Thesis for the Degree of Master of Engineering

Development of Flying Fish Roe Analogs and Their Physical Properties

by

Eun-Hee Jo

Department of Food Science & Technology

The Graduate School

Pukyong National University

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날치알 성형물의 개발 및 물리적 특성 해석

Advisor: Prof. Seon-Bong Kim

by

Eun-Hee Jo

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Eun-Hee Jo

Approved by:

(Chairman) Ji-Young Yang, Ph.D.

(Member) Dong-Hyun Ahn, Ph.D.

(Member) Seon-Bong Kim, Ph.D.

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부경대학교 대학원 식품공학과

요 약

본 연구는 이중노즐이 장착된 장치를 이용한 캡슐형의 날치알 성형물로부터 반응표면분석이라는 통계기법을 이용하여 제조 조건을 최적화하고 다양한 물리화학적 처리 효과를 알아보는데 그 목적이 있다. 제조 조건의 최적화에는 중심합성계획법이 사용되었으며 독립변수로는 알긴산나트륨의 농도 (X_1), 염화칼슘의 농도 (X_2), 반응조의 교반속도 (X_3) 및 낙하높이 (X_4)로 설정하였으며, 종속변수로는 날치알 성형물의 구형성능 (Y)이 선택되었다. 반응최적화에 의해 산출된 독립변수의 최적화 조건은 알긴산나트륨의 농도 1.66%, 염화칼슘의 농도 1.86%, 반응조의 교반속도 280 rpm과 낙하높이 17 cm로 나타났으며, 예상되는 날치알 성형물의 구형성능은 99.7%로 실제 제조한 결과 99.8±0.77%와 큰 차이를 보이지 않았다. 최적 조건에서 제조된 날치알 성형물의 다양한 물리화학적 처리

효과를 알아보기 위해 직경 (mm), 구형성능 (%), 파열강도 (kPa) 및 무게 (g)를 측정하였으며, 물리화학적 처리로는 열탕, 식염, 고압멸균 및 동결-해동처리를 실시하였다. 날치알 성형물의 물리적 특성을 알아보기 위해 측정한 직경, 구형성능, 파열강도와 무게를 물리화학적 처리 이전에 제조한 날치알 성형물의 물리적 특성과 비교한 결과, 95℃ 열탕 처리 이후 직경과 무게는 처리 시간 (0, 20, 40, 60, 및 80분)에 따라 그 값이 감소하였으며, 파열강도는 처리 시간 40분까지 증가하고 그 이후에 감소하였으며, 구형성능에서는 유의적인 차이를 나타내지 않았다. 0.5% 염화칼슘 열탕처리는 직경과 무게에서 처리 시간 (0, 5, 10, 15, 및 20분)에 따라 그 값이 감소하였으며, 파열강도는 처리 시간 15분까지 증가하고 그 이후에 감소하였으며, 구형성능에서는 초기 처리 시간 5분 동안 구형성능이 크게 감소하고 그 이상의 처리 시간에서는 구형성능이 떨어지지 않는 것으로 나타났다. 다양한 농도 (0, 0.5, 1.0, 1.5, 및 2.0%)에 따른 염화나트륨 처리는 10분의 침지 시간 이후 구형성능과 파열강도에서 농도 변화에 따라 그 값이 유의적으로 감소하였으며, 직경과 무게에서는 큰 차이를 나타내지 않았다. 고압멸균 (121℃, 1 atm) 처리는 직경, 구형성능, 파열강도와 무게에서 초기 처리 시간 5분 동안의 물리적 변화가 크게 나타났으며, 그 이상의

처리 시간에서는 변화 정도가 적게 나타났다. 반면에 구형성능은 처리 시간 (0, 5, 10, 15, 및 20분) 에 따라 꾸준히 감소하는 것으로 나타났다. 마지막으로, 5일 간의 날치알 성형물의 동결-해동 처리는 직경, 구형성능, 파열강도와 무게에서 현저한 감소를 나타내었다.

Introduction

Natural fish roes such as salmon roe, caviar, and flying fish roe and so on are decreasing in the production owing to marine pollution, overfishing, destruction of spawning grounds, and climate changes (Jackson et al., 2006). The supply of natural fish roe cannot meet the demand, so the demand to fish roe analogs is increasing on the market. Fish roe analogs are regarded as a high value added processing technology that enables a stable supply of goods, hygienic quality control, and development of various favorite foods. Encapsulation technology is applying for the production of fish roe analogs.

Encapsulation refers to the technology processing as bead or capsule types by entrapping active compounds in polymer matrixes, which are form of particulate (Lee et al., 2013). The size of the capsule can range from micrometers to millimeters and even if the ideal shape is spherical, the shape is greatly affected by the structure of the original material prior to encapsulation (Cho et al., 1997). The encapsulation technology is widely used for immobilizing, stabilizing, and controlling release of active compounds (Chan et al., 2009).

Alginic acid has been widely used as polymer materials for encapsulation. Alginic acid, which is a polysaccharide obtained from a brown algae, is a linear block copolymer in which β-D-mannuronate (M) and α-L-guluronate (G) are linked through a β-1,4 or a α-1,4 glycosidic bond (Pielesz et al., 2008; Gryshkov et al., 2014). It is capable of forming gel in a relatively mild condition in the presence of cations (Vandenberg et al., 2001). The gel is formed by an ionic interaction between a multivalent cations and G residues from alginate chain, and the mechanism can be explained by the 'egg-box' model (Hoad et al., 2011). The gel formation proceeds by cross-linkages of a three-dimensional structure between alginate and calcium ions when calcium ions are added to an alginate aqueous solution (Clark & Ross-Murphy, 1987; Rousseau et al., 2004).

The properties of alginate gels are strongly dependent on the ratio of uronic monomers, sequence, cation concentration in the reaction solution, and curing time (SmidsrØd., 1974; Draget et al., 1994; Velings. & Mestdagh., 1995; Ouwerx et al., 1998). In the food industry, alginate beads or capsules can be formed through the gelation reaction. Alginates are also used as a material for encapsulation in a variety of fields including bioprocess, pharmacology, and food and feed industries (Vandenberg et al., 2001; Pielesz et al., 2008; Chan et al., 2011; Gryshkov et al., 2014). Alginate gel is also used not only in immobilized cells, enzymes, and drug carriers of protein but also in the drug release control system due to the properties such as being an ingredient of natural origin, biocompatibility, and relatively low cost of production (Pielesz et al., 2008).

The production of fish roe analogs using alginate gel was often influenced on their physical properties such as size, sphericity, rupture strength etc. according to processing conditions. Ji et al. (2007) and Woo et al. (2007) reported on the sphericity optimization of beads using alginate gels and on the production of caviar analogs using calcium alginate gels with single nozzle. Furthermore, Jo et al. (2014) reported on the physical properties of the flying fish roe analogs such as size, sphericity, and rupture strength.

The present study aims to optimize sphericity for production of flying fish roe analogs with response surface methodology (RSM) analysis and then to explain physicochemical characteristics of flying fish roe analogs on boiling water, calcium chloride, sodium chloride, autoclaving, and freeze-thaw treatments for their processing quality.

