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Optimization of Physical Conditions for Caviar Analog Preparation Using Calcium-alginate Gel Capsules

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High prices, overfishing, and contamination have limited the availability of natural caviar as a food product. We attempted to apply encapsulation by calcium-alginate gel membranes to caviar analog preparation in an effort to produce a high-quality replacement for natural caviar. Physical conditions of stirring speed (X_1, rpm) and gelation time (X_2, min) as the independent variables for gelation were optimized by response surface methodology. Sphericity (Y_1, %), diameter (Y_2, mm), membrane thickness (Y_3, mm), rupture strength (Y_4, g), and rupturing deformation (Y_5, mm) were used as the dependent variables to compare characteristics of the capsules for caviar analogs with natural caviar. The values of the independent variables as evaluated by multiple response optimization were $X_1 = -0.1271$ (278 rpm) and $X_2 = 0.4436$ (12.2 min), respectively. Predicted values of the four dependent variables were $Y_1 = 97.7\%$, $Y_2 = 2.97$ mm, $Y_4 = 1{,}465$ g, and $Y_5 = 1.15$ mm. 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 experimental values prepared under the optimal conditions for verification nearly coincided with the predicted values and satisfied the conditions of natural caviar.

Key words: Caviar analog, Calcium-alginate gel capsule, Physical condition, Response surface methodology (RSM)

Introduction

Alginate polymer isolated from different alginate sources is widely used in food, pharmaceutical, textile, and chemical processing for its thickening, stabilizing, gel-forming, and film-forming properties. Under controlled conditions, alginate forms gels with several divalent cations (Skjåk-Bræk et al., 1989; Smidsrød and Haug, 1972), and the most important gels, particularly in food applications, are those formed with calcium ions.

Calcium-alginate gel capsules can be formed by dropping a divalent cationic solution into a solution of sodium alginate (Blandino et al., 1999; Miuraa et al., 1999). Polyvalent cations bind to the polymer whenever two neighboring guluronic acid residues are present. The egg-box model (Grant et al., 1973) is generally invoked to explain how the divalent cations, bound in the interchain cavities (essentially polyguluronate sequences), give rise to a rodlike cross-linked complex. The process of gelation, simply the exchange of calcium ions for sodium ions, is carried out under relatively mild conditions.

Encapsulation technology using calcium-alginate gel has several important advantages due to its good biocompatibility, simple operation, and low cost (Chai et al., 2004). Calcium-alginate gel capsules are often used in bioprocessing as a matrix to entrap molecules of biological significance, such as bioactive materials (Chang and Dobashi, 2003), enzymes (Blandino et al., 2001), whole microbes (Dembczynski and Jankowski, 2002), plant cells and tissues (Patel et al., 2002), and animal cells (Morre et al., 2000). They have been also investigated for utilizetion in controlled release systems (Hari et al., 1996), and may enable increased stability and controlled permeability of capsules (Gåserød et al., 1999).

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Caviar is the roe, or eggs, of fish preserved with salt (Power and Voigt, 1990). Originally, sturgeon, *Acipenser sinensis,* was the sole source of roe used in the preparation of caviar, but overfishing and degradation of spawning habitat for over a century have impacted worldwide sturgeon fisheries and caused a sharp decline in recent years (Wade and Fadel, 1997). In Russia, Iran, and the other countries surrounding the Caspian Sea, overfishing, combined with pollution, has led to rapidly declining populations of the highly prized sturgeons of the family Acipenseridae that produce most of the natural beluga, osetra, and sevruga caviars (Time, 2002). Therefore, sturgeon egg-like caviar analogs could be highly value-added products.

In the present study, we sought to apply encapsulation technology with calcium-alginate gel for caviar analog preparation. The encapsulation process was applied to the preparation of beluga-type caviar analogs. Caviar from the rare beluga sturgeon is acknowledged as superior by connoisseurs, but the beluga sturgeon is on the brink of extinction. It is so rare that the annual beluga catch does not exceed 100 units (Williot et al., 2001). As a result, availability problems, soaring prices, and unreliable quality are now associated with Caspian caviars. Therefore, encapsulation using calcium-alginate gel to produce caviar analogs could be an important food processing technology.

This study was designed to optimize the physical conditions of calcium-alginate gel capsules for caviar analog (beluga-type) preparation using response surface methodology (RSM), as well as to compare the mechanical properties and appearance of caviar analogs with those of natural caviar. For the processing of caviar analogs with calcium-alginate gel capsules, physical conditions of stirring speed and gelation time were the main parameters as well as the independent variables in the experimental design. To prepare analogs with similar appearance and physical properties to natural caviar, sphericity, diameter, and membrane thickness of the analogs were examined using an image analyzer, and rupture strength and rupturing deformation were assessed with a rheometer.

