# Physical and Chemical Effects on the Sonication Treatment of Chitosan Solution

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**As the first step of studies related to production of chitooligosaccharides by physical methods, chitosan solution were sonicated with 20 kHz and various treatment effects were examined to present fundamental data of sonicated chitosan solution. Intrinsic viscosity of chitosan solution sharply decreased from 3.76 dl/g to 2.90 dl/g until 5 minutes of sonication and then slowly decreased. With low volume of chitosan solution, sonication was very effective and temperature of chitosan solution slightly affected the efficiency of sonication. In case of changing the solvent, no significant differences were observed on the effect of sonication, however, acetate buffer had highest sonication effect among various solvents. The sonication effect was increased as the increasement of the value of pH, on the contrary, ionic strength and type of counterions showed no effect on sonication. With these results, we assumed that optimal sonication treatment would be as follows, solution volume was 10~20 ml, temperature range was 20~30℃, pH value of solution was 4.5 and type of solvent was acetate buffer.**

**Key words** : chitosan solution, sonication efficiency, physical and chemical effects

## Inrtoduction

Chitin, a natural abundant mucopolysaccharide, is known to be the $\beta$-1,4-glycan of N-acetyl-D-glucosamine, which is very similar with the structure of cellulose. And chitosan is a chemical derivative produced by deacetylation of chitin.

Recently, chitin and chitosan attract much attention in food industries, biochemical and medical fields as various oligosaccharides can be produced from them and its functions have been widely investigated. Many studies have been reported that chitin and chitosan oligosaccharides had activities as antibacterial agents, immuno-enhancers, lysozyme inducers and elicitors, and they could be used for food materials, accelerator for the growth of *Bifidus* and material substances for physiological activities (Aiba, 1994).

Chitin and chitosan oligosaccharides have been produced by chemical and enzymic methods. But the yield was very poor because of its heterogeneous conditions, and high oligomers are not obtained in good yields (Aiba, 1994). However, no studies related to production of chitin and chitosan oligosaccharides by physical method have been reported so as to utilize chitin and chitosan more generally in food.

Our objectives of this study were to examine lowering molecular weight of chitosan in a solution by physical method, ultrasonication treatment, and to examine various effects of treatment conditions such as temperature, salt concentration, type of counterions, ionic strength and volume of solution.

## Materials and Methods

### Materials

Materials used in this study were collected at a fishery processing plant located in YoungDuk afterprocessing the red snow crab shell, selected, and washed to remove extraneous substances, and then dried at 50℃ with hot air blast. Then, the material was grounded to approximate size of 20 mesh and their general components are shown in Table 1.

**Table 1. Ingredients and thickness of dried shell of red snow crab**

| Composition | Content |
|---|---|
| Moisture (%) | 9.4 |
| Nitrogen (%) | (8.3)[1] |
| Crude ash (%) | 46.2 |
| Crude fat (%) | Trace |
| Thickness (μm) | 224 ± 34[2] |

[1] numbers in parenthesis are crude ash free basis
[2] this value is the mean ± standard deviation for 10 samples

### Methods

1. Preparation of chitin and chitosan

Chitin was prepared by demineralization and by deproteinization of materials; demineralization was performed with 2N HCl for 3 hours at room temperature and deproteinization was followed with 1N NaOH for 3 hours at 100℃. And it was dried at 65℃ with hot air blast. Deacetylation of chitin (reaction was carried with 15 volumes of 47% NaOH for 3 hours at 130℃) was performed to produce chitosan.

2. Sonication of chitosan in a solution

Sonic dismembrator (Fisher Scientific, 550) was used to lower the molecular weight of chitosan in a solution. To prevent the rapid increase of solution temperature, pulse on and off time were controlled to 30 seconds, respectively, and intensity was stabilized to 20% and its output frequency was 20kHz.

Various solvent (0.2M HCl, 0,2M HOAc, 0.2M HOAc-0.1M NaOAc) were prepared and 1% chitosan solution (w/v) was made, and then it was sonicated for 5 minutes to examine the effect of solvents.

To make different type of counterions, NaCl, KCl, $NaNO_3$, and $CaCl_2$ were added to acetate buffer to be constant as 0.1 of ionic strength at all cases. Then, 1 % chitosan solution was prepared to sonicate.

Ionic strength was variously controlled such as 0.05, 0.1, 0.15, 0.2 adding 0.05, 0.1, 0.15, 0.2M of NaCl to acetate buffer, and then 1% chitosan solution was prepared.

