

Properties of Low-Molecular Alginate by Ultrasound

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Response Surface Methodology was applied for optimizing the processing parameters of ultrasound treatment in order to produce low-molecular alginate. The use of ultrasound significantly reduced viscosity of alginate solutions. Suggested parameters of ultrasound treatment for maximum reduction of alginate molecular weight were: specific intensity, 115.81 W/cm² at 20kHz frequency; treatment time, 35.55 min; temperature, 20.08°C; alginate concentration, 2.5%. Low-molecular alginate obtained by ultrasound had two peaks on Sepharose CL-6B gel filtration. The viscosities of control, fraction I, and fraction II at 0.1% concentration and 25°C were 3.07, 1.23, and 0.82cps, respectively. Molecular weights of control, fraction I, and fraction II alginates were 336,500, 70,400, and 52,800 daltons, and their solubilities were 3, 6, and 14%, respectively. The lower molecular weight of alginate, the lower the alcohol precipitation and the higher Ca²⁺ ion binding capacities. Heavy metal ion binding capacities of alginates were high in the following order of Pb, Cd, Zn, and Co.

Key words: Alginate, Ultrasound, Viscosity, Low-molecular, manufacture

Introduction

Alginate is a collective term for a family of co-polymers containing 1,4-linked β -D-mannuronic acid and α -L-guluronic acid residues in varying proportions and sequential arrangements. The major species used for alginate production are *Ascophyllum*, *Laminarium*, and *Macrocystis* of brown algae (*Phaeophyceae*). Alginate forms gel with divalent ions like calcium, and the gel-forming properties are strongly correlated with the proportion and lengths of the blocks of contiguous L-guluronic acid residues (G-block) in the polymeric chains. The viscosity of alginate solutions depends mainly on the molecular weight (Mw) of the alginate. As samples are normally polydisperse, knowledge of the distribution of Mw can be of importance in determining suitability of a particular alginate preparation for industrial applications. Alginates are widely used as thickening agents, gelling agents, and stabilizers in foods, medicinal

products and diagnostic products, biotechnological material, and other industrial products such as dye, paper, and textile (Sutherland, 1991). Ultrasound will depolymerize large molecules such as polystyrene, nitrocellulose starch, gum arabic, and nucleic acids. Biopolymers in solution may undergo intramolecular regrouping with either an increase or decrease of molecular weight as well as oxidation and reduction processes in the absence of a protective atmosphere (Alliger, 1975). The chain breaking reaction (weak bond) can be produced by the pulsation of bubbles as well as bubble collapse or cavitation. General applications include acceleration of conventional and decomposition reactions, degradation of polymers, and polymerization reactions (Henglein, 1987). In general, cavitation in liquids may accelerate chemical reactions and enhance polymerization and depolymerization reactions by temporarily dispersing aggregates or by permanently breaking chemical bonds in polymeric chains (Webster, 1963; Thakore et al., 1990). There are applications of alginate which limits the use of high-molecular-weight natural alginate preparations because of high viscosity

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and gelling property of their solution (Joo et al., 1995). Therefore, alginates with low viscosity in solution, weaker gelling formation, and high solubility are needed. For manufacturing the low-molecular-weight alginate, limited reports mention enzymatic hydrolysis (Joo et al., 1995; Takeuchi et al., 1994). In this study, we used ultrasound to manufacture the low-molecular alginate.

Materials and Methods

Raw material

Sodium alginate (Sigma product, U.S.A) extracted from *Macrocystis pyrifera*, which has a viscosity of approximately 3,500 cps at 2% concentration and 25°C, was used.