Materials and Methods

1. Materials

Sodium alginate and calcium chloride as main materials for flying fish roe analogs manufacture were purchased from Junsei Chemical Corporation (Tokyo, Japan). To inquire physical quality of salinity processing, sodium chloride was also purchased from Sigma Chemical Corporation (Steinheim, Germany). All chemicals and reagents used in this study were analytical grade.

2. Methods

2.1 Manufacturing process of the flying fish roe analogs with capsule type

The flying fish roe analogs with capsule type were manufactured as shown in Fig. 1. Sodium alginate solution and soybean oil were dropped into calcium chloride solution through the corresponding nozzles.

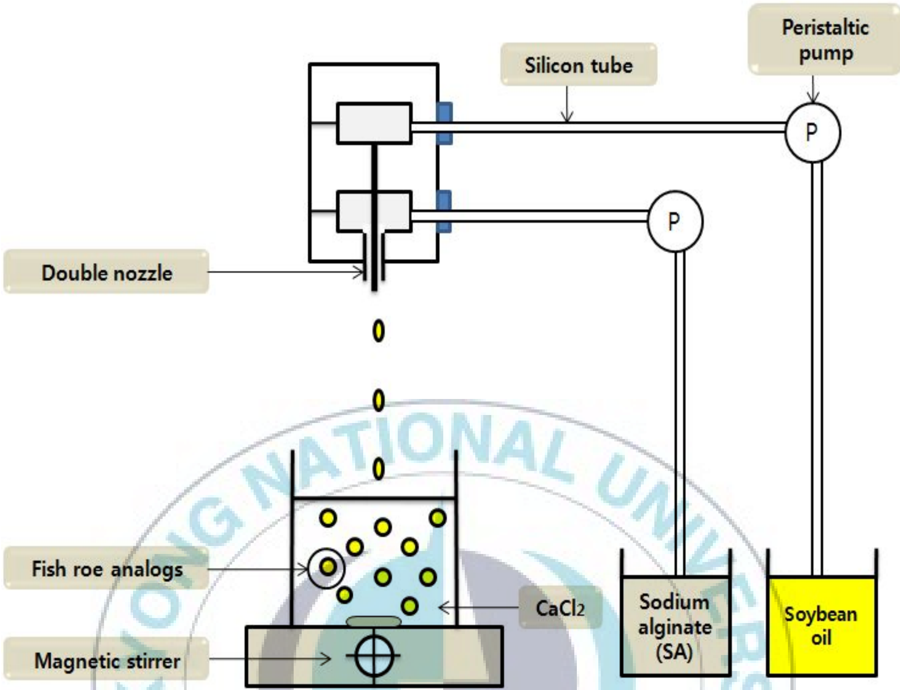


Fig. 1. Simplified schematic diagram for the processing of flying fish roe analogs with double nozzle.

The optimal conditions for manufacturing of the analogs were set up by manipulating sodium alginate concentration (%, w/v), concentration of calcium chloride (%, w/v), agitation speed of calcium chloride solution in the reactor (rpm), and dropping height between the tip of the nozzle and the surface of the reactor (cm). The sodium alginate solution was prepared at the ranges of 1.2, 1.5, 1.8, 2.1, and 2.4% (w/w). The sodium alginate solution was flowed into calcium chloride solution of a different concentration (0.5, 1.0, 1.5, 2.0, and 2.5%, w/w) through the outer nozzle (1 mm in inner diameter x 3 cm in length) of double nozzles at the speed of 1.5 mL/sec using peristaltic pump (Micro tube pump MP-3N, Eyela, Tokyo, Japan). Soybean oil was also flowed into calcium chloride solution of a different concentration through the inner nozzle (0.28 mm in inner diameter x 4.4 cm in length) of the double nozzles at the speed of 0.58 mL/sec using another peristaltic pump (Cassette tube pump SMP-23, Eyela, Tokyo, Japan). The calcium chloride solution was agitated at a different speed (150, 220, 290, 360, and 430 rpm) by a magnetic stirrer. The volume of calcium chloride solution in reactor was based on 250 mL and the reaction time was 3 min at an ambient temperature. The dropping height between the tip of the double nozzles and the surface of the calcium chloride solution was ranged from 5, 10, 15, 20, and 25 cm. After the reaction during 3 min in calcium chloride solution, the resultant analogs were separated from the calcium chloride solution using a sieve and then washed with distilled water. The analogs were kept at an ambient temperature until measuring their sizes and sphericity.

2.2 Measurement of size and sphericity

The size of the analogs was measured by an optical microscope (15x magnifications, BX-50, Olympus, Tokyo, Japan) with Motic Images Plus 2.0 program. The measurement was conducted by choosing five random analogs and then the average of their major diameters and minor diameters was determined as size of the analogs. The sphericity was indicated as the percentage ratio of the minor diameter to the major diameter of the five random analogs according to the following formula.

$$Sphericity\,(\%) = \frac{\text{minor diameter of the analogs}}{\text{major diameter of the analogs}} \times 100$$

2.3 Response surface methodology

Manufacturing process of the analogs with capsule type was estimated by using a central composite design and the optimization for sphericity of the analogs was conducted by response surface methodology. A central composite design was composed of the factorial portions of 24, the axial portions of 8, and the central point of triplicate repeats in experimental design (Table 2). In order to determine optimal sphericity conditions for manufacturing of the analogs, sodium alginate concentration (X_1, %), concentration of calcium chloride solution (X_2, %), agitation speed of calcium chloride solution in the reactor (X_3, rpm), and dropping height between the tip of the double nozzles and the surface of the calcium chloride solution (X_4, %) were used as independent variables. The portion values and ranges about 4 independent variables were based on the preliminary experimental (Table 1). It was randomized for experimental runs to minimize the effects of unsuspected variability in the surveyed responses. The response surface regression procedure for statistical analysis was carried out using SAS system (Version 9.1, SAS Institute Inc., USA). It was used to fit the following quadratic polynomial equation.

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j$$

Table 1. Experimental ranges and values of the independent variables in the central composite design for the processing of the flying fish roe analogs

Symbol	Independent variables	Coded levels				
		-2	-1	0	+1	+2
X_1	Sodium alginate concentration (%, w/v)	1.2	1.5	1.8	2.1	2.4
X_2	Calcium chloride concentration (%, w/v)	0.5	1.0	1.5	2.0	2.5
X_3	Agitation speed of calcium chloride solution (rpm)	150	220	290	360	430
X_4	Dropping height (cm)	5	10	15	20	25

Table 2. Central composite design and responses on dependent variables for processing of the flying fish roe analogs

Run order	Coded levels				Response
	X_1	X_2	X_3	X_4	Y
Factorial portion					
1	-1	-1	-1	-1	97.3
2	1	-1	-1	-1	97.8
3	-1	1	-1	-1	98.6
4	1	1	-1	-1	98.0
5	-1	-1	1	-1	97.6
6	1	-1	1	-1	95.7
7	-1	1	1	-1	98.4
8	1	1	1	-1	96.8
9	-1	-1	-1	1	98.5
10	1	-1	-1	1	96.9
11	-1	1	-1	1	98.7
12	1	1	-1	1	97.7
13	-1	-1	1	1	97.9
14	1	-1	1	1	96.0
15	-1	1	1	1	98.8
16	1	1	1	1	98.1
Axial portion					
17	-2	0	0	0	98.3
18	2	0	0	0	96.1
19	0	-2	0	0	98.2
20	0	2	0	0	98.9
21	0	0	-2	0	98.2
22	0	0	2	0	96.0
23	0	0	0	-2	98.2
24	0	0	0	2	98.7
Center portion					
25	0	0	0	0	99.3
26	0	0	0	0	99.2
27	0	0	0	0	99.1

Y, sphericitiy (%); X_1, sodium alginate concentration (%, w/v), X_2, calcium chloride solution (%, w/v), X_3, agitation speed of calcium chloride solution (rpm), X_4, dropping height (cm).

