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Two-stage microfluidization combined with ultrafiltration treatment for chitosan mass production and molecular weight manipulation

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ABSTRACT

The objectives of the study were to propose a two-stage microfluidization combined with an ultrafiltration (UF) treatment for chitosan mass production and the manipulation of molecular weight and its distribution. The proposed methods are based on the degradation rate and rate constant of various process variables studied. Results obtained were that the rate constants were faster during the earlier reaction period, were higher for those operating at a higher pressure, were better for using concurrent UF treatment to remove small degraded fragments, and the degradation rate constants were faster for 30 °C solutions than that for 50 or 0 °C. A two-stage microfluidization process is proposed. The first stage constitutes of the highest possible concentration solution with concurrent UF treatment at 50 °C, and recycled 5 times. The second stage consists of the highest possible concentration of solutions with concurrent UF treatment at 30 °C, and recycled 5 times.

1. Introduction

Chitinous materials, including chitin and chitosan, are considered to be versatile, environmentally friendly biomaterials. Chitin is composed of *N*-acetyl-glucosamine and glucosamine. Chitinous materials, considered to be the most widely distributed polycationic biopolymers, have huge resources, are non-toxic and are biodegradable. Chitinous materials can be applied in food processing, agriculture, biomedicine, biochemistry, wastewater treatment, paper, textiles, cosmetics, nanoparticles, hydrogel, liquid crystals, membranes, microcapsules, etc. (Chang, Chang, & Tsai, 2007; Ravi Kumar, 2000; Rinaudo, 2006; Tsai, Bai, & Chen, 2008).

The molecular weight of chitinous materials is a very important parameter which affects their applications; therefore, it is important to develop a method by which to manipulate their molecular weight and also to preserve their integral structure. Degradation methods concurrently used include: chemical (Huang, Zhuo, & Guo, 2008), enzymatic (Li, Du, Liang, Yao, & Wei, 2006), physical methods of ultrasonic (Chen, Chang, & Shyur, 1997; Tsaih & Chen, 2003; Tsaih, Tseng, & Chen, 2004), microfluidization (Kasaai, Charlet, Paquin, & Arul, 2003), mechanical shearing (Chen, Chang, & Shyur, 1998), and microbial methods (Chen & Chen, 1999).

Chemical methods are difficult to manipulate with the molecular weights of resultant chitosan. Enzymatic and microbial methods cannot be feasible for use in mass production. The ultrasonic method is an efficient mechanical method; however, the eroded metal ions of the horn might contaminate the products. The mechanical shearing method apparatus is inexpensive and easy to obtain, but the degradation efficiency is low. Microfluidization is a potentially promising method which can be used in mass production and continuous processing.

The microfluidization process is one of the physical methods used to manipulate the molecular weight of the polymers. The solution stream was accelerated to a very high speed and forced into a reaction chamber by an air compressor. The process stream separates in two, changes direction and collides into a single stream again, generating a powerful shear force, turbulence, impaction, and cavitation forces. Those forces cause the disintegration of the particles or the degradation of the polymers (Cencia-Rohan & Silvestri, 1993; Kasaai et al., 2003; Silvestri, Gabrielson, & Wu, 1991). The microfluidization process is the combination of ultrasonic radiation and mechanical shearing. Microfluidization has been applied in cell rupture, homogenization or preparing the unilamellar vesicle (Masson, 1989).

Kasaai et al. (2003) reported that chitosan was degraded with microfluidization. The average number of chain scission, $(M_0/M_t) - 1$, was used as the index for degradation. The average number of chain scission increased bi-linearly with increased operation pressure, number of passes, and the molecular weight of chitosan used. However, the average number of chain scission decreased bi-linearly with the increase in solution concentration. The effect of solution temperature was not significant. The efficiency of chitosan degradation by continuous process was higher than that of volume passes. Chitosan degraded by microfluidization process will narrow the molecular weight distribution of the resulting product.

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The degree of deacetylation (DD) of the resulting chitosan increased when 0.1 M acetic acid was used as a solvent, whereas the DD of the resulting chitosan did not change when 0.04 M hydrochloric acid was used as a solvent.

Tsaih et al. (2004) reported that the 84% DD chitosan was degraded with ultrasonic radiation with or without concurrent ultrafiltration (UF) treatment used to remove the small degraded fragments. The rate constant and degradation kinetics were higher for those using concurrent UF treatment to remove small degraded fragments during the ultrasonic operation than for those not using UF ones, i.e. UF treatment can improve the de-polymerization rate of chitosan.

