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Browning of chitooligomers and their optimum preservation

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Abstract

Chitooligomers have attracted much interest due to their unique biological activities. However, chitooligomers easily turn brown during shelf life. The factors influencing the browning of chitooligomers were investigated. The results indicated that the browning was attributed to the structure change of chitooligomers. The water-solubility, thermal stability and moisture–adsorption of chitooligomers decreased with the increase of browning. The time, temperature, pH, moisture, oxygen and reductant all had effect on the browning of chitooligomers. The optimal preservation condition for chitooligomers should be at low temperature and humidity, at pH below 4 or above 10, and in absence of oxygen.

Keywords: Chitosan; Chitooligomer; Browning; Preservation; Maillard reaction

1. Introduction

Chitosan, a linear polymer composed of β-1,4-linked glucosamine (GlcN) with various degrees of *N*-acetylated GlcN residues is a deacetylated derivative of chitin extracted from an abundant source of shellfish exoskeletons. Chitosan is the second natural polysaccharide after cellulose, and it is non-toxic, biocompatible and biodegradable (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Therefore, chitosan is widely used in food, pharmaceutics and cosmetics (Kumar, 2000). The use of chitosan as various functional materials, including biomedical materials (Khor & Lim, 2003; Liu & Yao, 2002; Suh & Matthew, 2000), has recently been developed. Chitosan with different molecular weight has different biological activities. Low molecular weight chitosan (LMWC) was shown to improve plant resistance to disease (Tokoro, Kobayashi, Tatewaki, Suzuki, & Okawa, 1989) and to stimulate murine peritoneal macrophages (Kuroiwa, Ichikawa, Hiruta, & Mukataka, 2002) to kill the tumor cells (Seo, Pae, & Chung, 2000). LMWC of 20 kDa were shown to prevent progression of diabetes mellitus (Muzzarelli, 1996).

Chitooligomers refer to chitosan with degree of polymerization (DP) <20, which can be achieved by depolymerization of chitosan. Some researchers have reported that chitooligomers have various biological activities (Kim & Rajapakse, 2005) such as antitumor and immune enhancing effects due to their good water-solubility and non-toxicity (Qin, Gao, Wang, & Zeng, 2006). Chitooligomers also have applications in cosmetics due to good properties of moisture–retention and moisture–adsorption. However, there exists a serious problem that browning of chitooligomers occurs easily during shelf life, which influences its properties and limits its applications in many fields. In order to preserve chitooligomers properly, some factors influencing their browning were investigated in this paper.

2. Experimental

2.1. Materials

Initial shrimp chitosan (degree of deacetylation DD = 85.5%) and crude hemicellusase solution were

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obtained from Hubei Yufeng Biology Engineering Co. Ltd. (Wuhan, China). Other reagents were of analytical grade.

2.2. Characterizations

The weight average molecular weight (Mw) of samples was measured by a gel permeation chromatography (GPC). GPC system incorporated a TSP P1000 instrument. Two columns in series (TSK G5000-PW and TSK G3000-PW) were used. The eluent was 0.2 M CH_3COOH/0.1 M CH_3COONa. The flow rate was maintained at 1.0 ml/min. The temperature of the columns was maintained at 30 °C. The eluent was monitored by a RI 150 refractive index detector. The sample concentration was ca.0.4% (w/v). The standards used to calibrate the column were TOSOH pullulan. All data provided by the GPC system were collected and analyzed using the Jiangshen Workstation software package.

FT-IR spectra were recorded with KBr pellets on a Nicolt Impact 380 spectrophotometer.

TG and DTA of samples (5.0 mg) in dynamic nitrogen atmosphere were performed by a Differential Thermal Analyzer Model WCT-1 (Beijing Optical Instruments Factory, China) from 20 to 596 °C at a heating rate of 10 °C/min.

The UV absorbance spectrum of chtiooligomers (COS) solution was measured by UV–Vis spectrophotometer Model WFZ900-D4 (Beijing the Second, Optical Instruments Factory, China).

