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Chitosan−Glucose Conjugates: Influence of Extent of Maillard Reaction on Antioxidant Properties

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Chitosan−glucose conjugates were prepared using Maillard reaction chemistry. Water-soluble and acid-soluble chitosan−glucose mixtures were heated at pH 4.9 and 6.0 at 98 °C. Mixtures at pH 6.0 containing acid-soluble chitosan gelled when heating was continued after reaching 98 °C and withstood gelation for only 30 min at pH 4.9. In contrast, mixtures containing water-soluble chitosan could be heated without gelation at pH 6.0 and 4.9. Examination of the extent of Maillard reaction and antioxidant properties showed that acid-soluble chitosan reacted for 30 min at pH 4.9 had the highest extent of reaction as judged by increased absorbance, the highest degree of modification to the amino group as evidenced by Fourier transform infrared and shifts of the endotherms by differential scanning calorimetry, and the highest antioxidant activity as indicated by ferric reducing power and oxygen radical absorbance capacity. There were significant correlations ($p < 0.05$) between indices of browning and antioxidant activity.

KEYWORDS: Chitosan; glucose; Maillard conjugation; reactivity; antioxidant activity

INTRODUCTION

The Maillard reaction chemistry that links the carbonyl group of reducing ends in carbohydrates with the amino groups of proteins has been exploited in food applications to enhance the functionality of proteins (*1*). This reaction also leads to the formation of antioxidant compounds, as well as the development of browning flavors that are desirable in some food products (*2*,*3*). The Maillard reaction takes place in three major stages (early, advanced, and final stage), and it is dependent upon factors such as pH, time, temperature, and concentration and type of reactants (*4*). A group of compounds is formed in the final products of the reaction including high molecular weight melanoidins, which are furan ring and nitrogen-containing brown compounds. These are complex structures, and their physical, chemical, and physiological properties have not been fully understood. This complexity in Maillard reaction product (MRP) structure limits the determination of antioxidant activity for each compound in the whole group of MRPs (*5*).

Chitosan is a cationic polysaccharide composed of β-1,4-linked glucosamine units obtained by deacetylation of chitin that is extracted from the exoskeleton of crustaceans, fungi, and insects. Chitosan is nontoxic, biocompatible and widely used for its antihypercholesteromic, antimicrobial, and antioxidant activities and as an encapsulation material in several food−pharmaceutical applications (*6*, *7*). Functional protein−polysaccharide conjugates of chitosan and soy protein were studied for reduced allergenicity of soy proteins (*8*). The Maillard reaction between chitosan and various carbohydrates such as glucose and cellobiose has also been studied in the dry state. The Maillard browning reaction rate was faster with a higher ratio of glucose and at higher water activity (*9*). Fourier transform infrared (FTIR) spectroscopy of the MRP indicated cleavage in sugar units of chitosan and formation of heterocyclic compounds (*10*).

Chitosan exhibits antioxidant properties due to its ability to form complexes with many of the transition metals, as well as some of those from groups 3−7 of the periodic table (*11*). The amount of iron bound by chitosan remained unchanged after the formation of Maillard conjugates, suggesting that the free amino group may not be involved in the absorption mechanism of iron (*9*). The antioxidant property of this chitosan−glucose complex has been found to be enhanced compared to pure chitosan (*12*). As the Maillard reaction is well-known to progress efficiently at higher pH in aqueous medium, we have chosen to compare the reactivity of a water-soluble chitosan to that of an acid-soluble chitosan. The effects of heat treatment (98 °C) at two pH values (4.9 and 6.0) on the extent of the Maillard reaction and the resulting antioxidant activity were examined. The acid-soluble chitosan was highly soluble in aqueous acetic acid medium with a pH value of 4.9. The aqueous solution of water-soluble chitosan had a pH value of 4.5. The Maillard reaction was performed for both chitosans at the initial solution pH (chosen as 4.9) and also at pH 6.0 to examine the enhanced reactivity.

EXPERIMENTAL PROCEDURES

Materials. Acid-soluble chitosan (degree of deacetylation (DD) > 90%; mol wt 810 kDa) was obtained from Swift, Australia. Water-soluble chitosan (DD = 86.2%) was purchased from Shandong AK Biotech Ltd., China. Dextrose monohydrate (glucose) was obtained from Penford Australia, Australia. Anhydrous ferric chloride ($FeCl_3$), 2,2′-azobis(2-amidopropane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 2,4,6-tripyridyl-*s*-triazine (TPTZ) were purchased from Sigma-Aldrich, Pty. Ltd., Australia.

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Ferrous sulfate heptahydrate ($Fe_2SO_4 \cdot 7H_2O$) was obtained from BDH Laboratory Supplies (England). All other chemicals used were of analytical grade.

Methods. *Preparation of Chitosan and Glucose Solutions.* Water-soluble and acid-soluble chitosan solutions (5%; w/w) were prepared by dissolving 75 g of chitosan in 1425 g of deionized water and 2% (w/v) aqueous acetic acid. respectively. The solutions were allowed to hydrate overnight. Glucose solution (5%; w/w) was prepared by dissolving 75 g of dextrose monohydrate in 1425 g of deionized water.

Preparation of Chitosan−Glucose (Ch−Gl) Conjugates. Equal weights of chitosan (acid-soluble and water-soluble) and glucose solutions were mixed (Ch/Gl weight ratio of 1:1, 5% (w/w) of total solids), and pH was adjusted to 4.9 and 6.0 using 1 M sodium hydroxide. Localized precipitation was observed on addition of sodium hydroxide. These precipitates were dispersed using a high shear mixer (Silverson Machines, Inc., USA). The mixtures (200 g) were sealed in metal cans and heated at 98 °C. An appropriate control sample of each of the reaction mixtures was left unheated, whereas the rest were sampled just after the reaction mixture reached 98 °C (i.e., 0 min) and at intervals of 30, 60, 90, and 120 min at 98 °C. Only samples that withstood gelation at 98 °C were analyzed further. These samples were diluted 1:1 (w/w) with warm deionized water to prevent gelation during cooling. The diluted solutions (2.5% total solids) were cooled immediately using an ice−water bath and stored at 4 °C until analysis for determination of the extent of Maillard reaction and antioxidant activity. A portion of the unheated and heated samples (up to 30 min) were freeze-dried for FTIR and DSC analysis.

