Journal of Metals, Materials and Minerals. Vol.15 No.1 pp. 7-11, 2005

Manipulations of the Molecular Weight and/or the Degree of Deacetylation of Chitosan by Mechanical Treatments

Rong Huei CHEN

Department of Food Science, National Taiwan Ocean University,
2-Pei-Ning Road, Keelung, Taiwan

Dedicated to the late Protessor Dr.Shigehiro Hirano

Abstract

The paper discuss why it is needed to manipulate the molecular weight (MW) and the degree of deacetylation (DD) of chitisan. Why the mechanical treatment methods were used to manipulate the above mentioned two most important characteristics of chitosan. The paper also discusses the factors effect the degradation rate of chitosan including: 1. type of mechanical treatment, 2. solution concentration, 3. reaction temperature, 4. removal of small degraded fragments, 5. the molecular weight and 6. the degree of deacetylation of chitosan, and 7. the other factors. Finaly the conclusion.

Keywords: ultrasonic radiation, mechanical shearing, microfluidization, molecular weight, degree of Deacetylation, degradation, polydispersion

Introduction

I. Why it is needed to manipulate the molecular weight and/or degree of deacetylation of chitosan

It is well known that functional properties of chitosans depend on intrinsic factors such as: degree of deacetylation (DD), distribution of the acetyl groups, molecular weight (MW) and its distribution etc. Different MWs chitosan were usually prepared by different hydrolysis methods. However, the MWs and/or DDs of chitosan produced by the most widely employed method, high temperature alkali hydrolysis, can not be controlled and the polydispersity of the resulted products are very wide because of the dual reactions on the bonds of acetamido and the β-1, 4 glucoside linkage occurred simultaneous during the chemical method and the random hydrolysis on the β-1, 4 glucoside linkage.

The MW of chitosan have been reported to affect the rheological properties and the conformation of solute in solution, water-holding capacity, antimicrobial capability, binding capacity with serum albumin, forming complex with DNA to protect from nuclease hydrolysis, to improve juiceness and overall acceptance of Chinese sausage (Tsaih and Chen, 2004). The DD also have been reported to affect the release of core materials, chain flexibility, immunoadjuvant, mechanical properties and pore size of membrane, bactericide effect, enzyme-binding ability, metal binding and biodegradation of chitosan film (Tsaih and Chen, 2003).

II. Why the mechanical treatment was used

There are many methods such as: chemical method of using high temperature alkali hydrolysis, enzymatic method of using chitinase or chitosanasc, oxidative reaction methods of using hydrogen peroxide, ozone, Fenten reaction, etc. (Tsaih and Chen, 2004) to manipulate the MW and DD of chitosan. Factors effect the choosing of a method to be employed are the operation cost, quality of the product, feasibility of mass production, and environmental impact. Each mentioned method has its pros and cons, for example the shortcoming of the commercial method, high temperature alkali hydrolysis, are that MW and/or DD of the products can not be controlled, their polydispersity are very wide, furthermore, it may cause enviromental stress. The MW and/or DD of the resulted product obtained by enzymatic method can be very specifically controlled but mass production may not be practical. The merits of using mechanical methods is that the DD of resulted chitosan can be maintained almost unchanged (Table 1) but the MW of the products can be controlled to the expected values, mass production is relatively easier, and it is enviromentally friendly. It may be due to the reaction rates for breaking the β-1, 4 glucoside linkage and for attacking the acetamido group are different. These changes occur as a result of several mechanisms such as: heating, structural effects, compression and rarefaction, turbulence, and cavitation. Cavitation is the formation, growth, and violent collapse of small bubbles or voids in liquids as a result of pressure fluctuation and/or a large negative pressure is created. Depolymerization may be due to shock wave energy, shear stress at cavitation interphase, local high press and temperature, the relative motion of the polymer segments and solvents, and elongation flow produces the stress etc. during the collapse of the cavity (Tsaih and Chen, 2003).