Ultrasound treatments

A Misonix Sonicator (Misonix, NY, U.S.A) to generate 20kHz ultrasound was used. Solution was cooled during treatment by using a Lauda RM6 circulator (Wobser GMBH & Co., Germany). Response Surface Methodology (Box and Behnken, 1960) was applied for determining the optimal parameters of ultrasound treatment. The spacings for each parameter were chosen on the basis of preliminary experiments: ultrasound intensity, 73.01~126.68 W/cm²; alginate concentration, 2~4%; temperature, 10~30°C; treatment time, 20~40 min. Sodium alginate of 2~4% was dissolved in distilled water and 10 mL of alginate solution was sonicated in 3 min pulses with 1 min pause between pulses. The percentage of solution viscosity, V, by ultrasound was determined by following equation:

$$V = \frac{\eta_0 - \eta_t}{\eta_0} \times 100$$

where, η_0 = the viscosity of control alginate

η_t = the viscosity of sonicated alginate

Gel filtration of low-molecular alginate produced by ultrasound

Alginate obtained by ultrasound treatment was chromatographed on Sepharose CL-6B with 0.1M NaCl and the fractions with above 50% maximum concentration of alginate were pooled and freeze-dried with Vacuum Freeze Dryer (SFDSM242, Samwon Freezing Engineering, Seoul, Korea).

Molecular weight determination of alginate

Relative viscosities of alginate solutions from 0.02

to 0.2% concentrations were determined with an Ubbelohde Capillary Viscometer at 25°C in 0.1 M NaCl solution. Intrinsic viscosity (η) was calculated from reduced viscosity estimated by relative viscosity. Molecular weight of alginate was estimated from the Mark-Houwink-Sakurada equation (Martinsen et al., 1991).

$$[\eta] = K M^a$$

where, $K = 7.3 \times 10^{-5}$ and $a = 0.92$ (Martinsen et al., 1991).

Viscosity measurement

The apparent viscosities of alginate solutions were determined with a coneplate viscometer (Brookfield DV-II%, MA, USA) at 25°C and 11.3 sec⁻¹ of shear rate.

Determination of alginate solubility

Solubilities of alginates were determined according to Joo et al.'s (1995) method. Different concentrations (1~14%) of alginate were dissolved in 100 ml of distilled water adjusted at 25°C in advance and mixed well. After holding for 30 min at 25°C, the beaker was inverted. Solubility of alginate was expressed as the concentration of solution which did not flow down the wall of beaker.

Alcohol precipitation capacity of alginate

Precipitation capacity of alginate in alcohol was determined according to Joo et al.'s (1995) method. Different concentrations of 95% alcohol were added to 3.0% alginate solution. The precipitates obtained by centrifuging were dried for 2 hrs at 105°C. All experiments were performed in triplicate. Alcohol precipitation capacity was expressed as the weight ratio of precipitated alginate to original amount in solution at each alcohol concentrations.

Ca²⁺ ion binding capacity

Ca²⁺ ion binding capacity was determined according to Joo et al.'s (1995) method. Alginate solutions of 1, 3, and 5% were prepared and CaCl₂ was added for making 500 mg% of CaCl₂-alginate solution. This solution was mixed well by stirring slowly with a glass rod for 30 min and then centrifuged to remove the precipitate. Ca²⁺ ion binding capacity was expressed as the ratio of the amount of Ca²⁺ ion in the precipitate to the amount of Ca²⁺ added.

Heavy metal ion binding capacity

Each 500 mg of control and sonicated alginates was added to 50 ml of 0.06N Pb (NO₃)₂, Cd (NO₃)₂, Zn (NO₃)₂, and Co (NO₃)₂ and then mixed well for 1hr at room temperature. The solution was filtered and the solution was adjusted to pH 4. The amount of heavy metal ion in the filtrate was titrated with 0.01M EDTA by using EBT (Erio Black T, pH range 7~11) as indicator. Heavy metal ion binding capacity of alginate was expressed as mmol/g sodium alginate.

Statistical analysis

The ranges and intervals of experimental parameters for response surface methodology (RSM) followed the designs of Box and Behnken (1960), and Box and Wilson (1951). Each experiment was duplicated for each treatment. Data analysis and graphic plotting were done with SAS programs (SAS, 1982). A quadratic model was used to create the 3-dimensional response surfaces. In response surfaces, independent variables are located along the traditional X and Y-axes, respectively, while the dependent variables were plotted in the Z-axis perpendicular to the X-Y axes.