Characterization of Chitosan and Its Conjugates. *FTIR.* FTIR analysis of pure and reacted chitosans (freeze-dried) solids was performed using a Shimadzu FTIR-8400S fitted with a MiRacle single-pass ATR cell with a zinc selenide crystal. A small amount of sample (1 mg) was placed on the window, and the sample was scanned between 700 and 4000 cm^{-1}. The resolution was set at 4 cm^{-1}, and 128 scans were collected for each sample.

DSC. The thermal transitional patterns were studied using a Perkin-Elmer series1020 DSC7 thermal analysis system and Pyris software (USA). The DSC instrument was equipped with an intercooler for programmed cooling. The instrument was calibrated against indium using a standard calibration method. The samples (1 mg) were placed in hermetically sealed aluminum pans, rapidly preheated from 25 to 100 °C, and cooled to 25 °C to eliminate moisture. The same samples were reheated from 25 to 300 °C at a constant rate of 20 °C/min with a constant purging of nitrogen at a rate of 20 mL/min. A sealed empty aluminum pan was used as a reference. DSC analysis of water-soluble and acid-soluble chitosan and glucose was also carried out using the same method.

Effect of Reaction Conditions on the Extent of the Maillard Reaction. *Total Color Change.* Hunter L^*, a^*, and b^* values were determined with a Minolta chromameter CR-300 (Minolta, Osaka, Japan). The system provides the values of three color components: L^* (0 = black and 100 = white), a^* (+ red to − green component), and b^* (+ yellow to − blue component). The chromameter was initially calibrated with a calibration plate. Chitosan−glucose samples (3 mL) were placed on an open Petri dish against a white background and scanned, and measurements were taken under artificial illumination. Total color change (ΔE) was calculated using the following equation (*13*):

$$\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)}$$

Absorbance at 294 and 420 nm. UV absorbance and browning of MRP samples were measured according to a reported method (*14*). Appropriate dilutions (to give an absorbance value of < 2 at 294 nm) were prepared using deionized water, and all of the samples were of the same dilution for comparison. Intermediate stages in the Maillard reaction were detected by measuring UV absorbance at 294 nm, and the intensity of brown color was measured at 420 nm using quartz cuvettes (with 10 mm path length × 2 mm width × 45 mm height). All measurements were obtained using a Shimadzu UV-1650PC model spectrophotometer (Shimadzu Corp., Kyoto, Japan) with deionized water as a blank reference.

FRAP Assay. The antioxidant properties of chitosan-based Maillard conjugates were measured in terms of their ferric reducing capacity. The FRAP (ferric reducing antioxidant power) assay was carried out according to an earlier method (*15*) with minor modifications. This method is based on the direct reduction of a Fe(III)−2,4,6-tripyridyl-*s*-triazine complex (Fe(III)−TPTZ) to the Fe(II)−TPTZ form by an antioxidant. In this assay, the reducing capacity was expressed as a nanomolar equivalent of Fe(II) per gram of solids in the sample.

$FeCl_3$ (20 mM, anhydrous) solution was prepared in deionized water, and 10 mM TPTZ solution was prepared in 40 mM HCl. The working FRAP reagent was prepared by mixing 2.5 mL of TPTZ solution and 2.5 mL of $FeCl_3$ solution with 25 mL of acetate buffer (0.3 M). $Fe_2SO_4 \cdot 7H_2O$ (10 mM) solution was prepared in deionized water, and 1.0 mL of this solution was further diluted to 100 mL to obtain a 0.1 mM solution. Standards were prepared by mixing different amounts of 0.1 mM $Fe_2SO_4 \cdot 7H_2O$ solution (0−800 μL) and 2.5 mL of TPTZ reagent with 25 mL of 0.3 M acetate buffer to obtain a range of 0−80 nM Fe(II) solutions. Two milliliters of each standard was taken into cuvettes with 10 mm path length, and the absorbance was measured at 593 nm using UV−vis spectrophotometer against acetate buffer blank. Appropriate dilutions of the samples were made using deionized water to obtain the absorbance within the linear range of calibration. Two hundred microliters of samples was mixed with 2 mL of TPTZ reagent, and the absorbance was measured after 10 min.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The antioxidant activity of the Ch−Gl conjugates was also studied by the ORAC assay. Trolox was used as the standard antioxidant in the assay (*16*, *17*). A stock solution of Trolox (100 μM) was prepared by dissolving 5 mg of Trolox in 200 mL of phosphate buffer (75 mM, pH 7.0). Trolox standard solutions were prepared by diluting the stock solution with phosphate buffer. The concentrations ranged between 6.25 and 75 μM. Fluorescein reagent (120 nM) was freshly prepared by diluting 120 μL of 0.05 mg/mL solution in 150 mL of phosphate buffer and stored in the dark until use. AAPH (360 mM) was prepared using phosphate buffer immediately prior to use and stored on ice.

Fluorescein (2.4 mL) and 0.3 mL of sample (standard or the Ch−Gl conjugate) were taken in a cuvette along with a stirrer bar. After the cuvettes had been loaded and placed into the multicell holder, they were equilibrated at 37 °C with rigorous stirring for a few minutes. Stirring was continued for the duration of the assay. AAPH (0.3 mL) was added as quickly as possible to each of the cuvettes to initiate the reaction. Fluorescence measurements were carried out at 495 nm (Ex) and 515 nm (Em) until the intensities remained constant at the baseline. Using the equation obtained from the calibration curve, the equivalent Trolox standard concentrations (or ORAC values) of the samples were deduced.

