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Enzymic digestibility of reduced-pressurized, heat-moisture treated starch

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The digestibility of the reduced-pressurized heat-moisture treated corn starches by α-amylase and glucoamylase was studied. By the treatment, regular and waxy corn starch granules were well digested by α-amylase without gelatinization, while the digestibility of the high amylose corn starch was reduced. Both regular and waxy corn starches, regardless of the treatment, were digested well by enzymes under the gelatinized condition. However, a drastic increase of indigestible portion was observed in the high amylose corn starch. Methylation analysis of the enzyme resistant moiety of the high amylose starch indicated that most of the indigestible moiety was composed of amylose. These findings suggested that the arrangement of regular or waxy corn starch molecules was made more random by the treatment while, in the high amylose corn starch molecule, crammed amylose formed the highly associated structure. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The heat-moisture treatment of starches gives remarkable changes in crystallinity of the starch molecules in the granules due to rearrangement or higher degree of association of the starch chains. The heat-moisture treated starch is also known to increase the gelatinization temperature and to decrease the gelatinization enthalpy, the viscosity peak in its Brabender diagram and the endothermic peak in its DSC curve. Although such changes may invest the starch with new physical properties potentially useful in the food industry, the heat-moisture treated starches have been produced only on a laboratory scale. Many means have been tried to avoid the main problem, which is the condensation of steam during the treatment to yield a mixture of gelatinized and heat moisture treated starch (Steeneken, 1984; Hagiwara *et al.*, 1991, 1992).

Recently, the conventional method for the heat-moisture treatment of the starch was improved to satisfy practical requirements for the industrial scale production. The new method, which is designated as the reduced-pressurized heat-moisture treatment (RP–HMT) consists of a combination of pressure reduction of a vessel containing the starch and subsequent heating of the starch with introduced live steam. The method is superior to the traditional one in that the materials can be processed on a large scale within short time (Maruta *et al.*, 1994). The RP–HMT corn starch thus prepared contains no gelatinized granule and possesses an excellent thermal stability potentially useful for the food industry. In this paper, we describe the enzymic digestibility of the RP–HMT starches.

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MATERIALS AND METHODS

Materials

Corn starches, from regular, waxy, and high amylose strains used in this study, were products of Sanwa Cornstarch Co., Ltd. (Nara, Japan). Enzymes, α-amylase (Spitase LH, 12 000 DUN g^{-1}, and Spitase HK, 18 000 DUN g^{-1}) and glucoamylase (Spitase XL-4, 4200 FSN g^{-1}), were purchased from Nagase and Co. Ltd. (Tokyo, Japan).

Reduced-pressurized heat-moisture treatment (RP–HMT)

Starches were subjected to RP–HMT at 120 and 125°C for 20 min according to the method previously reported (Maruta *et al.*, 1994). A starch sample was spread uniformly in a large dish (about 5 cm thickness) placed in a vessel and the vessel was evacuated to 30 Torr. Live steam was introduced to the vessel to maintain appropriate pressure for a predetermined time (2.02 kg

cm^{-2} at 120°C and 2.36 kg cm^{-2} at 125°C). After heating at 120 or 125°C for 20 min, the vessel was evacuated again to remove moisture, cooled and then opened. The dried samples were ground and stored at room temperature.

Enzymic digestion of RP–HMT starches

For determination of the enzyme digestibility of RP–HMT starches without gelatinization, the samples (0.1 g) were suspended in 10 ml of 0.1 M sodium acetate-1 mM calcium acetate-3 mM sodium chloride, pH 6.0, containing 0.1% Spitase LH and incubated at 40°C for 2 h. The digested sample solutions were centrifuged at 3 000 rpm for 5 min. The supernatant was collected and the total glucose content of the supernatant was determined by the phenol-sulfuric acid method (Dubois *et al*., 1956). Concurrent assays without enzyme indicated that the soluble portion of starches used in this study was negligible by the phenol-sulfuric acid method. To confirm the enzyme-resistant portion, the starches were digested by α-amylase and glucoamylase under the gelatinized condition according to the method of Sievert and Pomeranz (1989). A sample (0.5 g) was dispersed in 5 ml of 0.1% Spitase HK solution in 0.1 M sodium acetate buffer, pH6.0, containing 1 mM calcium acetate and 3 mM sodium chloride, and incubated for 30 min at 100°C. After cooling to 60°C, the reaction mixture was treated with 1 ml of 1% Spitase XL-4 solution in 0.1 M sodium acetate buffer, pH 4.1 and the mixture was incubated further at 60°C for 30 min. The mixture was then centrifuged at 15 000 rpm for 10 min and the precipitate was washed three times with water. The precipitate thus obtained was lyophilized and dissolved in 0.5 ml of 1 N NaOH. The sugar content of the resulting solution was determined by the phenol-sulfuric acid method. The insoluble portion obtained by digestion with α-amylase and glucoamylase under gelatinized conditions was designated as the enzyme-resistant portion in this study.

