Food Sci. Biotechnol. Vol. 16, No. 4, pp. 557 ~ 564 (2007)

Food Science and Biotechnology

The Processing Optimization of Caviar Analogs Encapsulated by Calcium-Alginate Gel Membranes

Cheong-Il Ji[1], Sueng-Mock Cho[2], Yeun-Suk Gu[3], and Seon-Bong Kim*

Department of Food Science and Technology/Institute of Food Science, Pukyong National University, Busan 608-737, Korea
[1]Gangneung Marine Bio Foundation, Gangneung, Gangwon 210-340, Korea
[2]Korea Food Research Institute, Seongnam, Gyeonggi 463-420, Korea
[3]Solae LLC, St. Louis, MO 63102, USA

Abstract We prepared caviar analogs encapsulated by calcium-alginate gel membranes as a means to replace higher priced natural caviars. Processing the caviar analogs (beluga type) was optimized by response surface methodology with central composite design. Concentrations of sodium alginate (X_1) and $CaCl_2$ (X_2) were chosen as the independent variables. In order to compare characteristics of the caviar analogs with the natural caviar, sphericity (Y_1), diameter (Y_2), membrane thickness (Y_3), rupture strength (Y_4), rupturing deformation (Y_5), and sensory score (Y_6) were used as the dependent variables. The sphericity of the caviar analogs showed a similar value to that of natural caviar (over 94%) in the range of independent variables. Generally, the $CaCl_2$ concentration (X_2) affected all dependent variables to a greater extent than the sodium alginate concentration (X_1). For the multiple response optimization of the 5 dependent variables (Y_1, Y_2, Y_4, Y_5, and Y_6), the desirability function was defined as the following conditions: target values (Y_1 = 100%, Y_2 = 3.0 mm, Y_4 = 1,470 g, Y_5 = 1.1 mm, and Y_6 = 10 points). Membrane thickness (Y_3) was eliminated from the dependent variables for multiple response optimization because it could not be measured with an image analyzer. The values of the independent variables as evaluated by multiple response optimization were X_1 = -0.093 (0.78%) and X_2 = -0.322 (1.07%), respectively.

Keywords: caviar analog, encapsulation, calcium-alginate gel membrane, physical property, response surface methodology (RSM)

Introduction

Alginate is a gelling polymer that becomes reversibly insoluble in the presence of metallic divalent cations (1, 2). It is composed of linear polymers of 1-4 linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues in varying proportions and sequential arrangements (3). Montero and Pérez-Mateos (4) reported that calcium ions make a stronger alginate gel than potassium and sodium ions. It has generally been accepted that in the gelation mechanism, cross-links are formed by coordination of the divalent metal cations (calcium ions) to interchain cavities made up of guluronic acid blocks, resulting in the development of a so-called 'egg-box' junction zone (5, 6).

Encapsulation technology using calcium-alginate gel has been widely applied to bioprocessing. Its application has many significant advantages due to its good biocompatibility, simple operation, and low cost (7). Calcium-alginate gel capsules have been investigated for utilization in immunoprotective applications such as containers in cell transplantation (*Lactobacillus rhamnosus* cells) (8), yeast cells (9), plant cells and tissues (10), animal cells (11), enzyme immobilization (6, 12), controlled release systems (13), encapsulation of bioactive materials (14), increased stability, and the controlled permeability of capsules (15).

Encapsulation technology, however, has never been performed in the production of fish roe analogs. Therefore, we prepared fish roe similar to costly caviar using calcium-alginate gel capsules. Caviar is the term used to describe fish roe used for human consumption, which has undergone processing and salting. The natural caviars used mainly as foods are produced by beluga, osetra, and sevruga sturgeons of the family *Acipenseridae*. In recent years, natural and aquacultural stocks of sturgeon have not been able to meet consumer demands. Since sturgeon fish require 15 to 20 years to mature and develop ripe ovaries for caviar production, the use of alternative roes for producing caviar have emerged onto world markets. These alternatives such as lumpfish, salmon, and trout are unique in the sense that they neither resemble nor taste like the sturgeon caviar. Therefore, encapsulation using calcium-alginate gel to produce sturgeon-like caviar analogs could be an important food processing technology. In the present study, the encapsulation process by calcium-alginate gel was applied to the preparation of beluga type caviar analogs. Caviar from the rare beluga sturgeon is most prized by connoisseurs, but the harvesting of this expensive delicacy is threatening the prehistoric fish with extinction.