2.3. Preparation of COS

Chitosan (20.0 g) was completely dissolved in 400 ml 2% acetic acid. After 3 h, the solution was neutralized to pH 5.5 and stirred at 50 °C. Crude hemicellusase solution (2.0 ml) was added to initiate the reaction. The reaction lasted for 8 h at 50 °C. Then, the mixture solution was filtrated by hollow-fiber membrances of 6 kDa. The ultra-filtrate was concentrated by nano-filtration membrance and COS product was collected after freeze drying.

2.4. Preparation of brown chitooligomer (BCOS)

Chitooligomers were set in desiccator with 81% relative humidity (RH) at 39 °C. Some samples, which had turned dark, were taken out after they had been stored for 11 days and 21 days, and put in vaccum dryer under P_2O_5.

2.5. Preparation of reduced chitooligomer (RCOS)

Chitooligomer (1.25 g) was dissolved in 100 ml distill water, then 20 ml solution containing 1.20 g $NaBH_4$ was added to the solution. The mixture was stirred for a night and the product was collected after freeze drying.

2.6. Factors influencing the browning of COS

2.6.1. Effect of time on the browning of COS and RCOS

Fifty milliliters of 0.5% COS and 50 ml of 0.5% RCOS were placed in water-bath at 60 °C, and their UV absorbance was measured by UV–Vis spectrophotometer at the predetermined time.

2.6.2. Effect of pH on the browning of COS

Seven fractions of 0.6% COS at pH 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 were placed in water-bath at 60 °C for 1 h, respectively. Their UV absorbance was measured at pH 6 by UV–Vis spectrophotometer.

2.6.3. Effect of temperature on the browning of COS

Seven fractions of COS solution at the concentration of 0.5% were placed in water-bath at 0, 28, 42, 48, 60, 70 and 84 °C for 1 h, respectively. After cooling down to room temperature, the UV absorbance of these solutions was measured at 278 nm by UV–Vis spectrophotometer.

2.6.4. Effect of oxygen on the browning of COS

Twelve fractions of 0.5000 g COS were weighed and six of them were placed in vacuum dryer filled with nitrogen atmosphere and anhydrous $CaCl_2$. The other six fractions were placed in another vacuum dryer filled with oxygen and anhydrous $CaCl_2$. At the predetermined time, these samples were dissolved in distill water with final volume at 100.0 ml. The UV absorbance of solution was measured at 278 nm by UV–Vis spectrophotometer.

2.6.5. Effect of moisture on the browning of COS

Twelve fractions of 0.5000 g COS were weighed. Six of them were placed in vacuum dryer at 24% RH and the other six fractions were placed in another vacuum dryer at 0.1% RH. At the predetermined time, these samples were dissolved in distill water with final volume at 100.0 ml. The UV absorbance of solution was measured at 278 nm by UV–Vis spectrophotometer.

2.7. Effect of browning on the water-solubility of samples

Twenty-two fractions of 0.5000 g COS were weighed and half of them were placed in refrigerator as blank group. The others were set in open air at 39 °C. At the predetermined time, the samples were added into 50.0 ml distill water with stirring for 2 h. The insoluble residues were separated off by filtration, dried under P_2O_5 for 3 days and weighed.

2.8. Moisture–absorption of COS and BCOS

Three fractions of dry COS and three fractions of dry BCOS samples (ca. 0.5000 g for each sample) stored for 21 days were weighed, respectively. They were placed in a desiccator containing saturated $(NH_4)_2SO_4$ solution with 81% RH. The weight of these samples was measured at the interval of 12 h. The moisture–absorption capacity of

samples was evaluated by the percentage of weight increase of dry sample: $Ra\,(\%) = 100\,(W_n - W_0)/W_0$, where W_0 and W_n were the weight of sample before and after being placed in desiccator.

