC was evaluated by a centrifuge method [28]. It was expressed as the percentage of the initial water remaining in the sample after centrifugation. Each value is the mean (standard deviation) of at least four measurements.

Response surface methodology

In order to determine the best conditions for the production of sardine surimi gel, Response surface methodolgy (RSM) was applied to derive mathematical models to estimate the effects design and the interactions of the independent variables: enzyme concentration (x_1), temperature (x_2) and incubation time (x_3) on the storage modulus (G′ y_1), loss modulus (G″ y_2), disulphide bond content (DB y_2), total sulfhydryl group content (TSH y_4) and water-holding capacity (WHC y_5) of surimi gel samples which were the selected responses for this research.

The experimental design followed a second-order factorial structure [29] with 4 replications at the centre point to estimate the experimental error, leading to 30 experiments, carried out in a random order and in triplicate.

Table 1 presents the variables used in the optimization of the setting process during sardine surimi production including fixed variables at usual values.

In order to estimate the response, an empirical model was constructed based on a second-order polynomial (1):

Variables		Unit	Nomenclature
Independent variables	Enzyme concentration	g/Kg	x1
	Temperature	°C	x2
	Incubation time	h	x3
Dependent variables	G'	Pa	y1
	G''	Pa	y2
	Disulphide bonds	%	y3
	Total sulfhydryl group	mmol/g proteins	y4
	Water holding capacity	%	y5

Table 1: Variables used for the experimental design.

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2 \qquad (1)$$

Where, y is the estimated response, β_0 the model constant, β_i the coefficients of the linear term, β_{ii} the coefficients of the quadratic term, β_{ij} the coefficients of interaction between the factors, x_i and x_1 the design variables, k the number of factors, and i and j the coded factors of the system. The experiments were assessed using the Design-Expert software (Statease Inc., Minneapolis, USA, version 7.0). For each response, the coefficients were calculated by regression analysis and the significance of the derived model equation was determined using the analysis of variance (ANOVA).

Results and Discussion

Parameter ranges studied

The main effects and the interactions of MTGase concentration, temperature and reaction time during setting of sardine surimi were assessed using the RSM. Many researchers apply RSM as a mathematical modelling tool in bioprocess optimization [30,31]. By using RSM, process variables could be controlled together to result in maximum product properties with desired characteristics [32,33].

Setting can be performed at low (0°C to 4°C), medium (25°C) or high (40°C) temperatures [34], and the choice of temperature may lead to different gelling properties, since gel setting behaviour is fish species and temperature dependent. At low temperature, setting takes longer, and so it is not commonly implemented in industry. So far, high-temperature setting is more widely used in Thailand, given the shorter time required, but there is a risk of protein degradation, due to modori-inducing proteinase, which is generally active at 50 to 60°C [35]. Therefore, a medium-temperature setting may be considered the best option for manufacturers to obtain the highest gel quality with negligible proteolysis.

In line with this, we studied temperatures between 25°C and 45°C, because 25°C is the temperature commonly recommended for setting in cold water species such as Alaska Pollock (*Theragra chalcogramma*) [36], while 45°C is the recommended maximum, to avoid the modori phenomenon in the range 50°C to 70°C [37]. Regarding reaction time, we collected data between 1 h and 5 h, because it has been reported that MTGase is active for between 2 h and 5 h in Alaska pollock at 25°C [38].

The range for the variable enzyme concentration (0 g/kg -10 g/kg) was chosen according to the studies which deal with the improvement of the mechanical properties of surimi produced from striped mullet (*Mugil cephalus*) and silver carp (*Hypophthalmichthys molitrix*) respectively [39,40].

Dynamic viscoelasticity measurements

The storage and loss modulus (G' and G') were considered as

responses, since research on surimi rheological properties has shown the importance of the viscoelastic moduli G' and G' in the nominal quality of surimi [41]. Moreover, the use of MTGase, through the cross-links it promotes, enables highly elastic and irreversible gels to be obtained in different substrates, even at relatively low protein concentrations [42,43].

Table 2 summarizes the operational conditions tested as well as the experimental and predicted response values determined for the dependent variables obtained from this experimental design. It can be observed that the experimental values for storage modulus G' (y_1) varied over a wide range (3121 Pa-63520 Pa).