Analysis of variance and least significant differences were used to ascertain significant effects at the 5% level.

Results and Discussion

Optimal parameters of ultrasound treatment

The effectiveness of low-molecular-weight alginate manufactured with combinations of specific ultrasound intensity, time of ultrasound treatment, temperature of ultrasound treatment, and con-

centration of alginate solution was determined to find the optimal parameters of ultrasound treatment. The spacings for each parameter were chosen on the basis of preliminary experiments. The quadratic model for viscosity reduction in alginate by ultrasound is:

$$V = 98.5958 + 0.3143X + 4.1550Y - 0.0859Z + 0.7368W + 4358X^2 - 0.8276XY - 3.3196Y^2 + 0.0675XZ + 0.3375YZ + 0.342Z^2 + 0.1075XW - 0.6200YW + 0.1425ZW - 0.7104W^2$$

(R²=0.9595, P<0.001)

where, V=viscosity reduction, %; X=temperature of ultrasound treatment, °C; Y=specific ultrasound intensity, W/cm² at 20kHz frequency; Z=concentration of alginate solution, %; and W=time of ultrasound treatment, min. Response surface analysis showed that the specific intensity of ultrasound influenced the viscosity of alginate solutions (Fig. 1). Specific intensity of ultrasound is defined as the amount of ultrasound tuned from the horn tip per unit area. The extent of viscosity reductions significantly increased up to a specific ultrasound intensity of 115.81 W/cm² at 20kHz frequency and 20.08°C (Fig. 1). The extent of viscosity reduction increased with increasing ultrasound treatment time up to 35.55 min and concentration of alginate solution up to 3.83% (Fig. 1). Therefore, specific ultrasound intensity, time of ultrasound treatment, concentration of alginate solution, and temperature of treatment are major parameters affecting the molecular-weight reduction by ultrasound treatment. However, 3.83% alginate solution

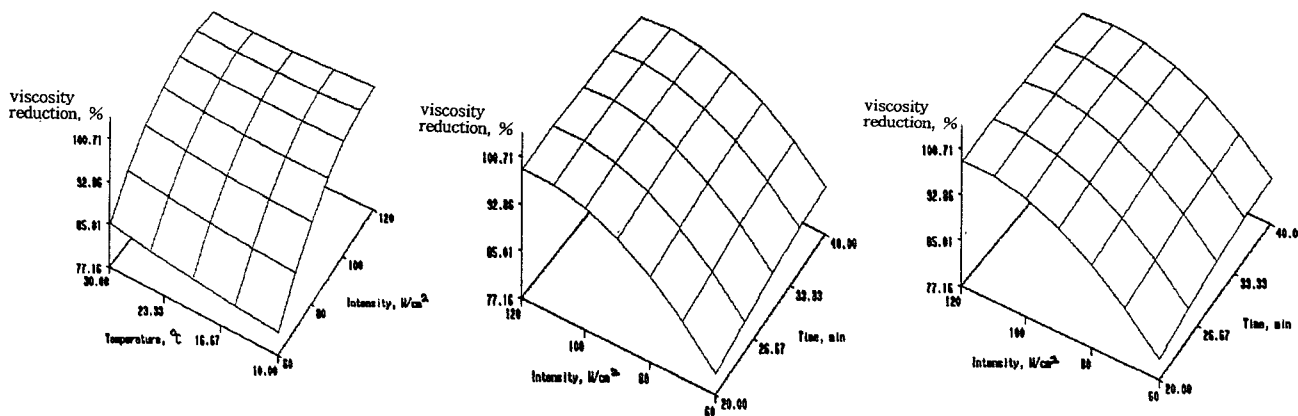


Fig. 1. Viscosity reduction of alginate solutions treated by ultrasound as a function of temperature, ultrasound intensity, alginate concentration, and treatment time.