3. Results and discussion

3.1. Gel permeation chromatography analysis

Chtiooligomers developed a yellow color and turned brown during storage. The solubility of sample after browning became weak, there were some insoluble residues when they were dissolved in distill water. Fig. 1 is the gel permeation chromatographic profiles of dissolved samples, which were stored at 81% RH and 39 °C for 0 day, 11 days and 21 days. A remarked difference among profiles of three samples was the intensity of peaks, which was due to the different water-solubility of stored samples. Initial COS was completely water-soluble. After COS sample was stored for 11 days, there were a few insoluble residues in the solution and the elution time of the dissolved sample brought forward 1.5 min. It indicated that there was polymerization among COS molecules (Thomas, Holger, Monika, & Theodor, 2001). The browning of COS sample increased as stored time prolonged to 21 days. Most of the stored sample was insoluble, so the height of peak was much lower than that of initial COS. The free amino groups and reducing sugar groups in COS molecules could react with each other (Muzzarelli, Terbojevich, Muzzarelli, & Francescangeli, 2002; Tressl, Wondrak, Garbe, & Rewicki, 1998) to form larger molecules, which further reacted to produce water-insoluble macromolecules.

3.2. UV–Vis spectra

Fig. 2 is the UV–Vis spectra of COS solution heated at 60 °C for different time. The peak at 212 nm did not change significantly, which was attributed to acetyl group. The absorbance peak at 278 nm became sharper with the increase of heating time, which indicated that COS was

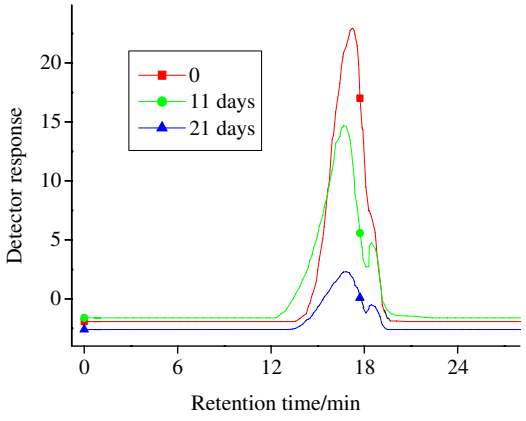

Fig. 1. GPC profiles of COS stored at 81% RH and 39 °C for different time.

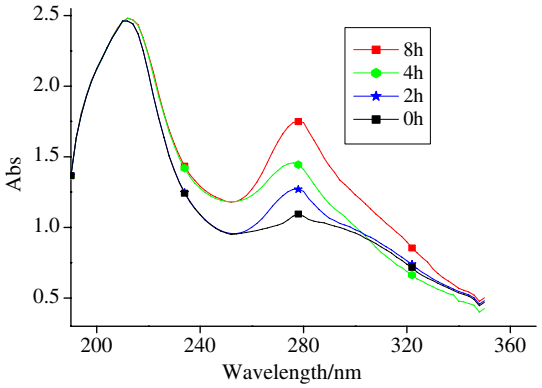

Fig. 2. UV–Vis spectra of COS solution heated at 60 °C for different time.

browning. Thus, 278 nm was selected as the analytical line in UV–Vis spectra of COS below. In fact, chitosan with high molecular weight had no absorbance at 278 nm (Kim, Petrisor, & Yen, 2004). However, COS contain much more reducing sugar groups, which are reactive groups. The reactions occurred and formed colored compounds even during preparation of COS, so the initial COS sample also exhibited absorbance at 278 nm.

3.3. Factors influencing the browning of COS

3.3.1. Effect of temperature

Fig. 3 shows the effect of temperature on browning of COS. The absorbance of COS solution increased with the increase of temperature. The rate of browning was faster at higher temperature. Manzocco and Maltini (1999) reported that color change due to Maillard reaction was associated with the formation of heat-induced antioxidants, and a positive correlation between browning and antioxidant activity was identified in some model system. Besides, high temperature could increase the rate of reaction and induce some radical reactions.