The analysis of the regression coefficients and the main experimental trends are presented in Table 3. It can be concluded that temperature and enzyme concentration have the most influence on G', followed by the interaction between temperature and time. Using the models, it is possible to predict the results and plot them as a response surface in three-dimensional graphs. The response surface for G' (y_1) is illustrated in the Figure 1 which describes the dependence of y1 on enzyme concentration and temperature at the different incubation times used. From the plot, it can be seen that increasing enzyme concentration and temperature allow us to reach higher values of G' at shorter times, but at longer times, high enzyme concentration and temperature lead to a decrease in y_1.

For loss modulus G' (y_2), a similar behaviour was observed, the experimental results varied within the wide range of 1845 to 13897 Pa (Table 2). Analysis of the regression coefficients and the main experimental trends suggest the same results as G' (y_1) (Table 3). Temperature and enzyme concentration have the most influence on G', followed by the interaction between temperature and time. The resulting variation pattern is shown in Figure 2 where MTGase concentration and temperature were the variables that most influenced the setting of sardine surimi gel.

Disulfide bond and total sulfhydryl group content

DB and TSH content were also used as responses because they are major parameters for evaluating the gelling properties of surimi. The presence of sulfhydryl groups in surimi is necessary for gel strengthening. High temperatures during heating led to further oxidation of sulfhydryl groups with a subsequent disulfide bond formation [44].

DB (y_3) and TSH (y_4) showed behaviour different to y_1 and y_2. The experimental results varied within the narrow ranges of 23.99% to 33.6% and 14.71 to 1.14 mmol/g proteins for DB and TSH, respectively. The most favourable conditions were defined by the maximum value of enzyme concentration, the highest value of temperature and the shortest time (experiment 28). Analysis of the regression coefficients for y3 and y4 showed that enzyme concentration did not significantly affect DB content. The variation of DB and TSH contents with

Test	Factors			Experimental responses					Predicted values				
	E	t	T	y1	y2	y3	y4	y5	y1	y2	y3	y4	y5
1	0	1	25	6692	2144	23.99	14.71	49.25	9501	3626	25.29	13.61	49.26
2	0	3	25	8186	4874	29.46	10.26	50.73	7841	3062	26.93	11.71	50.65
3	0	5	25	18602	4494	27.90	8.83	53.16	26153	6615	29.13	8.34	54.04
4	0	1	35	17760	4915	29.56	8.17	53.82	15812	5452	29.36	8.73	53.43
5	0	3	35	7080	4080	30.57	10.17	52.82	2753	2589	29.347	8.99	52.746
6	0	5	35	19710	4816	29.36	7.36	54.63	9666	3845	29.88	7.77	54.06
7	0	1	45	44242	12476	30.69	5.05	55.94	48641	12799	31.21	4.83	56.83
8	0	3	45	21763	7578	27.89	7.84	53.15	24183	7638	29.53	7.24	54.07
9	0	5	45	20213	6846	29.70	7.04	54.95	19697	6596	28.41	8.18	53.32
10	5	1	25	16396	4353	26.56	10.17	50.82	12611	3704	25.68	11.58	50.77
11	5	3	25	29630	5812	25.84	10.90	52.09	13209	3915	27.39	9.94	51.56
12	5	5	25	24096	7830	29.44	7.29	54.70	33780	8244	29.66	6.83	54.37
13	5	1	35	20363	7087	30.62	6.12	55.87	19704	5491	30.04	6.74	55.65
14	5	3	35	3121	1921	30.65	7.08	54.91	8903	3404	30.09	7.26	54.37
15	5	3	35	3263	3521	28.89	7.84	54.15	8903	3404	30.09	7.26	54.37
16	5	3	35	9353	3144	30.49	6.24	55.75	8903	3404	30.09	7.26	54.37
17	5	3	35	3333	1845	29.83	6.90	55.09	8903	3404	30.09	7.26	54.37
18	5	5	35	20340	6079	30.71	7.02	51.97	18074	5436	30.70	6.30	55.10
19	5	1	45	58330	13220	32.94	2.80	60.19	53314	12800	32.17	2.88	59.76
20	5	3	45	28850	7262	30.54	6.19	55.80	31114	8415	30.57	5.55	56.41
21	5	5	45	29230	7698	29.64	7.10	54.89	28887	8149	29.52	6.74	55.06
22	10	1	25	11010	4658	25.14	11.59	50.40	16839	5120	25.69	10.67	50.71
23	10	3	25	19703	4867	27.97	8.76	50.23	19695	6107	27.47	9.29	50.90
24	10	5	25	47840	12573	30.79	5.95	54.04	42524	11212	29.82	6.44	53.11
25	10	1	35	21926	7254	29.48	7.25	54.74	24712	6869	30.34	5.87	56.30
26	10	3	35	14610	4610	30.93	5.81	55.18	16170	5558	30.46	6.65	54.42
27	10	5	35	29243	7952	30.61	6.13	54.86	27599	8365	31.15	5.95	54.55
28	10	1	45	63520	13897	33.60	1.14	62.85	59103	14141	32.76	2.05	61.12
29	10	3	45	40846	11920	30.29	5.44	55.55	39162	10531	31.23	4.98	57.16
30	10	5	45	36302	11214	30.41	6.31	55.68	39193	11040	30.25	6.43	55.22