In this polynomial equation of quadratic, Y is the dependent variable, Xi and Xj are the normalized independent variables, β_0 is constant, and βi, βii, βij are regression coefficients. Response optimization was heuristically calculated by the desirability function of MINITAB statistical software (Version 14, Minitab Inc., State College, PA, USA), to find the conditions satisfying a dependent variable, sphericity (Y, %). The response surface plots were presented with a three-dimensional graph using Maple software (Maple 7. Waterloo Maple Inc., Canada) and represented connection between two independent variables and the dependent variable which is sphericity (Y, %).

2.4 Boiling water and calcium chloride, sodium chloride, autoclaving, and freeze-thaw treatments of the analogs

Boiling treatments of water and calcium chloride were performed by dipping the analogs in 95℃ water for different treatment times (20, 40, 60, and 80 min) and 0.5% (w/v) calcium chloride solution for 5, 10, 15, and 20 min. The physical properties including size, sphericity, rupture strength, and weight of the analogs treated by boiling water and calcium chloride were determined by Image-Pro Plus (Media Cybernetics, Inc., Bethesda, MD, USA), Bx-50 optical microscope (Olympus, Tokyo, Japan), and CR-100D rheometer (Sun Scientific Co., Ltd., Tokyo, Japan).

Sodium chloride treatment was carried out by dipping various concentrations (0.5, 1.0, 1.5, and 2.0%) for ten minutes and then the physical qualities were determined. Flying fish roe analogs were autoclaved at 121℃, 1 atm for 5, 10, 15, and 20 min with an autoclave apparatus (DW-AC 920, D.W. Industries, Busan, Korea). In addition, in order to examine change of physical condition after freeze-thaw process, flying fish roe analogs were in storage for 5 days on condition of freeze. After 5 days, the physical qualities of the thawed flying fish roe analogs were estimated by measurements of size, sphericity, rupture strength, and weight.

2.5 Statistical analysis

All the results were conducted to analysis of variance at a level of $p<0.05$ and the means were set apart using Duncan's multiple range tests (α=0.05). The analysis of data was subjected through the RSREG procedure of SAS software.

Results and Discussion

1. Diagnostic checking of the fitted models for the analogs

The response surface regression of SAS software was used to fit the quadratic polynomial equation about the results of the experiment for the analogs. All the coefficients of linear (X_1, X_2, X_3, X_4), quadratic (X_1X_1, X_2X_2, X_3X_3, X_4X_4), and interaction (X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4, X_3X_4) were estimated for significance based on t-statistic and the results for sphericity of the analogs were presented in Table 3. All the linear coefficients except for the X_4 (dropping height, cm) had a high significant difference ($p<0.01$). As the X_4 term was 0.1680, it was not significant ($p>0.05$). All of the quadratic coefficients were significant as $p<0.05$. On the other hand, all the interaction was not significant. Table 4 shows the fitted response surface model for manufacturing condition of the analogs. The model was explained as the equation which is eliminated all the insignificant coefficients ($p>0.05$) for fitness. The coefficient of determination was 0.9080, which means how suitable the selected parameters express the interrelation.

Table 3 Estimated coefficients of the fitted quadratic polynomial equation for different responses based on t-statistic for processing of the flying fish roe analogs

Parameters	Y (Sphericity, %)	
	Coefficient	P-value
Constant	99.37	0.0001
X_1	-0.55	0.0001
X_2	0.37	0.0003
X_3	-0.36	0.0030
X_4	0.14	0.1680
X_1X_1	-0.57	0.0001
X_2X_2	-0.23	0.0450
X_3X_3	-0.59	0.0001
X_4X_4	-0.25	0.0290
X_1X_2	0.06	0.6070
X_1X_3	-0.21	0.0980
X_1X_4	-0.10	0.4140
X_2X_3	0.15	0.2290
X_2X_4	0.04	0.7570
X_3X_4	0.14	0.2680

Y, sphericitiy (%); X_1, sodium alginate concentration (%, w/v), X_2, calcium chloride solution (%, w/v), X_3, agitation speed of calcium chloride solution (rpm), X_4, dropping height (cm).

Table 4. Response surface model for processing of the flying fish roe analogs

Response	Quadratic polynomial model equation	R^2	P-value
Y	Y =99.37-0.55X_1+0.37X_2-0.36X_3-0.57X_1^2-0.23X_2^2-0.59X_3^2-0.25X_4^2	0.9080	0.0003

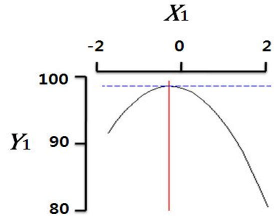
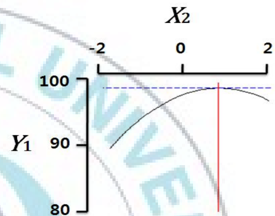
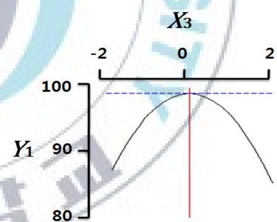
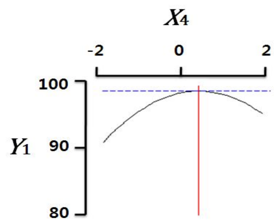
Y, sphericitiy (%); X_1, sodium alginate concentration (%, w/v), X_2, calcium chloride solution (%, w/v), X_3, agitation speed of calcium chloride solution (rpm), X_4, dropping height (cm).

When the value of R^2 is generally over 0.9, the response surface model has significant correlation for sphericity of the analogs. Thus, the quadratic polynomial model was significant within the limit of p<0.01.

2. Optimum conditions for manufacturing of the analogs

Table 5 shows the optimum conditions of independent variables for manufacturing process of the analogs. Four independent variables (X_1, X_2, X_3, X_4) were designated as important independent variables of central composite design through the preliminary experiment for processing of the analogs. Central values of independent variables established in the preliminary experiment were coded as zero, respectively. In order to optimize dependent variable (sphericity, Y) statistically, response optimizer function of SAS software was used. Predicted conditions of independent variables were reasoned as coded and uncoded values. As the actual values (uncoded values) of independent variables were X_1=1.66%, X_2=1.86%, X_3=280 rpm, and X_4=17 cm, the predicted value of the response was 99.7 %. In the four graphs relating to coded values (Table 5), the values of all the independent variables were calculated more high/low as compared with the experimental optimal conditions, 0.