3.3.2. Effect of pH

Fig. 4 shows the dependence of browning of 0.6% COS solution on pH. It was obvious that the browning of COS

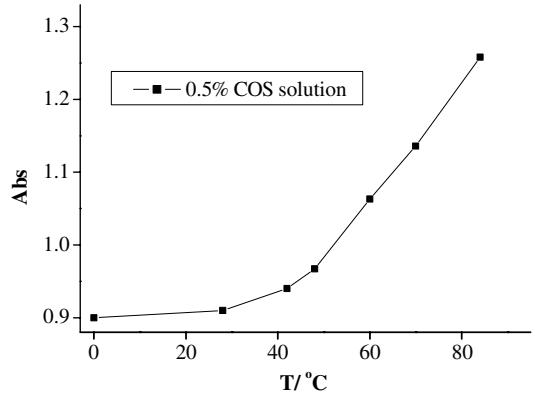

Fig. 3. Browning of 0.5% COS solution at different temperature.

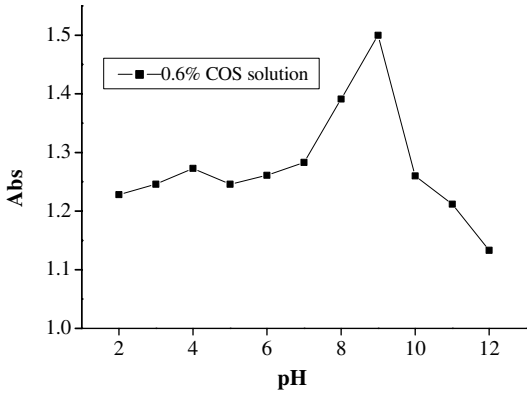

Fig. 4. Effect of pH on browning of 0.6% COS solution.

solution was related to pH. The absorbance at 278 nm increased slightly from pH 2 to 7 except pH 4. The absorbance increased sharply from pH 7 to 9 and then decreased after pH 9. An important factor leading to browning was Maillard reaction. Free amino groups in weak alkali condition were responsible for Maillard reaction. However, Maillard reaction could not occur in strong alkali condition, so the absorbance decreased after pH 9.

3.3.3. Effect of moisture

Chtiooligomers powder was stored at 24% RH and 0.1% RH for different time, respectively. The absorbance of tested sample at 278 nm was determined at the concentration of 0.1%. As shown in Fig. 5, the browning of COS increased much faster at higher RH as time prolonged. The $-NH_2$ and –CHO were imbedded in chain for solid COS powder, and had less opportunity to contact with each other, so the rate of reaction was very low for solid COS powder. Therefore, only a small change occurred with increase of time at 0.1% RH. However, at 24% RH, more moisture was absorbed by COS, which enabled COS to be partially dissolved in water and had more opportunities to react with each other. So, the reaction rate and absorbance increased with increase of RH.

3.3.4. Effect of oxygen

Chtiooligomers stored in oxygen atmosphere turns brown gradually. As shown in Fig. 6, oxygen affected the

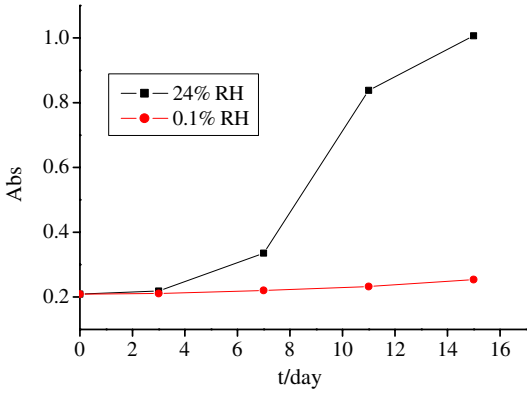

Fig. 5. Browning of COS after stored at 24% RH and 0.1% RH.