Materials and Methods

Materials

Sodium alginate (SA), calcium chloride ($CaCl_2$), and carboxymethyl cellulose (CMC), the major materials for caviar analog preparation, were purchased from Kimitsu Chemical Industries Co., Ltd., Tokyo, Japan; Nichia Corporation, Tokyo, Japan; and Katayama Chemical Industries Co., Kobe, Japan, respectively. According to the manufacturer's specifications, the viscosity of 1% (w/v) SA solution ranged from 350 to 400 cps, and the ratio of D-mannuronic acid to L-guluronic acid was 0.9. Squid ink powder was provided by BioAdd Co., Ltd. (Busan, Korea). All reagents used in this study were analytical grade.

Preparation of calcium-alginate gel capsules for caviar analogs

The calcium-alginate gel capsules were processed by allowing droplets of a complex solution containing $CaCl_2$ and CMC to disperse into the SA solution. The anionic SA solution (1%, 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 left undisturbed for 30 min to eliminate air bubbles that could later disturb formation of the capsules. To prepare the cationic complex solution containing $CaCl_2$ and CMC, CMC was dissolved in $CaCl_2$ solution (1%, w/v) to a 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 analogs. In preliminary work for 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 cps at 20°C.

The viscous complex solution containing $CaCl_2$ and CMC was formed into droplets using a 21G metal injection needle (0.52 mm internal diameter, 0.82 mm outside diameter, and 32 mm length) with a peristaltic pump (Casette tube pump SMP-23; Eyela, Tokyo, Japan) (Fig. 1). A dropping height of 10 cm was used to form spherical droplets. The SA solution was constantly stirred by a magnetic bar situated at the bottom of the vessel at a fixed dropping velocity (0.05 mL/s) to keep the droplets from sticking together and to minimize the external mass transfer resistance. The cationic droplets containing $CaCl_2$ and CMC were coated by the anionic solution containing SA and squid ink. An interfacial gel membrane formed instantaneously around each droplet due to the cross-linking of the interfacial alginate molecules by the calcium cations (Blandino et al., 2001). The droplets encapsulated by the SA solution were rinsed several times with distilled water. To stabilize and harden the gel membrane, the capsules were transferred to a 2% (w/v) $CaCl_2$ solution and soaked to mature for 20 min. Finally, the

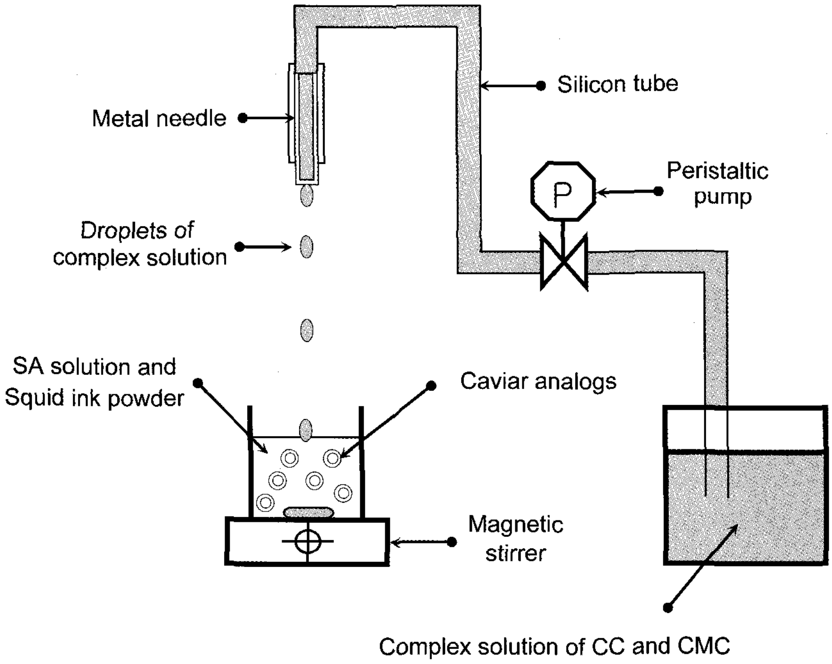

Fig. 1. Simplified schematic diagram of caviar analog preparation. The processing conditions are the followings: 21G metal injection needle (internal diameter of 0.52 mm, outside diameter of 0.82 mm, and length of 32 mm) and pump tube (internal diameter of 2.15 mm, outside diameter of 4.20 mm). CC, calcium chloride; CMC, carboxymethylcellulose; SA, sodium alginate.