Table 2: Operational conditions assayed with the independent variables and their experimental and predicted responses.

Co-efficients	Response				
	y1	y2	y3	y4	y5
β0	8902.69	3404.38	30.1	7.26	54.37
β1	6708.44	1484.56	0.33	-1.17	0.84
β2	8952.28	2250.33	1.59	-2.2	2.42
β3	-814.61	-27.89	0.56	-0.22	-0.28
β11	558.56	669.46	-0.19	0.56	-0.79
β22	13259.06	2760.79	-1.11	0.49	-0.38
β33	9986.4	2059.13	0.28	-0.74	1.01
β12	781.42	-37.92	0.29	0.038	0.71
β13	2258.17	775.75	0.073	0.26	-0.6
β23	-11398.92	-2298.08	-1.66	2.16	-2.07

Table 3: Regression co-efficients for the response surface models in terms of coded units.

enzyme concentration and temperature at several incubation times are presented in Figures 3 and 4 respectively. Notably, using a long incubation time did not induce oxidation of sulfhydryl groups and produce a surimi gel with low DB.
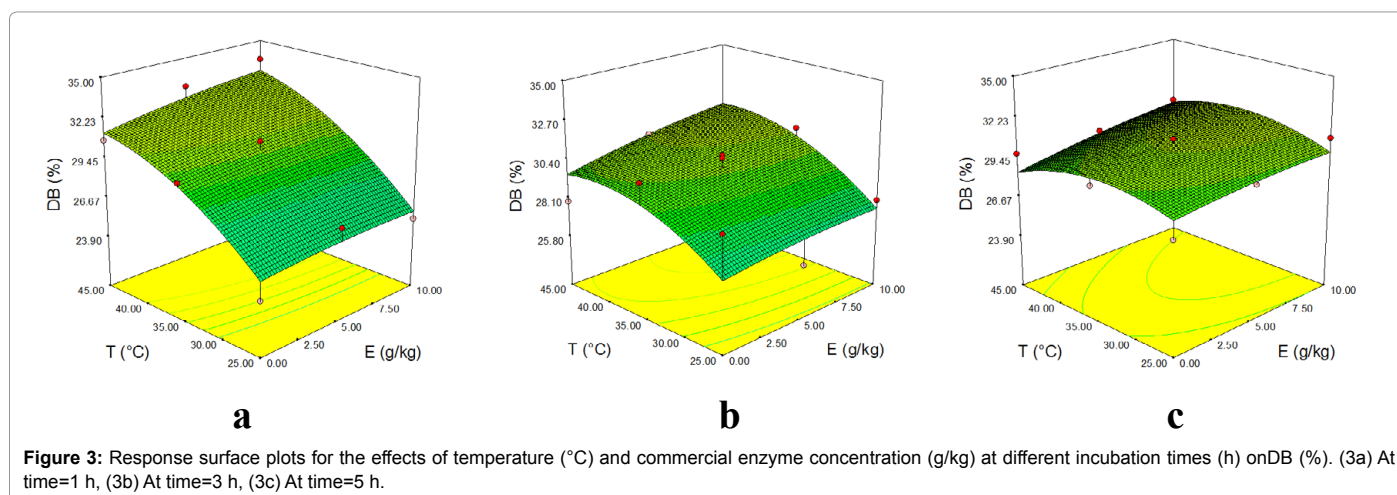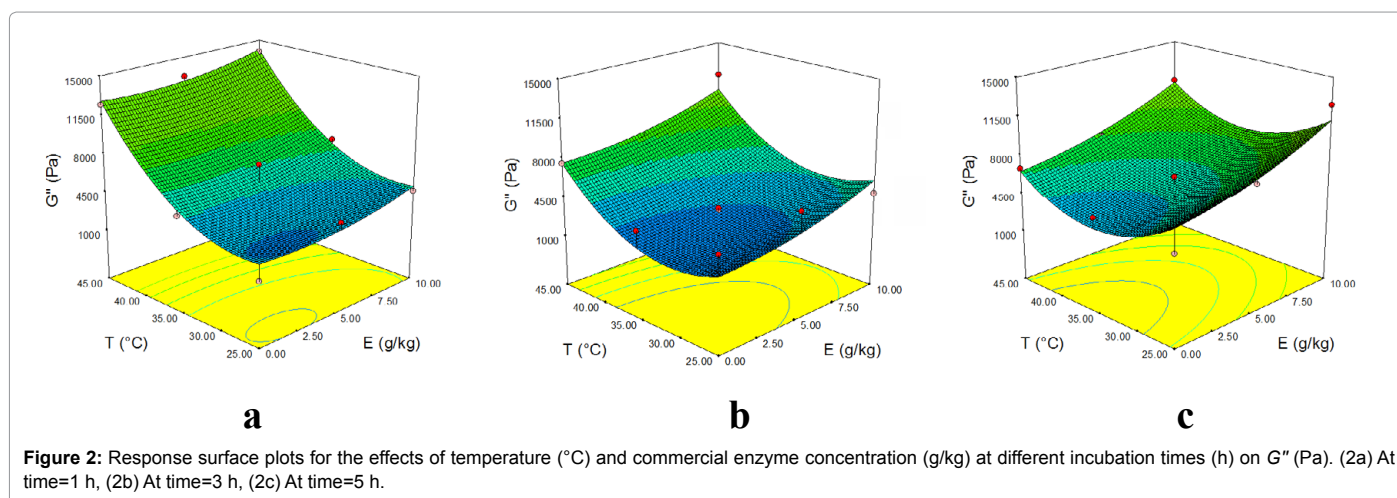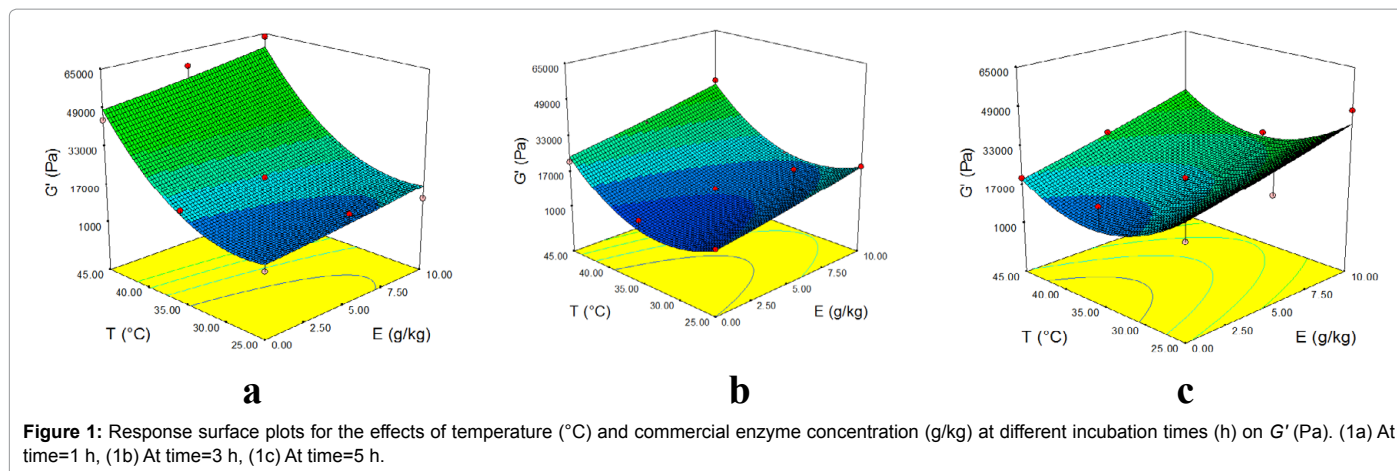
Water-holding capacity