Table 5 Optimum conditions for manufacturing process of the flying fish roe analogs

Responses				Y (Sphericity, %)
Optimum conditions	X_1	Coded value	-0.47	
		Actual value (%)	1.66	
	X_2	Coded value	0.72	
		Actual value (min)	1.86	
	X_3	Coded value	-0.07	
		Actual value (%)	280	
	X_4	Coded value	0.39	
		Actual value (rpm)	17	
Predicted value of response, Y				99.7

Y, sphericitiy (%); X_1, sodium alginate concentration (%, w/v), X_2, calcium chloride solution (%, w/v), X_3, agitation speed of calcium chloride solution (rpm), X_4, dropping height (cm).

The X_2 term (calcium chloride) was especially differential, which means concentration of calcium chloride solution was critical position in optimizing sphericity. Generally, a gelation occurs when alginate-droplet falls into a calcium chloride solution. The ionic interaction between G-blocks from two or more alginate chains and calcium cations formulates a three dimensional network of alginate molecules, and the formation of hydrogel network during cross-link is explained as 'Egg-box' model (Lee et al., 2013). High concentration of calcium chloride leads capsules with more homogeneous network. As the increase of Ca^{2+} ions concentration, the gelation kinetics is also accelerated, which makes more rapid stabilization and entrapment of capsule analogs (Chen et al., 2006). So, capsules with more spherical shape and uniform size are manufactured with increase of the concentration of calcium chloride (Chen et al., 2006; Smrdel et al., 2008).

3. Response surface plots for manufacturing of the analogs

Fig. 2 shows the three-dimensional graph created by using Maple software with the results of response surface analysis to examine the effect of four important factors (X_1, X_2, X_3, X_4) selected as independent variables on the sphericity (Y) of the analogs. As four independent variables have an effect on shape and size of the analogs, they are closely connected with sphericity (Y).

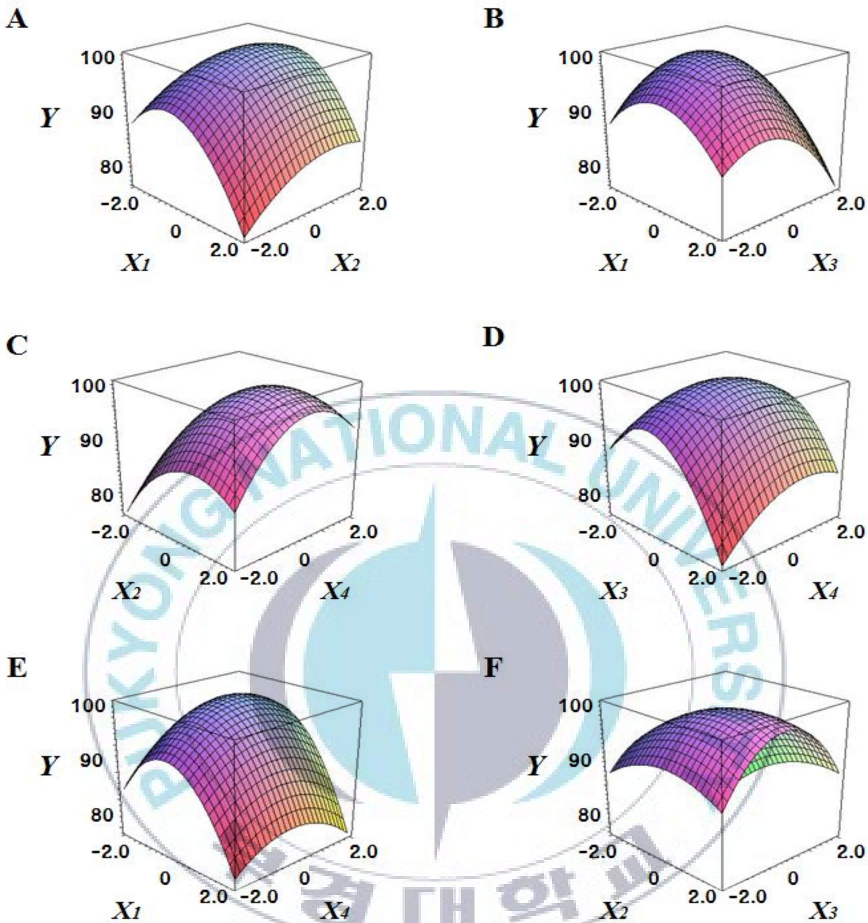


Fig. 2. Response surface plots for processing of flying fish roe analogs from sodium alginate and calcium chloride. ***Y (Sphericity, %), X_1 (Sodium alginate concentration, %), X_2 (Calcium chloride solution, min), X_3=Rotation speed of calcium chloride(rpm), X_4=Drop height between nozzle and calcium chloride solution(cm)***

To find which variables have a strong influence on sphericity among the independent factors of four, the mutual relations between two random independent variables and dependent variable were indicated as the response surface plots of six. As a result, influences of the independent variables on the sphericity of the analogs were different each other. Especially, concentrations of sodium alginate (X_1) and calcium chloride solution (X_2) had a strong influence on the sphericity of the analogs than other two independent variables (X_3, X_4). The sphericity remarkably decreased in the case of sodium alginate (0 to 2.0; Fig 3A and E) and calcium chloride (-2.0 to 0; Fig 3A and C), because concentration of two solutions affected gelation rate and capsule shape. Alginate solution which has low viscosity cannot able to keep their sphericity against the drag forces upon clash with the surface of the calcium chloride solution in the reactor, on the other hand, alginate solution which has high viscosity is not suitable to encapsulation between two solutions (Seifert & Philips., 1997). The increase of calcium chloride concentration also makes capsule more spherical according to the increase of gelation kimetics (Chen et al., 2006). Therefore, the concentration of X_1 and X_2 could become the critical factor on spherical shape of the analogs.

4. Verification experiment of the predicted value

Table 6 shows the results of verification experiment which was performed under the optimal conditions (concentration of sodium alginate=1.66%; concentration of calcium chloride=1.86%; agitation speed of calcium chloride solution=280 rpm; dropping height=17 cm) of the analogs processing. As compared to the predicted value, the experimental value was 99.8±0.77% through the triplicate repeats. Although the experimental value was a slightly higher than the predicted value, both are nearly identical. Accordingly, the estimated response surface model was suitable for optimization of manufacturing process of the analogs.

