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Effects of Chitosan on retrogradation properties of wheat starch

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ABSTRACT

Starch retrogradation could increase food's hardness and reduce food's digestion rate, which had bad influence on the food quality. Chitosan is the only natural cationic polysaccharide which could inhibit starch retrogradation. The effects of chitosan on retrogradation properties of wheat starch were studied and the possible influence mechanism of chitosan on wheat starch was investigated by X-ray diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FT-IR) in the paper. Results showed that iodine blue value increased while gel strength of wheat starch decreased with increasing chitosan content. Meanwhile the gelatinization temperature, retrogradation enthalpy and retrogradation rate of wheat starch reduced slightly from 62.71 to 55.85℃, 4.68J/g to 2.41J/g and 54.47% to 19.87% respectively. Distinctive differences between wheat starch gels without or with chitosan were observed. Furthermore the intensity of a peak close to 16.9° of wheat starch samples with chitosan were decreased and narrowed compared with the native starch, indicating the disappearance of the typical B-pattern, which implied that chitosan could retard the recrystallization or the retrogradation behavior of gelatinized starch. After blended with chitosan, the stretching vibration peak of hydroxyl of wheat starch shifted from 3427.78 to 3419.65, which indicated more hydrogen bonds formed between chitosan and wheat starch.

KEYWORDS

Wheat starch; Chitosan; Retrogradation; Properties; Effects.

INTRODUCTION

Starch is an important structural component of wheat flour, which strongly influences the texture and shelf life of the host products. Staleness of wheat flour products is a complex phenomenon involving many factors and processes, but the retrogradation of gelatinized starch during storage is believed to be an important contributor[1]. The term retrogradation refers to the changes that occur in gelatinized starch upon cooling, which implies fully reversible recrystallization in the case of amylopectin and partial irreversible recrystallization in the case of amylase[2]. Retrogradation results in loss of important sensory parameters of many food products made from wheat flour, like flavor and texture, and leads mainly to an increase of crumb firmness and loss of freshness. At present, many researchers are exploring the use of polysaccharides to control the retrogradation rate of starch-based products. There are a number of reports concerning the application of natural polysaccharides to modify the retrogradation behavior of starch, including mixtures of tea polysaccharides and pullulan, flaxseed gum and xanthan, soybean-soluble polysaccharide and gum arabic[1,3-6].

Chitosan[β-(1,4)-2-amino-2-deoxy-D-glucopyranose] is a carbohydrate derived by deacetylation of chitin, which is the second most abundant biopolymer in nature next to cellulose[7]. As a natural, nontoxic and biodegradable biopolymer, chitosan has received considerable attention in the food industry due to its physiological properties, nutritional and biochemical activities and has been approved as a food additive in many countries[8]. Wei xin-lin reported that chitooligosaccharides (average molecular weight of 1390, degree of deacetylation of 91%) had obvious resistance to starch aging[9]. Wuyue investigated the effect of chitooligosaccharides on the retrogradation of intermediate amylose rice starch and found the molecular size of chitosan determined its anti-retrogradation capability and chitosan with molecular weight between 5k and 10k had the best anti-retrogradation ability while chitosan with molecular weight under 5k had no effect[10]. Kerch G had the opposite conclusion that Chitosan oligosaccharides and low molecular weight chitosan increase bread crumb staling rate[11]. Chitosan as food additive was seldom applied in the wheat products. Furthermore application of chitosan in functional foods is an area of particular interest. The aim of this study is to investigate the effect of chitosan on the retrogradation properties of wheat starch and the possible mechanism involved chitosan and wheat starch.

MATERIALS AND METHODS

Materials

Wheat starch was purchased from Baoding brewing co., LTD (Shanghai,China). Soluble chitosan (average molecular weight of 5000, degree of deacetylation of 95%) was purchased from golden shell pharmaceutical co., LTD (Zhejiang province,China). All other analytical laboratory chemicals and reagents were purchased from Sinopharm Chemical Reagent Company (Shanghai, China).

Iodine blue value

The blue value (BV) was determined according to the method of Zeng Jie with some modifications[12]. The sample (0.10 g) was dispersed in boiling water for 10 min and cooled rapidly. Starch dispersion (5 ml) was taken in a 100 mL volumetric flask along with 50 mL of deionized water, 1mol/L acetic acid solution 1mL and iodine reagent 1mL (1.0 mg I_2/mL and 10.0 mg KI/mL). The volume was made up to 250 mL with distilled water and mixed immediately. Then standing 30 min and colored. Control solutions were made in the same way but without sample. All samples were scanned at 620 nm with an UV1100 spectrophotometer (Mapada instrument, Shanghai, China). The absorbance value of per gram of starch was recorded as the BV.