Volume of chitosan solution was also varied from 20 to 100 ml and temperature of the solution was controlled from 0 to 40℃ using refrigerated temperature bath (Brookfield Model No. TC-500).

And we defined a efficiency of sonication as "$V_o$-$V_t$/$V_o$×100" at our discretion, where $V_o$ indicated initial viscosity of chitosan solution, $V_t$ indicated viscosity of sonicated chitosan solution and t was sonication time.

3. Apparent and intrinsic viscosity measurement of sonicated chitosan solution

Apparent viscosity was measured with Viscometer (Brookfield c/p, DV-II+) at 25℃. Chitosan solutions of which solvent was acetate buffer were analyzed at 30℃ in Ubbelohde Capillary Viscometer and intrinsic viscosity were determined from the relative, specific and reduced viscosity following the Huggins and Kraemer equations. Molecular weights of chitosan solution were calculated by Mark-Houwink equation, which shows relationship between intrinsic viscosity and molecular weight.

$$[\eta] = K \times Mw^a$$

where [η] and Mw indicate intrinsic viscosity and molecular weight. And constant numbers "K" and "a" were $1.424\times10^{-5}$ and 0.96, respectively, according to Luyen (1994).

## Results and Discussion

1. Changes in intrinsic viscosity during the sonication time

Prepared chitosan showed intrinsic viscosity of 3.76 dl/g, molecular weight of approximately $4.44\times10^5$ and degree of deacetylation of 83.5%. And 1% chitosan solution 70 ml was sonicated for 1, 3, 5, 10 minutes respectively and intrinsic viscosities were measured. The results are shown in Fig. 1. Intrinsic viscosity of chitosan solution was sharply decreased until 5 minutes and then slowly decreased. And molecular weight of chitosan also decreased from $4.44 \times 10^5$ to $3.2 \times 10^5$ approximately.

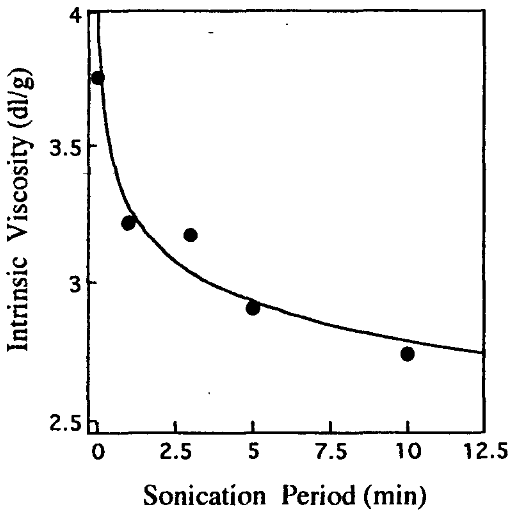


**Fig. 1. Changes in intrinsic viscosity during the sonication period.**

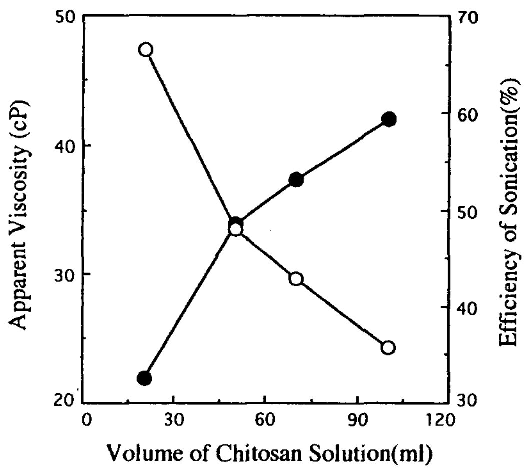


**Fig. 2. Effect of volume on the viscosity of sonicated chitosan solution (1%) for 5 min.**
**–●– Apparent viscosity**
**–○– Efficiency of sonication : Vo-Vt/Vo ×100**

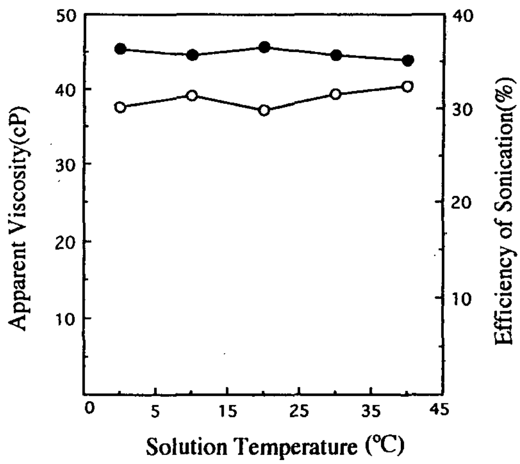


**Fig. 3. Effect of temperature on the viscosity of sonicated chitosan solution (1%) for 5 min.**
**–●– Apparent viscosity**
**–○– Efficiency of sonication : Vo-Vt/Vo ×100**