Table 6 Predicted and experimental values of the flying fish roe analogs manufactured at the optimum processing condition

Factors	Predicted value	Experimental value
Y, Sphericity (%)	99.7	99.8±0.77

5. Physical characteristics of the analogs

5.1 Changes in physical properties of the analogs by treatment of boiling water

The physical changes in the flying fish roe analogs by treatment of boiling as affected by different dipping time were shown in Fig. 3 which contains the changes of size, sphericity, rupture strength, and weight. Generally, boiling treatment is used to enhance storage stability based on sterilization. The size of the flying fish roe analogs somewhat decreased by boiling treatment at 95℃ from 20 min to 80 min excepting for 40 min (Fig. 3−A). Such decrease in diameter is considered due to dehydration resulting from denaturation of hydrocolloid during the boiling treatment. The dehydration process of hydrocolloid in pursuance of boiling treatment plainly was shown in weight loss of flying fish roe analogs (Fig. 3-D). The changes in sphericity of the flying fish roe analogs were shown in Fig.3-B. The sphericity (%) of the analogs produced in optimum was 99.4±0.21, suggesting that it was very close to a sphere. The sphericity of analogs was slightly decreased with increasing treatment time, but exhibited no significant differences (p<0.05). The changes in rupture strength of the flying fish roe analogs were shown in Fig. 3-C.

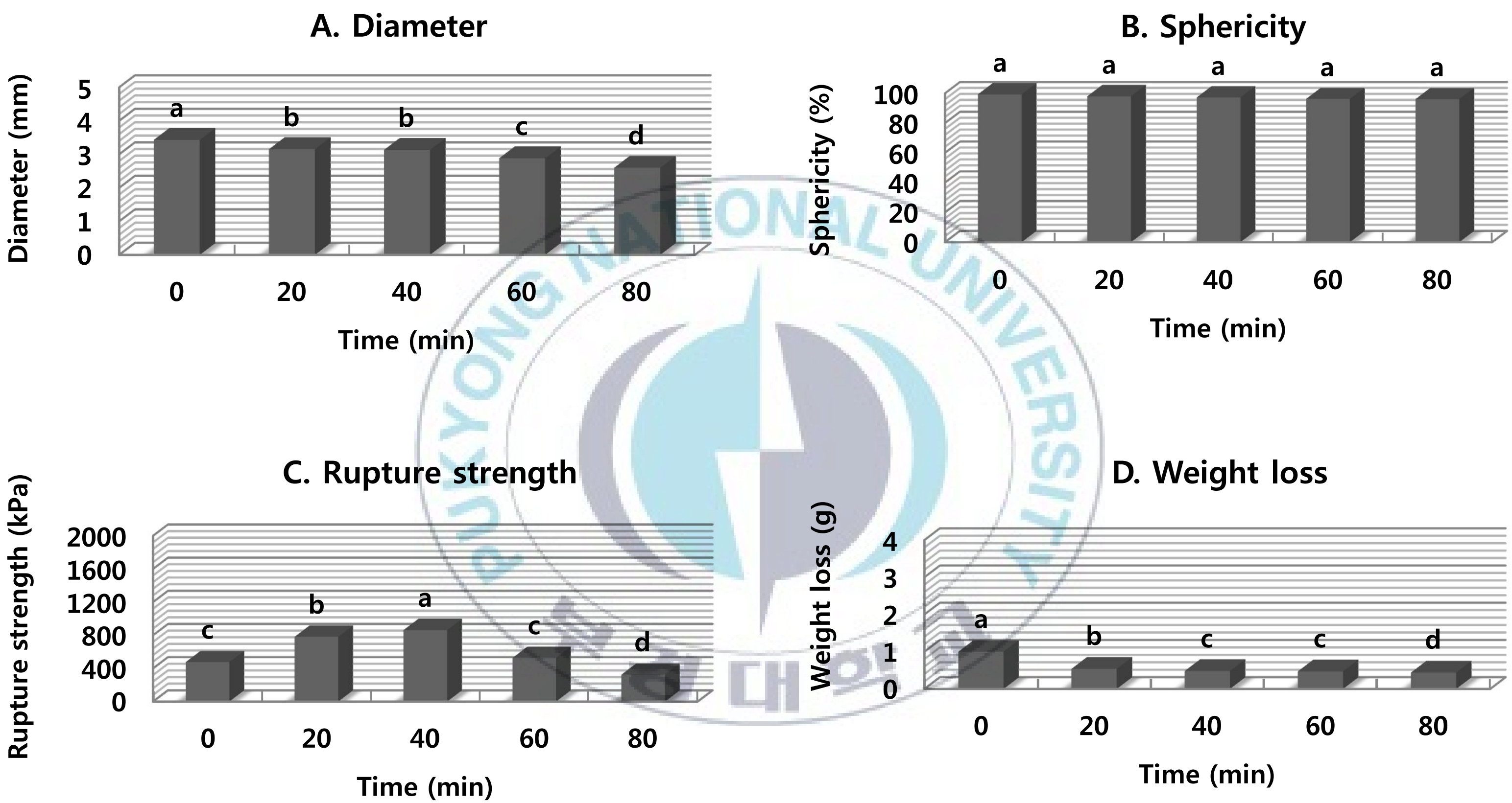


Fig. 3. Changes in physical properties of flying fish roe analogs heated at 95℃ in water.

The rupture strength (kPa) of the analogs produced in optimum was 476.32±40.84. The rupture strength (kPa) of the analogs heated at 95℃ water showed conspicuous increase until 862.99±50.12 by boiling treatment time of 40 min and then generally decreased since then by time of 80 min. Such increase in rupture strength was considered to be due to dehydration of hydrocolloid correlated with hardness increase of gel membrane. On the other hand, the rupture strength decrease of the analogs in the treatment time of 80 min was considered as soybean oil implanting at the center of alginate membrane was moved into outskirts of it during membrane contraction.

5.2 Changes in physical properties of the analogs by treatment of boiling calcium chloride

In general, the process of the calcium ion is an important factor in maintaining and improving the hardness by keeping the cell walls or cell structures and decreasing the biochemical changes during storage respectively (Konno et al., 1989; Nogata et al., 1993). Therefore, the calcium ion is added to examine texture change of flying fish roe analogs affected by boiling with 0.5% calcium chloride solution at the treatment time of 0, 5, 10, 15, and 20 min.