Table 1. The changes of degree of deacetyolation of chitosans after various microfluidizating treatments

Recycles	0	1	5	15	20
0.2 %	$73.01^{a,b**}$	$73.25^{a,b}$	74.93^{a}	74.91^{a}	74.94^{a}
0.8 %	$73.01^{a,b}$	$73.91^{a,b}$	74.43^{a}	$74.24^{a,b}$	75.01^{a}
1.4 %	$73.01^{a,b}$	$72.93^{a,b}$	$72.97^{a,b}$	$74.14^{a,b}$	$73.81^{a,b}$
2.0 % a~b	$73.01^{a,b}$	$73.44^{a,b}$	74.78^{a}	$73.12^{a,b}$	$74.22^{a,b}$

Means values (n = 3) followed by the same superscripts within the same block are not significantly different (P > 0.05 by Duncan's multiple range test). (Wu, 1998)

III. Factor effect on the reaction rate and/or rate constant

Factors affect the reaction rate and/or rate constant of breaking the β-1, 4 glucoside linkage include: 1. type of mechanical treatment, 2. solution concentration, 3. reaction temperature, 4. removal of small degraded fragments, 5. the molecular weight and 6. the degree of deacetylation of chitosan, and 7. the other factors such as: the solvent used and the reaction time etc.

3.1 Type of mechanical treatment

The types of mechanical treatment used in this report includes ultrasonic radiation, mechanical shearing, and microfluidization. They have different reaction rate and/or rate constant depending on the conditions used. The conditions used to compare the reaction rate and rate constant of breaking the β-1,4 glucoside linkage are that the chitosan-0.2 M acetic acid/ 0.1 M sodium acetate solutions were prepared and passed through a filter paper (55 mm # 1, Toyo Roshi Kaisha Ltd., Japan) to remove the insoluble materials. An aliquote of the filtrate in a stainless steel vessel was placed in a water bath at pre-setted temperature of 0~50°C. The pressurized solution (12,000~18,000 psi) enters the patented interaction chamber and directs into defined microchannels of a Microfluidizer (M-100Y cell Disruption, Microfluidics Corporation, U.S.A.) for 1~ 20 cycles. Where the solutions were accelerated to extreme velocities and intense cavitation occurs. An aliquote of the filtrate in a glass vessel at a pre-set temperature of 0~50°C was ultrasonic treatment at 300 watts for 2~120 min with a sonicator (VCX 600, Sonic & Materials Inc., USA) has a 1/2" extender (630-0410) or an aliquote of the filtrate in a glass vessel at pre-setted temperature of 0~50°C was shearing with a polytron Pt3000 (Polytron Kinematica AG, Switzerland) with PT-DA 3030/4t at 2,600~26,000 sec^{-1} for 2~120 min. An aliquote of the sample was pippetted out to analyze the molecular weight by SE-HPLC.

3.2 Effect of chitosan concentration

The changes of MW of treated chitosan was expressed as a molecular weight decrease ratio (MWDR). The MWDR was calculated as follows:

$$MWDR = 1 - Mi / M0$$

Here Mi and M0 is the MW of treated and untreated chitosan respectively. Larger values of MWDR indecates a large decrease in MW after mechanical treatment or great degradation occurred.

The effect of solution concentration shows that degraded reaction was faster in dilute solutions. Figure 1 shows the MWDR of microfluidizer treated chitosans increased fast after the first cycle treatment then slowed down during the rest of 20 cyclic treatments at 0°C, 18,000 psi. MWDRs were significantly different between 0.2% and 0.8% group to that of 1.4% and 2.0% group. MWDRs were significantly different between treated samples and untreated samples. The MWDR are 0.45, 0.41, 0.27, and 0.14 for 0.2, 0.8, 1.4, and 2.0% chitosan solution respectively at the first cycle treatment. The MWDR was near 0.69, 0.60, 0.50 and 0.36 for the solutions mentioned respectively at the end of 20 cycle treatment (Wu, 1998). The MWDR of ultrasonic (300 watts) treated chitosans increased fast before the first 10 min treatment then slowed down during the rest of the treatment time at 4°C. MWDR was 0.75, 0.56, 0.37, and 0.42 for 0.2, 0.8, 1.4, and 2.0% chitosan solution respectively at the first 10 min treatment. The MWDR was near 0.79, 0.69, 0.56 and 0.50 for the solutions mentioned respectively at the end of treatment (Chen *et al.*, 1996). The MWDRs of shearing treated chitosans increased with shearing time. MWDRs were 0.18, 0.16, 0.12, and 0.12 at the initial 10 min treatment and were 0.49, 0.43, 0.35, and 0.30 at the end of 30 min shearing treatment for 0.2, 0.8, 1.4, and 2.0% solution, respectively (Chen *et al.*, 1998).