Texture characteristics

Chitosan and wheat starch (0/12、1/48、1/36、1/24、1/12) were prepared and transferred to glass vials, covered with aluminum foil, and heated in a water bath at 95ºC for 20 min. After being heated, the formed gels were cooled and stored at 4ºC for 24 h before test. Prior to the gel strength measurement, gel samples were allowed to equilibrate at the room temperature (25±1ºC) for 30 min. Gels were penetrated with a flat-faced stainless steel probe (10 mm dia) attached to a Model TAXT2 texture analyzer (Stable Micro Systems Ltd., Surry, U.K.) at a crosshead speed of 0.3 mm/s, Pre- and post-test speed were controlled at 1.00 mm/s. The distance of the compression was set at 10 mm. The penetration force which was the peak force required to rupture the gels was expressed as the gel strength[8].

Differential scanning calorimetry (DSC)

The gelatinization and retrogradation properties of the samples were determined from the DSC curves. DSC measurements were carried out using a DSC-Q100 apparatus (TA instrument Co. Ltd., American). The weight ratio of dry wheat starch to water was maintained 1:2 and chitosan/wheat starch mixing ratios were 0/12、1/48、1/36、1/24、1/12. Samples were placed in a screw-top glass bottle and dispersed in distilled water by stirring for 2 h at room temperature using a magnetic stirrer. The calorimeter was calibrated with an indium standard. All samples were precisely weighed on an aluminum DSC pan. The pan was sealed using a sample-encapsulating press. The heating program increased the sample temperature from 30℃to 130℃at a rate of 5℃/min, followed by cooling to 30℃ at the same rate. Heating and cooling were performed in an atmosphere of nitrogen gas. Samples of the dispersions were weighed into an aluminum DSC pan. The onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c) were determined from the first-run heating DSC curves. The gelatinization enthalpy was evaluated based on the area of the main endothermic peak. After the first-run heating, the gelatinized samples were cooled to 30℃ in the instrument and stored at 4℃for 4 days. The stored samples were reheated

to examine the effects of chitosan on retrogradation. The retrogradation ratio was calculated by dividing the re-gelatinization enthalpy of the second heating run by the gelatinization enthalpy in the first heating run[1].

Microstructure

The wheat starch in the absence or presence of chitosan were pasted and aged for 7 days. Then the samples were freeze-dried in a freeze-dryer (Scientz-10N, China) for 24 hours. Microstructures of the freeze-dried samples were observed using SEM (Quanta-200, FEI, Netherlands) at an accelerating voltage of 25 KV[13].

X-ray diffraction

The recrystallization analysis of the gelatinized samples in the absence or presence of chitosan was carried out using an X-ray diffractometer (Gemini E, **Agilent**, England). The samples were stored for 2 weeks in desiccators containing P_2O_5 and pulverized before testing. The instrument was operated at 40 kV and 30 mA. Diffractograms were obtained using Cu-Ka radiation, scanning from 5°2θ to 35°2θ at a rate of 4°/min and a step size of 0.3mm. Origin Pro8.0 Software was used to analyze the diffractograms according to the method of Wuyue[2].

Fourier transform infrared spectroscopy

To perform FT-IR measurement, the dried retrograded wheat starches with and without chitosan (The ratio of chitosan: wheat starch 1:12) with weight of 2 mg were first ground into flour and then dispersed in 200 mg KBr (pellet procedure). KBr pelletized starch samples were analyzed using a Nicolet 5700 infrared spectrometer in the range 4000 to 400 cm^{-1}. The IR spectra for starch treated with and without alkali protease were recorded on a diamond plate with 32 scans and a resolution of 4 cm^{-1}[14].

Statistical analysis

All determinations were repeated in triplicate, and the mean values and SDs were reported.

RESULTS AND DISSICUSION

Effects of chitosan on iodine blue value of wheat starch

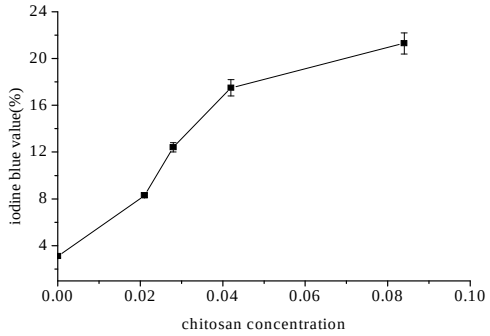


Figure 1 : Effects of chitosan addition on Iodine blue value of wheat starch

Figure 1 showed the blue value of wheat starch with different addition of chitosan. With the addition dose of chitosan increasing, the blue value added compared with the control. These results indicated that the content of free starch increased and chitosan inhabited the retrogradation of wheat starch.

