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Effect of single-, dual-, and triple-retrogradation treatments on *in vitro* digestibility and structural characteristics of waxy wheat starch

Xiao-Pei Hu [a], Yao-Yu Xie [a], Zheng-Yu Jin [b], Xue-Ming Xu [b], Han-Qing Chen [a,*]

[a] School of Biotechnology and Food Engineering, Hefei University of Technology, 193 Tunxi Road, Hefei, Anhui 230009, PR China
[b] State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi, Jiangsu 214122, PR China

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ABSTRACT

The effects of single-retrogradation (SR), dual-retrogradation (DR) and triple-retrogradation (TR) treatments on *in vitro* digestibility and structural characteristics of waxy wheat starch were investigated. The yield of slowly digestible starch in a DR-treated starch with retrogradation time interval of 48 h reached a maximum of 44.41%. The gelatinization temperature range and gelatinization enthalpy of DR-treated starch samples were the lowest. Moreover, compared with native starch, X-ray diffraction patterns of treated starches were altered from A-type to B-type and relative crystallinity was significantly decreased, which was responsible for the interaction between amylose–amylose and/or amylose–amylopectin chains that may generate more imperfect structures. Scanning electron micrographs revealed that compared with SR-treated and TR-treated starches, the surface of DR-treated starch with a retrogradation time interval of 48 h exhibited a net-like structure with numerous cavities. These results suggest that structural changes of waxy wheat starch by cycled retrogradation treatment significantly affect digestibility, and DR treatment can be used for preparing SDS product.

1. Introduction

Starch is not only the main energy storage in green plants such as wheat and maize, but also the main supply of energy and an important source of carbohydrates for humans. Based on the rate of glucose release and its absorption in the gastrointestinal tract, starch is classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). RDS is the starch fraction that causes a sudden increase in blood glucose level after ingestion, and SDS is the starch fraction that is digested completely in the small intestine, but much more slowly. RS has been defined as the starch portion that cannot be digested in the small intestine, but is fermented in the large intestine. Although SDS could be digested completely in the small intestine at a lower rate than RDS, and not like RS which cannot be further digested, SDS tends to provide a sustained supply of glucose with a low glycemic index (GI) that may contribute to the control and prevention of various hyperglycaemia-related diseases (Cummings, Beatty, Kingman, Bingham, & Englyst, 1996). In addition, SDS may maintain satiety and be beneficial in maintaining body weight if incorporated into foodstuffs marketed for weight-loss programmes (Jenkins et al., 2002; Ludwig, 2000). Moreover, SDS could be applied as a carbohydrate replenishment for athletes, especially for long-distance runners (Han & BeMiller, 2007). Therefore, SDS has attracted more attention as a new functional ingredient in novel food development in recent years.

Recently, a number of techniques have been developed to prepare SDS products from grain and tuber starches. These techniques include, but are not limited to, chemical modification by cross linking (Woo & Seib, 2002) or esterification (Han & BeMiller, 2007), enzymatic modification by debranching (Miao, Jiang, & Zhang, 2009; Shin, Choi, Chung, Hamaker, Park, & Moon, 2004), and physical modifications by thermal treatments (Chung, Liu, & Hoover, 2009; Lee, Kim, Choi, & Moon, 2012; Lee, Shin, Kim, Choi, & Moon, 2011; Shin, Kim, Ha, Lee, & Moon, 2005; Wongsagonsup, Varavinit, & BeMiller, 2008) and starch retrogradation (Park, Baik, & Lim, 2009; Tian, Zhan, Zhao, Xie, Xu, & Jin, 2013; Tian, Zhang, Xu, Xie, Zhao, & Jin, 2012; Zhang, Hu, Xu, Jin, & Tian, 2011; Zhou, Baik, Wang, & Lim, 2010; Zhou & Lim, 2012). Among these techniques, physical methods by controlling starch retrogradation are relatively simple and require no chemical reagents. Therefore, these methods have received more attention in recent years.

Starch retrogradation, including short-term and long-term retrogradation, is an unavoidable phenomenon and occurs readily during the storage of heat-processed starchy food. Through the process of retrogradation, the gelatinized starch is transformed

* Corresponding author. Tel./fax: +86 551 62901516.
E-mail address: hanqchen@hfut.edu.cn (H.-Q. Chen).

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from an amorphous state to a more ordered or crystalline state (Tian, Li, Jin, & Xu, 2009; Wang & Ding, 2005). This occurrence could reduce the digestibility of the starch to amylase. Park et al. (2009) reported that temperature-cycled retrogradation could induce a great amount of resistant starch from waxy maize starch gel. Zhou and Lim (2012) indicated that retrogradation increased SDS content in waxy and normal corn starch powders more effectively by temperature cycling (4/30 °C) than isothermal storage (4 °C). Zhang et al. (2011) successfully prepared SDS products with a high yield of 51.62% from waxy rice starch by temperature-cycled retrogradation. Moreover, Tian et al. (2012) further confirmed that temperature-cycled retrogradation significantly increased the SDS yield and the slow digestibility of waxy rice starch, with a maximum yield of SDS at 54.5%. In addition, Tian et al. (2013) reported that compared with single-retrogradation, the yield of SDS in rice starch was increased from 39.3% to 56.7% by dual-retrogradation treatment with the time interval of 36 h.

However, few studies have reported the effect of cycled retrogradation on the preparation and structural properties of the SDS product from waxy wheat starch. Therefore, the aims of the present study were to investigate the preparation of a SDS product from waxy wheat starch by single-retrogradation (SR) (gelatinization–retrogradation), dual-retrogradation (DR) (gelatinization–retrogradation–gelatinization–retrogradation) and triple-retrogradation (TR) (gelatinization–retrogradation–gelatinization–retrogradation–gelatinization–retrogradation) treatments. The structural characteristics and *in vitro* digestibility of the materials obtained were also analyzed.