Statistical Analysis. All analyses of samples from two independent preparations were carried out in triplicates, and mean values with standard deviations were reported. The significance of the differences between variables was determined using one-way ANOVA and Tukey HSD test using the Vassar Stats statistical

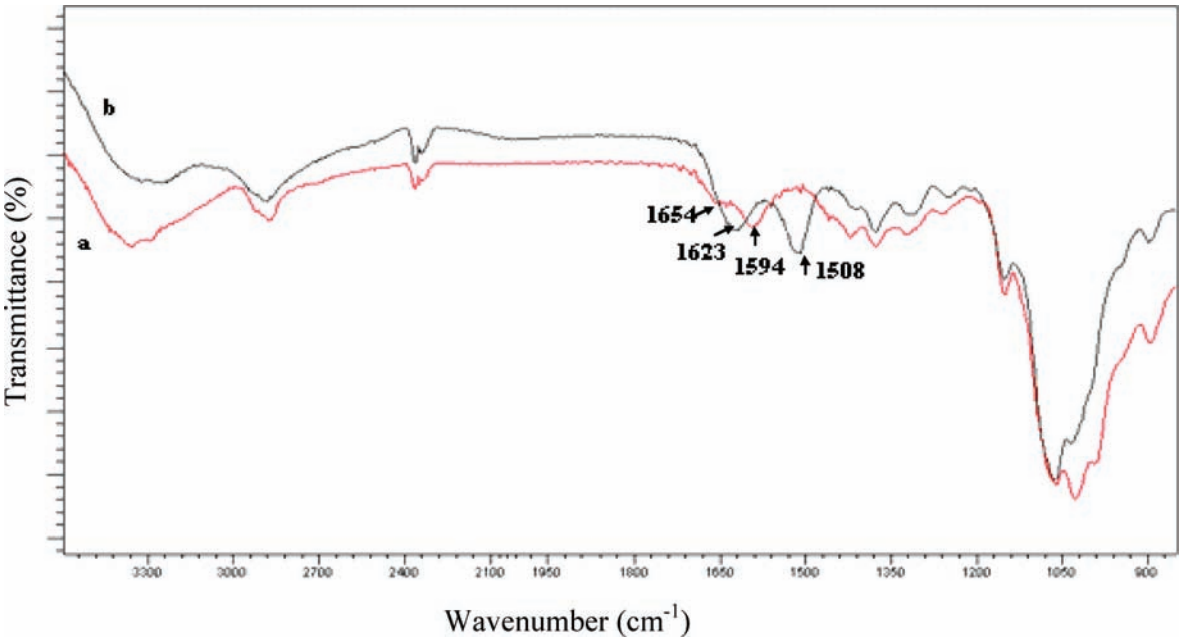

Figure 1. FTIR spectra of acid-soluble chitosan (a) and water-soluble chitosan (b).

computational Website (*18*)). A statistical difference at $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

FTIR and DSC of Acid-Soluble and Water-Soluble Chitosans. The FTIR spectra showed differences in water-soluble and acid-soluble chitosan (**Figure 1**). In the FTIR spectra, characteristic absorption bands between 4000 and 2500 cm^{-1} for −OH (3480−3440 cm^{-1}) and −NH (between 3260 and 3270 cm^{-1}) and −CH stretching regions (2960−2878 cm^{-1}) for acid-soluble chitosan were observed. The amide I band at 1654 cm^{-1} and the 1594 cm^{-1} band attributed to the primary amino group were observed for acid-soluble chitosan. The absorption bands at around 165, 1560, and around 1310 cm^{-1} were assigned to amide I, amide II, and amide III, respectively (*19*).

FTIR of water-soluble chitosan showed the amide I and II peaks at 1623 and 1508 cm^{-1}, respectively (**Figure 1**). These observations were in agreement with those reported by Muzzarelli (*20*), who attributed the absorption band at 1500 cm^{-1} to a protonated amine group, and Orienti et al. (*21*), who attributed bands at 1631 and 1522 cm^{-1} to the amide group. Commercially produced water-soluble chitosans are generally chitosan salts such as chlorides or glutamates. On the basis of our observations and the reported spectroscopic data, the water-soluble chitosan used in this study was considered to have undergone modifications to the amide/amino group due to the salt formation.

DSC data confirmed that there were differences between the acid-soluble and water-soluble chitosans (**Figure 2**). The enthalpy for the endothermic transition at 162 °C was much higher for the water-soluble chitosan when compared to the transitions for acid-soluble chitosan in that range (**Table 1**). Generally, these transitions are attributed to the loss of water in the polysaccharide. Endothermic transitions at 159, 173, and 186 °C were observed for chitosan formate, citrate, and malate films, respectively (*22*). The endothermic peaks that occur over a large range of temperatures (35−160 °C), attributable to water loss, represent the energy required to vaporize water present in the film samples (*23*). The sharp endothermic peak at 210 °C observed in acid-soluble chitosan may be due to the melting of low molecular weight chitooligosaccharides present in the biopolymer. The second endothermic

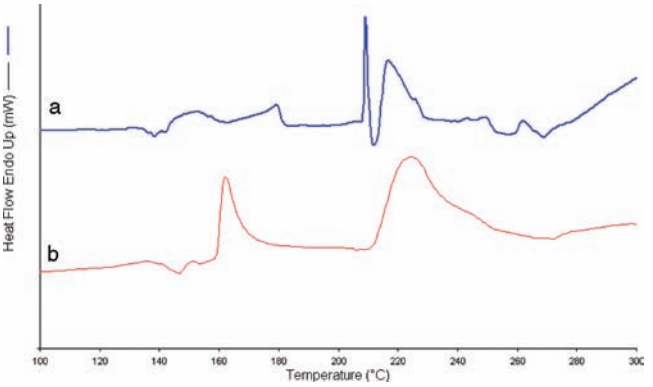

Figure 2. DSC scan of acid-soluble chitosan (a) and water-soluble chitosan (b).

transition appeared at 224 and 217 °C for water-soluble and acid soluble chitosans.