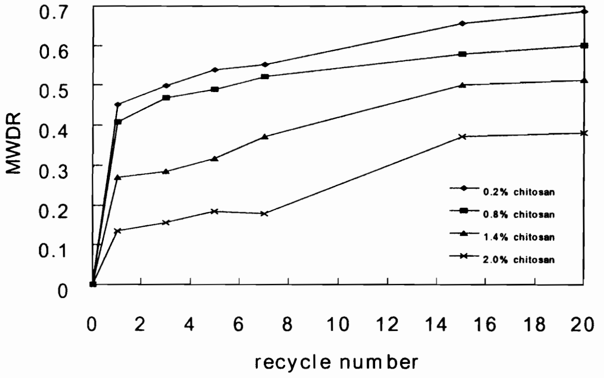

Figure 1. Effect of chitosan concentration (0.2%, 0.8%, 1.4% and 2.0%) on the changes of molecular weight decrease ratio (MWDR) of treated at 18,000 psi, 0°C for various recycle times (Wu, 1998)

The MWDR increased faster for dilute solutions than for concentrate solutions. It indicated the concentrate solution alleviated the depolymerization reaction. It may be due to solution viscosity was higher for higher concentration ones. The viscocity retarded the shearring force or the cavitation effect acting on the molecules in the solution because the higher the solution viscosity the lower the flow rate in turn the lower the shearring force or the cavitation effect. It may also be due to that at the same level of energy input, the energy needed to degraded the polymer was diluted by an increase of chitosan concentration. It may also due to the chance of re-polymerzation reactions occurring between degradaed molecules are higher for the higher concentration solutions.

3.3 Reaction temperature

The effect of solution temperature show that between 4 and 50°C, Chitosan was degraded faster in 30°C solutions by ultrasonic radiation and microfluidization but faster in 50°C solutions by mechanical shearing.

Tseng (2001) reported the effect of reaction temperature of 0, 30, and 50°C on the changes of MWDR of chitosan in 0.2 M acetic acid/0.1 M sodium acetate solution during ultrasonic (300 watts). MWDR of chitosan molecule increased faster for those treated at 30°C than those treated at 0 and 50°C during the beginning 30 min. Between 30 and 150 min, the effect of reaction temperature on MWDR of chitosan molecules were not significantly for 0.2% and 0.8% solutions. But for 1.4% solution, the increase of MWDR of chitosan molecules are in the order of 30 > 0 > 50°C and that of 2.0% solution are in the order of 30 > 0 > 50°C. The MWDR of 0.2%, 0.8%, 1.4%, and 2.0% solution after 150 min treatment were in 0.84, 0.90, 0.82, 0.87, 0.68, 0.78, and 0.65, 0.75 respectively. It indicated elavating the solution temperature was detrimental to the degradation process by ultrasound. It may be due to the cavitation bubble generated by the ultrasonic wave escaping out of the solution easier from higher temperature solutions than from lower temperature solutions. The shearing effect as the cavitation bubble collapsed is considered to be the major force degrading the polymer during ultrasonic treatment. Therefore, elavating solution temperature during ultrasonic treatment retarded the depolymerization effect.

Tseng (2001) also reported the effect of reaction temperature of 0, 30 and 50°C on the changes of MWDR of chitosan in 0.2 M acetic acid/0.1 M sodium acetate solution during shearring. For 0.2% or 0.8% chitosan solution, MWDR of chitosan molecule increased with increasing reaction temperature. MWDR of chitosan molecule for those treated at 50°C were higher than those treated at 30°C in turn higher than those at 0°C. For 1.4% chitosan solution, MWDR of chitosan molecule for those treated at 50°C were higher than those treated at 30°C or treated at 0°C but the difference between the later two temperature treatments are not significant before 120 min treatment. However, after 120 min treatment for those treated at 30°C was higher than those treated at 0°C. For 2.0% chitosan solution after 180 min treatment, MWDR of chitosan molecules for those treated at 50°C became higher than those treated at 30°C. The chitosan molecules in 0°C solution have the smallest MWDR. After 150 min treatment, the MWDR of chitosan molecules are 0.25, 0.50, and 0.62; 0.20, 0.38, and 0.54; 0.21, 0.41, and 0.52; 0.21, 0.30, and 0.46 in solution temperature in order of 0, 30, and 50°C for 0.2%, 0.8%, 1.4%, and 2.0% solution respectively

