



Effects of chitin nano-whiskers on the gelatinization and retrogradation of maize and potato starches



Na Ji, Chengzhen Liu, Shuangling Zhang, Jing Yu, Liu Xiong, Qingjie Sun*

School of Food Science and Engineering, Qingdao Agricultural University, Qingdao, Shandong Province 266109, China

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ABSTRACT

Starch is very prone to retrogradation after gelatinization. Inhibition of starch retrogradation has been an important factor in improving the quality of food. For the first time, we investigated the effect of nano-materials, represented by chitin nano-whiskers (CNWs), on the short- and long-term retrogradation of maize and potato starches. Rapid Visco-Analyser results showed that the addition of CNWs significantly decreased the setback values of maize and potato starches, which suggested that CNWs could retard the short-term retrogradation of starch. Differential scanning calorimetry and X-ray diffraction results showed that the percentage of retrogradation of maize and potato starches significantly decreased ($P < 0.05$), suggesting the inhibition of long-term retrogradation. The CNWs could be used as a new inhibitor of starch retrogradation to develop starch-based food with longer shelf life.

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1. Introduction

When starches are subjected to high temperatures (typically greater than 50 °C) in the presence of water, the granules swell and rupture due to disruption of amylopectin double helices (hydrogen bonds dissociation), while amylose preferentially leaches out of the swollen granules (Tester, 1989). These events, collectively known as “gelatinization”, are accompanied by a dramatic increase in the systems viscosity as granule structure is progressively ruptured (Yang & Rao, 1997). When gelatinized starch is stored, particularly at low temperature (Jang & Pyun, 1997), it undergoes “retrogradation” caused by re-crystallization of the polymer (dispersed amylose and amylopectin) chains.

Starch retrogradation occurs in cooked starch gels and starch-containing foods, affecting the sensorial attributes and shelf life of these foods (Xu, Zhao et al., 2012). The retrogradation is used to describe the changes that occur in gelatinized starch during storage, especially at low temperatures (Chen, Ren, Zhang, Tong, & Rashedb, 2015). The rate of starch retrogradation is mainly due to the amylose to amylopectin ratio (Fredriksson, Silverio, Andersson, Eliasson, & Aman, 1998). Retrogradation can be categorized as either short- or long-term retrogradation (Xu, Zhong et al., 2012). Short-term development of retrogradation or crystallization in starch gels is attributed to the gelation and crystallization of the

amylose fraction (Sievert & Wursch, 1993), while the long-term changes occurring during the storage of starch gels have been attributed to the amylopectin fraction (Chang & Lin, 2007). Starch retrogradation occurs readily during the storage of heat-processed starch-rich foods as a spontaneous process endeavoring to reach a thermodynamically low energy stable (crystalline) state (Fu, Wang, Li, Zhou, & Adhikari, 2013). In foods, the retrogradation of starch during processing or storage greatly affects the product texture, stability, quality, digestibility, and functionality (Mutungi, Passauer, Onyango, Jaros, & Rohm, 2012). Therefore, effective methods to solve this problem are urgently required. Previous research showed that the addition of polysaccharide (Banchathanakij & Suphantharika, 2009; Chen et al., 2015; Funami et al., 2005, 2008; Khanna & Tester, 2006; Xu, Zhao et al., 2012), tea polyphenols (Wu, Chen, Li, & Li, 2009), and amylases (Fadda, Sanguinetti, Del Caro, Collar, & Piga, 2014) inhibited the retrogradation of starch.

Chitin, naturally occurring polysaccharide, is the second most abundant naturally occurring polymer after cellulose (Zeng, He, Li, & Wang, 2012). It has many excellent properties, including biocompatibility, biodegradability, nontoxicity, and absorption properties, in addition to others. Thus, it is widely used in a variety of areas, such as biomedical applications, agriculture, water treatment, and cosmetics (Zeng et al., 2012). Chitin nano-whiskers (CNWs) of slender parallelepiped rods have been successfully prepared from different chitin sources, such as crab shells (Nair & Dufresne, 2003) and shrimp shells (Sriupayo, Supaphol, Blackwell, & Rujiravanit, 2005) under hydrolytic conditions with boiling HCl and vigorous stirring. The CNWs, with so many

* Corresponding author at: School of Food Science and Engineering, Qingdao Agricultural University, 266109, 700 Changcheng Road, Chengyang District, Qingdao, China.

E-mail address: phdsun@163.com (Q. Sun).

advantages such as low density, nontoxicity, biodegradability, biocompatibility and easy surface modification, and functionalization, are supposed to find extensive application in many areas including reinforcing nanocomposites, cosmetic, food industry, drug delivery and tissue engineering (Zeng et al., 2012). The CNWs have been widely used as substitutes for inorganic nanoparticles in reinforcing polymer nanocomposites (Zeng et al., 2012) and as raw materials to produce chitosan nanoscaffolds (Lertwattanaseri, Ichikawa, Mizoguchi, Tanaka, & Chirachanchai, 2009). Although many efforts have been made to investigate the applications of CNWs in thermoplastic starch-based biocomposites (Salaberria, Labidi, & Fernandes, 2014), there have been no reports about the effect of CNWs on the gelatinization and retrogradation behaviors of starch. The aim of this study is to investigate the effect of CNWs on the gelatinization and retrogradation behaviors of maize and potato starches. Multiple methods, including Rapid Visco-Analyser (RVA), differential scanning calorimetry (DSC), and X-ray diffraction (XRD) were applied, and the independent evidence derived from those methods could provide a comprehensive understanding of the effect of CNWs on both the gelatinization and retrogradation behaviors of maize and potato starches. The addition of CNWs could lead to new methods to affect the retrogradation of starch.

