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Bioresource Technology 96 (2005) 1473–1482
BIORESOURCE TECHNOLOGY

Preparation and important functional properties of water-soluble chitosan produced through Maillard reaction

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Received 14 January 2004; received in revised form 8 September 2004; accepted 1 December 2004
Available online 20 January 2005

Abstract

The objective of this research was to improve the solubility of chitosan at neutral or basic pH using the Maillard-type reaction method. To prepare the water-soluble chitosans, various chitosans and saccharides were used under various operating conditions. Biological and physicochemical properties of the chitosan-saccharide derivatives were investigated as well. Results indicated that the solubility of modified chitosan is significantly greater than that of native chitosan, and the chitosan-maltose derivative remained soluble when the pH approached 10. Among chitosan-saccharide derivatives, the solubility of chitosan-fructose derivative was highest at 17.1 g/l. Considering yield, solubility and pH stability, the chitosan-glucosamine derivative was deemed the optimal water-soluble derivative. Compared with the acid-soluble chitosan, the chitosan-glucosamine derivative exhibited high chelating capacity for Zn^{2+}, Fe^{2+} and Cu^{2+} ions. Relatively high antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* was noted for the chitosan-glucosamine derivative as compared with native chitosan. Results suggest that the water-soluble chitosan produced using the Maillard reaction may be a promising commercial substitute for acid-soluble chitosan.

Keywords: Chitosan; Maillard reaction; Antibacterial activity; Solubility

1. Introduction

Chitin is a major structural component of the fungal cell wall and of the exoskeletons of invertebrates, including insects and crustaceans (Jang et al., 2004). It is the second-most abundant biopolymer in nature. Chitosan is the collective name for a group of partially and fully deacetylated chitins. It has attracted tremendous attention as a potentially important renewable agricultural resource, and has been widely applied in the fields of agriculture, medicine, pharmaceuticals, functional food, environmental protection and biotechnology in the last 20 years (Kurita, 1998).

Chitosan is soluble in the acid pH range, but insoluble in the neutral or basic range (Koide, 1998). Additionally, it only dissolves in some specific organic acids including formic, acetic, propionic, lactic, citric and succinic acid, as well as in a very few inorganic solvents, such as hydrochloric, phosphoric, and nitric acid (Wang et al., 2004). The solubility of chitosan also depends on the pK_a of these acids and their concentrations. Furthermore, chitosan solution is very viscous even at low concentrations, and its applicability in a commercial context is thus often restricted (Sugimoto et al., 1998). Hence, improving the solubility of chitosan is crucial if this plentiful resource is to be utilized across a wide pH range.

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doi:10.1016/j.biortech.2004.12.001

Strategies for improving chitosan solubility can be divided into three methods based on preparation principles. Firstly, homogeneous phase reaction (Sannan et al., 1976) involves controlling the deacetylation process and results in water-soluble chitosan. However, the yield is not high (Kurita et al., 1991). Secondly, reducing the molecular weight of chitosan produces high solubility. This approach can be divided into physical, acid-hydrolysis and enzyme methods. Physical methods include the shear-force and ultrasonic variants, with respective molecular weights reduced to 1.1×10^5 and 1.4×10^5 (Chang, 1996). By combining the shear-force treatment and acid-hydrolysis, the molecular weight of the chitosan can be decreased from 8×10^5 to 7.5×10^4 (Austin et al., 1981). Although execution of these physical methods is not difficult, fast degradation rates and random reactions result in product variability and unstable solubility (Kurita et al., 2002). In acid hydrolysis, 10% acetic acid is generally used as a solvent, with 5% $NaNO_3$ added for the deacetylation reaction. This method can decompose chitosan, including thousands of *N*-acetylglucosamines, into units of six *N*-acetylglucosamines, and such products are prone to dissolution at pH 7 (Hirano et al., 1985). Where the molecular weight of the chitosan derivative is too low, however, almost all biological and/or chemical activity is lost (Liu et al., 2001; No et al., 2002). Reductions in chitosan molecular weight have been demonstrated using chitosanase, lysozyme, and papain (Ikeda et al., 1993; Nordtveit et al., 1996; Terbojevich et al., 1996), with higher solubility than that obtained with other methods. However, the relatively high cost of producing water-soluble chitosan remains an obstacle. The third and final method of improving solubility involves introducing a hydrophilic functional group to the chitosan, a technique also called the chemical modification method (Holme and Perlin, 1997). Many chitosan derivates—including CM-chitosan (carboxymethyl chitosan), *N*-sulfofuryl chitosan, 5-methyl pyrrolidinone chitosan, and dicarboxymethyl and quaternized chitosan—have been developed, with a solubility range of 3–10 g/l obtained (Delben et al., 1989; Muzzarelli, 1992; Watanabe et al., 1992; Dung et al., 1994; Jia et al., 2001). In a complex solvent system, however, a preparation process is typically required, and this becomes inconvenient and difficult to control (Ilyina et al., 2000; Kubota et al., 2000).

The Maillard reaction is a process involving the amino and carbonyl groups of different molecules (Jokic et al., 2004). It is characterized by the mildness of the reaction, ease of operation, and controllability (Tessier et al., 2003). Hence, high solubility, yield, and activity of water-soluble chitosan may be expected using the Maillard reaction. Recently, water-soluble chitosans, mainly derived from chitosan and disaccharides, have been produced and their rheological characteristics demonstrated (Yang et al., 2002). The results indicate that the Maillard reaction is quite promising for commercial production of water-soluble chitosan. The introduction of some monosaccharides (especially glucosamine) into the chitosan should be a feasible approach to improve solubility, because glucosamine, like chitosan, possesses active amino and hydrophilic hydroxyl groups. Thus, their metal-chelation capacity and microbe-inhibition activities merit examination.