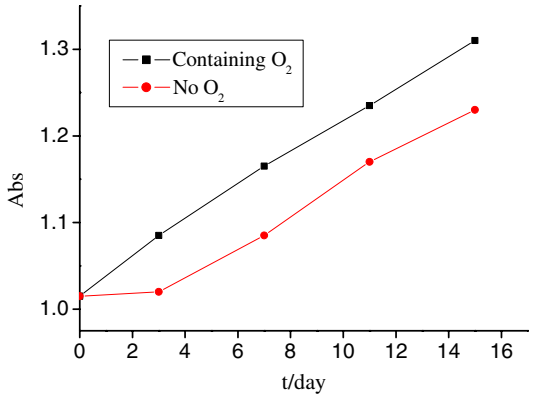

Fig. 6. Effect of oxygen on browning of COS sample.

absorbance of COS at 278 nm. The absorbance increased faster in the present of oxygen than in the absence of oxygen. It suggested that oxygen could activate the browning of COS. Therefore, COS should not be stored in the open air.

3.3.5. Effect of time

Fig. 7 describes the effect of heating time on absorbance of 0.5% COS solution at 60 °C. The absorbance of COS solution increased with increase of the heating time.

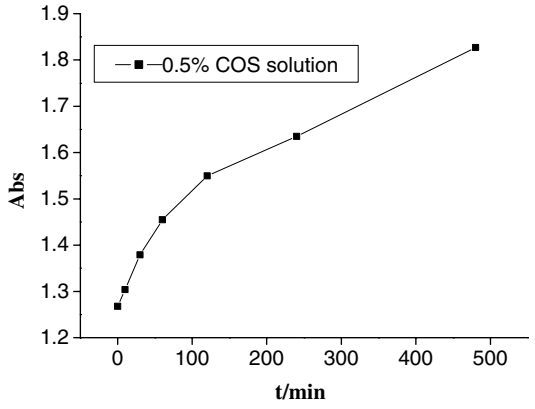

Fig. 7. Effect of heating time on browning of 0.5% COS solution at 60 °C.

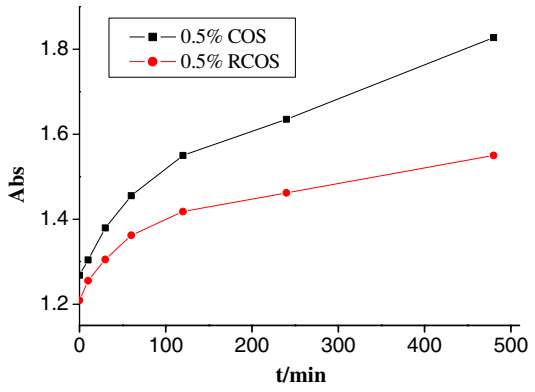

Fig. 8. Browning of 0.5% COS and 0.5% RCOS at 60 °C.

3.3.6. Effect of reducing end groups

Absorbance at 278 nm of 0.5% COS and 0.5% RCOS solution at 60 °C was depicted in Fig. 8. The browning rate of COS was much greater than that of RCOS. $NaBH_4$ could react with reducing end groups of COS, which inhibited the production of –C═N– and further reaction to form colored compounds. Thus, the color of RCOS was lighter, and the absorbance at 278 nm was lower than that of COS.

3.4. Water-solubility of browning COS

Fig. 9 shows the solubility-browning time dependence of sample stored at open air. The solubility of COS decreased with the increase of stored time. Especially, its solubility became very weak when the sample was severely browning. Some black sample was insoluble even in strong acidic condition, which was different from initial COS.

3.5. Moisture–adsorption of browning COS

Fig. 10 shows that the moisture–absorption capacity of COS was excellent while that of BCOS was poor. The moisture–absorption capacity was directly related to the amino content and the structure of molecule. The browning COS had less $–NH_2$ and –OH. Moreover, the powdered chitooligomers became small grain after further browning, which made $–NH_2$ and –OH to be imbedded in the inner place.