3.4 The effect of removing of small fragment

The results in Table 2 show that rate constant were higher for those solutions removing smaller fragment with ultrafiltration treatment than that without ultrafiltration treatment during sonolysis. It indicated that ultrafiltration treatment facilitate sonolysis reaction. This may be due to that either smaller fragment molecules in the solution reduced the chance of the cavitation energy degradation on larger molecular weight species or smaller fragments being activated by cavitation energy and become free radical molecules, then those free radical species interacted to form larger species. Therefore, removing smaller fragment species during sonolysis facilitate the degradation rate constant during sonolysis.

Table 2. Reaction rate constants (x 10^{-5} h^{-1}) of different concentrations chitosan solution degraded by ultrasonic radiation with or without ultrafiltration treatment at 0, 30 and 50°C

	0.2%	0.8%	1.4%	2.0%
0 °C	25.0	20.5	12.3	12.6
0 °C -UF	39.7	25.6	20.3	16.1
30 °C	36.2	29.1	15.3	13.8
30 °C -UF	48.9	29.9	23.9	25.2
50 °C	25.3	19.9	8.6	7.7
50 °C -UF	36.8	21.5	15.6	15.1

0°C: representing chitosan was degraded at 0°C without using ultrafiltration to remove smaller degraded fragments of chitosan

0°C-UF: representing chitosan was degraded at 0°C and concurrently using ultrafiltration to remove smaller degraded fragments of chitosans. (Tsaih et al., 2004)

3.5 Effect of molecular weight on the degradation of treated molecules

The effect of molecular weight show that all three mechanical treatments have the same trends of degradation rates and were proportional to the MW of chitosan. This may be due to the chances of being attacked by the cavitation energy generated by ultrasonic radiation or microfluization increased with increasing hydrodynamic volume species or longer chain failitate the molecule tearing by the the shearing force and may be due to smaller molecular weight species having a shorter relaxation time thus can alleviate the sonication or microfluidization stress easier or less chance of entanglement that is required duirng shearing (Tsaih and Chen, 2003).

3.6 Effect of DD of chitosan on the degradation of treated molecules

The effect of degree of deacetylation show that the degradation rate and rate constant of sonolysis increased with increasing DD of chitosan used. This mat be due to the bond energy of acetamido is higher than β-1, 4 glucoside linkage or hydrogen bonds, Breakage of hydrogen bonds might reform easily later. Breakage the β-1, 4 glucoside linkage will result in lower molecular weight and increasing reaction rate and rate constant.

The results in Table 3 show degradation rate and rate constant increased with increasing DD of chitosan used. $R_{0,\,0.5}$ are 71.7, 77.2, 82.8, 85.2 %/h and $k_{0,\ 1}$ are 7.0×10^{-5}, 11.8×10^{-5}, 49.5×10^{-5}, 156.1×10^{-5} 1/h for chitosans with DD of 67.9%, 81.3%, 90.5%, 92.2%, respectively. For entirely sonolysis reaction, $R_{0,\ 2}$ are 31.9, 33.2, 35.6, 35.8 %/h and $k_{0,\ 2}$ are 5.2×10^{-5}, 8.4×10^{-5}, 36.3×10^{-5}, 113.4×10^{-5} 1/h, respectively, for DD 67.9%, 81.3%, 90.5%, 92.2% chitosans.

Table 3. Ultrasonic degradation rate (R, %/h) and (k, 1/h, ×10^{-5}) of different DD chitosans

	DD (%)					DD (%)			
	67.9	81.3	90.5	92.2		67.9	81.3	90.5	92.2
$R_{0,0.5}$	71.7	77.2	82.8	85.2	$K_{0,1}$(x10^{-5})	7.0	11.8	49.5	156.1
$R_{0.5,1}$	65.7	63.5	72.7	72.4	$K_{1,2}$(x10^{-5})	1.8	5.1	23.1	70.6
$R_{1,2}$	16.1	19.9	22.9	22.4	$K_{0,2}$(x10^{-5})	5.2	8.4	36.3	113.4
$R_{0,2}$	31.9	33.2	35.6	35.8					