In this study, we have attempted to improve the solubility of chitosan in the neutral and basic range through utilization of the Maillard reaction. The factors that affected this reaction including pH level, reaction time, and the types and concentrations of the reducing sugar used were examined. Furthermore, the metal-ion chelating capacity and the antibacterial activity of the chitosan derivatives against *Escherichia coli* and *Staphylococcus aureus* were evaluated.

2. Methods

2.1. Materials

The α- and β-type chitosan were purchased from Shin Dar Biotechnology Company (Taipei, Taiwan). They originated from shrimp and squid, respectively. The α-type chitosans were prepared to 75% or 90% degree of deacetylation (DD), with the β-type chitosan only to 90% DD. The viscosity average molecular weights of these chitosans were $3–5 \times 10^4$. Two strains of waterborne pathogens, *E. coli* (ATCC 25922) and *S. aureus* (ATCC 27853), were obtained from the American Type Culture Collection (ATCC). Fresh inoculants for analysis of minimum inhibitory concentration (MIC) were prepared on nutrient agar at 37 °C for 72 h. Growth media were obtained from Difco Company. Monosaccharides and disaccharides, including glucose, fructose, glucosamine, and maltose, were purchased from Sigma Chemical Company. Unless otherwise stated, all reagents used in this study were reagent grade.

2.2. Preparation of water-soluble chitosan

To obtain commercially viable chitosan, α- or β-type chitosan at 90% DD was dissolved in 0.2 M CH_3COOH solution (pH 3.3) to give a final chitosan concentration of 1% (w/v). After that, glucose was dissolved in the chitosan solution to a final glucose concentration of 1% (w/v). A total of 15 samples (in triplicate) were reacted at 65 °C for 5 days. Every other day, three samples were withdrawn to determine yield and solubility. To produce the optimal water-soluble variant, α-type chitosan at 75% or 90% DD was dissolved in 0.2 M CH_3COOH solution, to give a final chitosan concentration of 1% (w/v), and then separately mixed with various amounts of glucose, glucosamine, maltose, and fructose

until dissolution by mild stirring. All the added saccharides were at a concentration of 1% or 2%, except for fructose which was added at 0.5% or 1%. The mixtures were reacted at 55, 65 or 75 °C for a specified period in an orbital shake incubator. Triplicate samples were drawn and centrifuged (8000 rpm, 15 min). The supernatant was dialyzed against distilled water by dialysis membrane with molecular weight cut-off 12,000–14,000 (Spectrum Laboratories Inc., USA) for 96 h and then freeze-dried.

2.3. Determination of yield, solubility, degree of deacetylation, and reactive extent of Maillard reaction

The yield of water-soluble chitosan (chitosan-saccharide derivative) was expressed as the ratio of water-soluble chitosan to total added chitosan and saccharides. To estimate solubility, 0.05 g of water-soluble chitosan was mixed with 5 ml distilled water, stirred for 5 h and then filtered through a 0.45-μm filter paper. Solubility was estimated from the change in filter-paper weight (Yalpani and Hall, 1984). To determine the degree of deacetylation of the water-soluble chitosan, 20 mg of the soluble variant was dissolved in 10 ml acetic acid (0.1 M) and completely stirred for 1 h at room temperature. The mixture was diluted with 40 ml distilled water, then 5 ml of the diluted solution was withdrawn and one drop of 1% toluidine blue added as an indicator. Potassium polyvinyl sulfate solution (PVSK, N/400) was successively added until the titration end point was reached (Toei and Kohara, 1976). To assess the reactivity of the Maillard reaction, 3 ml solutions from different chitosan-saccharide complexes were analyzed by measuring absorbance at 420 nm using a Beckman spectrophotometer (Liu et al., 2003). To examine the stability of the water-soluble chitosan, 0.3 g was dissolved in 10 ml distilled water and 2 M NaOH added drop-wise. When the absorbance of the solution at 600 nm was over 0.1, the solubility was deemed unstable (Yang et al., 2002).

2.4. Determination of metal-ion chelation capacity

The acid-soluble (DD 90%) and water-soluble chitosans, produced from the 1% α-type chitosan (DD 75%/90%) and the 1% glucosamine reacted at 65 °C for 2 days, were used to examine the chelating capacity for three metal ions. These ions, Cu^{2+}, Fe^{2+} and Zn^{2+}, were derived from copper sulfate, ferrous sulfate and zinc sulfate, respectively. The metal-ion chelation capacity of acid-soluble chitosan was examined at pH 5 and the water-soluble variant at pH 7. Two milliliters aliquots of acid-soluble and water-soluble chitosan (concentrations ranging from 0.1% to 0.6%) were separately mixed with 0.5 ml of 10 mM hexamine, 0.5 ml of 30 mM potassium chloride, 0.2 ml of TMM (tetramethylmurexide), and 1 ml of 3 mM of the metal ion for 5 min. The absorbance of the mixture was then determined at 485 nm using the Beckman spectrophotometer. The chelating capacity (CC) was calculated from the following equation (Shimada et al., 1992):

$$\begin{aligned} CC = \{[&(\text{OD value of control set}) \\ &- (\text{OD value of sample} \\ &- \text{OD value without TMM added})]/ \\ &(\text{OD value of control set})\} \times 100\%, \end{aligned}$$

where OD (Optical Density) is a representation of a material's light blocking ability.

