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Modification by α-D-glucan branching enzyme lowers the *in vitro* digestibility of starch from different sources

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ABSTRACT

Granular corn starch, waxy corn starch, potato starch and tapioca starch were modified using the α-D-glucan branching enzyme (1,4-α-D-glucan:1,4-α-D-glucan 6-α-D-(1,4-α-D-glucano)-transferase, GBE, EC 2.4.1.18) from *Geobacillus thermoglucosidans*. The GBE-catalyzed modification caused a time-dependent increase in the ratios of α-1,6 linkages to total glycosidic linkages, as well as reductions in the average chain length and relative crystallinity. These modifications lowered the *in vitro* digestibility of the starch. Modification with GBE caused varying degrees of change in the *in vitro* digestibility of starches obtained from different sources. The highest slowly digestible starch (SDS) and resistant starch (RS) contents were found in modified tapioca starch. After modification of tapioca starch with GBE for 10 h, the ratio of α-1,6 linkages to total glycosidic linkages was increased by 11.5%, while its relative crystallinity was decreased by 22.9%. Meanwhile, the SDS and RS contents of tapioca starch were increased by 47.3% and 13.5%, respectively. These results demonstrate that the digestibility of starch can be lowered through GBE modification, which may aid the development of modified starches that are digested more slowly.

1. Introduction

Plant starches are the main energy source in the human diet. Based on the rate and extent of digestion, starch has been classified into three types: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [1]. SDS is completely digested in the small intestine at a rate slower than that of RDS, leading to a lower glycemic response. RS, which cannot be digested in the small intestine, is fermented in the large intestine [2]. After the consumption of food, the starch component is hydrolyzed by salivary and pancreatic α-amylases to linear maltooligosaccharides (primarily maltose and maltotriose) and α-limit dextrins [3], and then digested to glucose by the intestinal brush border α-D-glucosidase: maltase–glucoamylase and sucrase–isomaltase [4]. Generally, the α-1,6 glycosidic bonds of starches are more difficult to hydrolyze than the α-1,4 bonds. Therefore, one strategy to produce SDS is to increase the number of α-1,6 branch points. However, many factors can affect the digestion of starch, especially the fine structure of the starch molecules. Starches isolated from different botanical sources display differences in structural properties [5]. Starches with A-type crystallinity, like corn starch, waxy corn starch and tapioca starch, have shorter average chain lengths than starches with B-type crystallinity, like potato starch. This is likely to cause differences in their rates of digestion. Various methods have been reported to slow the rate of digestion of different starch types. A number of physical [6], chemical [7,8], and enzymatic [9–16] methods have been used to modify the molecular and crystalline structure of starch. Among these, enzymatic modification is perhaps the most satisfactory method. It has the advantages of increased safety, substrate selectivity, and product specificity, compared with physical and chemical modifications [17].

α-D-Glucan branching enzyme (1,4-α-D-glucan:1,4-α-D-glucan 6-α-D-(1,4-α-D-glucano)-transferase, GBE, EC 2.4.1.18), a member of glycosyl hydrolase family thirteen, catalyzes the hydrolysis of the α-1,4-linked linear chains of amylose and amylopectin and promotes the formation of new α-1,6 linked branch chains [9,16,18]. Li et al. [19] suggested that partial branching enzyme treatment reduces the overall starch digestion rate, which is related to the starch's molecular weight, chain length, and branch density. A similar phenomenon was observed by Jo et al. [20] during a study of slowly digestible sweet potato Daeyumi starch prepared using GBE