The last dependent variable considered as a response variable in this study was WHC, that is, the protein's ability to take up water and retain it within a protein matrix, e.g., beef or fish muscle or a protein gel, through protein–water interactions. Hence, WHC is closely linked to gelation, emulsification and foaming properties [42]. In myofibrillar proteins, WHC is inversely correlated with enzyme concentration beyond a certain threshold concentration, because the higher the enzyme concentration, the greater the number of inter- and intra-chain peptide cross-links and the weaker the protein–water interaction. Thus, the amount of enzyme added should be carefully evaluated because, in appropriate concentrations, MTGase yields stable gels with relatively high porosity that are able to immobilize water more efficiently. As a result of this increase in WHC, protein gels obtained have better textural properties, in terms of bond strength, stiffness, cohesion, chewability and elasticity [45,46].

The experimental results of WHC varied within the narrow range of 49.25% to 62.85%. Analysis of the main experimental trends and the values of coefficients listed in Table 3 suggest that temperature has the strongest influence on WHC, followed by enzyme concentration and the interaction between temperature and incubation time. The most favourable conditions were defined by the maximum value of enzyme concentration, the highest value of temperature and the shortest time (experiment 28). In Figure 5, it can be observed that the model predicts that an increase in temperature and enzyme concentration would make it possible to reach higher values of $y5$ at short times, but that at long times, high temperatures lead to a decrease in y_5, so the effect of temperature and MTGase concentration are pronounced at shorter time.

Model fitting

It is important to assess the model fitted to ensure that it provides sufficiently close approximation to the results obtained under experimental conditions [47].
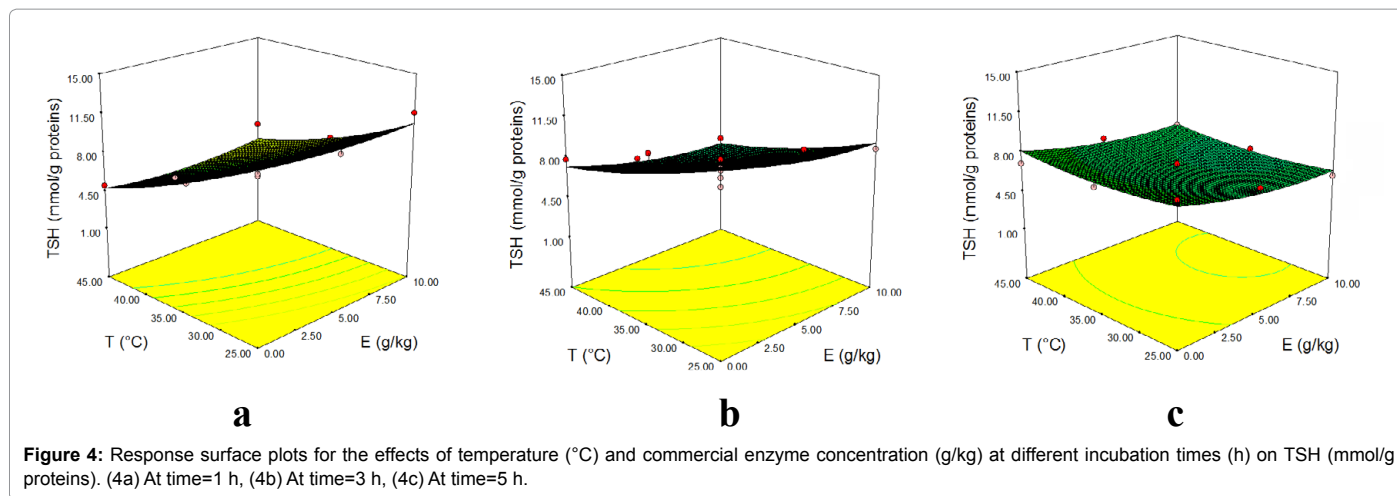
Figure 1: Response surface plots for the effects of temperature (°C) and commercial enzyme concentration (g/kg) at different incubation times (h) on G' (Pa). (1a) At time=1 h, (1b) At time=3 h, (1c) At time=5 h.

