RHEOLOGICAL PROPERTIES OF CHITOSAN AND ITS INTERACTION WITH MYOFIBRILLAR PROTEINS AS INFLUENCED BY CHITOSAN'S MOLECULAR WEIGHT AND CONCENTRATION

K. W. Lin[1] and Y. H. Lee[2]

[1] Providence University, Department of Food and Nutrition, Taiwan, ROC
[2] Shishin Technology Co., Ltd., Taipei City, Taiwan, ROC

Abstract –Biological functions of chitosan include antibacterial effect, intestinal lipid binding and serum cholesterol lowering effects, water binding ability, antioxidative and preservative abilities in muscle foods, and emulsifying capacity. Rheological properties of chitosan of different molecular weight (150 kDa, 400 kDa, or 600 kDa) and concentration (0.1%, 0.5%, 1.0%, 1.5%, or 2.0%) (dissolved in 1% lactic acid solution) mixed singly or in combination with myofibrillar proteins were investigated. Initial viscosity of chitosan solution at higher concentration (≥1.5%) increased with increasing chitosan molecular weight, and decreased during heating process under constant shearing speed. The magnitude of decrease in viscosity was more apparent with increasing chitosan concentration. The storage modulus (G') of porcine salt-soluble proteins (SSP) during thermal gelation process increased with rising temperature. Addition of chitosan increased the initial G' of chitosan/SSP mixture but also decreased the G' of SSP indicating an interference of chitosan with SSP gelation process and the gel strength. Regardless of molecular weight, the higher the chitosan concentration strengthened the interference and lowered the G' of chitosan/SSP mixture system. Tangent delta (tan δ) of chitosan/SSP mixture decreased with increasing temperature. Possible different gelation mechanisms between chitosan and SSP pertaining to molecular weight were proposed.

Key Words – chitosan, molecular weight, rheological properties, salt-soluble proteins

I. INTRODUCTION

Chitin is the second most abundant polysaccharide in the world with a structure similar to cellulose. Chitosan is a β-1,4-linked N-acetyl-D-glucosamine biopolymer and a naturally occurring component in shells of crustaceans and the cell walls of fungi [1]. After deacetylation in alkali, chitosan with a specific degree of deacetylation (dd) is obtained. The higher the dd, the higher the ratio of free amino group (NH^{+3}) on the chitosan molecule. Chitosan is soluble in certain acid solutions, such as formic acid, acetic acid, and lactic acid, and behaves like a non-Newtonian solution. When dissolved in acid solution, chitosan becomes a polycationic polymer, which possesses many functional properties in food applications [2]. These applications include antibacterial effect [3-4], intestinal lipid binding and serum cholesterol lowering effects [5-6], water binding ability [7], antioxidative and preservative abilities in muscle foods [8-9], and emulsifying capacity [10].

Many polysaccharides will gelatinize under heating into different gel textures, which contribute to improving functionality of meat products. However, Xiong and Blanchard [11] reported an interaction of SSP and polysaccharide via noncovalent bonding and thus interfered with SSP thermal gelation resulting in a decrease in gel strength. Protein gelation was interfered with at a pH near the pI of myofibrillar proteins due to electrostatic attraction among the molecules. Huang and Li [12] indicated that ionic interaction between the positively-charged amino group of chitosan and SSP affected SSP gelation in the lower temperature region (below 50 ºC) resulting in lower gel strength. The rheological properties of chitosan and its interaction with myofibrillar proteins as influenced by different molecular weight and concentration of chitosan have not been elucidated.

II. MATERIALS AND METHODS

Pork loin was trimmed of heavy connective tissue and subcutaneous fat and myofibrillar proteins were extracted followed by the procedure of Chen *el al.* [13]. The protein concentration was

determined by the BCA method. Chitosan (150 kDa, 400 kDa, 600 kDa; Fluka) was dissolved with gentle stirring in 1% lactic acid solution (pH 2.23) into various concentrations (0.1%, 0.5%, 1.0%, 1.5%, 2.0% w/w), and then mixed with SSP (2.5% protein concentration) at 1:1 ratio. The viscosity of chitosan and chitosan/SSP mixture solutions was measured with a Rapid-Visco Analyser (Model RVA-3D^{+}; Newport Scientific Pty. Ltd., Warriewood, Australia) [14]. Viscosity was calculated and expressed as cPs. Dynamic viscosity of chitosan/SSP mixture was measured by the dynamic rheometer (Rheolab Model 120; Physica Meβtechnik GmbH, Stuttgart, Germany). The settings for viscosity measurement were as follows: parallel plate apparatus= 50 mm, gap size= 1 mm, frequency= 0.1 Hz, strain= 0.02%, temperature increase rate= 1 ºC/min, temp sweep range= 25 to 80 ºC. Rheological parameters of storage modulus (G') and loss modulus (G") were determined loss tangent (tan δ) calculated. G' and G" are indications of elastic and viscous properties of a solution, respectively. Tangent delta was calculated as G"/G'.