The objectives of this study were to propose a two-stage microfluidization process combined with an UF treatment for chitosan mass production and molecular weight manipulation. The proposed methods were based on the degradation rate and rate constant of various process variables, such as: number of recycled treatments, solution temperature, operation pressure, solution concentration, and with, or without, concurrent removal of degraded fragments during microfluidization.

2. Experimental

2.1. Chitosan preparation

Chitin was prepared from shrimp (*Solenocera prominentis*) waste by modifying a method of Stanley, Watters, Chan, and Mercer (1975) and Chen, Lin, and Yang (1994). Ground shrimp waste was treated with 0.5 N NaOH at ambient temperatures to hydrolyze the surface flesh. The alkali-treated waste was washed until it was neutral, and then dried and disintegrated to obtain powder. The powder was passed though sieves of 40–60 meshes. The flake-free powder was soaked in 2 N HCl for 2 h to remove the minerals, until no CO_2 evolved. The de-mineralized powder was soaked in 2 N NaOH at 80 °C to hydrolyze the protein, and then washed with water until neutral. The alkali-treated powder was soaked in 1% $KMnO_4$ at room temperature for 1 h to oxidize the astaxanthin, and then soaked in 1% oxalic acid at 80 °C for 1 h to neutralize the $KMnO_4$. This was washed then dried to get a white chitin powder. Chitin powder was alkali-treated (50% NaOH) at 140 °C for 3 h to get about 80% DD chitosan. This was washed until neutral and dried at 50 °C to get the final product (Tsaih & Chen, 1999).

2.2. Degree of deacetylation measurement

Infrared spectrometry was used to determine the DD of the chitosans (Baxter, Dillon, Taylor, & Roberts, 1992). Chitosan powder was strained through a 200 mesh sieve and then mixed with KBr (1:100) and pressed into a pellet. The absorbance of amide I (1655 cm^{-1}) and hydroxyl band (3450 cm^{-1}) was measured using a Bio-Rad FTS-155 infrared spectrophotometer. The band of the hydroxyl group at 3450 cm^{-1} was used as an internal standard to correct for disc thickness and for differences in chitosan concentration in making the KBr disc. The percentage of the amine group's acetylation in a sample is given by $(A_{1655}/A_{3450}) \times 115$. Here, A_{1655}, A_{3450} are the absorbance at 1650 and 3450 cm^{-1}, respectively. The DD of the chitosan used in this study is 84%.

2.3. Molecular weight determination

A size exclusion high performance liquid chromatography (SE-HPLC) method of Tsaih and Chen (1999) was followed. A column (7.8 mm × 30 cm) packed with TSK gel G4000 PW_{XL} and G5000 PW_{XL} (Tosoh Co., Ltd, Japan) was used. The mobile phase consisted of 0.2 M acetic acid/0.1 M sodium acetate, and 0.008 M sodium azide. Sample concentration of 0.1% (w/v) was loaded and eluated with a flow rate of 0.6 ml/min by an LDC Analytical ConstaMetric 3500 pump. The elute peak was detected by an RI detector (Gilson Model M132, USA). The data was analyzed by the Chem-Lab software (Scientific Information Service Corporation, Taiwan). Chitosans with known molecular weight (determined by light scattering) were used as markers. The calibration curve of the elution volume and the molecular weight were established. The weight average molecular weights of the samples were calculated from the calibration curve with the Chem-Lab software. The molecular weight of the chitosan used in this study was 1790 kDa.

2.4. Microfluidization treatment without concurrent removal of small degraded fragments

The chitosan solution was prepared by dissolving 0.2%, 0.8%, 1.4%, and 2.0% (w/v) of chitosan in acetic acid buffer (0.2 M acetic acid/0.1 M sodium acetate, pH 4.3). The solution was passed through a filter (Toyo Roshi Kaisha Ltd., No. 1, 55 mm, Japan) to remove the insoluble materials. An aliquot of 300 ml filtrate in a stainless vessel was placed in a water bath (Firstek, B403, Taipei) at a pre-set temperature of 0 ± 1, 30 ± 1, 50 ± 1 °C and was treated with a microfluidizer (Microfluidizer, M-100Y cell Disruption, Microfluidics Corporation, USA) at a pressure of 82.7 or 117.2 MPa for 5, 10, 15, 20 and 25 passes. An aliquot of the sample was piped out to analyze the molecular weight by SE-HPLC immediately after each preset cyclic treatment.