Stability of Chitosan−Glucose Reaction Mixtures. The stability of chitosan−glucose mixtures to gelation depended on the type of chitosan and the pH of the reaction. Water-soluble chitosan−glucose mixture at pH 4.9 remained liquid until 120 min at 98 °C, but at pH 6.0, the reaction mixture became viscous after 90 min. After 120 min of reaction time, the mixture was very viscous and gelled while cooling.

Acid-soluble chitosan mixture at pH 4.9 gradually became viscous after 30 min and gelled at 60 min. At pH 6.0, the reaction mixture became viscous as it reached 98 °C and gelled when heating was continued. The observed gelling kinetics may be attributed to the extent of reaction undergone by the two varieties of chitosan used. The chemical modification of water-soluble chitosan as observed by FTIR would have led to the slow reactivity of water-soluble chitosan when compared to the acid-soluble chitosan.

Effect of Reaction Conditions on the Extent of the Maillard Reaction. The effect of reaction conditions on the formation of conjugates was studied by heating water-soluble and acid-soluble chitosan−sugar (1:1 w/w, 5% total solids) solutions at pH 4.9 and 6.0. The extent of the Maillard reaction (which is related to the formation of chitosan−glucose conjugate) of the ungelled products

Table 1. DSC Analysis Showing the Endothermic Transitions of Reactants and Chitosan−Glucose Conjugates

		peak onset (°C)	peak (°C)	peak end (°C)	ΔH (J/g)
water-soluble chitosan					
chitosan	I	159.5	162.1	169.1	123.6
	II	I212	224.4	240.4	431.7
chitosan−glucose (pH 4.9)	I	161.9	164.4	165.8	9.2
	II	I177.3	181.1	188.5	129.7
chitosan−glucose (pH 6.0)	I	158.3	160.1	166	158.1
acid-soluble chitosan					
chitosan	I	141.7	151.7	160.9	49.7
	II	I170.5	179.3	181.6	34.9
	II	I213.3	216.7	228.7	138.6
chitosan−glucose (pH 4.9)	I	149.4	154.7	160.7	33.7
	II	I174.9	176.7	180.7	200.5
glucose	I	82.19	86	90.5	113.5
	II	I198.8	201.3	206.7	25.3
	III	II219.0	221	225.8	106

was followed using the Hunter $L^*a^*b^*$ color scale and UV−vis spectrophotometry.

The total color change, absorbance patterns at both 294 and 420 nm, followed similar trends (**Figure 3**). Increasing duration of heat treatment increased all of these indices of the extent of Maillard reaction between chitosan and glucose. The Maillard reaction is known to be influenced by the pH of the medium (*24*). Huang et al. (*25*) reported that when chitosan was heated with a sugar (D-xylose), the rate of reaction has increased with increasing initial pH, but between pH 5.0 and 6.0, there was not much difference. In our work with water-soluble chitosan, the reactivity at pH 6.0 was higher ($p < 0.05$) compared to that at pH 4.9.

All of the chitosan−glucose samples had higher absorbance values at 294 nm than at 420 nm. Lerici et al. (*26*) detected the intermediate stages of the nonenzymatic browning reactions between glucose and glycine by recording the UV absorbance at 294 nm. They also found that heat treatment of a glucose−glycine mixture caused marked increase in absorbance at 294 nm. The absorbance values at 420 nm were used as an indicator of the browning development in the final stages of Maillard reaction of fructose and lysine (*14*). In our study, chitosan conjugates had higher levels of intermediate browning products than final-stage browning products. This may be due to the incomplete Maillard reaction that reduced the formation of final browning compounds. These compounds represent advanced glycation reaction. Kanatt et al. (*12*) also observed the intermediate and final browning products when 1% solutions of chitosan and glucose were reacted at 121 °C. An increase in browning of porcine plasma protein−sugar MRPs, as measured by absorbance at 420 nm, was observed as the heating time increased (*27*). A study on the effect of caramelization on the Maillard browning reaction revealed that heating glucose at 100 °C at pH 6 or 8 showed little or no browning, whereas the browning of a glucose and lysine mixture increased at pH 8 (*28*).

The results obtained demonstrate the higher reactivity of acid-soluble chitosan (pH 4.9, 30 min) than of water-soluble chitosan reacted at both pH values (4.9 or 6.0) for longer times (120 min). The limiting factor for the development of Maillard conjugates in aqueous systems containing acid-soluble chitosans was the gelling of the reaction mixture. The reaction mixture gelled after 30 min at pH 4.9, and the reaction could not be continued further. In the case of reaction at pH 6.0 with acid-soluble chitosan, the reaction mixture gelled when heating was continued beyond the initial stage (0 min of reaction time) at 98 °C. Despite the limitations of solubility with increased reaction times, acid-soluble chitosan reacted for 30 min at pH 4.9 had much higher reactivity than water-soluble chitosan reacted for longer reaction times at both pH values. These differences in the reactivity of chitosans clearly indicate that the Maillard reaction is markedly reduced with water-soluble chitosan. This is possibly due to the modification of the amide/amino groups in this chitosan.