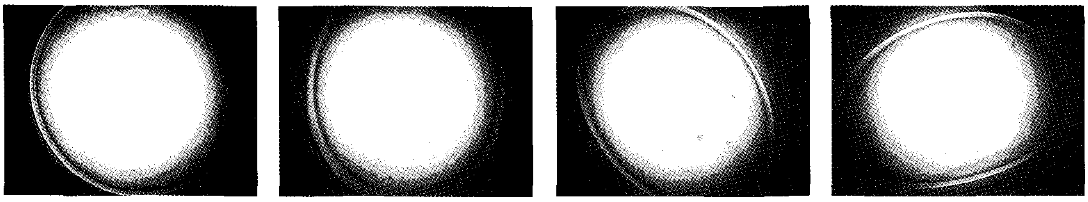
(a) 100% (b) 90% (c) 80% (d) 70%

Fig. 2. Microscopic photographs for determination of sphericity of the capsules for caviar analogs. (a)-(d) are standard photographs representing sphericity of 100-70%. These were produced at ×40 magnification. The membrane diameter was measured by an image analyzer (Image-Pro Plus, Media Sybernetics, Inc., USA) coupled to a microscope (B202 optical microscope, Olympus, Tokyo, Japan).

capsules were rinsed again with distilled water to remove any remaining $CaCl_2$. All of the above procedures were carried out at ambient temperature.

Determination of diameter, membrane thickness, and sphericity

The membrane diameter and thickness of the capsules were measured by an image analyzer (Image-Pro Plus; Media Cybernetics, Inc., Silver Spring, MD, USA) coupled to an optical microscope (B202; Olympus, Tokyo, Japan). The membrane thicknesses of the capsules for caviar analogs and natural caviars could not be measured due to their black color. Thus, the thickness measurements of the analogs were performed with hollow capsules prepared without squid ink powder. The image analysis of the capsules was produced at ×40 magnification. Ten capsules randomly sampled from each experimental condition were used for measurement. The sphericity, which measures the degree to which the analog approaches a spherical shape, was determined by the two linear dimensions (longest and shortest diameters) of the percent ratio of the minor diameter to the major diameter of the capsules (Eq. 1).

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

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 capsules 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) under 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.

Experimental design

A central composite design (CCD) was adopted for optimization of the physical conditions for caviar analog processing (Fig. 3). In the experimental design, CCD consisted of 2^2 factorial points, four axial points (α=1.414), and three replicates of the central point (Tables 1 and 2) (Box and Wilson, 1951). The stirring speed (X_1, rpm) and gelation time (X_2, min) were chosen as the independent variables. The range and center point values of the two independent variables were based on the results of preliminary experiments (Table 1). To compare the characteristics of the caviar analogs with those of natural caviar, responses of sphericity (Y_1, %), diameter (Y_2, mm), membrane thickness (Y_3, mm), rupture strength (Y_4, g), and rupturing deformation (Y_5, mm) were chosen as dependent variables. The experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

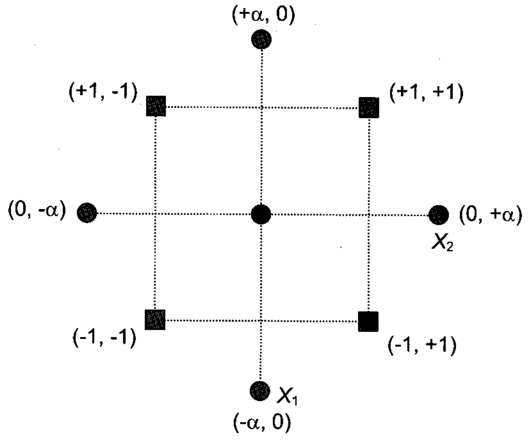

Fig. 3. Central composite design (CCD) for two independent variables at two levels.
■, Factorial points; ●, Axial points, ●, Central point.

Table 1. Experimental range and values of independent variables in central composite design for the physically optimal conditions of calcium-alginate gel capsules for caviar analog preparation

Coded levels	Independent variables	
	X_1 (stirring speed, rpm)	X_2 (gelation time, min)
-1.414	259	3
-1	265	5
0	280	10
1	295	15
1.414	301	17

Data analysis

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

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

where Y_i (i: 1 to 5) is a dependent variable, X_1 and X_2 are the dimensionless normalized independent variables, and α_1 to 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 College, PA, USA) to search for the condition simultaneously satisfying four dependent variables (Y_1, Y_2, Y_4, and Y_5; Y_3 was not included). The response surface plots were developed using Maple software (Maple 7; Waterloo Maple Inc., Waterloo, ON, Canada), and represented a function of two independent variables for five 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

Dropping height and velocity

The capsules for caviar analogs were prepared by allowing droplets of a complex solution containing $CaCl_2$ and CMC to disperse into the SA solution containing squid ink powder. In this process, the characteristics of the droplets depend on the internal diameter of the injection needle, dropping height, and velocity of the $CaCl_2$ and CMC complex solution

(Chai et al., 2004). Generally, the droplet size increases with increased needle size. A 0.52-mm internal diameter needle was selected to prepare capsules having a diameter of about 3 mm (data not shown). The effects of dropping height and velocity on properties of the capsules are shown in Tables 3 and 4, respectively.