2.5. Evaluation of antibacterial activity

Growth inhibition of the acid- and water-soluble chitosans (produced from 1% α-type chitosan at DD 90% and 1% glucosamine or 1% glucose) for *E. coli* and *S. aureus* at pH 5 and 7 were evaluated using agar plates. The cell suspension (0.1 ml; 10^8 cfu/ml) was added to 200 ml nutrient broth, and 0.1 ml acid- and water-soluble chitosans were simultaneously added at various concentrations (50–1600 ppm). The pH of the broth was immediately adjusted to 5 with 0.2 M HCl, or controlled at pH 7, and the broth was then incubated at 37 °C in a incubator for 72 h, with the minimum inhibitory concentration (MIC) evaluated subsequently (Tanaka et al., 1993).

2.6. Statistical analysis

All experiments were carried out in triplicate, and average values with standard deviation errors are reported. Mean separation and significance were analyzed using the SPSS software package.

3. Results and discussion

3.1. Yield and solubility of α- and β-type chitosan derivatives

To select the appropriate chitosan type, 1% α- and β-type chitosans at 90% DD were separately dissolved in 0.2 M CH_3COOH solution (pH 3.3) and reacted with 1% glucose at 65 °C for 5 days. The yield and solubility results for the α- and β-type chitosan derivatives are presented in Fig. 1A and B. Yields of the α- and β-type chitosan-glucose derivatives increased with reaction time, reaching maxima on the third day, with yield for the β-type chitosan derivative slightly higher than that for the α-type analog (51% and 46%, respectively). A similar tendency was observed analyzing the relationship between solubility and reaction time (Fig. 1B). However, the solubility of the α-type chitosan derivative was 1.37

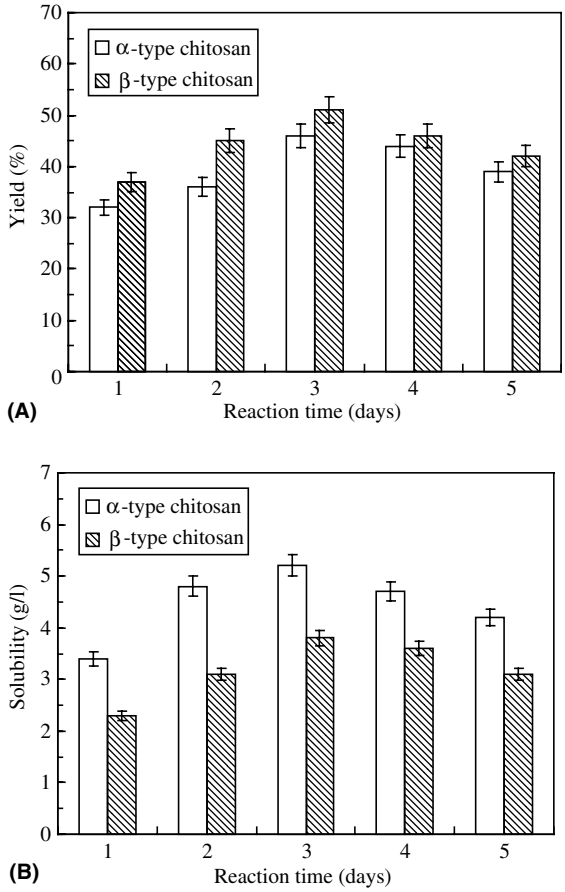

Fig. 1. Effect of α- and β-type chitosan derivatives on (A) yield and (B) solubility of chitosan-glucose derivative. The chitosan derivatives produced from 1% α- or β-type chitosan at 90% DD were reacted with 1% glucose at 65 °C for 5 days. The error bars indicate the standard deviation.

times higher than that of the β-type variant on the third day. Given the yield and solubility results, it seems reasonable to suggest that the α-type chitosan is a better candidate for preparation of a water-soluble chitosan.

In this study, the relatively long reaction time (>3 days) resulted in the formation of many precipitates during the dialysis process, producing a relatively low yield of the water-soluble chitosan. The occurrence of these precipitates may have been due to the increased complexity of the products produced during the longer reaction periods, or to the decrease in the ionic strength of the dialysis solution. Similarly, longer reaction times would result in the formation of crystalline variants during the freeze-drying process, and further reduce the solubility of water-soluble chitosan (Cabodevila et al., 1994). In short, reaction time is very important for successful production of water-soluble chitosan.