Effects of chitosan on gel strength of wheat starch gels

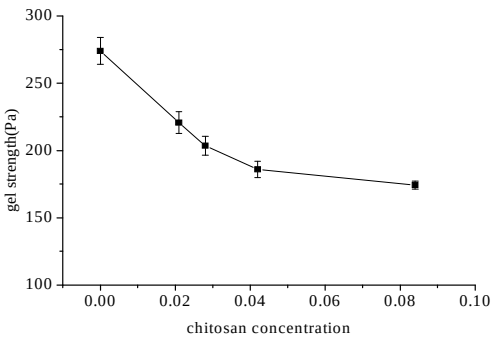


Figure 2 : Effects of chitosan addition on gel strength of wheat starch gels

To further elucidate the role of chitosan on gel properties of wheat starch, gels prepared with various concentration levels of chitosans were evaluated by gel strength. With increasing amount of chitosan added, gel strength of wheat starch gels decreased. Starch retrogradation could increase food's hardness and reduce food's digestion rate, which had bad influence on the food quality. Gel strength of wheat starch gels decreased proportionally with more chitosan incorporated in gels, which suggested possible wheat starch-chitosan interactions. Therefore, it was presumed that chitosan could enhance the texture characters and restrained the retrogradation of wheat starch.

Effects of chitosan on thermal properties of wheat starch gels

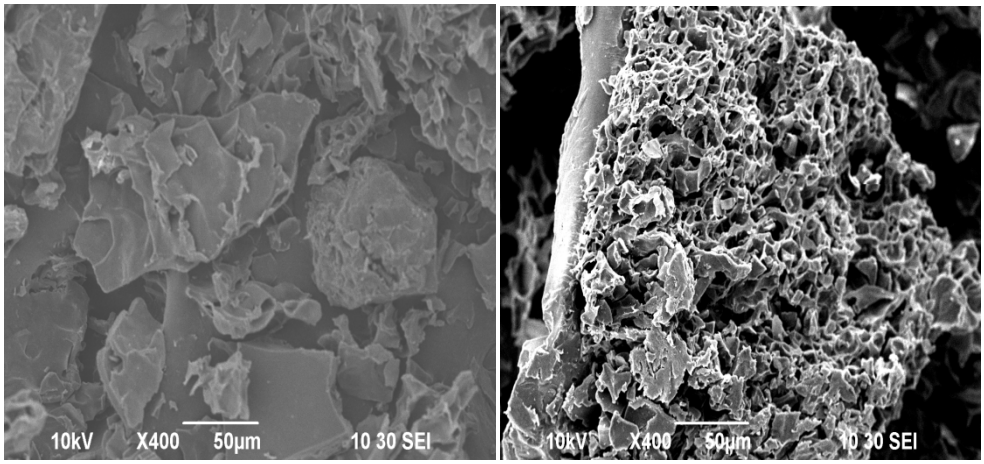
TABLE 1 : Effect of different chitosan addition on thermal properties of wheat starch during storage

samples	T。(℃)	Tp(℃)	Tc(℃)	ΔHg(J/g)	retrogradation ratio(%)
12/0	57.96±0.04	62.71±0.05	72.10±0.07	4.68±0.12	54.47±0.12
48/1	46.39±0.09	60.76±0.02	71.34±0.03	4.01±0.09	40.04±0.23
36/1	41.75±0.03	60.40±0.04	70.32±0.08	3.53±0.23	36.70±0.15
24/1	40.89±0.02	57.16±0.03	70.19±0.02	3.31±0.16	27.96±0.08
12/1	39.85±0.03	55.85±0.06	66.88±0.05	2.41±0.08	19.87±0.13

Differential Scanning Calorimetry (DSC) has proven to be one of the effective methods to determine the thermal behaviour of starch gelatinization and retrogradation. During the course of gelatinization, a certain quantity of heat expressed as gelatinization enthalpy is necessary to breakdown the crystalline areas of starch. As to retrograded starch, the value of melting enthalpy provides a quantitative measure of the energy used to melt the recrystallized starch. The ratio of melting enthalpy to gelatinization enthalpy is considered to be one useful indicator to determine the degree of starch recrystallization[2].

From TABLE 1, *To*, *Tp* and *Tc* were significantly decreased with increasing the addition dose of chitosan. And retrogradation ratio of wheat starch decreased. The decrease of gelatinization temperatures were consistent with the reports of other researchers and could be interpreted as follows: the available water was reduced by the hydration and chitosan could interact directly with starch by intermolecular hydrogen bonds to stabilize the crystalline regions of wheat starch gels[5,13]. Chitosan was efficient to retard starch retrogradation.