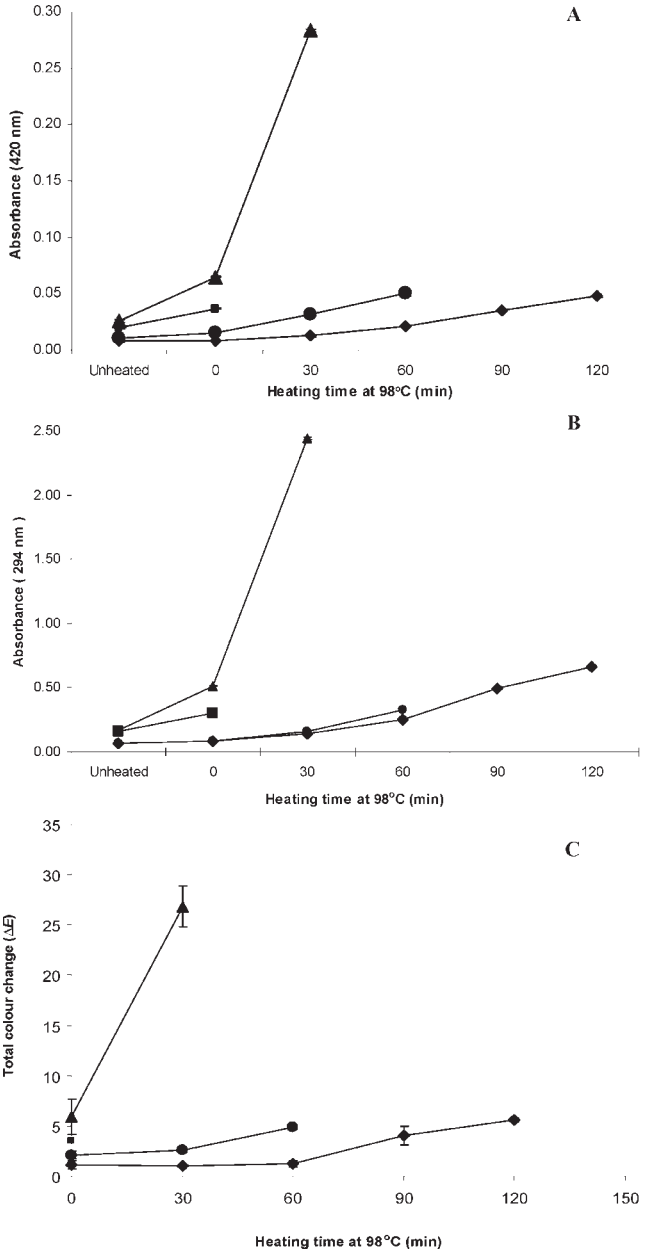

Figure 3. Extent of browning reaction by absorbance using UV−vis spectroscopy at 420 nm (**A**) and at 294 nm (**B**) and the total color change, ΔE (**C**), showing water-soluble Ch−Gl at pH 4.9 (◆), water-soluble Ch−Gl at pH 6.0 (●), acid soluble Ch−Gl at pH 4.9 (▲), and water-soluble Ch−Gl at pH 6.0 (■).

FTIR and DSC of the Conjugates. The FTIR spectra of the products were compared with the corresponding initial chitosans. In the case of water-soluble chitosan, the amide/amino bands were observed at 1623 and 1508 cm^{-1}, respectively. There was a shift of these amide/amino bands in the reaction product (**Figure 4A**),

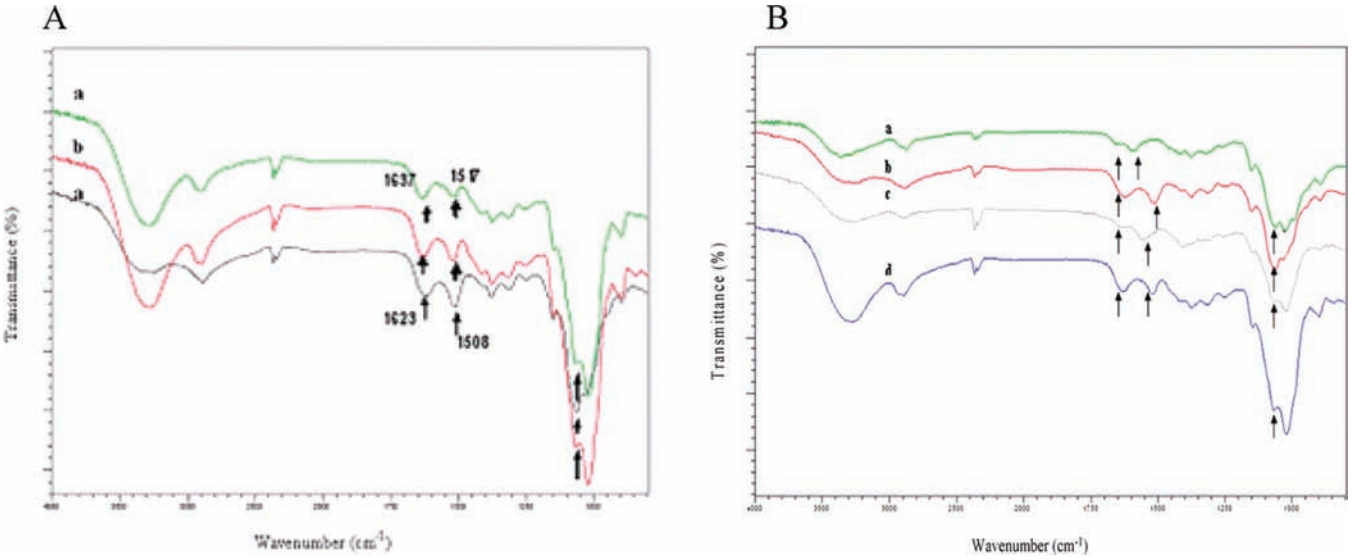

Figure 4. FTIR of water-soluble chitosan and its conjugates (**A**) showing water-soluble chitosan (black, a) and Ch−Gl conjugate with water-soluble Ch formed at pH 4.9 (red, b) and pH 6.0 (green, c) and comparison between acid-soluble and water-soluble chitosan-based conjugates (**B**) showing acid-soluble chitosan (green, a) and water-soluble chitosan (red, b) Ch−Gl conjugate formed at pH 4.9 with acid-soluble Ch (black, c) and with water-soluble Ch (blue, d).