This work was completed to optimize caviar analog processing using response surface methodology (RSM), as well as to compare the mechanical properties and appearance of caviar analogs with those of natural caviar. RSM is effective in the optimization and monitoring of food manufacturing processes (16-18). The basic principle of RSM is to determinate model equations that describe the interrelations between independent and dependent variables (19). For the processing of caviar analogs with calcium-alginate gel membranes, sodium alginate (SA) and $CaCl_2$ interacted electrostatically due to their opposite

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*Corresponding author: Tel: 82-51-620-6418; Fax: 82-51-622-9248
E-mail: owlkim@pknu.ac.kr

Received December 13, 2006; accepted March 21, 2007

charges (e.g., anionic and cationic for SA and $CaCl_2$, respectively). However, carboxymethyl cellulose (CMC) was used as a non-gelling agent to modulate the viscosity and density of the $CaCl_2$ solution, to ensure the spherical shape of the caviar analogs. Therefore, the 2 gelling agents, SA and $CaCl_2$ were the main parameters and were chosen as the independent variables in this experimental design. In order to prepare caviar analogs with similar appearance and physical properties of natural caviar, sphericity, diameter, and membrane thickness of the analogs were examined using an image analyzer, and rupture strength and rupturing deformation were examined by a rheometer. In addition to examining the physicochemical properties of the caviar analogs, sensory evaluations were performed to assess the bitterness of calcium chloride.

Materials and Methods

Materials Sodium alginate (SA), calcium chloride ($CaCl_2$), and carboxymethyl cellulose (CMC) were used as the major materials for caviar analog preparation. Squid ink powder was provided by BioAdd Co., Ltd. (Busan, Korea). All regents used in this study were analytical grade.

Processing of the caviar analogs The caviar analogs were fundamentally processed by allowing droplets of complex solution containing $CaCl_2$ and CMC to disperse into SA solution. The anionic SA solution (0.52-1.08%, w/v) containing 2% squid ink powder was agitated to prevent the formation of clumps. After the SA and squid ink powder were completely dissolved, the SA solution was undisturbed for 30 min to eliminate air bubbles that could later disturb formation of the caviar analogs. To prepare the cationic complex solution containing $CaCl_2$ and CMC, CMC was dissolved in different $CaCl_2$ solutions (0.63-1.77%, w/v) to the final concentration of 1.5%(w/v). As a non-gelling polymer, CMC was used to modulate the viscosity and density of the cationic solution, to ensure the spherical shape of the caviar analogs. In preliminary work to this study, the concentration of CMC was determined at 1.5% (data not shown), and the viscosity of the $CaCl_2$ and CMC complex solution was 500 cp at 20°C. The viscous complex solution containing $CaCl_2$ and CMC was produced to droplets using a 21G metal injection needle (internal diameter of 0.52 mm, outside diameter of 0.82 mm, and length of 32 mm) with a peristaltic pump (Casette tube pump SMP-23; Eyela, Tokyo, Japan). The SA solution was constantly stirred (280 rpm) by a magnetic bar situated at the bottom of the vessel at a fixed dropping velocity (0.05 mL/sec) to keep the droplets from sticking together, and to minimize the external mass transfer resistance. A dropping height of 10 cm was used to form spherical droplets. The cationic droplets containing $CaCl_2$ and CMC were coated by the anionic droplets containing SA and squid ink. An interfacial membrane formed instantaneously around each droplet due to the cross-linking of the interfacial alginate molecules by the calcium cations (6). The droplets encapsulated by SA solution were rinsed several times with distilled water. To stabilize and harden the gel membrane, the caviar analogs were transferred to a 2%(w/v) $CaCl_2$ solution and soaked to mature for 20 min. Finally, the caviar analogs were rinsed again with distilled water to remove any remaining $CaCl_2$. All of the above procedures were carried out at an ambient temperature.