The dropping height did not affect any characteristics of the caviar analogs except sphericity. Higher dropping height caused the capsules to have poor sphericity, and a dropping height of 12 cm was determined as the optimum condition to prepare capsules of good sphericity. The effects of the dropping velocity on the properties of the capsules were greater than those of the dropping height. As the dropping velocity (mL/s) increased, the diameter and membrane thickness of the capsules decreased. Rupture strength and rupturing deformation, which indicate the texture characteristics of the caviar analogs, also decreased. However, the sphericity of the analogs slightly increased. Chai et al. (2004) reported that a higher dropping velocity induces smaller droplets and thus smaller capsules when the needle diameter is fixed. The higher dropping velocity is adapted to prepare spherical capsules for caviar analogs; however, considering the other properties, a dropping velocity of 0.05 mL/s was determined as the most suitable condition.

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

No.	IVs		DVs				
	X_1	X_2	Y_1	Y_2	Y_3	Y_4	Y_5
Factorial portion							
1	-1	-1	91.2	2.45	0.186	1195	0.86
2	1	-1	83.6	2.4	0.179	1159	0.81
3	-1	1	90.2	3.12	0.232	1358	1.06
4	1	1	84.1	3.09	0.229	1371	1.19
Star portion							
5	-1.414	0	91.6	2.56	0.196	1213	0.99
6	1.414	0	75.6	2.71	0.203	1252	0.94
7	0	-1.414	94.2	2.12	0.161	1112	0.77
8	0	1.414	97.1	3.15	0.236	1499	1.25
Center portion							
9	0	0	97.5	2.88	0.220	1411	1.09
10	0	0	96.8	2.79	0.225	1424	1.08
11	0	0	97.7	2.84	0.222	1450	1.11

IVs (independent variables): X_1 (stirring speed, rpm), X_2 (gelation time, min).
DVs (dependent variables): Y_1 (sphericity, %), Y_2 (diameter, mm), Y_3 (membrane thickness, mm), Y_4 (rupture strength, g), Y_5 (rupturing deformation, mm).

Table 3. Changes in properties of the capsules for caviar analogs as affected by dropping height

Properties	Dropping height (cm)				
	4	8	12	20	30
Sphericity (%)	95.7 ± 0.88^{a}	96.5 ± 0.76^{a}	95.8 ± 0.86^{a}	88.2 ± 0.87^{b}	77.5 ± 0.91^{c}
Diameter (mm)	2.76 ± 0.05^{a}	2.74 ± 0.03^{a}	2.73 ± 0.03^{a}	2.73 ± 0.05^{a}	2.72 ± 0.04^{a}
Membrane thickness (mm)	0.218 ± 0.003^{a}	0.220 ± 0.006^{a}	0.221 ± 0.007^{a}	0.220 ± 0.003^{a}	0.220 ± 0.005^{a}
Rupture strength (g)	$1,431\pm10.5^{a}$	$1,439\pm9.7^{a}$	$1,434\pm10.1^{a}$	$1,430\pm11.7^{a}$	$1,432\pm12.3^{a}$
Rupturing deformation (mm)	1.10 ± 0.03^{a}	1.12 ± 0.02^{a}	1.12 ± 0.04^{a}	1.11 ± 0.02^{a}	1.12 ± 0.03^{a}

Different letters (a, b, c) indicate significant differences at an α level of 0.05.

Table 4. Changes in properties of the capsules for caviar analogs as affected by dropping velocity

Properties	Dropping velocity (mL/s)				
	0.03	0.04	0.05	0.06	0.07
Sphericity (%)	92.7 ± 0.92^{a}	93.5 ± 0.79^{a}	95.2 ± 0.84^{b}	95.8 ± 0.96^{b}	97.2 ± 0.93^{c}
Diameter (mm)	2.85 ± 0.03^{a}	2.79 ± 0.02^{b}	2.72 ± 0.02^{c}	2.68 ± 0.03^{c}	2.61 ± 0.04^{d}
Membrane thickness (mm)	0.227 ± 0.003^{a}	0.224 ± 0.003^{a}	0.221 ± 0.002^{b}	0.215 ± 0.003^{c}	0.211 ± 0.002^{d}
Rupture strength (g)	$1,469\pm10.5^{a}$	$1,462\pm9.7^{a}$	$1,449\pm8.1^{b}$	$1,445\pm8.7^{b}$	$1,433\pm9.3^{c}$
Rupturing deformation (mm)	1.15 ± 0.03^{a}	1.14 ± 0.02^{a}	1.10 ± 0.03^{b}	1.11 ± 0.02^{b}	1.06 ± 0.03^{c}

Different letters (a, b, c) indicate significant differences at an α level of 0.05.