Effects of chitosan on microstructure of wheat starch gels



wheat starch **chitosan-wheat starch mixture**

Figure 3 : Microstructure of wheat starch and chitosan-wheat starch mixture

SEM provided structural information about gels. As shown in Figure 3, distinctive differences between wheat starch gels without or with chitosan (1/12) were observed. The structure of wheat starch gels (left) showed granular aggregated structure within the matrix, which seemed less linkage and somewhat discontinuous. The micrograph of wheat starch gels with chitosan added (right) showed a well-structured matrix with a highly interconnected network of strands. These microstructural changes helped to explain these differences between wheat starch gels without or with chitosan. A fine, uniform structure would probably result in more absorptive capacity and better retention of water compared to coarse structure with large pores. These results showed that interaction possibly existed between chitosan and wheat starch and chitosan like a binder dispersed uniformly and tightly associated in the gel network.

Effects of chitosan on crystallinity of wheat starch gels

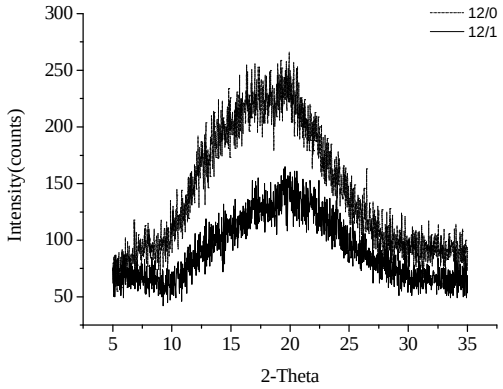


Figure 4 : X-ray diffraction spectra of wheat starch and chitosan-wheat starch mixture

Investigations using XRD were conducted to further and powerfully prove the preventing effect of chitosan on the retrogradation. The XRD patterns and corresponding crystallinity are shown in Figure 4. Once native RS is gelatinized, it develops a "B-type" diffraction pattern during aging. Retrograded starch gives a "B-type" diffraction pattern and this is accompanied by gradual increases in rigidity and phase separation between the polymer and the solvent (syneresis). B-type crystallinity is characterized by a well-defined peak at 16.9° (2θ). The formation of this peak was the result of the crystallization of the amorphous starch melt, mainly of the amylopectin fraction that increased during storage[2]. As shown in Figure 4, the intensity of a peak close to 16.9° of wheat starch samples with chitosan were decreased and narrowed compared with the native starch, indicating the disappearance of the typical B-pattern, which implied that chitosan could retard the recrystallization or the retrogradation behavior of gelatinized starch.

Effects of chitosan on infrared spectra of wheat starch gels

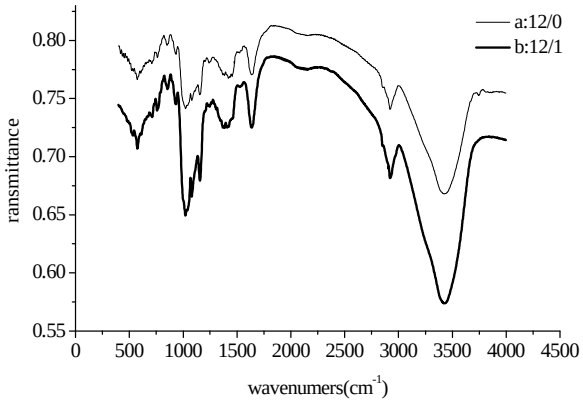


Figure 5 : Fourier infrared spectra of wheat starch and chitosan-wheat starch mixture

Figure 5 showed the IR spectra of retrograded wheat starches without and with chitosan. The band absorbance in starch have been assigned and matched with the vibrational modes of the chemical bonds and the structures of starch molecules[14]. IR spectra indicated that there was no difference between the wheat starch and chitosan-wheat starch mixture, which suggested that there was no significant effect of chitosan on the structure of wheat starch. But Hydrogen bonds possibly formed between wheat starch and chitosan during retrogradation. Formation of hydrogen bonds in retrograded wheat starch is identified by low-field shift of wave numbers for stretching vibration (3415 and 3387 cm^{-1} for –OH). After blended with chitosan, the hydroxyl stretching vibration peak shifted from 3427.78 to 3419.65 from IR spectra which suggested that more hydrogen bonds formed in these samples.

CONCLUSIONS

From the overall results, it could be concluded that the addition of chitosan to wheat starch could significantly retard retrogradation. Water migration contributed a lot to the starch retrogradation, which needs further study. Addition of chitosan

to wheat products creates an opportunity to combine beneficial technological properties with beneficial biological health promoting properties. Hence, chitosan could be suitable to add to wheat products and simultaneously enhance quality and nutrition. These studies are important for the development of chitosan as the food additive in wheat products to make them compete more effectively in the markets.

ACKNOWLEDGMENTS

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