Determination of diameter, membrane thickness, and sphericity The membrane diameter and thickness of the caviar analogs were measured by an image analyzer (Image-Pro Plus; Media Cybernetics, Inc., Bethesda, MD, USA) coupled to a microscope (B202 optical microscope; Olympus, Tokyo, Japan). The membrane thicknesses of the caviar analogs and natural caviars could not be measured due to their black color. Thus, the thickness measurements for the analogs were done with hollow capsules. The image analysis of the caviar analogs was produced at 40× magnification. Ten capsules randomly sampled from each experimental condition were used for measurement. The sphericity was determined by the 2 linear dimensions (longest and shortest diameters) of the percentage ratio of the minor diameter to the major diameter of the caviar analogs (Eq. 1).

$$\text{Sphericity (\%)} = \frac{\text{minor diameter of capsules}}{\text{major diameter of capsules}} \times 100 \quad \text{[Eq. 1]}$$

Measurement of rupture strength and rupturing deformation The rupture strength (g) and rupturing deformation (mm) imply the resistance, and the depth to resisting rupture, when the spherical shapes of the caviar analogs were completely broken by a penetrating plunger. The measurements of rupture strength and rupturing deformation were performed using a rheometer (Compac-100; Sun Scientific Co., Ltd., Tokyo, Japan) with the following conditions: round-disk stainless steel plunger, 10 mm diameter; penetration speed, 10 mm/min; and load-cell of 1 kN. Ten samples were used per experiment.

Sensory evaluation For the effect of $CaCl_2$ on caviar analog bitterness, sensory evaluations were performed by 10 panelists composed of Pukyong National University graduate students. Sensory scores were evaluated on a 0 to 10 point scale. When the degree of bitterness of the analogs was near that of the natural caviar (10 points), a higher point was assigned by the panelists.

Experimental design Central composite design (CCD) (20) was adopted for optimization of caviar analog processing. In the experimental design, CCD consisted of 2^2 factorial points, 4 axial points (α=1.414) and 3 replicates of the central point (Table 1 and 2). The concentrations of SA (X_1, %) and $CaCl_2$ (X_2, %) were chosen as the independent variables. The range and center point values of the 2 independent variables were based on the results of preliminary experiments (Table 1). In order to compare the characteristics of the caviar analogs with those of the natural caviar, responses of sphericity (Y_1, %), diameter (Y_2, mm), membrane thickness (Y_3, mm), rupture strength (Y_4, g), rupturing deformation (Y_5, mm), and sensory score (Y_6, point) were used as the dependent

Table 1. Experimental range and values of the independent variables in the central composite design for the processing of caviar analogs

Coded levels	Independent variables	
	Concentration of sodium alginate (SA)	Concentration of calcium chloride ($CaCl_2$)
	X_1 (%, w/v)	X_2 (%, w/v)
-1.414	0.52	0.63
-1	0.60	0.80
0	0.80	1.20
1	1.00	1.60
1.414	1.08	1.77

variables. The experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

Analysis of data The response surface regression (RSREG) procedure of the Statistical Analysis System software (Version 8.01, SAS Institute Inc., Cary, NC, USA) was used to fit the following second-order polynomial equation:

$$Y=\alpha_{0i}+\alpha_{1i}X_1+\alpha_{2i}X_2+\alpha_{3i}X_1^2+\alpha_{4i}X_2^2+\alpha_{5i}X_1X_2 \quad \text{[Eq. 2]}$$

where Y_i (i: 1-6) is a dependent variable, X_1 and X_2 are the dimensionless normalized independent variables, and α_1-α_5 are the coefficients obtained by RSREG of the experimental data. Multiple response optimization was heuristically calculated by the desirability function of MINITAB statistical software (Version 13, Minitab Inc., State Collage, PA, USA), to search for the condition simultaneously satisfying the 5 dependent variables (Y_1, Y_2, Y_4, Y_5, and Y_6). The response surface plots were developed using Maple software (Maple 7, Waterloo Maple Inc., ONT, Canada) and represented a function of 2 independent variables for 6 dependent variables.

Statistical analysis All experiments were analyzed with three repetitions per sample using one-way analysis of variance (ANOVA) ($p<0.05$). Means were separated using Duncan's multiple range tests (α= 0.05).