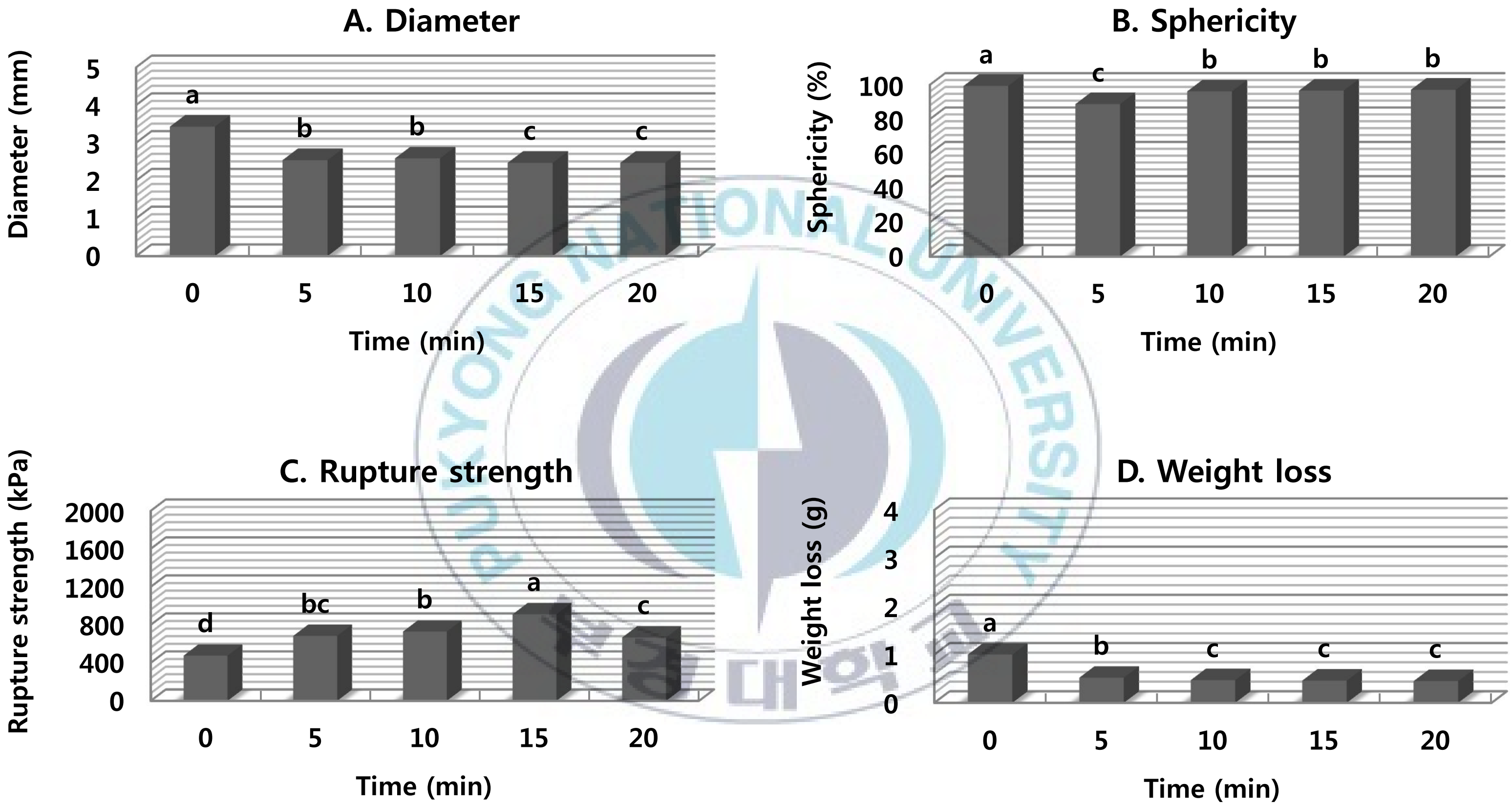


Fig. 4. Changes in physical properties of flying fish roe analogs heated at 95℃ in 0.5% calcium chloride.

Fig. 4 showed physical properties such as size, sphericity, rupture strength, and weight before and after heat treatment with calcium chloride solution. The changes in sphericity of the flying fish roe analogs were shown in Fig. 4-B. The sphericity of the analogs was slightly decreased from 99.4±0.21 to 96.17±1.30 for treatment time of 5 min, but exhibited no significant differences (p<0.05) more than 10 min. Such decrease in sphericity with heat treatment of 5 min is considered to be due to imbalance between dehydration amount of alginate gel and penetration amount of calcium ion. The weight loss of analogs as affected by heat treatment with 0.5% calcium chloride showed reduction until 5 min, but not more than 5 min, suggesting that the analogs maintained stability when the boiling treatment with 0.5% calcium chloride solution was continued in accordance with increasing treatment time over 5 min in common with Fig. 4-B. The changes in diameter of the flying fish roe analogs were measured in Fig. 4-A. The diameter of the analogs decreased or maintained by boiling treatment with 0.5% calcium chloride solution. Even if there was difference in diameter upon the change of treatment time with boiling solution, the steady difference was not remarkable. The changes in rupture strength of the flying fish roe analogs were shown in Fig. 4-C. When the analogs were heated with calcium chloride solution, the rupture strength continuously increased. The rupture strength (kPa) of the analogs mounted until 882.60±40.84 in boiling treatment affected by 0.5% calcium chloride for 15 min, but declined more than 15 min. It is seen that the increase in rupture strength was caused by maintaining equilibrium state when penetrating calcium ions into the tissue during moisture loss by denaturation of hydrocolloid (Kim, 2004). In addition, calcium ion generally forms the cross-linking with a part of alginate gel under a given heat treatment and consequently influences texture. On the other hand, the decrease of rupture strength of the analogs in the treatment time of 20 min was considered because soybean oil implanting at the center of alginate membrane was moved into outskirts of it in common with changes in the boiling treatment of water.

5.3 Changes in physical properties of the analogs by treatment of sodium chloride

The changes in physical properties of the analogs by sodium chloride treatment as affected by different concentrations of sodium chloride were shown in Fig. 5. Generally, sodium chloride treatment is used to improve taste of products. The diameter of the analogs was shown in Fig. 5-A. When the analogs were dipped in sodium chloride with different concentrations, the significant difference was not exhibited in the diameter of the analogs. On the other hand, the sphericity of analogs was slightly reduced in accordance with increased concentration (Fig. 5-B).