could not be subjected to the ultrasound treatment because its high viscosity would decrease ultrasound effectiveness. Therefore, we used 2.5% alginate to optimize the process of manufacturing low-molecular alginate. In the optimal ultrasound process, the maximum reduction of alginate viscosity was 96.98%. Joo et al. (1995) reported that the enzymatic hydrolysis for 60 min reduced alginate viscosity up to 90%. Generally, the yield of depolymerized alginate would increase with increasing temperature. However, in ultrasound treatment, high temperature rise will increase vapor pressure inside the cavitation bubbles and reduce the violence of the bubbles' collapse, because gas molecules within the bubbles provide a cushion to shock (Sirotyuk, 1966). Suggested parameters for maximum viscosity reduction of alginate by ultrasound treatment were: specific ultrasound intensity, 115.81 W/cm² at 20kHz frequency; temperature of ultrasound treatment, 20.08°C; concentration of alginate solution, 2.5%; and time of ultrasound treatment, 35.55 min.

Chromatography pattern of alginate by ultrasound treatment

Chromatography patterns of alginate solutions on Sepharose CL-6B are shown in Fig. 2. Control (unsonicated) alginate showed only one peak, while the sonicated alginate had two peaks. The amount of alginate in peak II was more than that in Peak I. Our results show that ultrasound resulted in only 2 isolated breaks in the alginate molecule.

Properties of alginates treated by ultrasound

Apparent solution viscosities, yield, molecular weights, and solubilities of sonicated alginate fractions separated by Sepharose gel filtration are shown in Table 1. The viscosities of solutions produced from control, fraction I, and fraction II isolates at 0.1% concentration and 25°C were 3.07, 1.23 and 0.82cps, respectively ($p < 0.05$). Yields of alginate fraction I and fraction II were 5 and 40%, respectively, in which fraction II was 8 times more than fraction I in amount. In order to calculate the molecular weight of alginate by the Mark-Houwink-Sakurada equation (Martinsen et al., 1991), it is necessary to use the Newtonian fluid. The slopes of straight linear line were successively high in the order: control, fraction I, and fraction II. Reduced viscosities of control, fraction I, and fraction II at different alginate concentrations are shown in Fig. 3. All alginate solutions showed a

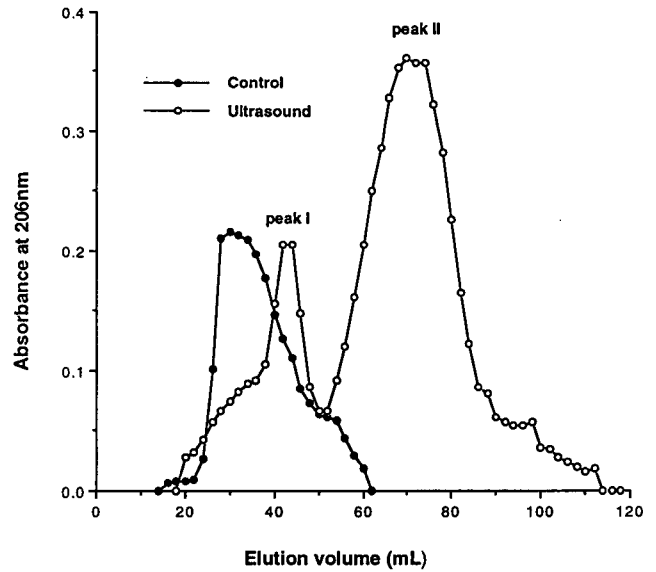


Fig. 2. Gel filtration of alginate on Sepharose CL-6B. Column, 1.4 x 40 cm; Eluent, 0.1 M NaCl; Flow rate, 6 ml/hr; Fraction size, 2 ml.