Diagnostic checking of the fitted models

The RSREG procedure was performed to fit a 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 5. The linear coefficient X_1 (stirring speed) of the independent variables was not significant ($P>0.05$), with the exception of X_1 in terms of Y_1 (sphericity, %). However, the linear coefficient X_2 (gelation time) was significant ($P<0.05$), with the exception of X_2 in terms of Y_1 (sphericity, %). The quadratic coefficients, X_1^2 and X_2^2, showed no significance at Y_2 (diameter) and Y_3 (membrane thickness), and Y_1 and Y_2, respectively. All nonsignificant terms ($P>0.05$) were eliminated to develop the fitted response surface model equation, and the fitted models are shown in T 6. The response model equations of all dependent variables had high coefficients of determination ($R^2>0.95$), 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 extremely high and significant at a 99% probability level. The reason the values of R^2 were so 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. When a model was selected, the ANOVA was calculated to assess how well the model represents the data. ANOVA values for the models of all dependent variables are presented in Table 7 (Y_1, Y_2, and Y_3) and 8 (Y_4 and Y_5). The total regression model of all dependent variables presented very low *P*-values ($P<0.01$) and was significant at the 99% probability level. The linear terms (X_1 and X_2) for all dependent variables were significant at the 95% probability level, with the exception of X_1 and X_2 in terms of Y_2. In contrast, the *P*-values of all cross-product terms (X_{12}) were very high and not significant, with the exception of Y_5. In the results for the lack-of-fit test, which indicates the fitness of the model, Y_1 was not significant at the 95% probability level. However, the lack-of-fit test for other dependent variables showed significant *P*-values ($P<0.05$).

Table 5. Estimated coefficients of the fitted quadratic polynomial equation for different response based on *t*-statistic

Responses		Intercept	X_1	X_2	X_1X_1	X_1X_2	X_2X_2
Y_1	Coefficient	97.3333	-4.5409	0.4501	-7.4541	0.3750	-1.4291
	P-value	0.0001	0.0021	0.5871	0.0005	0.7465	0.1826
Y_2	Coefficient	2.8366	0.0165	0.3520	-0.0683	0.0050	-0.0683
	P-value	0.0001	0.6591	0.0002	0.1643	0.9240	0.1643
Y_3	Coefficient	0.2223	-0.00001	0.02525	-0.0095	0.0010	-0.0100
	P-value	0.0001	0.9956	0.001	0.0139	0.7571	0.0114
Y_4	Coefficient	1,428.33	4.0192	115.2876	-97.4794	12.2500	-60.9792
	P-value	0.0001	0.7405	0.0002	0.0008	0.4844	0.0066
Y_5	Coefficient	1.0933	0.0011	0.1573	-0.0660	0.04500	-0.0435
	P-value	0.0001	0.9183	0.0001	0.0036	0.0317	0.0193

IVs: X_1 (stirring speed, rpm), X_2 (gelation time, min).

DVs: Y_1 (sphericity, %), Y_2 (diameter, mm), Y_3 (membrane thickness, mm), Y_4 (rupture strength, g), Y_5 (rupturing deformation, mm).

Table 6. Response surface model equations for the physically optimal conditions of calcium-alginate gel capsules for caviar analog preparation

Responses	Second-order polynomial equations	R^2	*P*-value
Y_1	$Y_1 = 97.3333 - 4.5409X_1 - 7.4541X_1^2$	0.9526	0.003
Y_2	$Y_2 = 2.8366 + 0.3520X_2$	0.9542	0.002
Y_3	$Y_3 = 0.2223 + 0.0252X_2 - 0.0095X_1^2 - 0.01X_2^2$	0.9695	0.001
Y_4	$Y_4 = 1428.33 + 115.28X_2 - 97.4794X_1^2 - 60.9762X_2^2$	0.9694	0.001
Y_5	$Y_5 = 1.0933 - 0.1573X_2 - 0.1573X_1^2 + 0.045X_1X_2 - 0.0435X_2^2$	0.9806	0.001

IVs: X_1 (stirring speed, rpm), X_2 (gelation time, min).