Results and Discussion

Diagnostic checking of the fitted models The RSREG procedure was performed to fit the second-order polynomial equation to the experimental data. The linear, quadratic, and interaction coefficients were calculated for significance with the t-statistic, and the estimated coefficients of each model are presented in Table 3. All the linear coefficients (X_1, X_2) of the independent variables were significant ($p<0.05$), with the exception of X_1 and X_2 in terms of Y_1 (sphericity, %). On the other hand, the quadratic (X_{11}, X_{22}) and interaction (X_{12}) coefficients of Y_1 were significant, but the other independent variables were not significant ($p<0.05$). In order to develop the fitted response surface model equations, all insignificant terms ($p<0.05$) were eliminated, and the fitted models are shown in Table 4. The response model equations of all dependent variables had high coefficients of determination (R^2), over 0.95, which indicates the model was suitable for representing the real relationships among the selected reaction parameters. The p-values of all the response models were

Table 2. Central composite design matrix and experimental results of dependent variables

No.	Independent variables[1]		Dependent variables[2]					
	X_1	X_2	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
Factorial portion								
1	-1	-1	95.5	2.76	0.212	1224	0.87	10.0
2	1	-1	97.9	2.87	0.226	1434	1.09	10.0
3	-1	1	97.4	3.07	0.237	1331	1.16	7.8
4	1	1	95.7	3.28	0.255	2260	1.37	5.0
Star portion								
5	-1.414	0	96.1	2.84	0.217	1205	0.96	10.0
6	1.414	0	98.2	3.01	0.235	2342	1.31	9.0
7	0	-1.414	94.7	2.87	0.218	1032	0.93	10.0
8	0	1.414	94.2	3.39	0.257	1802	1.29	5.9
Center portion								
9	0	0	100.0	3.03	0.235	1590	1.22	9.8
10	0	0	100.0	3.07	0.238	1579	1.19	9.7
11	0	0	99.9	3.04	0.234	1592	1.18	9.8

[1] X_1 (concentration of SA, %), X_2 (concentration of $CaCl_2$, %).

[2] Y_1 (sphericity, %), Y_2 (diameter, mm), Y_3 (membrane thickness, mm), Y_4 (rupture strength, g), Y_5 (rupturing deformation, mm), and Y_6 (sensory score, point).

Table 3. Estimated coefficients of the fitted quadratic polynomial equation for different response based on *t* statistics[1]

Responses		Intercept	X_1	X_2	X_1X_1	X_1X_2	X_2X_2
Y_1	Coefficient	99.931	0.457	-0.126	-1.185	-1.025	-2.535
	p-value	0.0001	0.099	0.602	0.007	0. 0001	0. 024
Y_2	Coefficient	3.047	0.070	0.182	0.069	0.025	0.034
	p-value	0.001	0.001	0.001	0.002	0.129	0.034
Y_3	Coefficient	0.236	0.007	0.014	0.005	0.001	0.001
	p-value	0.001	0.001	0.001	0.002	0.305	0.194
Y_4	Coefficient	1,587.007	343.392	252.761	85.022	179.750	-93.282
	p-value	0.001	0.001	0.001	0.055	0.007	0.041
Y_5	Coefficient	1.197	0.116	0.135	-0.031	-0.003	-0.043
	p-value	0.001	0.001	0.001	0.013	0.806	0.003
Y_6	Coefficient	9.767	-0.527	-1.625	-0.265	-0.7	-1.040
	p-value	0.0001	0.0224	0.0002	0.2269	0.0279	0.0029

[1] Independent variables: X_1 (concentration of SA, %), X_2 (concentration of $CaCl_2$, %); Dependent variables: Y_1 (sphericity, %), Y_2 (diameter, mm), Y_3 (membrane thickness, mm), Y_4 (rupture strength, g), Y_5 (rupturing deformation, mm), and Y_6 (sensory score, point).