Table 1. Properties of alginate fractions obtained by ultrasound treatment

Alginates	Viscosity ^r (cps)	Yield* (%)	Mw (daltons)	Solubility (%)
Control	3.07 ^a	100	336,500 ^a	3 ^a
Fraction I	1.23 ^b	5	70,400 ^b	6 ^b
Fraction II	0.82 ^c	40	52,800 ^c	14 ^c

^{a,b,c}Means in the same column with the same superscripts are not significantly different ($p < 0.05$). *Yield was calculated from the weight ratio of ultrasound alginate to control alginate.

linear relationship between reduced viscosity and alginate concentrations up to 0.15% in control and 0.3% in sonicated alginates. Intrinsic viscosities of control, fraction I, and fraction II were 8.87, 2.11, and 1.61 cps, and their relative molecular weights were 336,500, 70,400, and 52,800 daltons, respectively (Table 1). Solubilities of control, fraction I, and fraction II were 3, 6, and 14%, respectively (Table 1). Therefore, the lower the molecular weight of alginate, the higher solubility. This was similar to the results of Joo et al. (1995) in which the partially degraded alginate by enzyme hydrolysis had higher solubility.

Alcohol precipitation capacity

Generally, alginate precipitates by the dehydration reaction with alcohol (Mizuno et al., 1983). The precipitation capacity of alginate solutions at

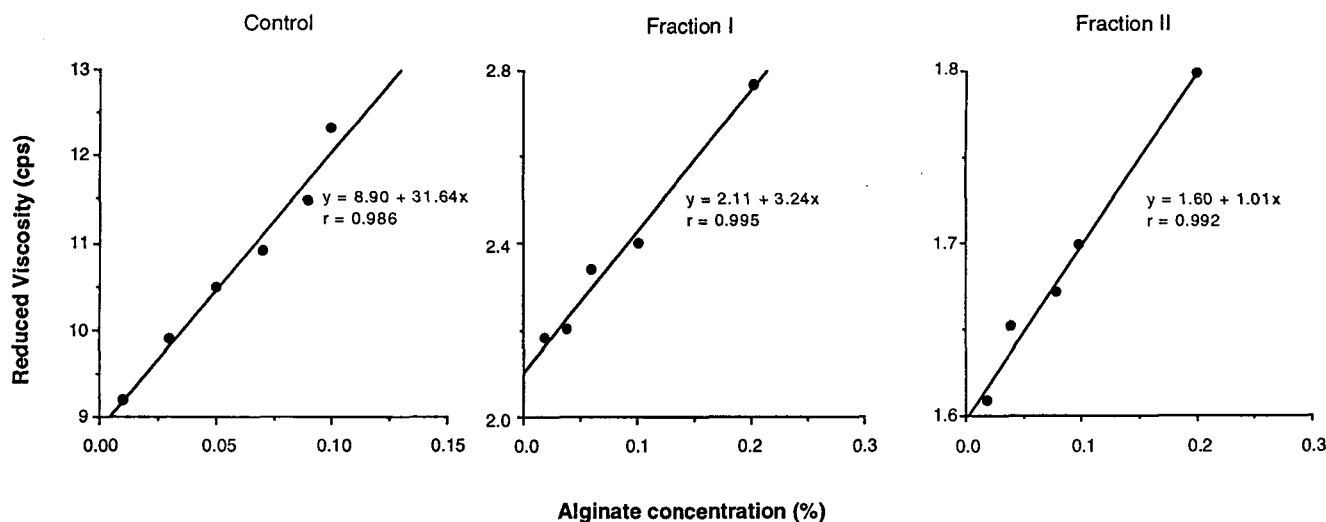


Fig. 3. Reduced viscosities of control, fraction I and fraction II alginate.

Table 2. Effects of ethyl alcohol on precipitation capacity of alginates treated by ultrasound (Unit : Precipitation capacity*, %)

Ethyl alcohol concentration, %	1	3	5	10	20	30	40	50	60	70	80	90
Control (2.5%)	0	2.26	3.51	4.48	15.10	30.14	36.46	56.50	66.06	72.46	92.80	96.76
Fraction I (3%)	0	2.10	4.00	6.60	15.60	23.30	33.66	37.33	40.00	49.30	57.00	79.30
Fraction II (3%)	0	0.03	1.30	3.00	11.60	15.60	27.00	30.30	36.60	41.00	42.00	73.60

*Precipitation capacity was expressed as the weight ratio of the precipitated alginate to the original amount.