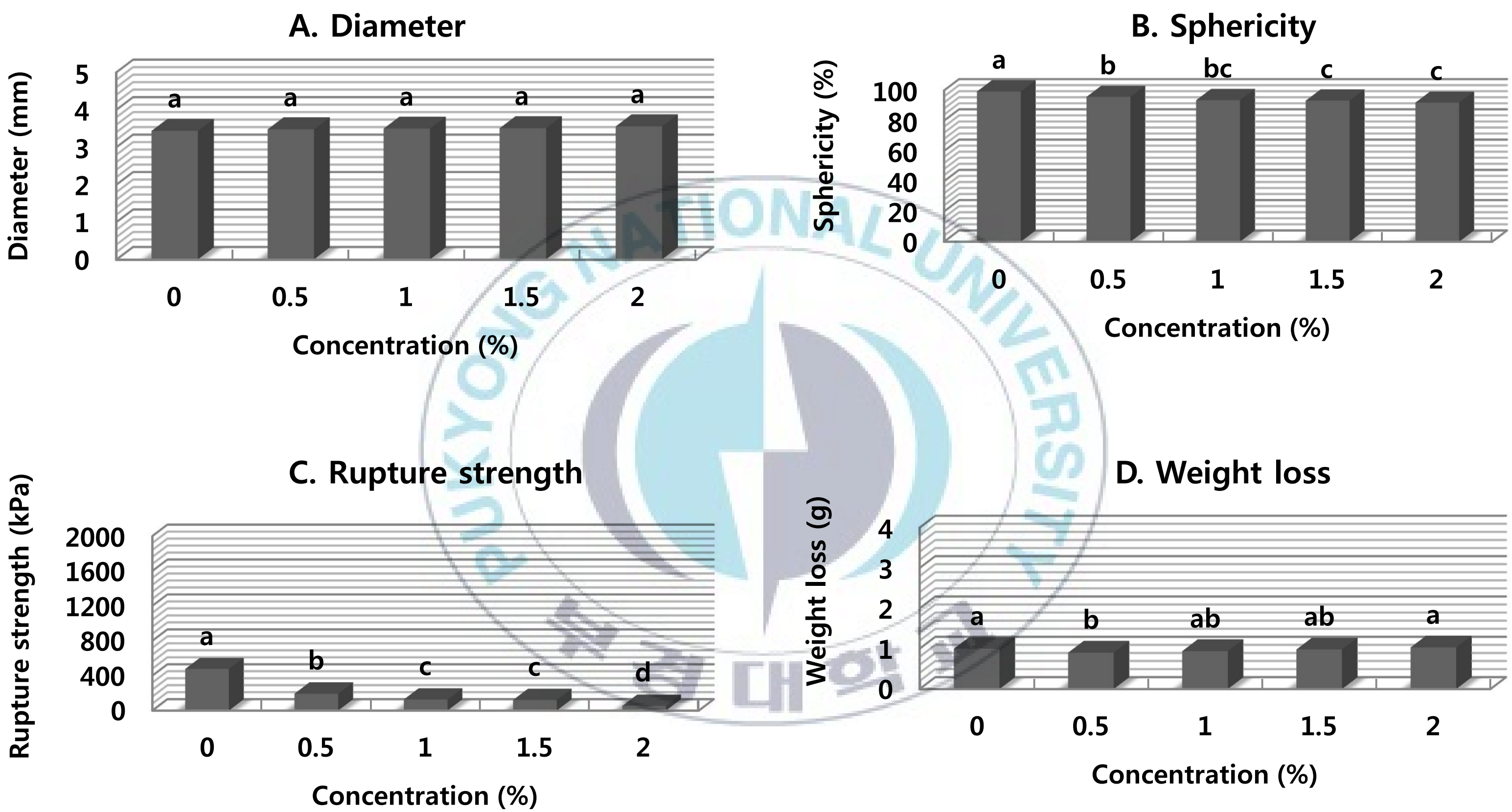


Fig. 5. Changes in physical properties of flying fish roe analogs treated with different concentrations of sodium chloride.

The changes in rupture strength of the analogs treated to diverse concentration of sodium chloride were shown in Fig. 5-C. The rupture strength in intact analogs was 476.32±40.84 kPa and showed a sharp decline until 193.33±42.49 kPa by sodium chloride treatment with 0.5 %. This is caused by the liberation of calcium ion which came away from bounded G residues of calcium-alginate gel when performing salt treatment. To preserve reduction phenomenon of rupture strength in the analogs, the prevention from separation of calcium ion is needed for storage stability of flying fish roe analogs.

5.4 Changes in physical properties of the analogs by autoclaving

Generally, autoclaving is a pressurized thermal process to enhance the shelf-life of foods through the sterilization. The changes in physical properties of the analogs by different autoclaving time were shown in Fig. 6. The diameter (mm) of analogs produced by the optimum was 3.43±0.06 (Fig. 6-A). The diameter (mm) of the analogs was distinctly declined until 2.06±0.02 by autoclaving treatment for 5 min but there was no more decrease significantly when the autoclaving treatment time was continued more than 5 min. Such initial decrease of diameter appeared similar aspect in weight loss of analogs (Fig. 6-D).

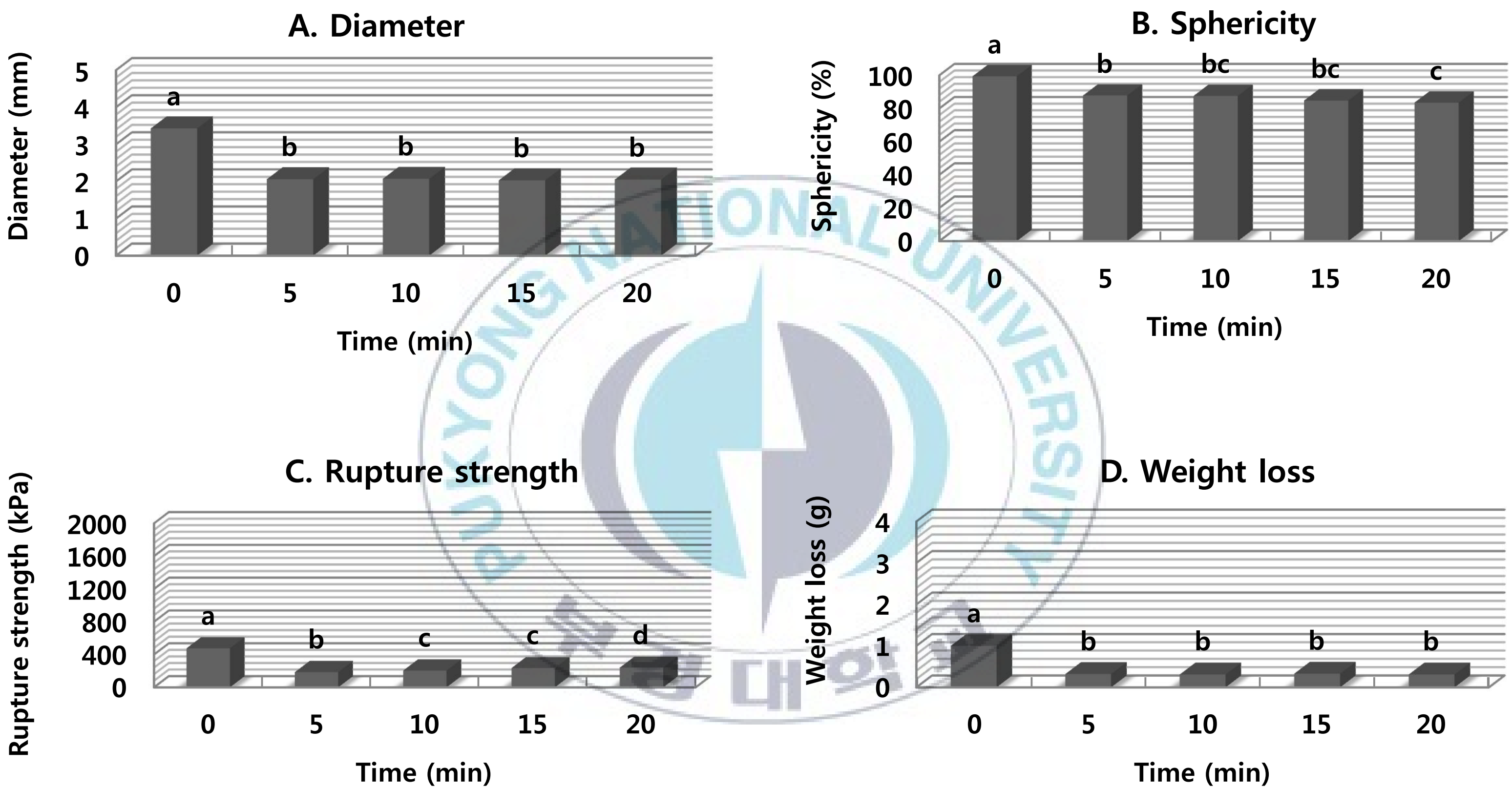


Fig. 6. Changes in physical properties of flying fish roe analogs treated by autoclaving.