III. RESULTS AND DISCUSSION

For the same chitosan's molecular weight, decreasing chitosan concentration resulted in lower viscosity of chitosan solution. Chitosan with higher molecular weight showed higher viscosity at any chitosan concentration (Figure 1). When heated alone, the G' and G" of chitosan solution was not significantly changed. Generally, chitosan did not gelate upon cooling. The tangent delta (tan δ) of chitosan solution with varying molecular weight and concentration revealed that chitosan solution was more towards viscous than elastic property (Figure 2).

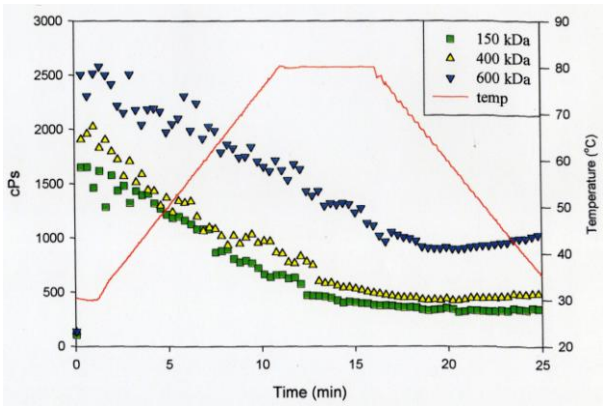

Figure 1. Viscosity profile of various molecular weight chitosan solution at 2.0% concentration.

When subjected to temperature variation (from 25 to 80 ºC) (Figure 3), storage moduli (G') of salt-soluble protein solution (2.5%) increased sharply at 50 ℃ and continued rising till the end of heating process (80 ℃), while only minute change of loss modulus (G") were detected. When G' and G" intersected at approximately 52 ℃, tan δ became 1 and SSP changed from a sol to a gel, and G' continued to rise rapidly. As a result, tan δ decreased with increasing temperature suggesting a transfer from a viscous solution to an elastic gel.

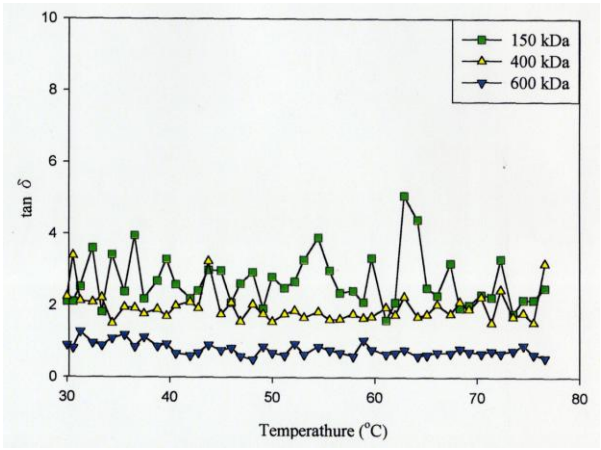

Figure 2. Effect of molecular weight on tangent delta of 2% chitosan solution during thermal process.

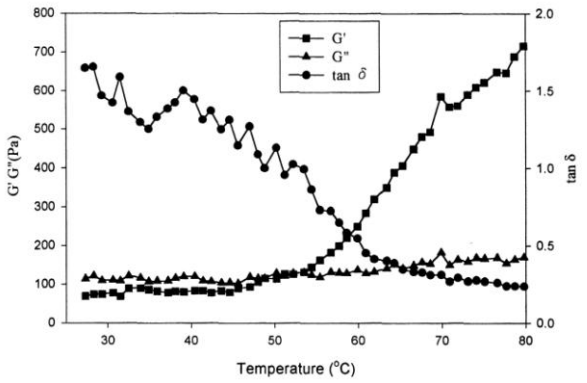

Figure 3. Temperature variation of SSP solution (containing 2.5% protein) during thermal gelation.

After mixing SSP (2.5% protein) with chitosan, G' of chitosan/SSP mixture was higher than SSP during the beginning of heating process regardless of molecular weight; nonetheless, the results were reversed (Figure 4). These results suggested that chitosan with varying molecular weight could interfere SSP thermal gelation.