2. Effect of treatment conditions (physical effect)

2-1. Effect of solution volume

As shown in Fig. 2, the efficiency of sonication was much higher in case of 20 ml of solution volume (66 %) than that of 100 ml of solution volume (35%). In case of 100 ml of solution volume, frictions between chitosan residues and interwinding of chitosan residues presumably prevented the circulation of chitosan solution so that only parts of solution were treated by sonication. However, when the chitosan solution volume was 20 ml, the treated surface area was relatively wider and circulation was well performed than that of 100 ml solution resulted in good sonication efficiency.

2-2. Effect of solution temperature

Chitosan solution temperature was controlled from 0℃ to 40℃ in order to examine the effect of temperature on the efficiency of sonication. And the results are shown in Fig. 3. It was well established that viscosity of solution tended to decrease as the increasement of temperature. From these results, we supposed that sonication treatment at high temperature (40

℃) was more effective. However, the differences were very small, so we concluded that the effect of solution temperature could be disregarded within the temperature range of 0~40℃.

3. Effect of treatment conditions (chemical effect)

3-1. Effect of solvent

To examine the effect of solvent, various solvent-0.2 M HCl (pH 0.7), 0.2 M HOAc (pH 2.65), 0.2M HOAc-0.1M NaOAc buffer (pH 4.5)-were prepared and 1% chitosan solutions were sonicated for 5 minutes. As shown in Fig. 4, viscosity of 1% chitosan solution increased from 45 to 65 cP as the increasement of pH value. This was probably due to destruction of chitosan structure by high acidity and agitation when it was dissolved. No significant differences on viscosities of each sonicated chitosan solution were observed, so we concluded that acetate buffer showed highest effect on sonication than any other solvents.

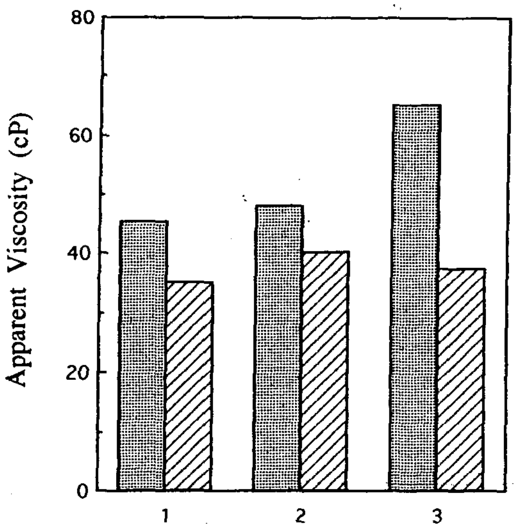


**Fig. 4. Viscosity of 1% chitosan solution with various solvents.**
**1 : 0.2M HCl (pH 0.7)**
**2 : 0.2M HOAc (pH 2.65)**
**3 : 0.2M HOAc-0.1M NaHOAc (pH 4.5)**
**▦ Sonication time : 0 min**
**▨ Sonication time : 5 min**

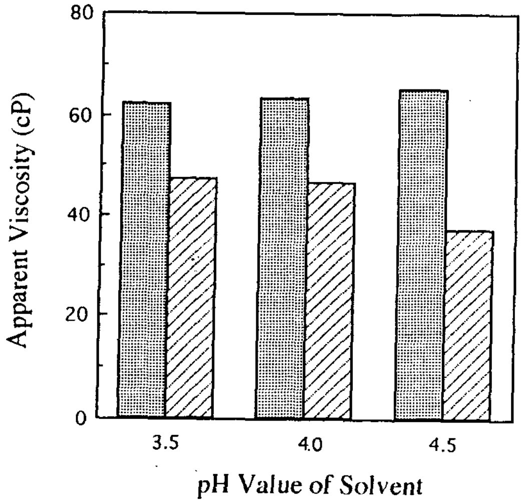


**Fig. 5. Effect of the solvent pH on the viscosity of 1% chitosan solution.**
**▦ Sonication time : 0 min**
**▨ Sonication time : 5 min**