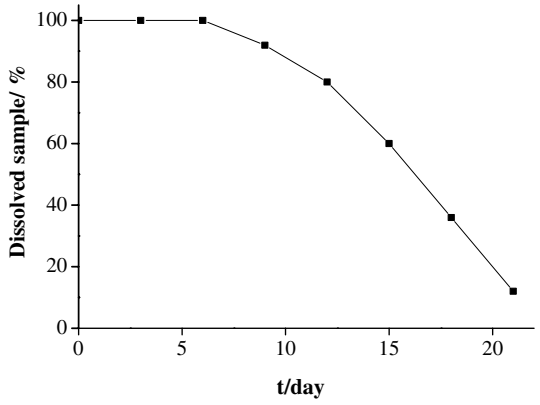

Fig. 9. Effect of preservation time on the solubility of COS powder.

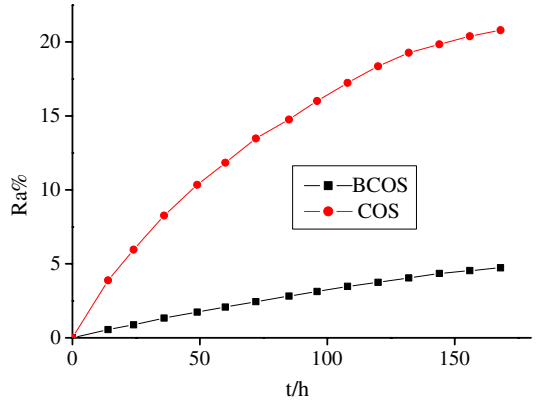

Fig. 10. Moisture–absorption of COS and BCOS at 81% RH.

3.6. Thermogravity analysis

Fig. 11 is the TG curves of COS and BCOS in dynamic nitrogen atmosphere. COS and BCOS both had two steps of weight loss during the process of heating. The first step in the range of 40–110 °C was connected with loss of moisture–absorbed and crystalline water (Kittur, Prashanth, Sankar, & Tharanathan, 2002). BCOS had a small quantity of water in contrast to COS. The second step from 150 to 340 °C was due to decomposition of main bone in COS and BCOS. BCOS had slightly lower decomposition temperature than COS. It indicated that browning led to the decrease of thermal stability of COS.

3.7. IR spectra

Fig. 12 shows the differences of structure between initial COS and BCOS. The adsorption bands at 1647.3, 1585.6 and 1326.2 cm^{-1} in COS are, respectively, attributed to amide I(C═O), free $–NH_2$ and amide III(Qin, Du, Xiao, & Gao, 2002). The strong adsorption bands of BCOS at 1641.3 and 1320.1 cm^{-1} shifted to low wave number by 6 cm^{-1}, compared to the corresponding adsorption bands of COS. Another significant difference was that the adsorption of COS at 1585.6 cm^{-1} shifted to 1591.8 cm^{-1}. The absorption at 1418.9 and 1384.0 cm^{-1} was attributed to $\delta_{CH_3} + \delta_{CH_2}$, which changed dramatically according to Fig. 12. It suggested that $–NH_2$, amide and –OH in COS changed during the process of browning.

3.8. Discussion

Maillard reaction plays an important role in the process of browning. It occurs in most food upon heating or during storage (Knerr, Lerche, Pischetsrieder, & Severin, 2001). Some researchers conclude that analogous structures and chromophores are part of more complex melanoidins and contribute to their color (Hofmann, 1998a, 1998b). The reaction products have a great impact on the quality of

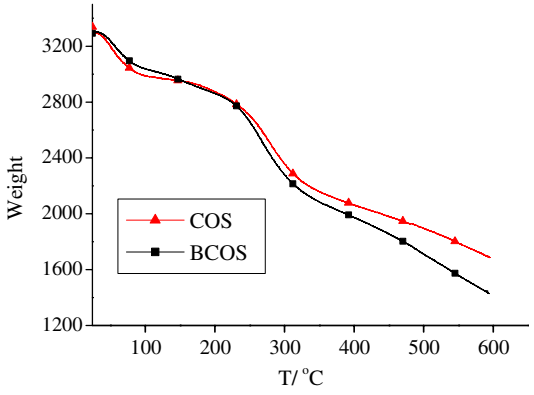

Fig. 11. TG curves of COS and BCOS.