Figure 2: Response surface plots for the effects of temperature (°C) and commercial enzyme concentration (g/kg) at different incubation times (h) on G'' (Pa). (2a) At time=1 h, (2b) At time=3 h, (2c) At time=5 h.

Figure 3: Response surface plots for the effects of temperature (°C) and commercial enzyme concentration (g/kg) at different incubation times (h) onDB (%). (3a) At time=1 h, (3b) At time=3 h, (3c) At time=5 h.

After checking the normality of the data using a normal probability plot of the residuals and the difference between the observed values and those predicted from the regression, we found that the experimental points were normally distributed around the line, indicating that the normality assumption was satisfied.
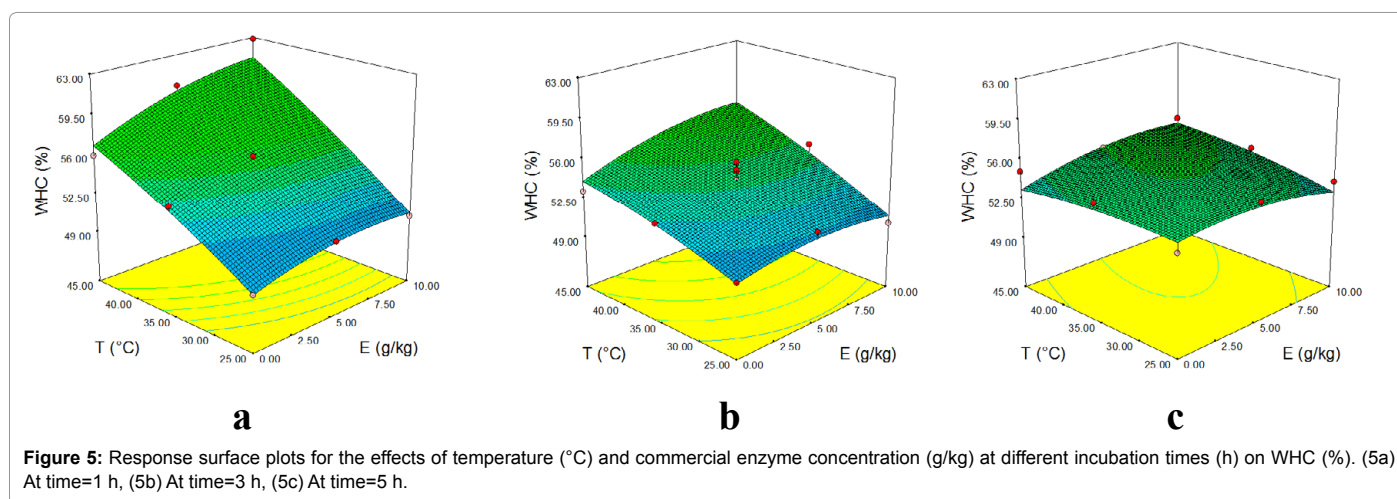
ANOVA and regression analysis were used to examine the statistical significance of the terms and to check the model adequacy (Table 4).

It was suggested that for a good fitted model, the coefficient of determination (R^2) should not be less than 80%. The lower values of R^2 show the inappropriateness of the model to explain the relation between variables [48].

Figure 4: Response surface plots for the effects of temperature (°C) and commercial enzyme concentration (g/kg) at different incubation times (h) on TSH (mmol/g proteins). (4a) At time=1 h, (4b) At time=3 h, (4c) At time=5 h.

Figure 5: Response surface plots for the effects of temperature (°C) and commercial enzyme concentration (g/kg) at different incubation times (h) on WHC (%). (5a) At time=1 h, (5b) At time=3 h, (5c) At time=5 h.

The R^2 values for the regression model predicting the storage modulus G′, the loss modulus G′, DB content, TSH content and WHC were found to be 0.8824, 0.9015, 0.7845, 0.9057 and 0.8671 respectively, indicating a good suitability between the observed and predicted response values.

The lack of fit is an indication of the failure of a model representing the experimental data at which points were not included in the regression or variations in the models cannot be accounted for random error [49]. When a lack of fit is significant, the response predicted is discarded.

Data indicated the insignificance of the lack of fit of all dependent variables ($F_{cal} < F_{tab}$) confirming that the quadratic model was valid for this study (Table 4).