3.2. Effect of pH value on yield and solubility

The Maillard reaction generally takes place at neutral or slightly basic pHs (Tessier et al., 2003), but dissolving

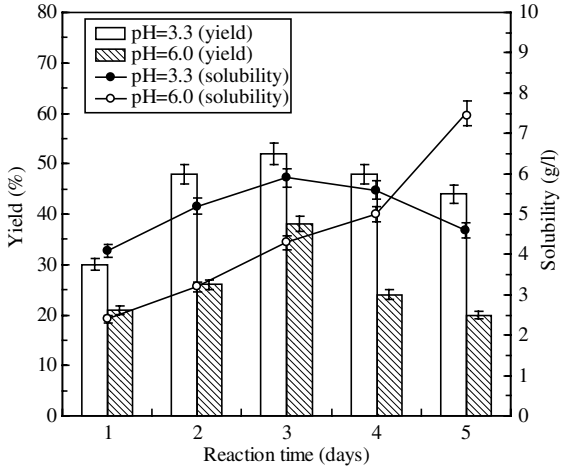

Fig. 2. Effect of pH value on yield and solubility of chitosan-glucose derivative. The chitosan derivatives produced from 1% α-type chitosan at 90% DD were reacted with 2% glucose at pH 3.3 or pH 6.0 for 5 days, with the reaction temperature controlled at 65 °C. The error bars indicate the standard deviation.

chitosan typically requires an acid solution. Therefore, we examined the effect of pH value on the yield and solubility of the chitosan derivative in this study. The 1% α-type chitosan (90% DD) was dissolved in 0.2 M CH_3COOH solution (pH 3.3) or adjusted to pH 6 using 0.1 N NaOH, and then mixed with 2% glucose at 65 °C for 5 days. Analysis of the effect of pH value and the yield and solubility of the chitosan-glucose derivative (depicted in Fig. 2) reveals that at pH 3.3, both yield and solubility increased with reaction time, reaching a maximum on the third day. A similar effect on yield was observed at pH 6.0, but the solubility of chitosan-glucose derivative at pH 6.0 continued to increase with reaction time. Generally, the yield and solubility of the chitosan derivatives were higher at pH 3.3 than pH 6.0, with a statistically significant difference demonstrated ($P < 0.05$). The maximal yield and solubility at pH 3.3 on the third day were 52% and 5.9 g/l, respectively, while the analogous values at pH 6.0 were 38% and 4.3 g/l. The improved solubility of chitosan derivatives at pH 3.3 compared with pH 6.0 may be due to the protonation of amine groups at this pH. Considering solubility, yield and operating cost, a pH of 3.3 was superior for the production of water-soluble chitosan even though solubility of chitosan derivative at pH 6 continued to increase with time.

3.3. Yield and solubility of various chitosan derivatives

The 1% α-type chitosan (75%/90% DD) was separately mixed with various quantities of glucose, glucosamine, maltose, or fructose and reacted at 55, 65 or 75 °C for a predetermined interval. Yield increased with longer reaction time, reaching a maximum on a particular day (the 2nd, 3rd or 6th day) depending on the saccharide

used (data not shown). The maximal mean average yields for the chitosan-fructose, chitosan-glucose, chitosan-maltose, and chitosan-glucosamine derivatives were 42%, 46%, 52%, and 48%, respectively, at 65 °C (Table 1). The 10-day yields of the chitosan-fructose derivatives at 65 °C (Fig. 3A) indicate that higher chitosan deacetylation was associated with higher yield at the same saccharide concentration (the data for saccharides, apart from fructose, not shown). Furthermore, high concentrations of fructose resulted in high yields at the same level of chitosan deacetylation. Similar results were also observed with glucose and glucosamine, but not maltose (data not shown). Since maltose is a disaccharide derived from a combination of two glucose molecules, the same concentration may provide more reactive locations (e.g. carbonyl group or potential carbonyl group) than a monosaccharide. Hence, excessive maltose will result in an inappropriate Maillard reaction and a low yield of water-soluble chitosan. Fig. 3B maps yield for 1% chitosan (90% DD) reacted with 1% fructose at different temperatures for 10 days, with the maximum achieved at 65 °C. Relatively low temperatures resulted in a slower Maillard reaction, with relatively high temperatures leading to formation of insoluble variants (Cabodevila et al., 1994).

Similarly, when chitosan reacted with various saccharides, the solubility of the chitosan derivatives increased with reaction time, reaching a maximum on a particular day, and then gradually decreased (data not shown). The optimal solubility of the chitosan-saccharide derivative was achieved at 65 °C (data not shown). The solubility of the chitosan derivatives was profoundly affected by the degree of chitosan deacetylation (data not shown). However, no significant relationship between saccharide concentration and the solubility of the chitosan derivatives was determined. The solubility of chitosan-fructose derivatives at 65 °C is depicted in Fig. 4, with high-DD chitosan producing relatively high-solubility chitosan-fructose derivatives at the same fructose concentration. In addition, the highest solubility (17.1 g/l) was noted on the sixth day. After six days, the chitosan derivatives consisted of micro-crystals formed during the freeze-drying process, resulting in decreased solubility (Cabodevila et al., 1994).

