

Branched limit dextrin impact on wheat and waxy starch gels retrogradation



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ABSTRACT

The effect of branched limited dextrin (BLD) on starch retrogradation was investigated to explain the anti-firming mechanism of α -amylase. The influence of BLD on the gelatinized wheat and waxy rice starch retrogradation was characterized by differential scanning calorimetry (DSC) and wide-angle X-ray diffraction (WXR). DSC and WXR results showed that retrogradation of wheat and waxy rice starch gels were reduced with the addition of branched limit dextrans (F_{75} and F_{88}). Avrami equation was used to analyze the enthalpies of retrograde wheat starch gels, and the value of k indicated that F_{75} and F_{88} reduced the kinetics of starch retrogradation. In addition, molecular dynamic (MD) simulation was adapted to predict the interaction of BLD and starch fraction, and the results showed that the BLD reduced starch retrogradation by the interplay between starch and BLD.

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1. Introduction

The issue of starch retrogradation is a hotspot (Cappa, Lucisano, & Mariotti, 2013; Goñi, Escribano, & Merodio, 2008; Yao, Zhang, & Ding, 2003), as it decreases consumer acceptance and shelf life of cooked starchy foods. Furthermore, stale starchy foods are considered to be one of the most difficult technical and economic challenges. In particular, bread staling, of which crumb firming and loss of crumb resilience are an integral part, is an important problem of starchy foods (Goesaert, Leman, Bijttebier, & Delcour, 2009). It is generally accepted that the retrogradation of starch, specifically the retrogradation of amylopectin, plays a critical role in bread firming (Gray & Bemiller, 2003). Therefore, bread staling and associated crumb firming phenomena have received a considerable amount of attention in cereal science research (Escalada Pla, Rojas, & Gerschenson, 2013; Purhagen, Sjöö, & Eliasson, 2011; Ziobro, Korus, Juszczak, & Witczak, 2013). It is well known that α -amylases are starch hydrolyzing enzymes usually added to bread formulae in order to reduce the firming rate (Caballero, Gómez, & Rosell, 2007; Gomes-Ruffi, Cunha, Almeida, Chang, & Steel, 2012; Gujral, Haros, & Rosell, 2003; León, Durán, & Benedito de Barber,

2002; Min et al., 1998; Palacios, Schwarz, & D'Appolonia, 2004; Purhagen et al., 2011). However, despite the efforts of many investigators, the basic mechanism of α -amylase inhibiting bread staling is controversial. Many different and sometimes conflicting views on the anti-firming action of amylases have appeared in the literature.

Degradation of starch by α -amylase leads to changes in starch structure and production of low and intermediate molecular weight dextrans. Thus, it is conceivable that the anti-staling effect of enzymes is due to changes of the starch structure or the formation of dextrans (Hug-Iten, Escher, & Conde-Petit, 2003). Every, Mann, and Ross, (1992) suggested that the anti-firming effect of α -amylases was caused by the reduced ability of the degraded starch to retrograde and indicated that the dextrans were just an expression of the structural changes produced by the enzymes in the starch. Morgan et al. (1997) concluded that the anti-firming effect of the α -amylase was due to the degradation of starch. Gerrard, Every, Sutton, and Gilpin, (1997) also concluded that the anti-staling effect of α -amylases is not due to the production of dextrans but to a modification of the starch, interfering with the starch–gluten interactions. Furthermore, Hug-Iten, Escher, and Conde-Petit (2001) indicated that the changes of starch being important for the anti-firming effect. In contrast, León, Durán, and de Barber (1997) confirmed that the anti-firming effect was not due to modifications of the starch but to dextrans produced by starch hydrolysis, since the effect did not occur when dextrans

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were removed by glucoamylase. Martin and Hosene (1991) reported that bread supplemented with either bacterial or fungal α -amylase contained large quantities of low molecular weight dextrans with a degree of polymerization of 3–9, maltohexaose in particular, hindered the formation of gluten–gluten and gluten–starch cross-links, which they considered to be the cause of bread firming. In model studies on starch gels with the addition of low-molecular weight dextrans it was observed, that they restricted starch gelatinization and reduced the values of retrogradation enthalpy (Durán, León, Barber, & Benedito de Barber, 2001) as well as reduced gel firmness both after preparation and during storage (Rojas, Rosell, & Benedito de Barber, 2001). The application of maltodextrins as antistaling agents for bread was already studied. Witczak, Korus, Ziobro, and Juszczak (2010) concluded that maltodextrins with higher DE, especially 18.0 and 21.8, had a beneficial influence on crumb hardening during storage. Furthermore, Miyazaki, Maeda, and Morita (2004) reported that the retrogradation of wheat bread during storage was significantly retarded, if lower molecular weight dextrans were used as compared with high molecular weight dextrans. Defloor and Delcour (Isabelle & Delcour, 1999) also observed that maltodextrin preparations with average degree varying between 4 and 66 reduced DSC staling endotherm in baked and stored bread doughs. All in all, the conflicting views on the anti-firming mechanism of starch-degrading enzymes reflect the still incomplete understanding of the staling mechanism itself.