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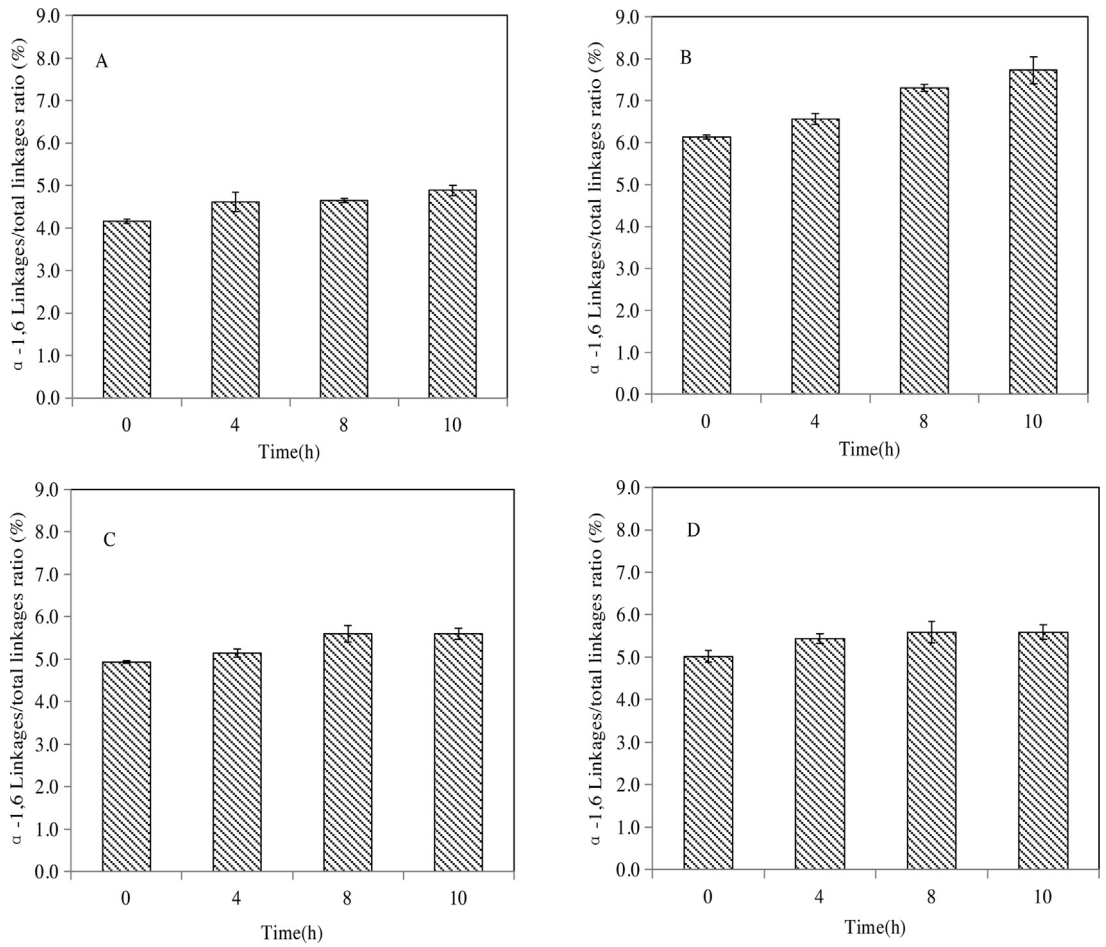

Fig. 1. ^{1}H NMR analyses of the glycosidic linkage ratios of starch samples. (A) The α-1,6 glycosidic linkage ratios of corn starch treated with GBE for 0 h (control), 4 h, 8 h, or 10 h; (B) The ratios of α-1,6 linkages to total glycosidic linkages of waxy corn starch treated with GBE for 0 h (control), 4 h, 8 h, or 10 h; (C) The ratios of α-1,6 linkages to total glycosidic linkages of potato starch treated with GBE for 0 h (control), 4 h, 8 h, or 10 h; (D) The ratios of α-1,6 linkages to total glycosidic linkages of tapioca starch treated with GBE for 0 h (control), 4 h, 8 h, or 10 h. Ratios were determined by dividing the area under the peak at 4.96 ppm by the sum of the areas under the peaks at 5.37 and 4.96 ppm, then multiplying the ratio by 100 to get a percentage.

and amylosucrase. GBE was used in the modification of gelatinized starches in previous studies [10,21]. However, these modified gelatinized starches are usually used at low concentrations in industrial applications because of their high cost and high viscosity. Incubation of granular starch with GBE could lead to a starch product with a higher starch concentration (usually 30% in industrial applications) and lower viscosity than gelatinized starches. This would also reduce production costs, since it eliminates the pasting process, enhancing its utility in industrial applications. Therefore, studying the enzymatic modification of granular starch with GBE is potentially valuable.

In this study, granular corn starch, waxy corn starch, potato starch and tapioca starch were modified using the GBE from *Geobacillus thermoglucosidans* STB02. The structural properties and the *in vitro* digestibility of the GBE-modified starches were investigated, and the relationships among them are discussed. These results provide additional information that will aid the development of modified starches with reduced rates of digestion.

2. Materials and methods

2.1. Materials

Corn starch was purchased from Zhucheng Xingmao Corn Developing Co., Ltd. (Shandong, China). Potato starch was purchased from Inner Mongolia Nijlen Co., Ltd. (Inner Mongolia, China). Waxy corn starch and tapioca starch were obtained from Cargill Inc. (Beijing, China). The GBE from *G. thermoglucosidans* STB02 was produced as a recombinant protein by *Escherichia coli* BL21(DE3) harboring plasmid pET-20b(+)/*gbe* [22]. Porcine pancreatic α-amylase, amyloglucosidase, pepsin and isoamylase were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All chemical were reagent grade and were purchased from China Medicine Group (Shanghai, China).

2.2. Preparation of GBE-modified starch

Corn starch, waxy corn starch, potato starch and tapioca starch (30%, w/v) were suspended separately in 50 mM phosphate buffer (pH 7.5). The starch slurries were preheated at 50 °C for 15 min, then GBE (200 U/g dry weight of starch) was added and the mixtures were placed in a water bath at 50 °C for 0 h, 4 h, 8 h, or 10 h. Immediately after incubation, the starch samples were washed with 10 volumes of deionized water, which removed the enzyme. The collected solids were dried at 50 °C for 12 h, ground, and sieved through a 100-mesh sieve. The controls were corn starch, waxy corn starch, potato starch and tapioca starch treated in the absence of GBE.

2.3. Determination of glycosidic linkage ratio

The glycosidic linkage ratios of the starches obtained from different botanic sources, after modified with GBE, was measured