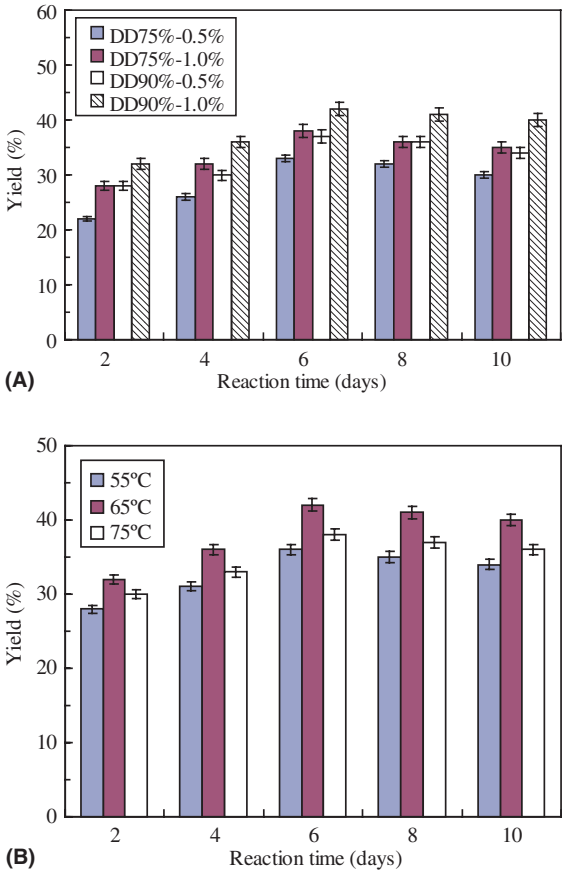

Fig. 3. (A) Effect of degree of chitosan deacetylation and fructose concentration on yield of chitosan-fructose derivative at 65 °C for 10 days. (B) Effect of reaction temperature on yield of chitosan-fructose derivative. The error bars indicate the standard deviation.

Table 1 indicates the basic properties of the chitosan derivatives at the optimized reaction conditions for the Maillard reaction. The optimal temperature for all saccharides was 65 °C, and, with the exception of fructose, the best results were produced with reaction periods ranging from 2 to 3 days. The yields of chitosan-glucosamine derivative and chitosan-glucose derivative did not show any statistically significant difference. It was determined that, in ascending order, derivative solubility increased for the chitosan-glucose, chitosan-maltose, chitosan-glucosamine, and chitosan-fructose

Table 1
Yield, solubility, degree of deacetylation (DD), and pH stability of chitosan derivatives at optimal reaction conditions for Maillard reaction

Optimal reaction set			Property of chitosan derivative			
α-type chitosan	Saccharide	Operating condition	Yield (%)	Solubility (g/l)	DD (%)	pH stability*
DD 90%, 1%	1%, Fructose	65 °C, 6 days	42 ± 0.40^{c}	17.1 ± 0.2^{a}	63.9 ± 1.62	<9
DD 90%, 1%	1%, Glucose	65 °C, 3 days	46 ± 1.45^{b}	6.4 ± 0.2^{b}	60.2 ± 1.81	<8
DD 90%, 1%	1%, Maltose	65 °C, 3 days	52 ± 0.60^{a}	13.2 ± 0.6^{c}	63.2 ± 1.75	<10
DD 90%, 1%	1%, Glucosamine	65 °C, 2 days	48 ± 0.95^{b}	16.2 ± 0.3^{d}	80.4 ± 1.38	<9

The values of yield and solubility with different superscripts within a column indicate significant differences ($P < 0.05$).
* pH stability represents the pH range for stable solubility of chitosan derivative.

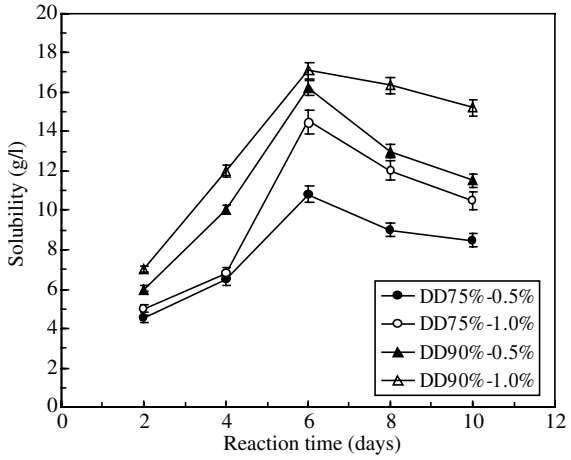

Fig. 4. Effect of degree of chitosan deacetylation and fructose concentration on solubility of chitosan-fructose derivative at 65 °C for 10 days. The error bars indicate the standard deviation.

variants. Compared with other chitosan derivatives (e.g. 2-mercaptoacetyl-chitosan, 6-deoxy-6-mercapto-chitosan) produced by alkaline treatment or other chemical modification methods, the chitosan derivatives produced using the Maillard reaction in this study exhibited higher solubility and yield (Sannan et al., 1976; Kurita et al., 1993). Additionally, these chitosan-saccharide derivatives required fewer solvents, processes, and operating skills in comparison to other chemical treatments. Compared with the chitosan derivatives produced using ultrasonic treatment, higher solubility was also demonstrated for the chitosan-saccharide derivatives (Chu, 1995; Chang, 1996).

3.4. Effect of reaction time, reaction temperature, degree of deacetylation of chitosan, and concentration of saccharide on Maillard reaction

The extent of the Maillard reaction in the chitosan and saccharide mixture was determined from the absorption at 420 nm using a spectrophotometer. The results indicate that absorbance increased with the concentration of the added saccharide (data not shown). Furthermore, absorbance increased with reaction time, leveling off at a specific reaction time. The time taken to reach maximum absorbance resembled that taken to achieve optimum solubility and yield of chitosan-derivative (Table 1). Fig. 5A indicates the change in absorbance for the chitosan derivatives produced from the reaction of 1% chitosan and fructose at 65 °C. Analysis of the results reveals that the degree of chitosan deacetylation did not have a significant effect ($P > 0.05$) when saccharide concentration remained the same. Conversely, saccharide concentration was an important factor in terms of the effectiveness of the Maillard reaction. It was determined that doubling the concentration resulted in a doubling of the effects on