Table 4. Response surface model equations for processing conditions of caviar analogs[1]

Responses	Second-order polynomial equations	R^2	p-value
Y_1	$Y_1 = 99.931 - 1.1854X_1^2 - 1.025X_1X_2 - 2.5354X_2^2$	0.9549	0.0022
Y_2	$Y_2 = 3.0466 + 0.07X_1 + 0.1819X_2 - 0.0689X_1^2 + 0.03355X_2^2$	0.9893	0.0001
Y_3	$Y_3 = 0.2356 + 0.0071X_1 + 0.0136X_2 - 0.0046X_1^2$	0.9926	0.0001
Y_4	$Y_4 = 1{,}587.0066 + 343.3916X_1 + 252.7606X_2 + 179.75X_1X_2 - 93.2821X_2^2$	0.9812	0.0003
Y_5	$Y_5 = 1.1966 + 0.1156X_1 + 0.1349X_2 - 0.0308X_1^2 - 0.0433X_2^2$	0.9930	0.0001
Y_6	$Y_6 = 9.7668 - 0.5268X_1 - 1.6249X_2 - 0.7X_1X_2 - 1.0399X_2^2$	0.9679	0.0001

[1] Independent variables: X_1 (concentration of SA, %), X_2 (concentration of $CaCl_2$, %); Dependent variables: Y_1 (sphericity, %), Y_2 (diameter, mm), Y_3 (membrane thickness, mm), Y_4 (rupture strength, g), Y_5 (rupturing deformation, mm), and Y_6 (sensory score, point).

extremely high and significant at a 95% probability level. The reason the values of R^2 were quite high is that the experimental design was based on an adequately performed preliminary test.

Analysis of variance The statistical significance of the second-order polynomial model equation was evaluated by ANOVA (analysis of variance). When a model was selected, ANOVA was calculated to assess how well the model represents the data. ANOVA values for the models of each dependent variable are presented in Table 5 and 6. The quadratic terms for all dependent variables were significant at a 95% probability level, whereas the linear term of Y_1 (sphericity) and the cross-product terms of Y_2 (diameter), Y_3 (membrane thickness), and Y_5 (rupturing deformation) were not significant ($p<0.05$). The total regression model of all dependent variables presented very low p-values under 0.01, and was significant at a 99% probability level. In the results for the lack-of-fit test, which indicates the fitness of the model, the dependent variables Y_1, Y_4, and Y_6 were significant at a 95% probability level. However, the lack-of-fit test for Y_2, Y_3, and Y_6 did not show significant p-values ($p<0.05$).

The effects of SA and $CaCl_2$ concentration on the caviar analogs In this study, the sphericity (Y_1) of the caviar analogs was selected as a dependent variable to investigate the caviar's commercial value. Sphericity has been proven to be a useful property of particles (greater than sand diameter), and measures the degree to which a particle approaches a spherical shape. It was defined by Wadell (21) as the ratio between the diameter of a sphere (with the same volume as the particle) to the diameter of the circumscribed sphere. The sphericity of the natural caviar was approximately 95%, and those of the caviar analogs in the range of independent variables showed similar values (over 94%). Overall, the caviar analogs had sphericities ranging from 90 to 100%, which were measured by the image analyzer of the microscope, and could hardly be differentiated with the naked eye. Figure 1A presents the effects of the SA (X_1) and $CaCl_2$ (X_2) concentrations on caviar analog sphericity. The sphericity decreased greatly with an increase in $CaCl_2$ concentration (X_2) from 0 (1.20%) to 1.5 (1.77%). This decrease of sphericity with an increase in $CaCl_2$ concentration was attributed to the fact that droplets in a high concentration of $CaCl_2$ would not completely agitate in the SA solution maintained under constant stirring (280 rpm).

Studies on the diameter and membrane thickness of calcium-alginate gel capsules/membranes have been

Table 5. Analysis of variance (ANOVA) for response of dependent variables: Y_1 (sphericity, %), Y_2 (diameter, mm), and Y_3 (membrane thickness, mm) [1)]