2.5. Microfluidization treatment with concurrent removal of small degraded fragments

During microfluidization treatment, the solution was circulated through an UF spiral-wound cartridge with a cut-off size of 1000 Da (Amicon CH2PRS system, Beverly, Mass.) to remove the degraded molecules (Fig. 1). The retentates were returned to the reactor for continuous treatment. Fresh solution equal to the volume of the eluate was added at the pre-determined time to make up the reaction solution at constant volume. An aliquot of the sample was piped out to analyze the molecular weight by SE-HPLC immediately after each preset cyclic treatment.

2.6. Calculation of the rate constant

The degradation reaction by microfluidization treatment is a first-order reaction. Its rate constant (k) can be obtained from

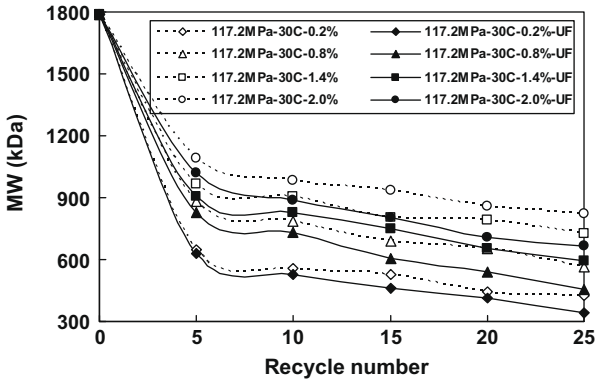

Fig. 1. Effect of solution concentration on the changes of molecular weight of chitosan treated by microfluidization with or without concurrent removal of small degraded fragments by ultrafiltration (UF) treatment over different cyclic treatments at 117.2 MPa, 30 °C.

Eq. (1) (Lii, Chen, Yeh, & Lai, 1999; Tsaih & Chen, 2003; Tsaih et al., 2004):

$$1/M_t = 1/M_0 + kt/m = 1/M_0 + k't, \quad (1)$$

where k is the rate constant (s^{-1}) of molecular weight degradation during microfluidization, k' is in mol g^{-1} s^{-1}, t is the microfluidization time, M_0, and M_t are the weight average molecular weight of the chitosans before microfluidization and after microfluidization treatment, respectively, and m is the molecular weight of the chitosan monomer, $m = 161 + 42\ (1 - DD)$.

Since microfluidization treatment is a batch type operation, the reaction time is controlled by the number of batch volumes that pass through the reaction chamber; therefore, the reaction time is changed to the number of batch volumes passing through, and Eq. (1) changes to Eq. (2) as follows:

$$1/M_t = 1/M_0 + kN/m = 1/M_0 + k'N \quad (2)$$

where k is the rate constant (recycle^{-1}) of molecular weight degradation during microfluidization, k' is in mol g^{-1} recycle^{-1}, N is the number of passes of the microfluidization process.

3. Results and discussion

3.1. Effect of the number of passes of the microfluidization process

Fig. 1 and Table 1 show that the molecular weight of chitosan decreased over the number of passes for four different concentrations, either with UF or without using UF to remove small degraded fragments during the microfluidization process at 117.2 MPa, 30 °C. The decrease was faster during the early operation periods. Similar results were observed for chitosan solutions (Kasaai et al., 2003) and xanthan gum (Lagoueyte & Paquin, 1998).