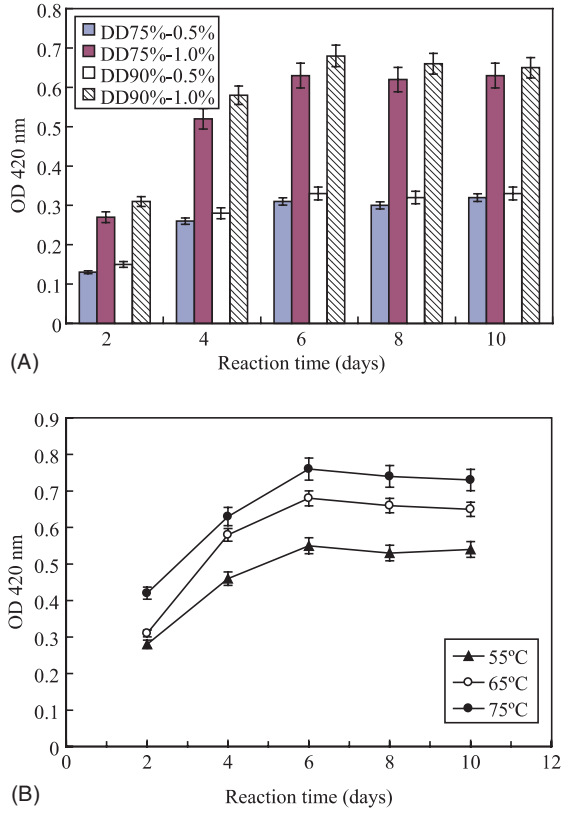

Fig. 5. (A) Effect of degree of chitosan deacetylation and fructose concentration on absorbance of chitosan-fructose derivative at 65 °C for 10 days. (B) Effect of reaction temperature on absorbance of chitosan-fructose derivative. The error bars indicate the standard deviation.

the absorbance of chitosan derivatives or the rate of Maillard reaction at the same degree of chitosan deacetylation (see Fig. 5A). However, the results were not similar when other saccharides were reacted with chitosan. It is suggested that 0.5% or 1% fructose was sufficient to completely react with the chitosan using the Maillard reaction. At 1% or 2% concentrations, however, the other saccharides did not completely react with the chitosan (data not shown). Fig. 5B depicts the effect of reaction temperature on the absorbance of the chitosan-fructose derivatives over a period of 10 days, with the rate of Maillard reaction strongly associated with reaction temperature. Although high reaction temperatures favour development of the Maillard reaction (Demyttenaere et al., 2002), this does not mean that the maximum yield or solubility of chitosan-saccharide derivatives achieved is proportional to the rate of the Maillard reaction (see Table 1). The ratios of soluble product or derivatives through Maillard reaction appear to be decisive factors in terms of both the yield and the solubility of the chitosan-saccharide derivatives. In our study, the optimal reaction temperature in term of producing water-soluble chitosan was 65 °C (as detailed in the previous section). The maximum absorbances for

the chitosan-glucosamine, chitosan-fructose, chitosan-glucose and chitosan-maltose derivatives at 65 °C were 1.52, 0.68, 0.63 and 0.46, respectively, with these results in accordance with the theory of Kato et al. (1989). It is presumed that the relatively high rate of the chitosan-glucosamine Maillard reaction was due to the contribution of the extra amino groups from the glucosamine in addition to those from the chitosan. Although the rate of the Maillard reaction for the chitosan and fructose was much lower than that for glucosamine, relatively high solubility was demonstrated for the chitosan-fructose derivative (Table 1). As fructose is a ketose, the products of the Heyn's rearrangement and isomerization were resistant to formation of crystal blocks in molecules (Whistler and BeMiller, 1996). Thus, production of a chitosan-fructose derivative of high solubility was relatively simple. Conversely, glucosamine, maltose and glucose are aldoses. Crystals would form during the freeze-drying process because their products were derived from the Amadori's rearrangement and isomerization (Whistler and BeMiller, 1996). Hence, relatively low solubility was determined.

The degree of chitosan deacetylation typically affects its physical, chemical and even biological properties or activities (Chen et al., 2002; Chung et al., 2003). Hence, it is necessary to determine the degree of deacetylation of the chitosan derivatives, which is related to increased reaction time, temperature and saccharide concentration. Under optimal reaction conditions, the average degree of deacetylation of the chitosan-glucosamine, chitosan-fructose, chitosan-maltose and chitosan-glucose derivatives was 80.4%, 63.9%, 63.2%, and 60.2%, respectively (Table 1). The change in the degree of deacetylation of the chitosan-glucosamine derivatives at 65 °C over five days is depicted in Fig. 6. The results indicate that, for all tested conditions, the degree of chitosan-glucosamine deacetylation first decreased and then leveled off on the second day. Compared with the other chitosan-saccharide derivatives, the chitosan-glucosamine variant possessed the highest degree of deacetylation (80.4%) at the optimum reaction conditions (see Table 1). Since colloid titration was used to determine the numbers of free amino groups in this study, the amino groups on both chitosan and glucosamine were estimated. Hence, the chitosan-glucosamine derivative possessed the highest degree of deacetylation.