Responses	Sources	DF	SS	MS	F-value	p-value
Y_1	Model	5	43.5092	8.7018	21.20	0.0022
	Linear	2	1.8103	0.9051	2.20	0.2063
	Quadratic	2	37.4964	18.7482	45.67	0.0006
	Cross-product	1	4.2025	4.2025	10.24	0.0241
	Residual	5	2.0527	0.4105	-	-
	Lack of fit	3	2.0260	0.6753	50.65	0.0194
	Pure error	2	0.0267	0.0133	-	-
	Total	10	45.5618	-	-	-
Y_2	Model	5	0.35129	0.070258	92.43	0.0001
	Linear	2	0.304029	0.152014	199.99	0.0001
	Quadratic	2	0.044762	0.022381	29.44	0.0017
	Cross-product	1	0.0025	0.0025	3.29	0.1294
	Residual	5	0.0038	0.00076	-	-
	Lack of fit	3	0.002934	0.000978	2.26	0.3219
	Pure error	2	0.000867	0.000433	-	-
	Total	10	0.355091	-	-	-
Y_3	Model	5	0.002066	0.000413	134.91	0.0001
	Linear	2	0.001902	0.000951	310.55	0.0001
	Quadratic	2	0.00016	0.00008	26.06	0.0023
	Cross-product	1	0.000004	0.000004	1.31	0.3048
	Residual	5	0.000015	0.000003	-	-
	Lack of fit	3	0.000007	0.000002	0.51	0.7142
	Pure error	2	0.000009	0.000004	-	-
	Total	10	0.002081	-	-	-

[1)] SS, sum of squares; DF, degrees of freedom; MS, mean squares.

previously performed (6, 7). We found that the concentration of SA (X_1) had only a slight influence on the diameter (Y_2) and membrane thickness (Y_3) of the caviar analogs, and Y_2 and Y_3 had a tendency to decrease or remain constant in SA concentrations over 0 (0.8%) (Fig. 1B and C). When the cationic $CaCl_2$+CMC solution containing Ca^{2+} was dropped into the anionic SA solution, a spherical gel membrane was immediately formed around the droplet. The instantaneous diffusion of Ca^{2+} through the membrane surface resulted in a progressive build-up to form a calcium-alginate layer surrounding the core, and increased the droplet diameter and membrane thickness until the Ca^{2+} contained within the droplet was completely utilized to form cross linkages (22, 23). The amount of Na-alginate per unit volume around the droplet, as well as the binding sites for Ca^{2+}, increased with increasing SA concentration (X_1) (7, 22). An increase in SA concentration lead to the formation of a denser gel membrane, but the droplet diameter (Y_2) and membrane thickness (Y_3) did not increase significantly. On the other hand, by increasing the concentration of $CaCl_2$ (X_2), diameter (Y_2) and membrane thickness (Y_3) increased remarkably. This result can be explained by the fact that as Ca^{2+} mass per unit volume inside the liquid core increases, more Ca^{2+} binds to the alginate chains in the capsule membrane (6, 7).

In order to process caviar analogs with a texture like natural caviar, we investigated the physical properties (rupture strength and rupturing deformation) of the caviar. Both rupture strength (Y_4) and rupturing deformation (Y_5) increased with increases in SA and $CaCl_2$ concentrations. In particular, the effect of $CaCl_2$ was greater than SA (Fig. 1D and 1E). A higher concentration of cross-linkers leads to the formation of more compact and dense caviar analog membranes, which have resistance against external pressure. This result was also observed in a study on the compression intensity of calcium-alginate gel capsules by Chai *et al.* (7).

To investigate $CaCl_2$ bitterness, we evaluated the effect of its concentration on the sensory score (Y_6). Here, an increase in SA concentration did not influence the score, whereas increasing the concentration of $CaCl_2$ over 0 (1.2%) caused the caviar analog sensory score bitterness to increase greatly (Fig. 1F). The panelists, however, could not taste bitter for analogs prepared at $CaCl_2$ concentra-

Table 6. Analysis of variance (ANOVA) for response of dependent variables: Y_4 (rupture strength, g), Y_5 (rupturing deformation, mm), and Y_6 (sensory score, point)[1]