Results in Fig. 2 show that the effect of recycle numbers on the pseudo-rate constant of microfluidization treatment on 0.2% chitosan at 117.2 MPa, 30 °C, and the concurrent removal of small degraded fragments by UF treatment. Line 1 is the regression line of the pseudo-rate constant of microfluidization treatment from recycle #0 to recycle #25, line 2 is the regression line of the pseudo-rate constant of treatment from recycle #0 to recycle #5, and line 3 is the regression line of pseudo-rate constant of treatment from recycle #5 to recycle #25. Line 1 obviously deviated from its origin. The data were re-plotted to obtain line 2 and line 3 and Eq. (2) was used to calculate the rate constants before and after the number 5 recycle, as listed in Table 2. The results show that all of the $k_{0,5}$ for all different conditions were larger than those of all $k_{5,25}$ of the corresponding conditions. The results indicate that the constant rate of chitosan decreased with the increase in the number of recycle treatments and that the decreases in molecular weight were more efficient during earlier reaction periods. Tsaih and Chen (2003) had similar results and they reported that the molecular weight of chitosan decreased in correlation to the sonolysis time, and that the decreases in molecular weight were more pronounced during earlier reaction times. Kasaai et al. (2003) reported that when chitosan was treated with the microfluidization process, the average number of chain scission increased bi-linearly (the slope was higher at early periods of operation and lower at the later operation periods) with the increased number of passes, i.e. decreases in molecular weight was more pronounced in the beginning. This may be because in the earlier stage (higher degradation rate) (Fig. 2), the rate constant was the sum of the results of cavitation, free radical effect, and entanglement tearing force. However, in the later stage (lower degradation rate), the rate constant was the sum of the results of cavitation and free radical effect only, because the higher molecular weight chitosan species were consumed in the earlier stage. The mechanism of the polymer entanglement tearing most likely occurred in the earlier state of degradation during the high rate of shearing, via the strong elongated flow encountered by the polymer, which may entail sufficient energy to disrupt the molecules (Casale & Porter, 1978).

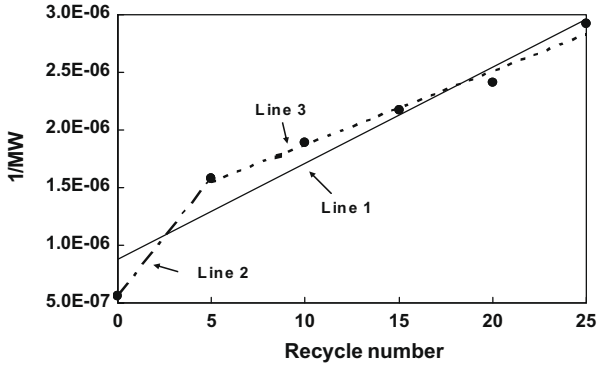

Fig. 2. Plot of the reciprocal molecular weight and recycle number of microfluidization treatment of 0.2% chitosan solution at 117.2 MPa, 30 °C, and concurrent removal of small degraded fragment by UF treatment. Line 1 is the regression line of the pseudo-rate constant of treatment from recycle #0 to recycle #25, line 2 is the regression line of the pseudo-rate constant of treatment from recycle #0 to recycle #5, and line 3 is the regression line of the pseudo-rate constant of treatment from recycle #5 to recycle #25.

Table 1
The change of molecular weight (kDa) of 0.2% chitosans solution during microfluidization (117.2 MPa) treatments under various temperatures and recycle numbers (UF, ultrafiltration treatment).

Condition	Recycle number					
	0	5	10	15	20	25
0 °C	1790[a]	867[b,t]	716[c,t]	705[c,t]	650[d,t]	558[e,t]
30 °C	1790[a]	651[b,x,y]	559[c,y]	526[c,x]	442[d,w]	415[e,x]
50 °C	1790[a]	729[b,u]	624[c,u]	571[d,u]	482[e,u]	452[f,u]
0 °C-UF	1790[a]	675[b,w]	596[c,w]	564[d,w]	480[e,u]	431[f,w]
30 °C-UF	1790[a]	633[b,y]	530[c,z]	460[d,z]	415[e,x]	343[f,z]
50 °C-UF	1790[a]	656[b,w,x]	561[c,x]	511[d,y]	445[e,w]	397[e,y]

[a–f] Means value ($n = 2$) followed by the same superscripted within the same row are not significantly different ($p > 0.05$ by Duncan's multiple range test).
[t–z] Means value ($n = 2$) followed by the same superscripted within the same column are not significantly different ($p > 0.05$ by Duncan's multiple range test).

Table 2
Rate constants (recycle^{-1}) of chitosan solution degraded with different microfluidization conditions (k, the rate constants of 0–25 recycle; $k_{0,5}$, the rate constants of 0–5 recycle; $k_{5,25}$, the rate constants of 5 to 25 recycle).