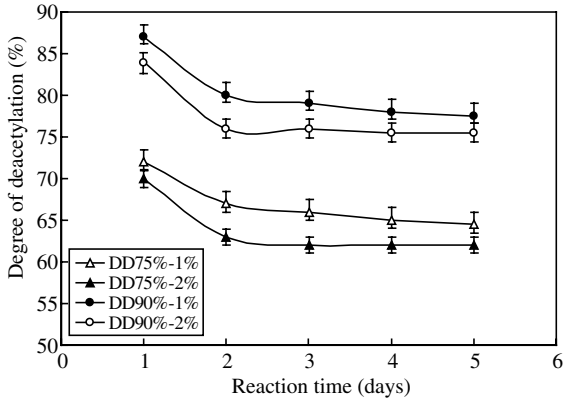

Fig. 6. Effect of Maillard reaction on degree of deacetylation of chitosan-glucosamine derivatives. The chitosan derivatives produced from 1% α-type chitosan at 90% or 75% DD were reacted with 1% or 2% glucosamine at 65 °C for 5 days. The error bars indicate the standard deviation.

3.5. Solution stability of various water-soluble chitosan derivatives at varying pHs

Since chitosan itself is only soluble in some specific acid solvents, its usage has often been restricted in practical applications (Sugimoto et al., 1998). Moreover, acid-soluble chitosan must first be dissolved in acid solvent before application. Its preservation period in acid solvents, however, is short (Ottoy et al., 1996). Hence, the development of a water-soluble chitosan and examination of its stability characteristics at various pHs is a prerequisite to successful implementation in a real-world environment. The pH stabilities for various water-soluble chitosan derivatives are presented in Table 1. The chitosan-disaccharide (maltose) derivative appeared to possess higher pH stability than the chitosan-monosaccharide (glucose, fructose or glucosamine) variants. The results were in agreement with the previous study of Yalpani and Hall (1984). Chitosan is generally only soluble below pH 6 (Koide, 1998); however, these derivatives were soluble at pH 8–10. The results were superior to the pH 7 analogs presented by Yang et al. (2002). The difference may be due to the higher degree of deacetylation demonstrated for the chitosan derivatives in the present study compared to those in previous work, resulting in more hydrophilic groups, and producing higher solubility over a relatively wide pH range. Obviously, these chitosan derivatives produced through the Maillard reaction enhanced the solubility of the native chitosan, overall and in terms of relative pH, from acidic to slightly basic.

3.6. Chelating capacity of various chitosans for metal ion

Chitosan, a polycationic biopolymer, possesses high chelating capacity for various metal ions (including Ni^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Mg^{2+} and Cu^{2+}) in acid conditions, and it has been widely applied for the removal or recovery of metal ions in different industries (Kurita, 1998). However, not all fluid bodies, foods, drinks or other liquid materials are acidic. Hence, it was necessary to examine the chelating capacity of chitosan derivatives for metal ions where the pH was neutral. The chelating capacity of chitosan and chitosan-glucosamine derivatives for Cu^{2+} was evaluated over a chelating agent concentration range of 0.1–0.6%, and the results are plotted

in Fig. 7. The results indicate that the chelating capacities of chitosan and its derivatives increased with greater concentration and leveled off to a saturated chelating capacity at a 0.3% sample concentration. The maximal average chelating capacities for the chitosan-glucosamine produced from chitosan at 90% and 75% DD and the acid-soluble chitosan were 76.3%, 58.1%, and 43.4%, respectively. High-deacetylation chitosan derivatives were associated with a high chelating capacity for Cu^{2+}. In addition, water-soluble chitosan exhibited higher chelating capacity than the acid-soluble chitosan. This may be attributable to the introduction of an extra functional group (e.g. amino group) from the saccharides (Muzzarelli, 1992). Similar results were determined for various metal ions (Table 2). It appears that chitosan and its derivatives most readily chelated Cu^{2+}, then Fe^{2+}, but that Zn^{2+} adsorption was relatively difficult. This was attributed either to potential difference or to the effect of the spatial distribution of the chitosans and the metal ions (Wijewickreme et al., 1997). From the standard plots for TMM-chelation capacity and metal-ion concentration (data not shown), the maximum chelating capacities of the chitosan derivative-2 for Cu^{2+}, Fe^{2+} and Zn^{2+} were 321, 238 and 53 mg/g chitosan, respectively. Relative to the crosslinked chitosan beads (250 mg/g), chitosan flakes (176 mg/g), chitosan powder (45 mg/g) and prawn shell (17 mg/g) (Chu, 2002), the highest chelating capacity for Cu^{2+} was demonstrated by the chitosan derivative-2.

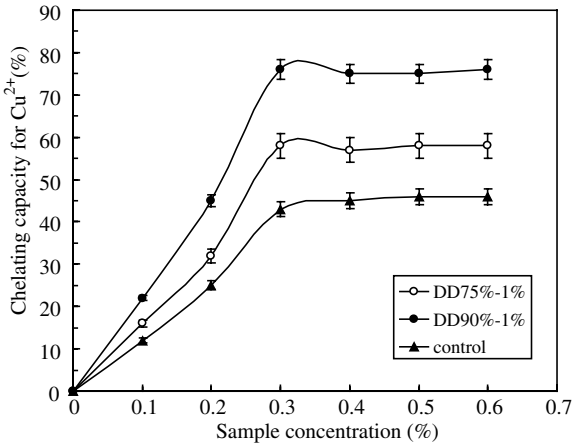

Fig. 7. Plot of the chelating capacity of the acid-soluble chitosan and chitosan-glucosamine derivatives for Cu^{2+} at different concentrations of chitosan or derivatives. The chitosan derivatives produced from 1% α-type chitosan at 90% or 75% DD were with 1% glucosamine at 65 °C for 2 days. Acid-soluble chitosan (DD 90%) was used as the control. The error bars indicate the standard deviation.