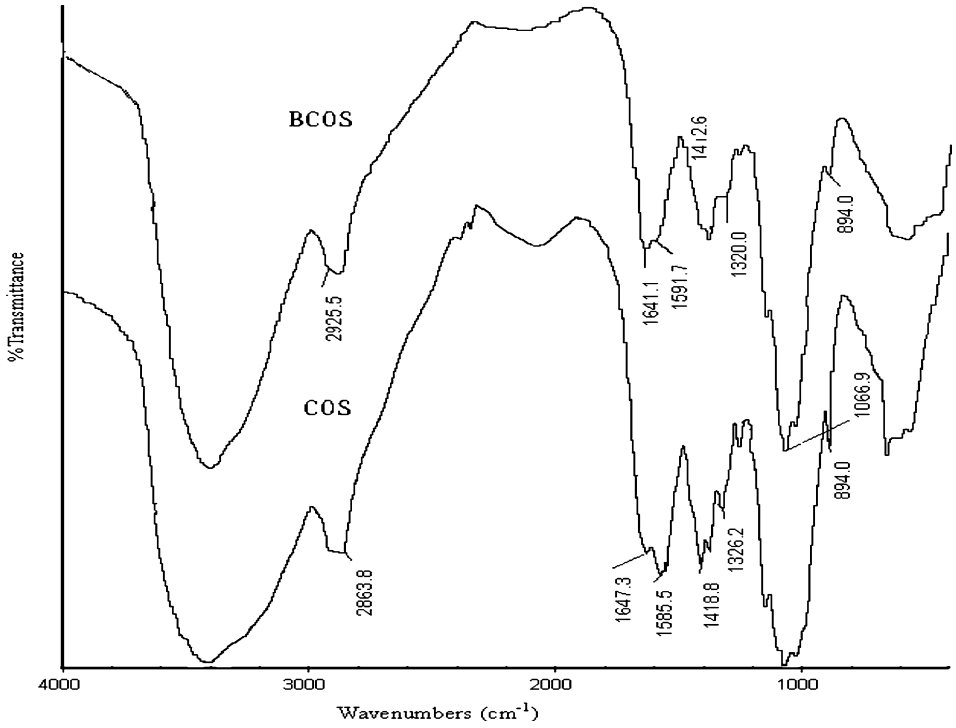

Fig. 12. FT-IR of COS and BCOS.

food, by browning, flavor formation (Kim et al., 2004) or increase of antioxidative activity. However, there are some negative effects for food. For example, lysine modification by Maillard products leads to a decrease of nutritional value (Hofmann, 1998a, 1998b), textural properties and stability of foods (Manzocco & Maltini, 1999). The reductive sugar and free amino groups in COS can react with each other resulting in melanoidins, which is commonly referred as Maillard reaction (Tomas, Karin, Stephanie, & Imre, 2005). The Maillard reaction between reducing sugars and compounds containing amino groups leads to the development of a yellow color, which turns brown during prolonged storage. A weak basic pH favors occurrence of Maillard reaction (Hodge, 1953; Martins, Jongen, & Boe-