Moreover, to validate the model, the plots of the residual versus the predicted values should present residuals scattered randomly around zero and did not reveal any outliers.

Our results (plots not shown), showed that all the values lie within the accepted range (-3 and +3) [23] for all the response variables. Thus, all the model assumptions being satisfied, the variance analysis indicated that the model was valid.

When analysing the parity plots (plots not shown) showing the distribution of predicted values versus the actual ones of the storage modulus G′, the loss modulus G′, DB content, TSH content and WHC, it can be seen a satisfactory correlation between the experimental and predictive values since the points are clustered around the diagonal line which indicates a good agreement between the polynomial regression model and the experimental results.

Optimization of conditions using the response surface methodology

Based on the results, it was observed that the optimum region of enzyme activity of MTGase was obtained for an MTGase concentration of 10 g/kg with 1h of incubation at 45°C (Table 5). This means that the highest degree of fish protein cross-linking occurring during sardine surimi gel production was obtained under these conditions. From the models, it can be concluded that the optimal temperature for MTGase (40°C to 45°C) is higher than that for endogenous TGase (35°C to 40°C) extracted from sardine muscle, established in a preliminary study (data not shown), and is close to the optimal temperature (50°C) for the catalysis of MTGase [50].

Finally, in order to compare the behaviour of the setting phenomenon with the addition of MTGase, Table 6 shows the results of related studies of surimi gels from different species. The optimal setting temperature and enzyme concentration for sardine were both higher than those reported for the other fish species. This could be explained

Response	R²	p-value Prob>F	F value	Lack of Fit	Adeq. Precision	Adj. R²	Pred. R²
y1	0.8824	<0.0001	16.67	0.1039	15.15	0.8295	0.7279
y2	0.9015	<0.0001	20.35	0.2362	15.34	0.8572	0.7625
y3	0.7845	<0.0001	8.09	0.2738	11.24	0.6876	0.4450
y4	0.9057	<0.0001	1.33	0.2679	20.94	0.8632	0.7636
y5	0.8671	<0.0001	14.50	0.1478	16.92	0.8073	0.6739

Table 4: Statistical parameters measuring the correlation and significance of models.

Test	Process variables			Predicted values					Desirability
	E	T	t	G'	G"	DB	TSH	WHC	
1	10	45	1	59103	14141	32.77	2.05	61.12	0.928
2	10	44.98	1.02	58747	14071	32.75	2.09	61.07	0.925
3	10	44.82	1	58248	13960	32.74	2.11	61.04	0.923

Table 5: Predicted optimum conditions for optimal MTGase activity.

Fish species	Setting conditions			References
	Enzyme concentration (g/kg)	Temperature (°C)	Incubation time (h)	
Alaska pollock *Theragra chalcogramma*	2	25	3	Lee et al. [36]
Striped mullet *Mugil cephalus*	5	34.5	1	Ramírez et al. [39]
Silver carp *Hypophthalmichthys molitrix*	8.8	39.6	1	Ramírez et al. [40]
Sardine *Sardina pilcharus*	10	45	1	The present study

Table 6: Comparison of optimal setting conditions of surimi gels from different fish species.

by the optimal temperature for setting being related to fish habitat temperature [51] and to the thermal stability of myosin in each fish species [52]. Furthermore, the cross-linking reaction induced by TGase occurs when protein molecules and the enzyme become associated in a highly-oriented and conformation-dependent fashion during the catalytic process [53]. Therefore, optimal enzyme concentrations and setting temperatures should be determined for each type of surimi to obtain the best gel quality.

Conclusion

In conclusion, the application of the RSM is a practical and effective tool in optimizing the parameters of transglutaminase activity in sardine surimi gel during setting.

The enzymatic treatment considerably ameliorated the gelling properties of the surimi gel obtained, due to the high-molecular-weight polymers formed during the cross-linking reaction induced by TGase.

Results suggest that setting is dependent on enzymatic activity and protein denaturation/aggregation, both processes occurring during the setting phenomenon. Hence, MTGase can be considered a useful additive for the production of surimi gels from sardine. Sardine surimi gel was predicted to have optimal properties for setting under the following conditions: 45°C/1 h using 10 g of microbial TGase/kg of surimi. All the mathematical models derived from the various responses were found to be a good fit to predict the data.

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