Spring dextrin (SD) is a linear, poly-disperse saccharide, featuring a repeating (1-4)- α -D-glucose unit (see reference (Xu, Zhao, Ning, Bashari, Wu, et al., 2013) and (Xu, Zhao, Ning, Bashari, Jin, et al., 2013) for a through overview). Different from SD, branched limit dextrin (BLD) can be made by treating gelatinized starch with pure α -amylase and β -amylase. It is composed of linear chain of α -(1-4)-D-glucose residues connected together through α -(1-6)-linkages (Fig. 1). In recent studies, we had found that SDs with lower molecular weight disturbed the short-term retrogradation of amylose (Xu et al., 2012) by non-bonded interaction and also reported on SDs impacted on the long-term retrogradation of wheat and corn starches (Xu, Fan, et al., 2013). Reports on BLD impact on starch retrogradation, especially the impacting mechanism, however, are unknown and so far little attention has been paid to the role of the BLD in starch retrogradation. To the best of our knowledge, this approach has not been used elsewhere to investigate the anti-firming functionality of amylases, allowed us to gain greater insight into the anti-firming action of amylases. Therefore, the main focus of present study was to investigate the influence of BLDs on the gelatinized wheat and waxy starches retrogradation by wide-angle X-ray diffraction (WAXRD), differential thermal analysis (DSC) and molecular dynamics (MD) simulations.

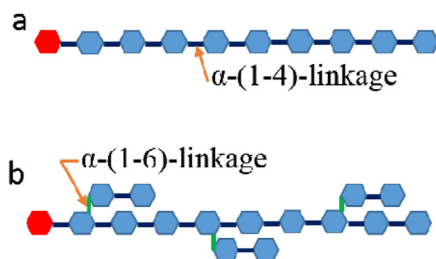


Fig. 1. Schematic representation of spring dextrin (a) and branched limit dextrin (b). The red ring structure represents a reducing glucose residue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Materials

Barley β -amylase (from barley) and α -amylase (from *Bacillus* sp.) were purchased from Sigma–Aldrich Co. LLC. (Shanghai, China). Commercial wheat starch was purchased from Tianjin Tingfeng Starch Development Co., Ltd. (Tianjin, China) and waxy rice starch was obtained from Jiangsu Baby Group Co. Ltd. (Suqian, China). All other reagents and chemicals were analytical grade.

2.2. Preparation of BLD

BLDs were prepared from gelatinized wheat starch solution by the hydrolysis action of α -amylase and β -amylase. Briefly, wheat starch was defatted in 85% methanol by refluxing for 24 h. The total gelatinized wheat starch (1 L, starch/deionized water: 5/95) was hydrolyzed using α -amylase (5 mL, 100 U/mL) for 12 h, and then the α -amylase hydrolyzate was hydrolyzed with β -amylase (20 mL, 50 U/mL) for 24 h (seeing illustrative diagram, Fig. 2).

The BLD hydrolyzate of wheat starch were fractionated by ethanol precipitation (Defloor, Vandenreyken, Grobet, & Delcour, 1998). Ethanol was added under continuous stirring to obtain a final concentration of 75% (v/v) and 88% (v/v) ethanol. The precipitated material was recovered by centrifugation (at room temperature, 30 min, 10,000 g) and dried by washing with ethanol. It was finally dissolved in water and recovered by freeze drying. The precipitates (final concentration of 75% (v/v) and 88% (v/v) ethanol) were referred to as F₇₅ and F₈₈, respectively.

2.3. SE-HPLC analysis

Dried powder of BLD (0.1 g) was dispersed in NaOH (0.5 mol/L, 2 mL) and mildly shook. The volume was later diluted to 100 mL before incubation at 45 °C for 2 h. The prepared solution (2 mL) was centrifuged at 10,000 g for 15 min and filtered with a 0.22 μ m filter. The chromatograph system (Shimadzu Corp., Tokyo, Japan) consisted of an LC-20AT pump, a SIL-10A injection valve with a 10 μ L loop and RID-10A differential refractive index detector (Shimadzu Corp., Tokyo, Japan). The analysis of the BLD was achieved on Shodex OHPark SB-804 HQ (8.0 $\times$ 300 mm, i.d. 6 μ m) column (Shodex China Co., Ltd., Shanghai, China).

2.4. Preparation of wheat starch/BLD and waxy rice starch/BLD blends

Starch/BLD blends were prepared by adding starch (4 g) to BLD solution (10 mL, 20 mg/mL; 10 mL, 40 mg/mL and 10 mL, 80 mg/mL) in a cylindrical plastic (Φ 3 cm) and sealed, and then the suspension were gently shaken in boiling water for 1 h. The samples were cooled and stored at 4 °C and were prepared in triplicate. Samples without BLD undergoing identical treatment were used as control.

2.5. Thermal analysis

The retrogradation properties were analyzed using SII X-DSC7000 (SII NanoTechnology Inc., Japan) under an ultrahigh-purity nitrogen atmosphere. An empty pan was used as a reference. Three milligrams of the each prepared BLD-starch physical mixture (w/w: 5/95; 10/90; 20/80) and 6 μ L of distilled water were sealed into aluminum pans (volume: 40 μ L). The sealed samples were heated from 20 °C to 120 °C in the calorimeter at 10 °C/min. Subsequent to the heating, the samples were stored for 1, 3, 5, 15 and 30 days at 4 °C before rescanning. DSC measurements were performed again in the same condition (20 °C–120 °C, 10 °C/min) to

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