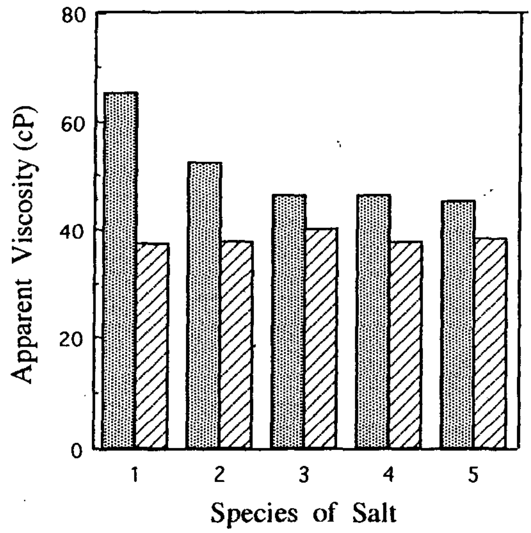


**Fig. 6. Effect of the type of counterions on the viscosity of 1% chitosan solution.**
**1 : Salt free**
**2 : KCl**
**3 : NaCl**
**4 : $NaNO_3$**
**5 : $CaCl_2$**
**▦ Sonication time : 0 min**
**▨ Sonication time : 5 min**

3-2. Effect of pH value

Value of pH of acetate buffer was adjusted to 3.5, 4.0 and 4.5 respectively and 1% chitosan solutions were prepared. In case of pH value of 5.0, chitosan was not to be dissolved sufficiently. Viscosity of chitosan solution, which was sonicated for 5 minutes, had the minimum value of 45 cP at pH 4.5 (Fig. 5). And the sonication showed the hightest effect at pH value of 4.5. As the increase of pH from 3.5 to 4.5, coil overlap between chitosan molecule chains was presumably decreased, and in this condition chitosan chains could easily be treated by sonication, which resulted in effective sonication treatment compared to pH 3.5 and 4.0.

3-3. Effect of counterion type

Various types of counterions, NaCl, KCl, $NaNO_3$, $CaCl_2$, were added to 1% chitosan solution, and their ionic strength was controlled to be constant as 0.1. Fig. 6 shows that the effect of types of counterions. Generally, viscosity of chitosan solution decrease with adding salt. But after sonication, viscosity of chitosan solution showed similar value with each other. So the effect of counterions was considered very small.

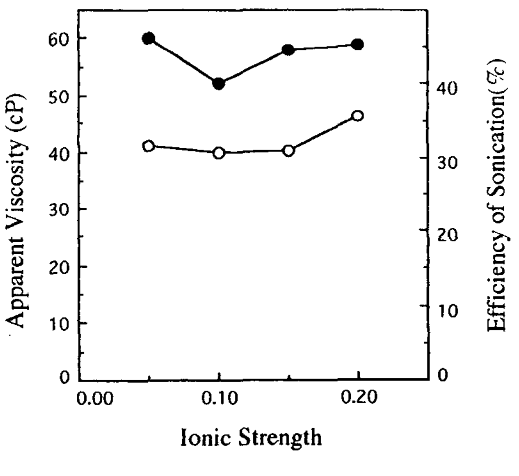


**Fig. 7. Effect of ionic strength of NaCl on the viscosity of 1% chitosan solution.**
**—●— Apparent viscosity**
**—○— Efficiency of sonication : Vo-Vt/Vo ×100**

3-4. Effect of ionic strength

Chitosan solution (1%), in which NaCl was added to make ionic strength value of 0.05, 0.1, 0.15, 0.2, was sonicated for 5 minutes and their results are shown in Fig. 7. Rodriguez-Sanchez (1982) reported that ionic strength affected on the intrinsic viscosity of high molecular electrolytes as a important factor, that is to say viscosity of dilute solution decreased as the increasement of ionic strength. But, in case of 1% chitosan solution, it showed that ionic strength had no notable effect on the sonication.

From these facts, we assumed that sonication treatment was not sufficient to produce chitin and chitosan oligosaccharides, as their structure have very hard bonds to destroy. And above experiments were carried without knowing the best condition for sonication, so the best condition for sonication including volume of solution, sonication intensity, temperature and concentration of chitosan solution, and sonication time should be investigated at further study for the most effective sonication treatment. And if we carry out two methods, physical and enzymic treatment side by side, it would be more effective way to produce chitin and chitosan oligosaccharides.

## Acknowledgements

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