Table 2
Chelating capacities of chitosan and chitosan derivatives for various metal ions (Cu^{2+}, Fe^{2+}, Zn^{2+})

	Chelating capacity (%)		
	Cu^{2+}	Fe^{2+}	Zn^{2+}
Chitosan derivative-1[a]	58.1% ± 2.3%	49.9% ± 1.8%	42.2% ± 1.1%
Chitosan derivative-2[b]	76.3% ± 2.8%	59.3% ± 2.3%	51.2% ± 2.4%
Acid-soluble chitosan	43.4% ± 1.1%	34.4% ± 1.4%	23.3% ± 0.6%

[a] Water-soluble chitosan derived from 1% α-type chitosan at 75% DD and 1% glucosamine and reacted at 65 °C for 2 days.
[b] Water-soluble chitosan derived from 1% α-type chitosan at 90% DD and 1% glucosamine and reacted at 65 °C for 2 days.

3.7. Antibacterial activity of various chitosans

The antibacterial activity of chitosan has been widely studied, and its feasibility as a natural antibacterial agent proven after much research (Song et al., 2002). Generally, there is a strong association between chitosan antibacterial activity and the cationic amino group (NH_3^+). When water-soluble chitosan has been prepared using the Maillard reaction, there is a loss of partial amino groups, which leads to low antibacterial activity. Thus, in this study the antibacterial activities of the chitosan derivatives were examined and further compared with acid-soluble chitosan. Table 3 lists the minimum inhibitory concentration (MIC) data for water-soluble and acid-soluble chitosans against *E. coli* and *S. aureus* at pH 5 or 7. Of these chitosans, the strongest antibacterial activity was demonstrated for chitosan derivative-1, produced from chitosan and glucosamine. Acid-soluble chitosan possesses greater antibacterial activity than chitosan derivative-2 (produced from chitosan and glucose) at pH 5; however, the inverse was true at pH 7. As Table 1 reveals, the degrees of deacetylation of chitosan derivative-1 and chitosan derivative-2 were 80.4% and 60.2%, respectively. Hence, low antibacterial activity was noted for the latter. The antibacterial activity of the chitosan derivative-2 was higher than that of the acid-soluble chitosan at pH 7 because of acid-soluble chitosan's limited applicability in acid conditions. Thus, the antibacterial activity of the acid-soluble chitosan at pH 5 was greater than at pH 7. This may be due to the fact that more amino groups (NH_3^+) are formed at pH 5 than

Table 3
Minimum inhibitory concentration (ppm) of water- and acid-soluble chitosans against *E. coil* and *S. aureus* at pH 5 or 7

	Minimum inhibitory concentration (ppm)		
	pH condition	*E. coli*	*S. aureus*
Chitosan derivative-1[a]	pH 5	100 ± 5	140 ± 5
	pH 7	180 ± 5	200 ± 5
Chitosan derivative-2[b]	pH 5	550 ± 10	750 ± 25
	pH 7	700 ± 25	900 ± 45
Acid-soluble chitosan	pH 5	450 ± 18	600 ± 25
	pH 7	>1500 ± 50	>1500 ± 50

[a] Water-soluble chitosan derived from 1% α-type chitosan at 90% DD and 1% glucosamine and reacted at 65 °C for 2 days.
[b] Water-soluble chitosan derived from 1% α-type chitosan at 90% DD and 1% glucose and reacted at 65 °C for 3 days.

at pH 7, as determined from the pK_a (6–6.5) of the amino group in chitosan and the cooperative effect of acetic acid (Jia et al., 2001). Yun et al. (1999) have demonstrated optimal chitosan MIC values of 500 and 400 ppm for *E. coli* and *S. aureus*, respectively. Furthermore, Jia et al. (2001) have determined MIC values for quaternized chitosan against *E. coli* in water and 0.25% acetic-acid medium of 500 and 250 ppm, respectively. No et al. (2002) have reported an MIC value of 800 ppm against *E. coli* and *S. aureus* for optimal chitosan oligomer. Compared with these results, chitosan derivative-1 (chitosan-glucosamine derivative) appeared to be more effective than other chitosans or chitosan derivatives as a natural bactericidal agent.

4. Conclusions

Considering its solubility, the α-type chitosan is more suitable for preparing water-soluble chitosan than β-type chitosan. The high degree of chitosan deacetylation favours production of water-soluble chitosan. The optimal pH for production of the water-soluble variant was pH 3.3, with an optimal reaction temperature of 65 °C in this study. The optimal yield results for chitosan derivatives obtained on a given day (from 2 to 6 days) depended on the saccharide used. Results indicated that chitosan solubility was significantly improved by using the Maillard reaction method, and that all the chitosan derivatives were soluble in water. Based on the results with respect to yield, solubility, degree of deacetylation and pH stability, the most potentially water-soluble chitosan was the chitosan-glucosamine derivative. This derivative exhibited higher metal-ion chelating capacity and antibacterial activities compared with acid-soluble chitosan. These results suggest that the chitosan-glucosamine derivative produced using the Maillard reaction is a promising commercial substitute for acid-soluble chitosan.

Acknowledgements

The authors would like to express their thanks to the National Science Council, Project No. NSC 93-2211-E-157-002-, for the partial financial support that made this work possible.

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