2. Materials and methods

2.1. Materials

The waxy wheat used was provided by the Yangzhou Academy of Agricultural Sciences, China. The enzymes used in the starch digestion were α-amylase type VI-B from porcine pancreas (EC 3.2.1.1, A3176, Sigma, St. Louis, MO, USA) and amyloglucosidase (EC 3.2.1.3, Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China). Other chemical reagents were of analytical grade.

2.2. Isolation of starch from waxy wheat

Waxy wheat flour was fully milled in a laboratory mill (CHOPIN CD1, France) and sifted. The flour was dissolved in 0.03 M NaOH (1:3, w/w) and stirred with a propeller stirrer (JJ-1, Ronghua Corporation, Jiangsu, China) at 1200 rpm for 1.5 h at room temperature. The soft top layer was discarded, then the remaining slurry was centrifuged at 3500 rpm for 5 min in a benchtop centrifuge (TDL-40B, Anke, Shanghai, China) and the upper greyish layer was discarded. Sediment containing starch was washed with distilled water over a filter cloth. Washings were centrifuged (3500 rpm for 5 min) and the upper grey layer of proteins was removed with a spatula. White starch sediment was washed with distilled water and centrifuged for several times until the grey layer disappeared. Washed starch was neutralised with 1 M HCl and centrifuged once more. The residue was air-dried, milled (Model LG-04, Baixin Yaoji Corporation, Ruian, China) and passed through a sieve with 100 μm openings.

2.3. SR, DR, and TR treatments of waxy wheat starch

Starch (5.0 g) was dispersed with 5 ml distilled water and autoclaved at 110 °C for 30 min. The resultant gels were cooled to room temperature, then hermetically sealed and stored at 4 °C for 12, 24, 36, 48, 60 and 72 h, respectively, to perform SR treatment. The retrograded starch samples were autoclaved again at 110 °C for 30 min. After cooling to room temperature, they were hermetically sealed and stored at 4 °C for 12, 24, 36, 48, 60 and 72 h, respectively, to perform DR treatment. Under the same conditions as above, waxy wheat starch was treated repeatedly to perform TR treatment. Afterwards, the starch samples were freeze-dried and milled to pass through a 100-mesh sieve.

2.4. Determination of in vitro digestibility of starch

In vitro digestibility of starch samples was determined according to the procedure of Englyst et al. (1992) with a minor modification. The enzyme solution used was a mixture of pancreatic α-amylase solution (54 ml), amyloglucosidase solution (4 ml) and distilled water (4 ml). Pancreatic α-amylase solution was prepared as follows: Enzyme (1.4 g) was mixed with 80 ml distilled water, incubated at 35 °C with shaking (150 rpm) for 20 min and centrifuged at 1500 rpm for 15 min. The supernatant was the α-amylase solution required in this study. Similarly, amyloglucosidase (2.25 g) was suspended in distilled water (50 ml), then prepared following the same procedure as mentioned above to obtain the amyloglucosidase solution.

Subsequently, phosphate buffer (15 ml, pH 5.2) and seven glass balls (10 mm diameter) were added to a starch sample (200 mg), and equilibrated in a water bath at 37 °C for 5 min. They were then mixed with the enzyme solution (5 ml), and shaken (150 rpm) at 37 °C in a water bath. At time intervals of 20 and 120 min, aliquots of hydrolysed solution (0.5 ml) were taken and uniformly mixed with absolute ethanol (4 ml) and centrifuged (2000 rpm) for 10 min to deactivate the enzyme. The glucose content in the supernatant was measured using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). The percentage of hydrolysed starch was calculated by multiplying the glucose content by a factor of 0.9. Each sample was determined in triplicate.

The values of RDS, SDS and RS fractions in each sample were obtained by the following equations:

$$\text{RDS}\ (\%) = [(G_{20} - \text{FG})/\text{TS}] \times 0.9 \times 100$$
$$\text{SDS}\ (\%) = [(G_{120} - G_{20})/\text{TS}] \times 0.9 \times 100$$
$$\text{RS}\ (\%) = [(\text{TS} - G_{120})/\text{TS}] \times 0.9 \times 100$$

where, G_{20} and G_{120} are the amounts of glucose released within 20 and 120 min of hydrolysis, respectively, and FG is the amount of free glucose in starch and TS was total starch weight.

2.5. Differential scanning calorimetry (DSC)

Thermal characteristics of starch samples were investigated using DSC (Q200, TA, New Castle, DE, USA). Approximately 3 mg starch sample was mixed with 6 μl deionized water and hermetically sealed in an aluminium pan, equilibrated at 4 °C for 24 h, equilibrated at 20 °C for 10 min and then heated from 20 to 85 °C at a rate of 8 °C/min. An empty aluminuim pan was used as the reference. The transition temperatures (T_o, T_p and T_c) and gelatinization enthalpy (ΔH) were analysed and calculated using TA Universal Analysis Software (TA Instruments, New Castle, DE, USA). Experiments were conducted in triplicate.

2.6. X-ray diffraction (XRD)

An X-ray diffractometer (D/MAX 2500V, Rigaku Corporation, Japan) operating at 40 kV and 40 mA with Cu Kα radiation was used to perform X-ray diffraction analysis. The starch samples were dried at 40 °C for more than 6 h, and then scanned at 2θ values from 3° to 30° at room temperature. The degree of relative crystallinity was calculated using MDI-Jade 6.0 software (Material