Responses	Sources	DF	SS	MS	F-value	p-value
Y_4	Model	5	1,710,738	342,148	52.15	0.0001
	Linear	2	1,454,257	727,128	110.82	0.0001
	Quadratic	2	127,241	63,620	9.7	0.0191
	Cross-product	1	129,240	129,240	19.7	0.0068
	Residual	5	32,806	6,561	-	-
	Lack of fit	3	32,708	10,903	222.51	0.0045
	Pure error	2	98	49	-	-
	Total	10	1,743,544	-	-	-
Y_5	Model	5	0.26516	0.053032	142.73	0.0001
	Linear	2	0.252509	0.126254	339.81	0.0001
	Quadratic	2	0.012627	0.006313	16.99	0.0059
	Cross-product	1	0.000025	0.000025	0.07	0.8057
	Residual	5	0.001858	0.000372	-	-
	Lack of fit	3	0.000991	0.00033	0.76	0.6104
	Pure error	2	0.000867	0.000433	-	-
	Total	10	0.267018	-	-	-
Y_6	Model	5	31.4128	6.2826	30.1	0.0014
	Linear	2	23.3394	11.6692	55.91	0.0004
	Quadratic	2	6.1135	3.0567	14.65	0.0081
	Cross-product	1	1.9600	1.9600	9.39	0.0279
	Residual	5	1.0435	0.2087	-	-
	Lack of fit	3	1.0369	0.3456	103.69	0.01
	Pure error	2	0.0067	0.0067	-	-
	Total	10	32.4564	-	-	-

[1] SS, sum of squares; DF, degrees of freedom; MS, mean squares.

tions ranging from -1.414 (0.63%) to 0 (1.20%).

When considering the 6 response surface plots, the $CaCl_2$ concentration (X_2) affected all the dependent variables more greatly than the concentration of SA (X_1), and this result can be confirmed by the coefficients of independent variables in Table 4.

Optimization for the processing of the caviar analogs The ranges of the 2 independent variables, concentrations of SA (X_1) and $CaCl_2$ (X_2), were determined by a preliminary study for the optimization of caviar analog processing. The optimal conditions included the coded and uncoded (actual) values of all the dependent variables except Y_3 (membrane thickness, mm), which are shown in Table 7. The membrane thicknesses (Y_3) of the natural caviar and the caviar analogs could not be measured with the image analyzer of the microscope because they were tinted with black. Moreover, as stated in the results for the effects of SA and $CaCl_2$ concentrations on the analogs, membrane thickness tended to increase in proportion to diameter (Y_2), rupture strength (Y_4), and rupturing deformation (Y_5). Therefore, membrane thickness (Y_3) was eliminated from the dependent variables of multiple response optimization. According to the canonical analysis of the RSREG procedure, all the eigen-values of Y_1, Y_5, and Y_6 were negative, therefore, the stationary points were maxima. However, the stationary points of Y_3 and Y_4 were saddle points. Each dependent variable was optimized individually to satisfy the natural caviar's conditions. The optimized values of SA (X_1) and $CaCl_2$ (X_2) concentration had the coded ranges of -0.162 to 0.224 (actual value: from 0.70 to 0.84%) and -0.416 to -0.071 (actual value: from 1.03 to 1.17%), respectively. To optimize the five dependent variables (Y_1, Y_2, Y_4, Y_5, and Y_6) simultaneously, we used the desirability function of MINITAB statistical software; the conditions were defined as the following: target values (Y_1 = 100%, Y_2 = 3.0 mm, Y_4 = 1,470 g, Y_5 = 1.1 mm, and Y_6 = 10 points). The coded values of the independent variables calculated by multiple response optimization were X_1 (SA, %) = -0.093 and X_2 ($CaCl_2$, %) = -0.322, respectively. The actual values against the coded values were X_1 = 0.78 and X_2 = 1.07%, respectively. The predicted values of the dependent variables under the multiple response optimal conditions for the independent