2. Materials and methods

2.1. Materials

Normal maize starch (with an amylose content of approximately 28.0%) and potato starch (with an amylose content of approximately 24.3%) was obtained from the Zhucheng Xingmao Corn Development Co., Ltd. (Shandong, China). Chitin was supplied by Zhejiang Aoxing Biotechnology Co., Ltd. (China) with a degree of N-acetylation of 0.96 from shells of adult *Penaeus schmitti*, as determined with elemental analysis. All other chemicals used in the present study were of analytical grade.

2.2. Preparation of CNWs

The CNWs were prepared using the method described by Nair and Dufresne (2003), with some modifications. Briefly, 3 mol/L H_2SO_4 aqueous solution and chitin powder, at a weight ratio of 30:1, was added to a round-bottomed flask fitted with magnetic stirring at 95 °C for 12 h. After acid hydrolysis, the suspensions were diluted with deionized water, followed by centrifugation (3000g) and decanting of the supernatant. This process was repeated several times until a neutral pH was reached. Finally, the chitin whisker slurry was collected and lyophilized to obtain the dried CNWs.

2.3. Characterizations of CNWs

A scanning electron microscope (Akishima, JEOL JMS 7600F, Japan) with a secondary electron detector was used to obtain images of the CNWs. The dried CNWs powder were evenly coated on a double-sided adhesive tabs (Leit-Tabs, 12 mm, Christine Groepel, Tulln, Austria) mounted on aluminum stubs (Stiftprobenteller, Christine Groepel, Tulln, Austria). The as-obtained specimen were coated with gold. The voltage applied was 3.0 kV.

The sample was also measured with a Hitachi (Tokyo, Japan) 7650 transmission electron microscope at an acceleration voltage of 80 kV. The CNWs suspensions (0.25%, w/w) were sonicated at 25 °C for 15 min. A droplet of the aqueous dispersion was placed on Formvar coated grids. Prior to complete drying, Formvar coated grid was freeze-dried.

The zeta potentials of the CNWs were measured using a Malvern Zetasizer Nano ZS at 25 °C. The X-ray diffraction of samples was determined using an X-ray diffractometer (AXS D8 ADVANCE, Bruker, Karlsruhe, Germany) with $Cu K\alpha$ radiation ($\lambda = 1.543$) at 40 kV and 100 mA.

2.4. Preparation of starch/CNWs systems

The percentage of CNWs was set at 0%, 2%, 4%, 6%, and 8% levels on a dry base of starch in the starch/CNWs systems. The CNWs was dispersed in 50 mL deionized water by ultrasonic mixing. The starch was mixed with CNWs by stirring for 30 min at room temperature using a magnetic stirrer. Then the mixed samples were dried using an air oven at 40 °C for 30 h, ground in an unglazed porcelain mortar and sieved through a 150 μm nylon sieve.

2.5. Characterizations of starch/CNWs systems

2.5.1. Pasting properties

The pasting properties of the starches and starch/CNWs systems were determined using Rapid Visco Analyser (RVA-4 Newport Scientific Pvt. Ltd., Australia) according to the method described by Zhang, Yin, Zheng, and Shen (2016). A suspension of 3 g (12% moisture basis) samples (normal maize starch, potato starch and starch/CNWs system) in 25 g of accurately weighed distilled water underwent a controlled heating and cooling cycle under constant shear. The rotating speed was maintained at 160 rpm throughout the process. Parameters including peak viscosity (PV), trough viscosity (TV), final viscosity (FV) at the end of cooling, breakdown ($BD = PV - TV$), setback ($SB = FV - TV$), and pasting temperature were recorded.

2.5.2. Differential scanning calorimetry (DSC)

The DSC measurements were carried out using a differential scanning calorimeter (DSC1, Mettler Toledo, Schwerzenbach, Switzerland) equipped with a thermal analysis data station and data recording software (STAR@ SW 9.20). The gelatinization and retrogradation properties of the samples were determined according to the method described by Guo et al. (2015). The calorimeter was calibrated with an indium standard. Samples of starch/CNWs systems (about 3 mg) were accurately weighed into aluminum DSC pans, and deionized water was added by micropipette in order to achieve a water-sample ratio of 2:1. The sample pans were sealed and equilibrated at room temperature for 24 h before analysis. The DSC runs were operated under a nitrogen gas (30 mL/min). The samples were heated at a rate of 10 °C/min in a temperature range of 10–100 °C, using an empty pan as a reference. The onset temperature T_o , peak temperature T_p , and conclusion temperature T_c were determined from the first-run heating DSC curves. Gelatinization enthalpy change (ΔH) was estimated from the area of the main endothermic peak and was expressed in terms of Joules per gram of dry starch. Then, the gelatinized samples were stored at 4 °C for 1 day, 3 days, 5 days, and 7 days. For the study of retrogradation, these stored samples were scanned anew under the same conditions, and retrogradation enthalpy change (ΔH_R) was determined from the second-run heating. In addition, the percentage of retrogradation (R%) was calculated as the ratio of the ΔH_R to the ΔH in run heating (Rodríguez-Sandovala, Fernández-Quinterob, Cuvelier, Relkinc, & Bello-Pérez, 2008).

2.5.3. X-ray diffraction

Ten grams of maize starch, potato starch and starch/CNWs systems with 20 mL of deionized water were gelatinized for 20 min in a closed thermostat water bath. These samples were cooled to room temperature, stored at 4 °C for 0 day and 7 days, respectively, and then freeze-dried. The freeze-dried samples were ground and then

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