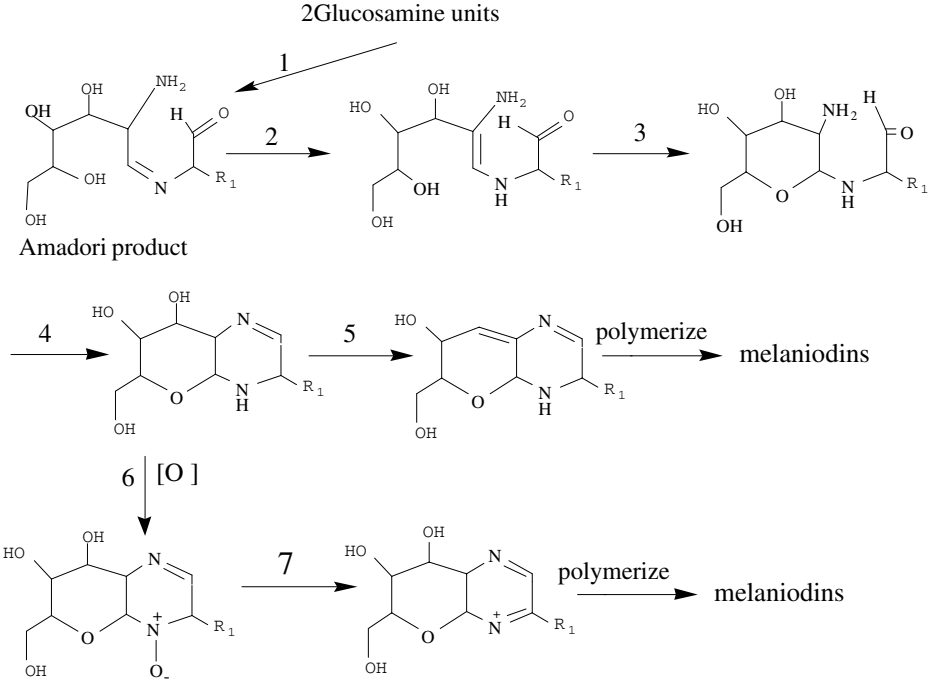

Fig. 13. Proposed reaction mechanism for browning of COS.

kel, 2001), which may be explained by decreased reactivity of the amine group at lower pH due to its protonation. Colored compounds are formed from Amadori intermediate of the Maillard reaction by 1,2-enolization, and followed by dehydration (Meynier & Mottram, 1995). This tautomerism is believed to be favored by lower pH (Ames, Guy, & Kipping, 2001), and pH 4 is the most optimal for it, but 2,3-enolization is more dependent on higher pH. This may provide an explanation for the effect of pH on the browning of COS. The proposed mechanism is described in Fig. 13. Step 1 can occur easily at higher pH, but step 2 and step 3 take place difficultly in basic condition. Therefore, the absorbance of COS only increased slightly from pH 2 to 7 except pH 4, and increased sharply from pH 7 to 9. An important factor leading to browning was Maillard reaction. Alkali or acidic condition plays an important role in forming melanoidines. The free amino group was formed in weak alkali condition, which was responsible for Maillard reaction. However, the Maillard reaction could not occur in strong alkali condition, so the absorbance decreased after pH 9. Although $NaBH_4$ could react with the initial reactant and intermediate, it could not react with the products of step 4. The products of step 4 still existed and could proceed with the next reaction to form colored compounds. Oxygen might react with the product of step 4 in radical manner to form colored products. Temperature had relation with radical reaction, and high temperature activated radical reaction. Therefore, the browning of COS was faster at higher temperature.

4. Conclusion

It is a complex process for browning of chitooligomers, the structure and properties had been changed in the process. Moisture–absorption and water-solubility were weakened. The temperature, pH, moisture, time, oxygen and reductant all influenced the browning of chitooligomers. The rate of browning was faster with the increase of temperature. Moisture was critical to the browning of chitooligomer because it could activate greatly the reaction. As time prolonged, the degree of browning became much remarkable. So, we must consider the factors influencing browning so as to take some steps to inhibit it during production and preservation of COS. The optimal preservation condition for chitooligomers should be at low temperature and humidity, in absence of oxygen, and at pH below 4 or above 10.

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