Therefore, initial process of management with regard to autoclaving treatment induced variable property of membrane. The changes in sphericity of the analogs by autoclaving treatment were shown in Fig. 6-B. The sphericity (%) of the analogs decreased from 99.4±0.21 to 87.82±3.43 during 5 min of autoclaving treatment and then slightly decreased by the longer autoclaving time. The changes in rupture strength over the 5 min intervals of autoclaving treatment were shown in Fig. 6-C. The rupture strength of the optimum analogs treated in autoclaving was clearly decreased during initial 5 min in common with other physical properties such as diameter and weight, but increased little in treatment for more than 5 min. Such increase in rupture strength of the analogs was thought to be due to dehydration of hydrocolloid correlated with hardness increase of alginate gel membrane.

5.5 Changes in physical properties of the analogs by freeze-thaw process

The changes in physical properties of the analogs by freeze-thaw process as affected by storage period (5 day) were shown in Fig. 7. The diameter (mm), sphericity (%), and rupture strength (kPa) of the analogs produced in optimum were 3.43±0.06, 99.4±0.21, 476.32±40.84, respectively. When the analogs were treated by freeze-thaw process for 5 day, the physical texture was overall reduced.

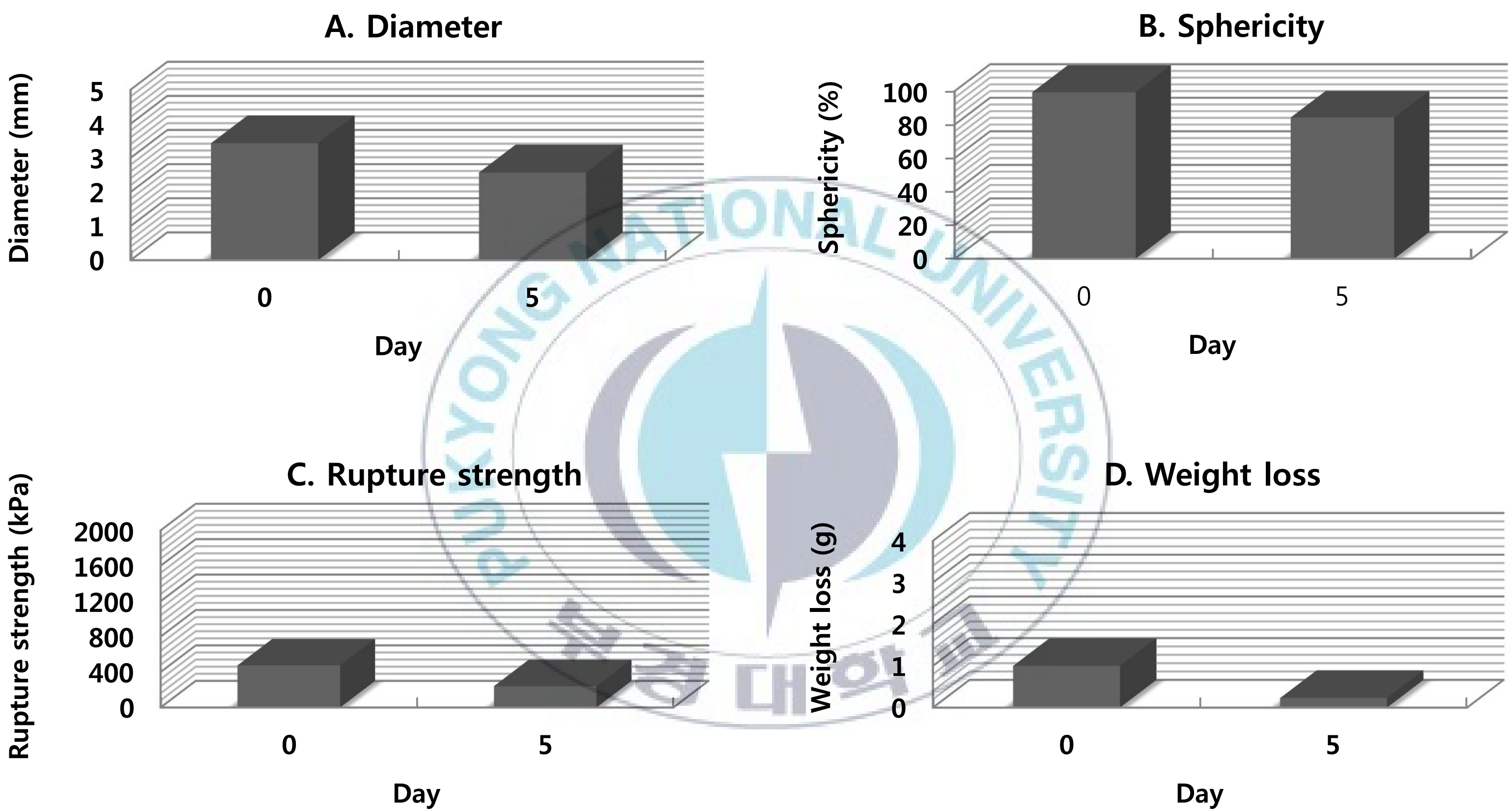


Fig. 7. Changes in physical properties of flying fish roe analogs as affected by freeze-thaw process.

The diameter (mm) of the analogs was 2.57±0.06 after 5 days and remarkably decreased as compared with the control analogs (Fig. 7-A). The sphericity (%) and rupture strength (kPa) also showed the significant decrease (Fig. 7-B, C). Such decrease in physical texture of the analogs was a result of the drip generated from water got out from a membrane and oil escaped from encapsulated soybean oil on the inside. It is considered freeze-thaw process which was known for forming a drip greatly influences the physical properties of the flying fish roe analogs. As shown in Fig. 7-D, it was found to have passed drip through the weight loss (g) of the analogs produced by freeze-thaw process.

Conclusion

The present study set four major factors such as sodium alginate concentration, calcium chloride concentration, agitation speed, and dropping height that affect to production of the flying fish roe analogs as independent variables. The optimum conditions for the analogs processing were investigated using response surface methodology with central composite design. The optimum conditions were 1.66% sodium alginate, 1.86% calcium chloride, agitation speed of calcium chloride solution at 280 rpm, and dropping height at 17 cm between the tip of nozzle and the surface of calcium chloride solution in reactor. As a result, the predicted value for the sphericity of the response was 99.7% and the experimental value was 99.8±0.77%. Sphericity is a very important factor for processing of the flying fish roe analogs. The manufactured analogs showed high sphericity near a complete spherical shape. Moreover, under optimal conditions, the changes of size, sphericity, rupture strength, and weight in the analogs treated by boiling water and calcium chloride, sodium chloride, autoclaving, and freeze-thaw treatments were investigated. The diameter (mm), sphericity (%), rupture strength (kPa), and weight loss (g) of the analogs showed differences in various treatment process in accordance with a increasing treatment time or concentration. Especially, the rupture strength of the analogs was affected by physicochemical treatments.

The study elucidated manufacturing conditions of flying fish roe analogs with a high sphericity similar to natural ones using double nozzles and showed physical property changes of the analogs in various treatments. The results are considered to play a role for a potential application to substitutes of natural ones and development of other analog foods.

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