DVs: Y_1 (sphericity, %), Y_2 (diameter, mm), Y_3 (membrane thickness, mm), Y_4 (rupture strength, g), Y_5 (rupturing deformation, mm).

Table 7. Analysis of variance (ANOVA) for response of dependent variables, Y_1 (sphericity, %), Y_2 (diameter, mm), Y_3 (membrane thickness, mm)

Responses	Sources	DF	SS	MS	*F*-value	*P*-value
Y_1	Model	5	484.521	96.904	20.10	0.003
	Linear	2	166.58	157.641	32.71	0.001
	Quadratic	2	317.378	158.689	32.92	0.001
	Cross-product	1	0.562	0.562	0.12	0.747
	Residual	5	24.100	4.820	–	–
	Lack of fit	3	23.654	7.885	35.30	0.028
	Pure error	2	0.447	0.223	–	–
	Total	10	508.622	–	–	–
Y_2	Model	5	1.03472	0.206943	20.82	0.002
	Linear	2	0.99386	0.013173	1.33	0.345
	Quadratic	2	0.04075	0.020376	2.05	0.224
	Cross-product	1	0.00010	0.000100	0.01	0.924
	Residual	5	0.04970	0.009940	–	–
	Lack of fit	3	0.04564	0.015212	7.48	0.120
	Pure error	2	0.00407	0.002033	–	–
	Total	10	1.08442	–	–	–
Y_3	Model	5	0.005946	0.001189	31.74	0.001
	Linear	2	0.005104	0.000257	6.87	0.037
	Quadratic	2	0.000838	0.000419	11.18	0.014
	Cross-product	1	0.000004	0.000004	0.11	0.757
	Residual	5	0.000187	0.000037	–	–
	Lack of fit	3	0.000175	0.000058	9.19	0.100
	Pure error	2	0.000013	0.000006	–	–
	Total	10	0.006133	–	–	–

SS, sum of squares; DF, degrees of freedom; MS, mean square.

Table 8. Analysis of variance (ANOVA) for response of dependent variables, Y4 (rupture strength, g), Y5 (rupturing deformation, mm)

Responses	Sources	DF	SS	MS	*F*-value	*P*-value
Y_4	Model	5	167172	33434.3	31.73	0.001
	Linear	2	106459	27020.3	25.64	0.002
	Quadratic	2	60112	30056.2	28.52	0.002
	Cross-product	1	600	600.2	0.57	0.484
	Residual	5	5269	1053.8	–	–
	Lack of fit	3	4481	1493.5	3.79	0.216
	Pure error	2	789	394.3	–	–
	Total	10	172441	–	–	–
Y_5	Model	5	0.234415	0.046883	50.53	0.001
	Linear	2	0.198090	0.016136	17.39	0.006
	Quadratic	2	0.028225	0.014113	15.21	0.007
	Cross-product	1	0.008100	0.008100	8.73	0.032
	Residual	5	0.004639	0.000928	–	–
	Lack of fit	3	0.004172	0.001391	5.96	0.147
	Pure error	2	0.000467	0.000233	–	–
	Total	10	0.239055	–	–	–

SS, sum of squares; DF, degrees of freedom; MS, mean square.

Response surface plots and the effects of stirring speed and gelation time

The five three-dimensional response surface plots in Fig. 4 present the estimated response function and the effects of stirring speed (X_1) and gelation time (X_2) on the characteristics (Y_1, Y_2, Y_3, Y_4, and Y_5) of the capsules. In this study, the sphericity (Y_1) of the capsules was chosen as a dependent variable to examine their commercial value. Fig. 4a presents the effects of stirring speed (X_1) and gelation time (X_2) on sphericity. The sphericity of the natural caviar was approximately 95%, and the capsules for caviar analogs showed a range of 75.6 to 97.7%. Gelation time (X_2) did not significantly influence the sphericity of the capsules. However, the effect of stirring speed