Pressure (MPa)	Temp. (°C)	Conc. (%)	UF*	k (E-06)	$k_{0,5}$ (E-06)	$k_{5,25}$ (E-06)
117.2	30	0.2	+	14.0	34.2	10.7
117.2	30	0.8	+	9.98	21.6	8.29
117.2	30	1.4	+	6.78	18.3	5.03
117.2	30	2.0	+	5.85	14.1	4.43
117.2	30	0.2	–	11.1	32.7	7.45
117.2	30	0.8	–	7.18	19.5	5.12
117.2	30	1.4	–	4.73	15.9	2.87
117.2	30	2.0	–	3.62	12.0	2.31
117.2	0	0.2	+	10.3	31.0	6.99
117.2	0	0.2	–	7.04	20.0	4.76
117.2	0	2.0	+	4.31	10.7	3.02
117.2	50	0.2	+	11.6	32.4	8.24
117.2	50	0.2	–	10.6	27.3	7.23
117.2	50	2.0	+	5.05	15.0	3.40
82.7	30	0.2	+	10.6	19.1	9.04

* + and – representing with or without concurrent UF treatment to remove small degraded fragments during the microfluidization process, respectively.

3.2. Effect of removing small degraded fragments by UF

Fig. 1 shows that the effect of concurrent UF treatment to remove small degraded fragments during microfluidization resulted in lower molecular weight products than for those without using UF treatment, i.e. increasing the efficiency or rate constant of microfluidization treatment.

Data in Table 2 show that the degradation rate constant was higher for those using concurrent UF treatment to remove small degraded fragments during the microfluidization process than for those without using UF treatment. Fig. 3 shows that the $(k - \mathrm{UF})/k$ ratio increased with the increased concentration of chitosan. The use of concurrent UF to remove small degraded fragments during microfluidization will increase the rate constant from 1.26 times (0.2% chitosan solution) to 1.62 times (2.0% chitosan solution). In other words, UF treatment can alleviate the crowded effect caused by the higher chitosan concentration, consequently increasing the degradation rate constant. Microfluidization in combination with UF treatment enhanced the degradation rate of chitosan more than did degradation by ultrasonic radiation (Tsaih et al., 2004). This may be because during microfluidization processing, the small molecules remaining in the reaction vessel will reduce the chance of the higher molecular weight molecules being degraded by the forces of cavitation, turbulence, shear force, etc. or perhaps because the small degraded molecules become free radicals species by interacting with the free radicals generated by the cavitation effect. The free radical species might interact with other molecules in the solution to generate a higher molecular weight polymer, thus increasing the average molecular weight of the products and decreasing the degradation rate constant (Portenlänger & Heusinger, 1994).

3.3. Effect of solution temperature

Data in Table 1 shows the effects of solution temperature on the changes of the molecular weight of chitosan. The molecular weights of chitosan in solutions of 30 °C were the lowest among three different solution temperatures studied.

Data in Table 2 also shows that the degradation rate constants were the highest for 30 °C solutions among the three different solution temperatures studied. The degradation rate constant of 30 °C solutions were higher than those of 50 °C, which were in turn higher than those of 0 °C.

Chen et al. (1997) reported using ultrasonic radiation to degrade the chitosan molecules in solutions of 4 or 50 °C. Using the molecular weight decrease ratio (MWDR) as an index of degradation rate, the MWDR of the chitosan solution at 4 °C were higher than those of 50 °C. Tsaih et al. (2004) reported that the degradation rate constant of chitosan by ultrasonic radiation in solutions of 30 °C was higher than that of 0 °C, which was in turn higher than that of 50 °C. Chen et al. (1998) reported that a degradation rate constant of chitosan by mechanical shearing in solutions of higher temperatures was higher than that at lower temperatures. Higher solution temperature facilitates the degradation reaction with mechanical shearing.

Kasaai et al. (2003) reported that an average number of chain scissions were all about 0.8 for chitosan degraded by microfluidization process at 25, 40, and 50 °C. This may be because higher solution temperature will lower the solution viscosity and in turn, increase the thermal motion and the expansion factor. Higher thermal motion may reduce the degradation efficiency; however, higher expansion factors may increase the degradation efficiency. Those two effects will neutralize each other and nullify the effect of the solution temperature.