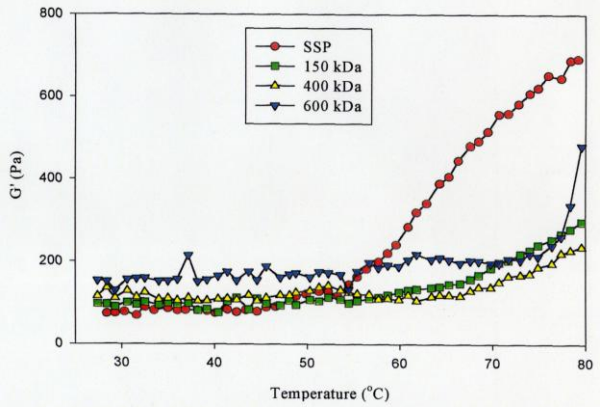

Figure 4. Storage modulus (G') of 2.0% chitosan/SSP mixture during thermal gelation as related to chitosan's molecular weight.

When 0.1% chitosan was mixed with SSP, transformation temperature of SSP from sol to gel (i.e., gelation) changed from 58 ℃ for 150 kDa, 58 ℃ for 400 kDa, to 63 ℃ for 600 kDa (Figure 5).

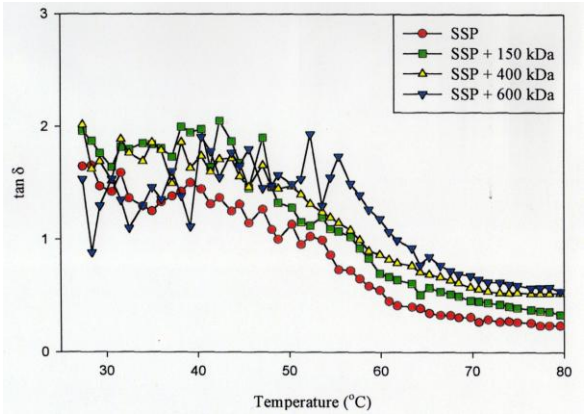

Figure 5. Tangent delta of 0.1% chitosan/SSP mixture during thermal gelation as related to chitosan's molecular weight.

The gelation temperature increased with increasing chitosan to 2.0% to approximately 70 - 76℃ for 150-600 kDa (Figure 6) indicating that phase transition temperature of chitosan/SSP was affected by chitosan concentration. At the same chitosan concentration, tan δ was found to be the highest for 600 kDa, followed by 400 kDa, 150 kDa and SSP being the lowest. Results suggested that SSP had the highest gelling strength and addition of chitosan would interfere with the thermal gelation of SSP. At the acidic environment of chitosan/SSP mixture (pH 3.45~4.99), both SSP and chitosan carried positive charges which caused repulsion.

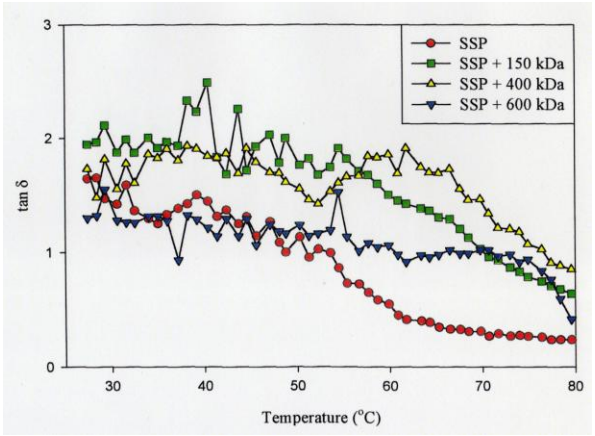

Figure 6. Tangent delta of 2.0% chitosan/SSP mixture during thermal gelation as related to chitosan's molecular weight.

IV. CONCLUSION

This study demonstrated the effects of molecular weight and concentration on the rheological and thermal gelation properties of chitosan and chitosan/myofibrillar protein mixtures. Possible different gelation mechanisms between chitosan and SSP pertaining to molecular weight exist. When chitosan was applied to precooked or cooked meat products, interaction between chitosan and myofibrillar proteins could have varying influences on product characteristics. Our previous work had demonstrated the possibility of applying chitosan with varying molecular weights into reduced-fat Chinese sausage [14]. Current findings will enable the meat industry to utilize this "functional food" more effectively.

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