(Tsaih and Chen, 2003)

Those results indicated the higher the DD the easier the molecular degradation occurred during sonolysis. Good exponential relationship between sonolysis rate constant and DD of treated chitosan has been reported (Tsaih and Chen, 2003). Molecular weight of different chitosans decreased with increasing their DD values. In previous sections the larger the molecular weight chitosans the higher reaction rate and rate constant will be. However, resulted showed opposite resulted e.g. lower molecular weight species ones (higher DD species) having higher reaction rate and rate constant. Those results implied that the effects of DD overwhelmed the effect of molecular weight on sonolysis. To avoid the effect of molecular weight of treated chitosan, data of similar molecular weight chitosan were used, their sonolysis reaction rate and rate constant were compared and listed in Table 4. Results show that higher DD ones had a higher reaction rate and rate constant for all four groups of chitosans. It implied that degree of deacetylation of chitosan affect degradation rate prominently. The reasons may be due to the flexible molecules of higher DD chitosans being more vulnerable to the shear force of elongation flow generated by the cavitation field or maybe due to the bond energy of acetamido, β-1, 4 glucoside linkage, and hydrogen bonds are different and during sonolysis the energy of cavitation may preferential reacted at β-1, 4 glycoside linkage. Breakage of the β-1, 4 glucoside linkage will result in lower molecular weight and increasing reaction rate and rate constant.

Table 4. Ultrasonic degradation rate and rate constant of similar molecular weight but different DD chitosans

Groups	DD (%)	Mw (KDa)	Degradation rate (%h)	Rate constant ($1/h \times 10^{-5}$)
1	67.9	2040	65.7	8.4
	81.3	2020	77.2	11.8
2	67.9	1370	16.1	1.8
	81.3	1240	63.6	12.7
3	81.3	846	19.9	5.1
	90.5	563	82.8	41.4
4	90.5	210	22.9	23.1
	92.2	183	85.3	133.4

(Tsaih and Chen, 2003)

3.7 The other factors

The other factors such as the effect of solvent used show that in solvent systems in which chitosan's conformation is more extended both by ultrasonic radiation and mechanical shearing. The effect of reaction time show that the degradation rate and rate constant of sonolysis decreased with increasing sonolysis time (Chen *et al.*, 1996).

IV. Conclusions

Mechnical methods have merits the molecular weight of chitosan can be manipulated at the mean time the degree of deacetylation can be preserved in a limited range. The efficiency of mechanical treatment can be increased by the condition employed.

V. References

Chen, R. H., Chang, J. R. and Shyur, J. S. 1996. Effect of ultrasonic conditions and storage in acidic solution on changes in molecular weight and polydispersity of treated chitosan. *Carbohydr. Res.* **299**: 287-294.

Chen, R. H., Chang, J. R. and Shyur, J. S. 1998. Effect of shear conditions and storage in acidic solution on changes in molecular weight and polydispersity of treated chitosan. *J. Fish Soc. Taiwan* **25(3)** : 219-229.

Tsaih, M. L. and Chen' R. H. 2003. The effect of degree of deacetylation of chitosan on the kinetics of ultrasonic degradation of chitosan. *J. Appl. Polym. Sci.* **90(13):** 3526-3531.

Tsaih, M. L. and Chen, R. H. 2003. The effect of reaction time and temperature during heterogenous alkali deacetylation on degree of deacetylation and molecular weight of resulted chitosan. *J. Applied Polym. Sci.* **88(13)**: 2917-2923.

Tsaih, M. L. Tseng, L. Z. and Chen' R. H. 2004. Effects of removing small fragment with ultrafiltration treatment and ultrasonic conditions on degradation kinetics of chitosan. *Polym. Degrad. Stab.* **86**: 25-32.

Tseng, L. Z. 2000. *Effects of Different Types and Powers of Mechanical Forces on Changes in Molecular Weight and Limited Molecular Weight of Treated Chitosan.*Master thesis, National Taiwan Ocean University.

Wu, C. L. 1998. *Effect of Microfluization Treatment on the Physichemical Properties of chitosan and Its Application on Producing Water Soluble Chitosan.* Master thesis, National Taiwan Ocean University.