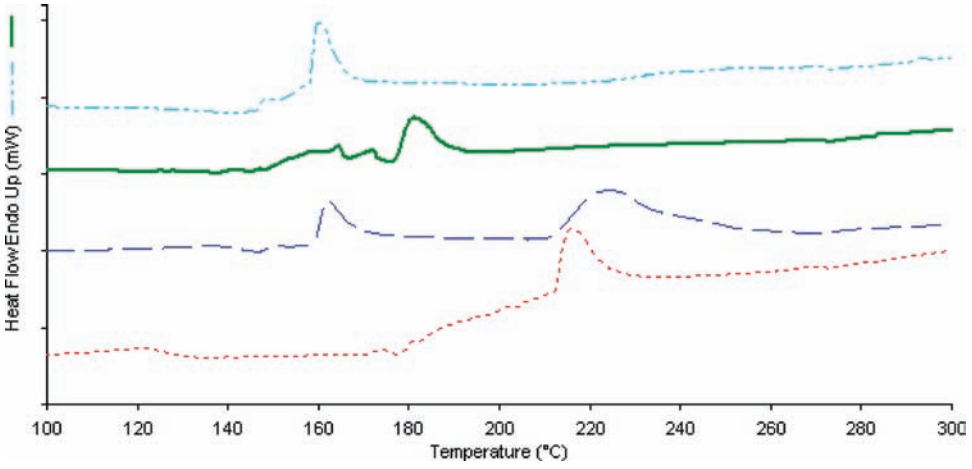

Figure 5. DSC scans of glucose (red, dot), water-soluble chitosan (blue, dash), and Ch−Gl conjugate formed with water-soluble chitosan formed at pH 4.9 (green, solid) and pH 6.0 (blue, dash dot dot).

indicating the progress of the Maillard reaction. This may be attributed to the consumption of amino groups due to formation of a Schiff base during the reaction. The absorption band at 1594 cm^{-1}, attributed to the primary amino group, was observed along with the amide I peak at 1654 cm^{-1} in acid-soluble chitosan. These peaks were shifted to 1554 and 1637 cm^{-1}, respectively, after the reaction (**Figure 4B**).

Earlier studies on chitosan reacted with hemicellulose compounds reported that the absorption band at 1644 cm^{-1} would represent the spectral overlap of the C═O group and C═N linkage (*29*). They also indicated the conversion of primary amino groups during Maillard reaction to result in secondary amine (1558 cm^{-1}) formation and subsequent reduction of peak intensity due to the formation of tertiary amines. The absorption bands at 1060, 1020, and 898/894 cm^{-1} can be assigned to the C−O stretching and the β-D-configuration, respectively. The band at 1060 cm^{-1} decreased in intensity with an increase in intensity of the absorption band at 1020 cm^{-1} after the reaction with glucose. This was observed for both water-soluble and acid-soluble chitosans.

Water-soluble chitosan-based conjugates formed at pH 4.9 showed two endothermic transitions with a shift of the second endothermic transition when compared to unreacted water-soluble chitosan (**Table 1**; **Figure 5**). This peak shift to a lower temperature indicates its reaction with glucose. A peak with higher enthalpy change for the first endothermic transition could be observed after the pH 6.0 reaction. The second endothermic peak could not be seen as a peak in the conjugates formed at pH 6.0 with water-soluble chitosan. This transition appeared to be broadened and was seen as a shift of the baseline initiating at a higher temperature. The observed peak shifts of the endothermic transitions indicated insignificant reactivity at both pH values with slightly higher reactivity at pH 6 compared to pH 4.9. These results corroborate the results obtained with the analysis of the browning reaction to the extent that there was a greater change measured at pH 6.0 compared to pH 4.9.

In the case of acid-soluble chitosan−glucose conjugate formed at pH 4.9, there were two endothermic transitions observed (**Table 1**; **Figure 6**). There was a shift of these endothermic transitions, with the second transition having much higher enthalpy when compared to the same transition for water-soluble chitosan-based conjugate. Similar peak shift was observed in the conjugates formed between chitosan and whey protein in our earlier study (*30*). The results obtained corroborate the FTIR data and demonstrate the higher reactivity of acid-soluble chitosan compared to water-soluble chitosan.

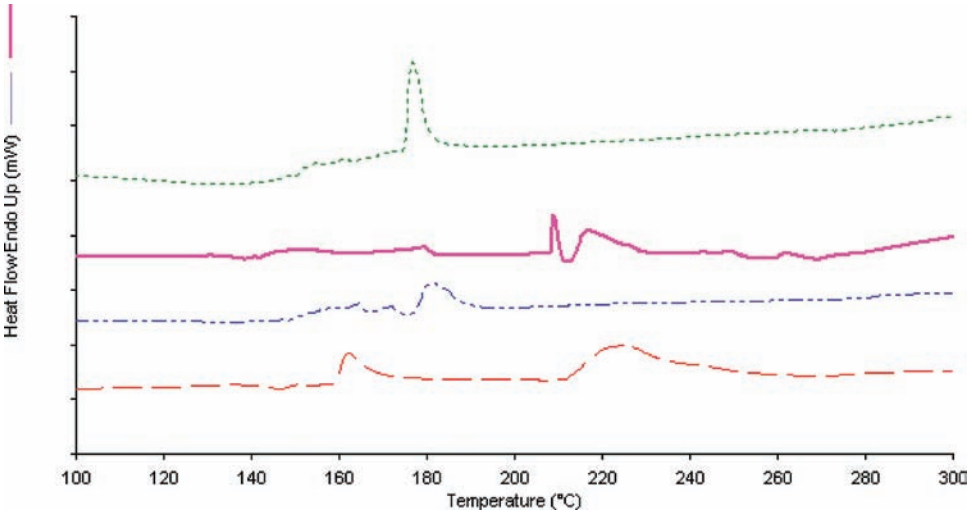

Figure 6. DSC scans of water-soluble chitosan (red, dash), acid-soluble chitosan (blue, dash dot dot), and Ch−Gl conjugate formed at pH 4.9 with water-soluble chitosan (pink, solid) and acid-soluble chitosan (green, dot).