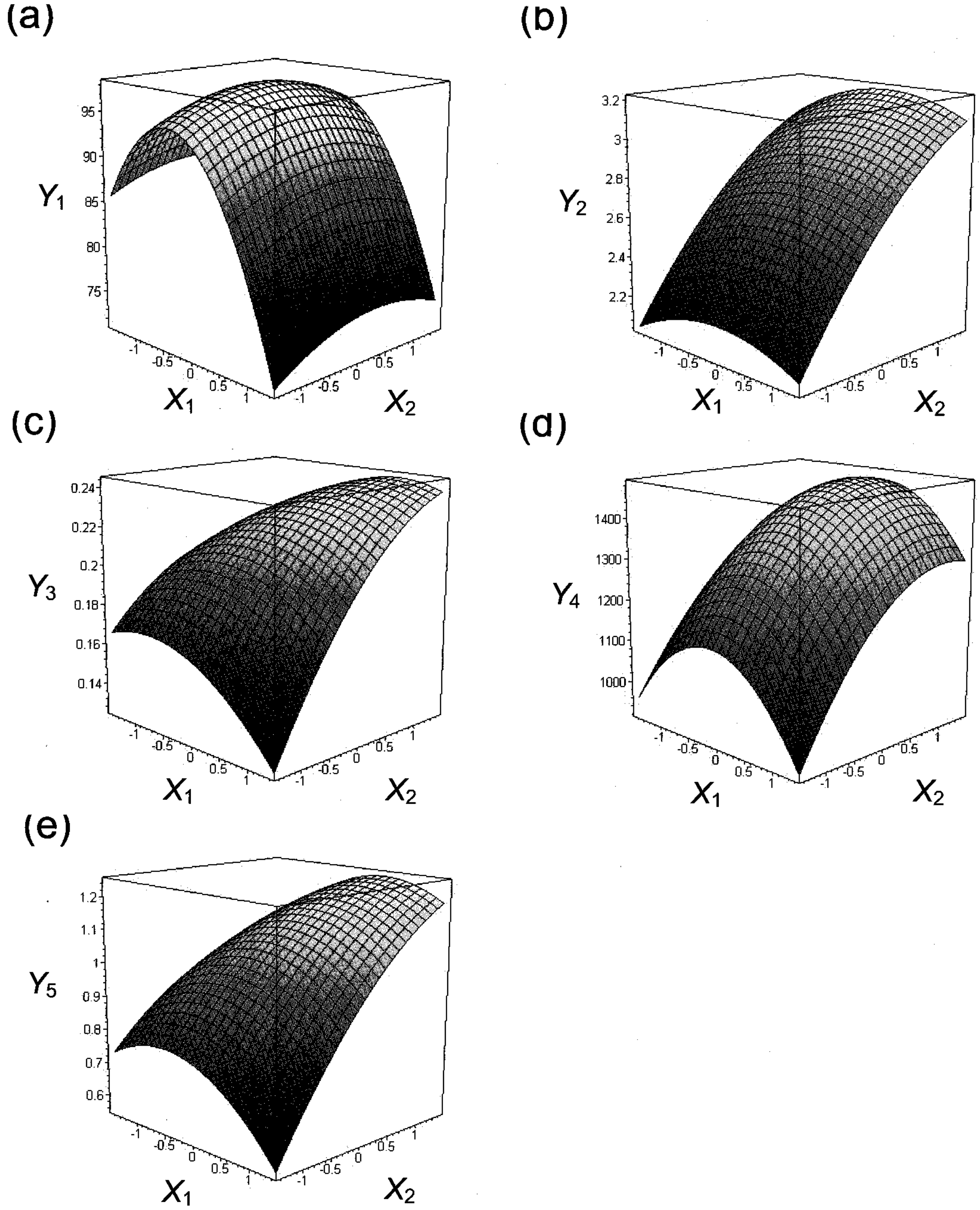

Fig. 4. Effects of X_1 (stirring speed, rpm) and X_2 (gelation time, min) on the Y_1 (sphericity, %), Y_2 (diameter, mm), Y_3 (membrane thickness, mm), Y_4 (rupture strength, g), and Y_5 (rupturing deformation, mm).

(X_1) was quite great, and sphericity greatly decreased in the range of 0 to 1.414 (280-301 rpm). This decrease was attributable to stirring speeds in excess of 280 rpm. The effects of stirring speed (X_1) and gelation time (X_2) on the diameter and membrane thickness of the capsules are shown in Fig. 4b and c. Stirring speed (X_1) had a very small influence on diameter (Y_2) and membrane thickness (Y_3). Diameter and membrane thickness increased with increases of gelation time (X_2). As our previous investigation indicated, they initially (until 20 min) increased and gradually stabilized (20-60 min) at a maximum value (data not shown). This result can be explained by the transport of Ca^{2+} in the capsules. When the cationic $CaCl_2$ and CMC solution containing Ca^{2+} is dropped into the anionic SA solution, all binding sites present in Na-alginate are unoccupied. Accordingly, Ca^{2+} can cross-link rapidly with the polymer, and the diameter and membrane thickness increase sharply. Instantaneous diffusion of Ca^{2+} through the surface of the membrane results in progressive buildup to form a calcium-alginate layer surrounding the core. This increases the diameter of the droplet and membrane thickness until Ca^{2+} contained within the droplet is completely utilized to form cross-linkage (Grant et al., 1973). However, once the capsule membrane is formed, Ca^{2+} must diffuse through the gel film to react with unoccupied binding sites on the Na-alginate chains. Furthermore, Ca^{2+} faces greater resistance with increasing membrane thickness (Chai et al., 2004).