However, results of this report show that the degradation rate constant of chitosan solutions of 30 °C was higher than that of 50 °C solutions which, in turn, was higher than that of 0 °C solutions. The effect of solution temperature on the degradation rate may not only be because of the effect of temperature on thermal motion, expansion factor of the chitosan molecules in solution but may also be because of the effects of the cavitation formation and transportation. Since the elevation temperature facilitates the loss of cavitation energy (Chen et al., 1997; Ohta, Kato, & Kawahara, 1983), reaction rates were higher at 30 °C than at 50 °C. Lii et al. (1999) reported that a high viscous solution hinders the effect of cavitation energy because solutions at lower temperatures have a higher viscosity than do higher temperature ones; thus a reaction rate at 0 °C was lower than that at 30 °C. As a result, the mechanisms of the microfluidization process are the combined effects of ultrasonic radiation and mechanical shearing that generate powerful shear, turbulence, impaction, and cavitation forces (Cencia-Rohan & Silvestri, 1993; Kasaai et al., 2003; Silvestri et al., 1991). In regard to temperature affecting the cavitation formation, transportation should have an effect on degradation among different solution temperatures.

3.4. Effect of chitosan concentration

Data in Fig. 1 shows the effect of solution concentrations on the efficiency of the degradation rate of treated chitosan. The higher the solution concentration, the higher the molecular weight of the end products of degraded chitosan. The molecular weights of 0.2% chitosan solutions have the lowest molecular weight while the 2.0% solutions have the highest molecular weight.

Data in Table 2 shows that the degradation rate constant decreased in correlation to the increased solution concentration. The results indicated that the higher concentration solution hindered the microfluidization degradation. These trends have been observed in chitosan degradation by ultrasonic radiation (Chen et al. 1997; Tsaih & Chen, 2003; Tsaih et al., 2004), and by mechanical shearing (Chen et al., 1998); perhaps under the same operation pressure, the output energy level is the same but an increased solution concentration will increase the number of molecules. This will reduce the energy level received by each molecule, and therefore will decrease the degradation rate. It may also be because the higher the solution concentration, the higher the solution viscosity. A high viscous solution retards the cavitation formation and transportation (Lii et al., 1999). Kasaai et al. (2003) reported that when using microfluidization to degrade the chitosan, the average number of chain scission of chitosan decreased bi-linearly with an increasing concentration of chitosan solution.

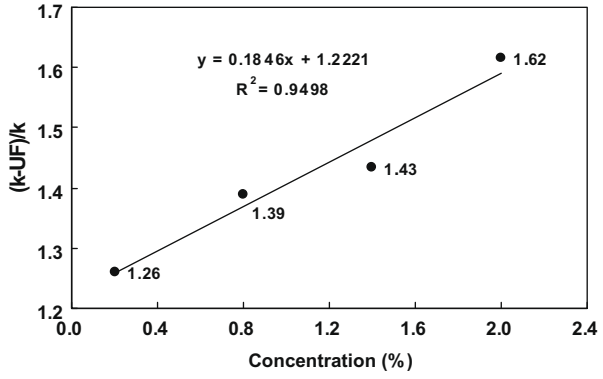

Fig. 3. Effect of chitosan concentration on rate constant ratio of $(k - \mathrm{UF})/k$ by microfluidization treatment at 117.2 MPa, 30 °C over different cyclic treatments. The $k - \mathrm{UF}$ and k representing the rate constant of chitosan solution degraded by microfluidization with or without concurrent removal of small degraded fragments by UF treatment, respectively.

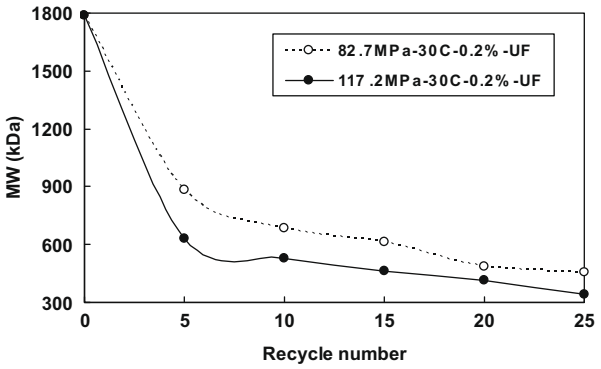

Fig. 4. Effect of operation pressure of microfluidization treatment on the changes of molecular weight of 0.2% chitosan solution at 30 °C, and concurrent removal of small degraded fragments by UF treatment over different cyclic treatments.

3.5. Effect of pressure of microfluidization process

Fig. 4 showed that the molecular weight of the resulting chitosan was smaller for those treated at a higher operation pressure (117.2 MPa) than that of lower pressure treatment (82.7 MPa).