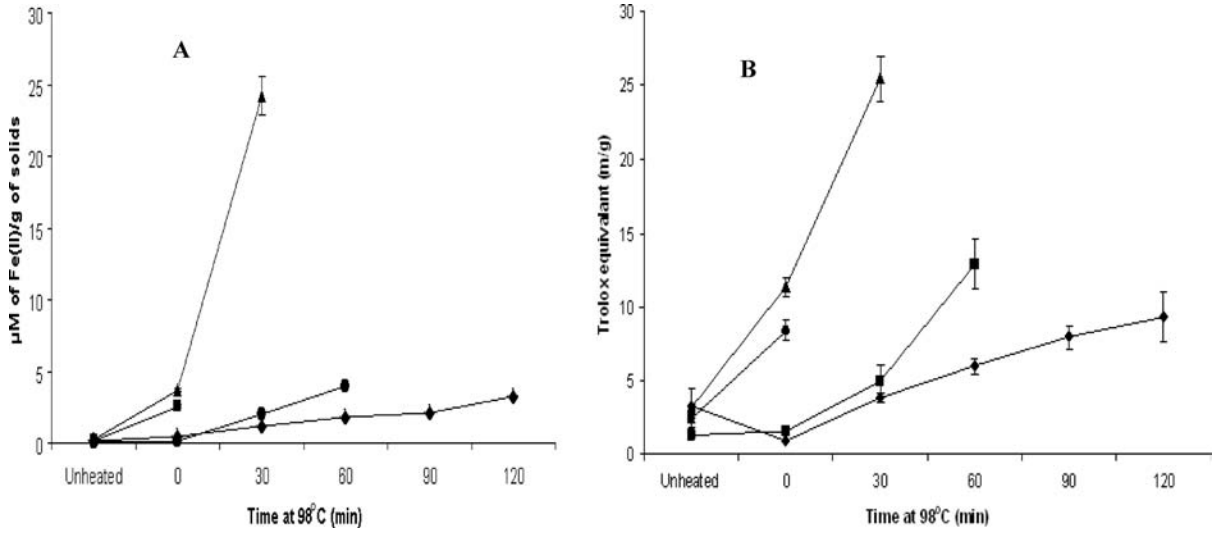

Figure 7. Antioxidant activity of chitosan−glucose conjugates by FRAP assay (**A**) and by ORAC assay (**B**) for water-soluble Ch−Gl at pH 4.9 (◆), water-soluble Ch−Gl at pH 6.0 (●), acid soluble Ch−Gl at pH 4.9 (▲), and water-soluble Ch−Gl at pH 6.0 (■).

This can be attributed to the availability of unmodified amide/amino groups in acid-soluble chitosan.

Antioxidant Activity. The development of antioxidants was measured using FRAP and ORAC methods. The antioxidant activity determined by FRAP assay (**Figure 7A**) followed a pattern similar to that of color development. The antioxidant activity of Ch−Gl conjugates prepared using two different chitosans indicated that the conjugates formed with acid-soluble chitosan had much higher activity than the water-soluble chitosan. The reaction mixture at pH 6.0 with acid-soluble chitosan gelled at 98 °C and hence could not be compared. The reaction at pH 4.9 and 6.0 with water-soluble chitosan did not show marked differences in activity even after 60 min of reaction time. The higher antioxidant activity obtained can be explained due to higher reactivity of acid-soluble chitosan and was correlated with the total color change of the conjugates formed. Antioxidant activity is related to the development of reductones, which are terminators of free radical chain reactions (*31*). Earlier studies on chitosan−glucose conjugates have reported insignificant reducing power (*12*). This may be due to the degree of deacetylation and molecular weight of the chitosan used in that study compared to this work. It may also be possibly due to differences in the reaction conditions and the concentration of the reactants used in the two studies. Another study (*27*) showed that MRPs of plasma protein−galactose conjugates exhibited much greater reducing power compared to the glucose-based MRPs. The results discussed in these studies reflect the variation of the Maillard reaction intermediates/products formed depending on the reactants involved and the reaction conditions employed in these studies.

The antioxidant activity obtained by ORAC (**Figure 7B**) also demonstrated that acid-soluble chitosan based conjugates exhibit much higher activity when compared to the water-soluble chitosan based conjugates formed at pH 4.9. The activities obtained also indicated the influence of reaction time on the antioxidant activity with water-soluble chitosan-based conjugate formation. Histidine−glucose mixtures were found to exhibit higher antioxidant activity when the reaction time was increased at 100 °C (*5*). As the reaction time increases, the conjugate formed exhibited higher activity. Due to the higher viscosity of acid-soluble chitosan, reaction time could not be extended beyond 30 min at pH 4.9, and the reaction mixture at pH 6.0 formed gels when heating was

continued after the reaction temperature reached 98 °C. The activities of the chitosan-based conjugates followed almost similar trends as obtained by FRAP assay.

The results obtained clearly demonstrate that the initial stage of Maillard reaction between acid-soluble chitosan and glucose generates antioxidant activity. On the basis of the FTIR of the conjugates, the generated antioxidant activity may be attributed to the formation of secondary and tertiary amines.

Comparative studies on the reactivity of acid/water-soluble chitosans to Maillard reaction with glucose indicated that acid-soluble chitosan has much higher reactivity, as confirmed by the various indicators of the Maillard reaction. The chitosan−glucose mixtures of acid-soluble chitosan gelled on heating beyond 30 min reaction time at pH 4.9. Water-soluble chitosan was found to be slightly more reactive at pH 6.0 than at pH 4.9. At pH 6.0, the acid-soluble chitosan and glucose reaction mixture gelled even before heating was begun and hence could not be compared with water-soluble chitosan. Acid-soluble chitosan conjugates formed at pH 4.9 also displayed significantly higher ($p < 0.1$) antioxidant activity than the conjugates formed by water-soluble chitosan at both pH conditions examined. The initial stage of Maillard reaction between acid-soluble chitosan and a low molecular weight carbohydrate such as glucose has good potential as an antioxidant coating in food applications.

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LITERATURE CITED

(1) Handa, H.; Kuroda, N. Functional improvements in dried egg white through the Maillard reaction. *J. Agric. Food Chem.* **1999**, *47*, 1845−1850.

(2) Monti, S. M.; Ritieni, A.; Graziani, G.; Randazzo, G.; Mannina, L.; Segre, A. L.; Fogliano, V. LC/MS analysis and antioxidative efficiency of Maillard reaction products from lactose−lysine model system. *J. Agric. Food Chem.* **1999**, *47*, 1506−1513.