different ethyl alcohol concentrations are shown in Table 2. Alginate solution of 2.5% in control started precipitating from 3.0% ethyl alcohol concentration and precipitation capacity at 80% alcohol concentration was 92.80%. Even though 3.0% of fraction I and II alginate solutions was also precipitated from 3.0% of ethyl alcohol concentration, their precipitation capacities at 80% alcohol concentration were 57.0 and 42.0%, respectively. Therefore, low-molecular alginates obtained by ultrasound showed lower alcohol precipitation capacity, which was similar to the results of Joo et al. (1995). Especially, precipitation capacities of 3.0% fraction I and II alginate solutions were 3.00 and 6.60%, respectively at 10% ethyl alcohol concentration, which indicated low-molecular alginate obtained by ultrasound treatment could be used in foods containing alcohol up to 10%.

Ca²⁺ ion binding capacity of alginate

Alginate forms gels with divalent ions like calcium, and the gel-forming properties are strongly correlated with the proportion and lengths of the blocks of contiguous L-guluronic acid residues (G-blocks) in the polymeric chains (Martinsen et al., 1989). The Ca²⁺ ion binding capacities of fraction

I alginate at 1, 2 and 3% concentrations were 28.6, 45.8, and 66.5%, respectively, while those of fraction II were 64.6, 68.3, and 71.5% (Table 3). According to these results, fraction II alginate showed the higher Ca²⁺ binding capacity than fraction I. But, there were no significant differences in Ca²⁺ binding capacities between 3% fraction I and, 1 and 2% fraction II solutions. This was similar to the result of Joo et al. (1995) in which Ca²⁺ binding capacity of partially degraded alginate by enzyme hydrolysis increased with increasing its concentration.

Heavy metal ion binding capacity of alginate

Alginate can selectively bind alkaline earth metal ions. The selectivity increases with the guluronic acid content of the alginate (Smidsrod and Haug, 1968). Polymannuronic acid is essentially nonselective. Ba²⁺, Sr²⁺, and Ca²⁺ ions are strongly bound with alginate than other cations. This property is utilized in a number of industrial applications (Sutherland, 1991). Alginate has been recently recognized as a kind of dietary fiber because of its biological functionalities such as heavy metal ion binding capacity. Heavy metal ion binding capacities of alginates are shown in Table 4. Zn ion binding capacities were high in the following

Table 3. Ca²⁺ ion binding capacities of alginates treated by ultrasound

Alginates	Concentration (%)	Ca ²⁺ ion binding capacity* (%)
Fraction I	1	28.6 ^a
	2	45.8 ^b
	3	66.5 ^c
Fraction II	1	64.6 ^c
	2	68.3 ^d
	3	71.5 ^d

^{a,b,c,d}Means in the same column with same superscripts are not significantly different ($p < 0.05$).

*Ca²⁺ binding capacity was expressed as the ratio of the amount of Ca²⁺ bound to alginate to the amount of Ca²⁺ added.

Table 4. Heavy metal ion binding capacities of alginates treated by ultrasound

Alginates Heavy metal ion	Heavy metal ion binding capacity* (mmol/g)		
	Control	Fraction I	Fraction II
Zn	2.30 ^a	1.40 ^c	1.65 ^b
Pb	3.20 ^c	3.84 ^b	4.37 ^a
Cd	2.60 ^c	2.82 ^b	3.00 ^a
Co	0.83 ^c	1.07 ^b	1.23 ^a

^{a,b,c}Means in the same column with the same superscripts are significantly different ($p < 0.05$).

*Heavy metal capacity was expressed as the ratio of heavy metal content bound to alginate to total amount in solution.

order: control, fraction II, and fraction I. Pb, Cd, and Co ion binding capacities were high in order of fraction II, fraction I, and control. Among 4 heavy metal ions, Pb ion showed the highest alginate binding capacity and next was in order of Cd, Zn, and Co ions. This was similar to the result of Kim and Park (1985) in which metal ion binding capacity of alginate was high in the following order of Pb, Cu, Zn, and Co.

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