Physical properties such as rupture strength and rupturing deformation were investigated to prepare caviar analogs with texture characteristics similar to those of natural caviar. Rupture strength (Y_4) and rupturing deformation (Y_5) were influenced by both stirring speed (X_1) and gelation time (X_2) in contrast to the other Ivs [Independent Variables]. Because diameter and membrane thickness of the capsules increase with increasing gelation time, the effect of gelation time (X_2) was greater than that of stirring speed (X_1).

Optimization of physical conditions for gelation

The membrane thicknesses of both the natural caviar and the capsules for caviar analogs could not be measured with the image analyzer of the microscope because of their black color, so colorless capsules were prepared for this purpose. The membrane thickness (Y_3) tended to increase in proportion to diameter (Y_2). Thus, the membrane thickness was eliminated from the dependent variables used for multiple response optimizations. The range values of stirring speed (X_1, rpm) and gelation time (X_2, min) were determined by our preliminary investigations for optimizing the preparation of caviar analogs. Table 9 presents the optimal conditions (coded and uncoded values) for the dependent variables (Y_1, Y_2, Y_4, and Y_5). Individual optimizations for Y_1, Y_2, Y_4, and Y_5 to satisfy the conditions of natural caviar were performed using MINITAB statistical software. The optimal values of X_1 (stirring speed, rpm) and X_2 (gelation time, min) had the coded range of -0.0053 to 0.6597 (actual values, 279-289 rpm) and 0.1630 to 5148 (actual values, 10.8-12.6 min), respectively. To optimize four dependent variables (Y_1, Y_2, Y_4, and Y_5) simultaneously, the desirability function of MINITAB statistical software was defined as follows: target values (Y_1=100%, Y_2=3.0 mm, Y_4=1,470 g, and Y_5=1.1 mm). Optimal conditions of X_1 (stirring speed, rpm) and X_2 (gelation time, min) estimated by multiple response optimization, were -0.1272 (278 rpm) and 0.4436 (12.2 min), respectively. Predicted values of the dependent variables (Y_1, Y_2, Y_4, and Y_5) under optimal conditions were Y_1=97.7%, Y_2=2.97 mm, Y_4=1,465 g, and Y_5=1.15 mm.

Table 9. Physically optimal conditions of calcium-alginate gel capsules for caviar analogs

Dependent variables	Target value Predict value	Independent variables	Critical value	
			Coded	Uncoded
Y_1	100 %	X_1 X_2	-0.0053 0.1630	279 10.8
Y_2	3.00 mm	X_1 X_2	0.0097 0.5148	280 12.6
Y_4	1,470 g	X_1 X_2	0.0087 0.4853	280 12.4
Y_5	1.10 mm	X_1 X_2	0.6597 0.1940	289 11.0
Multiple response optimization				
Y_1 Y_2	97.7% 2.97 mm	X_1	-0.1272	278
Y_4 Y_5	1,465 g 1.15 mm	X_2	0.4436	12.2

IVs: X_1 (stirring speed, rpm), X_2 (gelation time, min).
DVs: Y_1 (sphericity, %), Y_2 (diameter, mm), Y_4 (rupture strength, g), Y_5 (rupturing deformation, mm).

Verification of predicted values

Verification experiments were conducted under the optimal conditions (stirring speed=278 rpm, gelation time=12.2 min) to compare the predicted and actual values of the dependent variables (Table 10). The experimental values were repeated ten times and nearly coincided with the predicted values and satisfied the conditions of natural caviar. Therefore, the estimated response surface model was adapted to prepare the caviar analogs.

Table 10. Experimental and predicted results of verification under the optimal conditions

DVs	Predicted value	Experimental value	Natural caviar
Y_1 (%)	97.7	96.88 ± 1.54	95.33 ± 0.86
Y_2 (mm)	2.97	2.95 ± 0.09	3.02 ± 0.23
Y_4 (g)	1,465	1,461 ± 10.92	1,470 ± 8.41
Y_5 (mm)	1.15	1.14 ± 0.06	1.11 ± 0.07

Optimal conditions: stirring speed (X_1) = 278 rpm, gelation time (X_2) = 12.2 min.
DVs: Y_1 (sphericity, %), Y_2 (diameter, mm), Y_4 (rupture strength, g), Y_5 (rupturing deformation, mm).

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