(3) Ho, C. T. Thermal generation of Maillard aroma. In *The Maillard Reaction: Consequences for the Chemical and Life Science*; Ikan, R., Ed.; Wiley: New York, 1996; pp 27−53.

(4) Ames, J. M. The Maillard reaction. In *Biochemistry of Food Proteins*; Hudson, B. J. F., Ed.; Elvesier Applied Science: London, U.K., 1992; Vol. 4, pp 99−153.

(5) Yilmaz, Y.; Toledo, R. Antioxidant activity of water-soluble Maillard reaction products. *Food Chem.* **2005**, *93*, 273−278.

(6) Weerakkody, R.; Fagan, P.; Kosaraju, S. L. Chitosan microspheres for encapsulation of α-lipoic acid. *Int. J. Pharm.* **2008**, *357*, 213−218.

(7) Kumar, M. N. V. R.; Muzzarelli, R. A. A.; Muzzarelli, C.; Sashiwa, H.; Domb, A. J. Chitosan chemistry and pharmaceutical perspectives. *Chem. Rev.* **2004**, *104*, 6017−6084.

(8) Usui, M.; Tamura, H.; Nakamura, K.; Ogawa, T.; Muroshita, M.; Azakami, H.; Kanuma, S.; Kato, A. Enhanced bactericidal action and masking of allergen structure of soy protein by attachment of chitosan through Maillard-type protein−polysaccharide conjugation. *Nahrung* **2004**, *48*, 69−72.

(9) Tanaka, M.; Huang, J. R.; Chiu, W. K.; Ishizaki, S.; Taguchi, T. Effects of the Maillard reaction on functional properties of chitosan. *Nippon Suisan Gakkai Shi* **1993**, *59*, 1915−1921.

(10) Umemura, K.; Kawai, S. Modification of chitosan by the Maillard reaction using cellulose model compounds. *Carbohydr. Polym.* **2007**, *68*, 242−248.

(11) Muzzarelli, R. A. A. *Natural Chelating Polymers: Alginic Acid, Chitin and Chitosan*; Pergamon Press: Oxford, U.K., 1973.

(12) Kanatt, S. R.; Chander, R.; Sharma, A. Chitosan glucose complex − a novel food preservative. *Food Chem.* **2008**, *106*, 521−528.

(13) HunterLab. Hunter Lab Colour Scale, 1996; Vol. 2005 (http://www.hunterlab.com/appnotes/an08_96a.pdf).

(14) Ajandouz, E. H.; Tchiakpe, L. S.; Ore, F. D.; Benajiba, A.; Puigserver, A. Effects of pH on caramelization and Maillard reaction kinetics in fructose−lysine model systems. *J. Food Sci.* **2001**, *66*, 926−931.

(15) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant microcapsules": the FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.

(16) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and validation of an improved oxygen radical absorbance capacity using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* **2001**, *49*, 4619−4626.

(17) Prior, R. L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *5*, 4290−4302.

(18) One-way ANOVA for 4 independent samples, http://faculty.vassar.edu/lowry/VassarStats.html, Sept 8, 2010.

(19) Sannan, T.; Kurita, K.; Ogura, K.; Iwakura, Y. Studies on chitin: 7. IR spectroscopic determination of degree of deacetylation. *Polymer* **1978**, *19*, 458−459.

(20) Muzzarelli, R. A. A. Carboxymethylated chitins and chitosans. *Carbohydr. Polym.* **1988**, *8*, 1−21.

(21) Orienti, I.; Cerchiara, T.; Luppi, B.; Bigucci, F.; Zuccari, G.; Zecchi, V. Influence of different chitosan salts on the release of sodium dichlofenac in colon-specific delivery. *Int. J. Pharm.* **2002**, *238*, 51−55.

(22) Ritthidej, G. C.; Phaechamud, T.; Koizumi, T. Moist heat treatment on physicochemical change of chitosan salt films. *Int. J. Pharm.* **2002**, *232*, 11−22.

(23) Cervera, M. F.; Heinamaki, J.; Krogars, K.; Jorgensen, A. C.; Karjalainen, M.; Colarte, A. I.; Yliruusi, J. Solid-state and mechanical properties of aqueous chitosan-amylose starch films plasticized with polyols. *J. Pharm. Sci. Technol.* **2004**, *5*, 1−6.

(24) Ashoor, S. H.; Zent, J. B. The Maillard browning of common amino acids and sugars. *J. Food Sci.* **1984**, *49*, 1206−1207.

(25) Huang, J.; Huang, C.; Huang, Y.; Chen, R. Shelf-life of fresh noodles as affected by chitosan and its Maillard reaction products. *LWT−Food Sci. Technol.* **2007**, *40*, 1287−1291.

(26) Lerici, C. R.; Barbanti, D.; Manzano, M.; Cherubin, S. Early indicators of the chemical changes in foods. 1. Study on heat treated model systems. *Lebensm. Wiss. Technol.* **1990**, *23*, 289−294.

(27) Benjakul, S.; Lertittikul, W.; Bauer, F. Antioxidant activity of Maillard reaction products from a porcine plasma protein−sugar model system. *Food Chem.* **2005**, *93*, 189−196.

(28) Ajandouz, E. H.; Puigserver, A. Non-enzymatic browning reaction of essential amino acids. Effect of pH on caramelization and Maillard reaction kinetics. *J. Agric. Food Chem.* **1999**, *1*, 928−943.

(29) Umemura, K.; Kawai, S. Preparation and characterisation of Maillard reacted chitosan films with hemicellulose model compounds. *J. Appl. Polym. Sci.* **2008**, *108*, 2481−2487.

(30) Zhang, L.; Dudhani, A.; Lundin, L.; Kosaraju, S. L. Macromolecular conjugate based particulates: preparation, characterisation and evaluation of controlled release properties. *Eur. Polym. J.* **2009**, *45*, 1960−1969.

(31) Singh, N.; Rajini, P. S. Free radical scavenging activity of an aqueous extract of potato peel. *Food Chem.* **2004**, *85*, 611−616.

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