Data in Table 2 shows that the rate constant was higher for those operated at a pressure of 117.2 than that at 82.7 MPa. This indicates that higher operation pressure will result in higher degradation efficiency, and that the higher pressure of the microfluidization process facilitates the degradation reaction. Kasaai et al. (2003) reported similar results; they revealed that the average number of chain scission increased with increased operation pressure. It indicated that the higher the operation pressure, the higher the degradation efficiency. This may be because increasing the operation pressure will enhance the fluid velocity and increase the energy flow, thereby enhancing the efficiency of degradation and resulting in rapidly lowering the molecular weight of chitosan, so that the rate constant increases.

3.6. Rationale for proposing a two- stage process method

The increasing rate constant from using concurrent UF to remove small degraded fragments was more pronounced for using a higher concentration solutions than for a lower concentration ones (1.62 times of 2.0% solution to 1.26 times of 0.2%, respectively) (Fig. 3).

For 2.0% solution, the $k_{0.5}$ at 50 °C was higher than that of 30 °C (15.0×10^{-6} vs. 14.1×10^{-6}). This may be because the viscosity of a solution at 50 °C is lower than that found at 30 °C. A lower viscosity solution facilitates entanglement and thus stretched entangled molecules each other to generate the effective shearing needed to tear apart the molecules; therefore, the degradation rate constant increased.

At 30 °C, the k value of 0.2% was 14.0×10^{-6} recycle^{-1}, whereas the k value of 2.0% was 5.85×10^{-6} recycle^{-1}. The solution concentration increased 10-fold, whereas the rate constant only decreased 41.8% (Table 3). Under the same output energy of microfluidization, the efficiency of treating the 2.0% solution was 4.18 times higher than that of treating the 0.2% solution. In considering operational cost, degradation rate and productivity, the highest concentration solution would be preferred.

Table 3
Effect of chitosan concentration on degraded efficiency of microfluidization combined with UF and treated at 117.2 MPa, 30 °C.

Chitosan concentration (%)	Ratio of concentration[a]	Ratio of k[b]	Efficiency[c]
0.2	1	1.00	1.00
0.8	4	0.71	2.84
1.4	7	0.48	3.36
2.0	10	0.42	4.20

[a] Ratio of concentration, $C_x/C_{0.2}$, x are concentration of chitosan.
[b] Ratio of k, $k_x/k_{0.2}$, x are concentration of chitosan.
[c] Efficiency = (ratio of concentration) × (ratio of k).

Based on the above analysis, a two-stage micofluidization process is proposed. Using the highest possible concentration solution, the first stage constitutes of concurrent UF treatment at 50 °C, and recycled 5 times. The second stage consists of concurrent UF treatment at 30 °C, and recycled 5 times. The reason for using the highest possible concentration solution with concurrent UF treatment was illustrated in Fig. 3 and Table 3. The reason for proposing microfluidization at 50 °C, and recycled 5 times for the first stage was based on that for 2.0% solution, the $k_{0.5}$ at 50 °C was higher than that of 30 °C (15.0×10^{-6} vs. 14.1×10^{-6}) shown in Table 2. Whereas, the reason for proposing microfluidization at 30 °C, and recycled 5 times for the second stage was illustrated in Table 3.

4. Conclusions

A two-stage microfluidization combined with an ultrafiltration (UF) treatment for chitosan mass production and the manipulation of molecular weight and its distribution was proposed. The proposed methods are based on the degradation rate and rate constant of various process variables studied. The proposal constitute using the highest possible concentration solution in either the first or the second stages and microfluidization at 50 °C, and recycled 5 times for the first stage, then microfluidization at 30 °C, and recycled 5 times for the second stage.