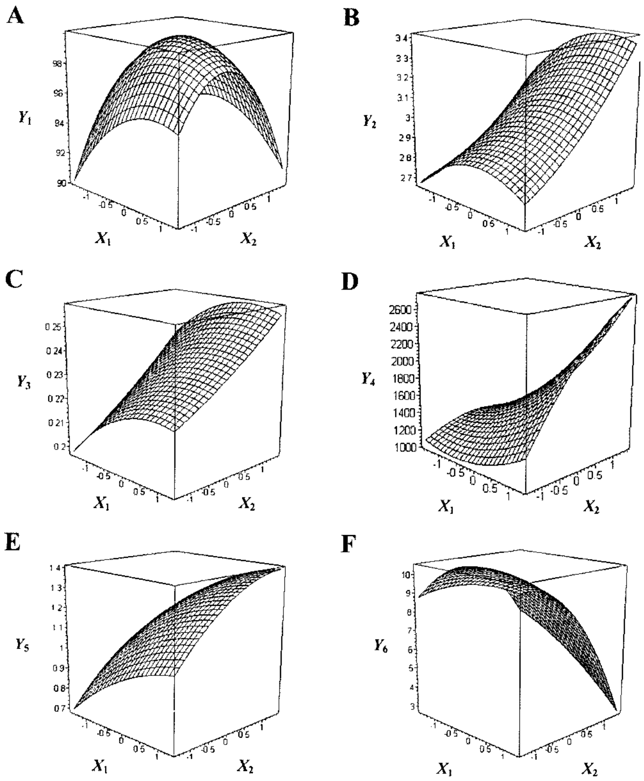

Fig. 1. Effects of X_1 (concentration of SA, %), X_2 (concentration of $CaCl_2$, %) on Y_1 (sphericity, %), Y_2 (diameter, mm), Y_3 (membrane thickness, mm), Y_4 (rupture strength, g), Y_5 (rupturing deformation, mm), and Y_6 (sensory score, point).

Table 7. Optimal conditions for the processing of caviar analogs[1)]

Dependent variables	Target value Predict value	Independent variables	Critical value	
			Coded	Uncoded
Y_1	100 %	X_1	0.224	0.84
		X_2	-0.071	1.17
Y_2	3.0 mm	X_1	-0.196	0.76
		X_2	-0.175	1.13
Y_4	1,470 g	X_1	-0.265	0.75
		X_2	-0.141	1.14
Y_5	1.1 mm	X_1	-0.265	0.75
		X_2	-0.416	1.03
Y_6	10 points	X_1	-0.162	0.70
		X_2	-0.107	1.16
Multiple response optimization				
Y_1	99.64 %	X_1	-0.093	0.78
Y_2	2.99 mm			
Y_4	1,472 g			
Y_5	1.13 mm	X_2	-0.322	1.07
Y_6	9.9 points			

[1)] Independent variables: X_1 (concentration of SA, %), X_2 (concentration of $CaCl_2$, %); Dependent variables: Y_1 (sphericity, %), Y_2 (diameter, mm), Y_4 (rupture strength, g), Y_5 (rupturing deformation, mm), and Y_6 (sensory score, point).

Table 8. Experimental and predicted results of verification under optimized conditions[1)]

Dependent variables	Predicted value	Experimental value	Natural caviar
Y_1 (%)	99.64	98.33±0.38	95.33±0.86
Y_2 (mm)	3.99	2.99±0.13	3.02±0.23
Y_4 (g)	1,472	1,474±7.51	1,470±8.41
Y_5 (mm)	1.13	1.12±0.17	1.11±0.07
Y_6 (points)	9.9	9.8±0.12	10±0.00

[1)] Optimized conditions: concentration of sodium alginate = 0.78%(w/v); Concentration of calcium chloride = 1.07%(w/v); Dependent variables: Y_1 (sphericity, %), Y_2 (diameter, mm), Y_4 (rupture strength, g), Y_5 (rupturing deformation, mm), and Y_6 (sensory score, point).

variables were Y_1 = 99.64%, Y_2 = 2.99 mm, Y_4 = 1,472 g, Y_5 = 1.13 mm, and Y_6 = 9.9 points, respectively.

Verification of predicted values Verification experiments were conducted under the optimal conditions [concentration of SA = 0.78%(w/v), concentration of $CaCl_2$ = 1.07%(w/v)] to compare the predicted and actual values of the dependent variables (Table 8). The experimental values were repeated 10 times and nearly coincided with the predicted values and satisfied the conditions of the natural caviar. Therefore, the estimated response surface model was adapted for the analog processing of natural caviar.

Acknowledgments

This research was supported in part by a Special Grants Research Program from the Ministry of Maritime Affairs and Fisheries (MOMAF), Korea. The authors gratefully acknowledge the financial support of MOMAF.

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