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References

Baxter, A., Dillon, M., Taylor, K. D. A., & Roberts, G. A. F. (1992). Improved method for i.r. determination of the degree of N-acetylation of chitosan. *International Journal of Biology Macromolecules, 14*, 166–169.
Casale, A., & Porter, P. (1978). *Polymer stress reactions*. New York: Academic Press. Vol. 1.
Cencia-Rohan, L., & Silvestri, S. (1993). Effect of solvent system on microfluidization-induced mechanical degradation. *International Journal of Pharmaceutics, 95*, 23–28.
Chang, J. S., Chang, K. L. B., & Tsai, M. L. (2007). Liquid-crystalline behavior of chitosan in malic acid. *Journal of Applied Polymer Science, 105*, 2670–2675.
Chen, R. H., Chang, J. R., & Shyur, J. S. (1997). Effects of ultrasonic conditions and storage in acidic solutions on changes in molecular weight and polydispersity of treated chitosan. *Carbohydrate Research, 299*, 287–294.
Chen, R. H., Chang, J. R., & Shyur, J. S. (1998). Effect of shear conditions and storage in acidic solution on changes in molecular weight and polydispersity of treated chitosan. *Journal of the Fisheries Society of Taiwan, 25*(3), 219–229.
Chen, R. H., Lin, J. H., & Yang, M. H. (1994). Relationships between the chain flexibilities of chitosan molecules and the physical properties of their casted films. *Carbohydrate Polymers, 24*, 41–46.
Chen, S.-H., & Chen, H.-C. (1999). Effect of oral administration of *Cellulomonas flavigena* NTOU 1-degraded chitin hydrolysate on physiological changes in rats. *Food Science and Agricultural Chemistry, 1*(3), 186–193.
Huang, Q. Z., Zhuo, L. H., & Guo, Y. C. (2008). Heterogeneous degradation of chitosan with H_2O_2 catalysed by phosphotungstate. *Carbohydrate Polymers, 72*, 500–505.
Kasaai, M. R., Charlet, G., Paquin, P., & Arul, J. (2003). Fragmentation of chitosan by microfluidization process. *Innovative Food Science and Emerging Technologies, 4*, 403–413.
Lagoueyte, N., & Paquin, P. (1998). Effects of microfluidization on the functional properties of xanthan gum. *Food Hydrocolloids, 12*, 365–371.
Li, J., Du, Y. M., Liang, H. B., Yao, P. J., & Wei, Y. A. (2006). Effect of immobilized neutral protease on the preparation and physicochemical properties of low molecular weight chitosan and chito-oligomers. *Journal of Applied Polymer Science, 102*, 4185–4193.
Lii, C.-Y., Chen, C.-H., Yeh, A.-I., & Lai, V. M.-F. (1999). Preliminary study on the degradation kinetics of agarose and carrageenans by ultrasound. *Food Hydrocolloids, 13*, 477–481.

Masson, G. (1989). Advanced techniques for preparation and characterization of small unilamellar vesicles. *Food Microstructure, 8*, 11–14.
Ohta, K., Kato, S., & Kawahara, K. (1983). Ultrasonic degradation of dextran in solution. *Kobunshi Ronbunshu, 40*, 417–423.
Portenlänger, G., & Heusinger, H. (1994). Polymer formation from aqueous solutions of α-D-glucose by ultrasound and γ-rays. *Ultrasonics Sonochemistry, 1*, S125–S129.
Ravi Kumar, M. N. V. (2000). A review of chitin and chitosan applications. *Reactive & Functional Polymers, 46*, 1–27.
Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science, 31*, 603–632.
Silvestri, S., Gabrielson, G., & Wu, L. L. (1991). Effect of terminal block on the microfluidization induced degradation of a model A–B–A block copolymer. *International Journal of Pharmaceutics, 71*, 65–71.
Stanley, W. L., Watters, G. G., Chan, B. G., & Mercer, J. M. (1975). Lactose and other enzymes bound to chitin with glutaraldehyde. *Biotechnology & Bioengineering, XVII*, 315–326.
Tsai, M. L., Bai, S. W., & Chen, R. H. (2008). Cavitation effects versus stretch effects resulted in different size and polydispersity of ionotropic gelation chitosan–sodium tripolyphosphate nanoparticle. *Carbohydrate Polymers, 71*, 448–457.
Tsaih, M. L., & Chen, R. H. (1999). Molecular weight determination of 83% degree of deacetylation chitosan with non-Gaussian and wide range distribution by high-performance size exclusion chromatography and capillary viscometry. *Journal of Applied Polymer Science, 71*, 1905–1913.
Tsaih, M. L., & Chen, R. H. (2003). The effect of degree of deacetylation of chitosan on the kinetics of ultrasonic degradation of chitosan. *Journal of Applied Polymer Science, 90*, 3526–3531.
Tsaih, M. L., Tseng, L. Z., & Chen, R. H. (2004). Effects of removing small fragment with ultrafiltration treatment and ultrasonic conditions on degradation kinetics of chitosan. *Polymer Degradation and Stability, 86*, 25–32.