Methylation analysis

The sample was methylated by powdered NaOH and iodomethane in dimethylsulfoxide based on the conventional method (Isogai *et al*., 1985). After three runs of the methylation, the methylated sample was hydrolyzed, reduced with $NaBH_4$, acetylated, and analyzed by GC/MS (Shimadzu GC-QP1000, Kyoto, Japan) as the partially methylated glucitol acetates (Jansson *et al*., 1976).

RESULTS AND DISCUSSION

Enzymic digestibility of ungelatinized RP–HMT starches

Non-treated and RP–HMT starches were digested with Spitase LH under the ungelatinized condition to confirm the α-amylase- digestible portion included in starch granules. The result is listed in Table 1. While α-amylase could digest 14% of the non-treated regular corn starch, the digestibility increased to 35 and 60% by RP–HMT at 120 and 125°C, respectively. The waxy corn starch showed almost the same tendency of the enzymic digestibility by RP–HMT as that of the regular corn starch. On the other hand, the resistant portion against α-amylase increased in the RP–HM high amylose corn starch. The result indicates that RP–HMT works in a different manner in each starch strain. The effect seen in the regular and waxy corn starches seems to be that crystalline structures in starch granules were broken down yielding an increase of the digestible portion of α-amylase. Moreover, in the high amylose corn starch granule, the tighter structure is probably due to the rearrangement of amylose molecules formed by RP–HMT. Thus it is considered that the amylose moiety forms a higher degree of association during RP–HMT. In the case of amylose chains surrounded by amylopectin as seen in regular starch granules or few amylose chains in waxy corn starch granules, the amylose and amylopectin molecules may change their structure to form more susceptible to α-amylase.

Enzyme resistance caused by RP–HMT

The enzyme-resistant portion caused by RP–HMT was determined by digestion of starches under gelatinized conditions with α-amylase and glucoamylase. As shown in Table 2, the enzyme resistant portions among non-treated, RP–HMT regular and waxy corn starches are a very small amount (less than 1%). However, the increase of the enzyme-resistant portions observed in these RP–HMT starches was obvious and 2–3 times more than those of non-treated starches. In the case of the high amylose starch, while 24.3% of the non-treated high amylose starch was not digested by enzymes, the enzyme-resistant portion was drastically increased to 44.3 and 55.7% by RP–HMT at 120 and 125°C, respectively. These results indicate that the rearrangement of the starch molecule to the enzyme-resistant structure by RP–HMT is very small for the regular or waxy corn starch, but RP–HMT strongly affects the high amylose corn starch to form a highly associated structure. These results coincide with the assumption of a change of the enzyme-digestible portion of RP–HMT starches under non-gelatinized condition. The characteristic distinguishing the three starch strains is

Table 1. Digestibility of RP–HMT starches by α-amylase

Starch	Non-treated	RP–HMT	
		120°C	125°C
	(%, digestible portion/sample)		
Regular	13.7	34.7	59.8
Waxy	4.8	35.4	45.9
High amylose	14.4	12.2	8.4

Table 2. Enzyme-resistant moiety of RP–HMT starches

Starch	Non-treated	RP–HMT	
		120°C	125°C
	(%, insoluble portion sample)		
Regular	0.31	0.32	0.75
Waxy	0.06	0.16	0.16
High amylose	24.3	44.3	55.7

Table 3. Methylation analysis of enzyme-resistant moiety of RP–HMT high amylose starch

Glucitol	High amylose	Enzyme–resistant portion	
		Non-treated	RP–HMT at 125°C
	(mole %)		
2, 3, 4, 6 Me_4	1.5	5.9	6.3
2, 3, 6, Me_3	96.1	89.8	90.9
2, 3 Me_2	2.4	4.3	2.3

the amylose content, negligible for waxy, 25% for regular and 75% for high amylose. Therefore, it is reasonable to expect that highly condensed amylose molecules, not surrounded by amylopectin, would undergo strong intermolecular interactions by RP–HMT.

Methylation analysis of enzyme-resistant portion

For the further confirmation of the structure of the indigestible portion, the enzyme-resistant portion of the RP–HMT high amylose corn starch at 125°C was subjected to methylation analysis. The result is shown in Table 3. The increase of the non-reducing end residue (2,3,4,6-tetra-*O*-methyl glucitol) is supposed to be due to a lowered degree of polymerization of the starch molecule caused by enzymic digestion. Comparing the enzyme-resistant portions of non-treated and RP–HMT high amylose starches, the decrease of 2,3-di-*O*-methyl glucitol suggests degradation of the amylopectin moiety or increase of amylose moieties in the latter. These results indicate that RP–HMT caused the higher association of the amylose structure. Therefore, the main constituent of the enzyme-resistant portion of the RP–HMT starch is supposed to be the amylose moiety.

As is known well, amylose contents of starches among regular, waxy and high amylose corn starches are quite different. These starches are affected oppositely by RP–HMT. One effect is the destruction of the starch molecule observed as a higher enzyme digestibility and the other is the high degree of association shown as the enzyme resistance. Comparing the enzyme digestibility or resistance and analysis of compositions by methylation, the amylose moiety seems to play the main role in RP–HMT. According to our knowledge, no structural effect of the heat-moisturized treatment on the starch molecule has yet been reported. We conclude that RP–HMT converts the structure of the roughly distributed amylose or amylopectin in the starch granule to a more random state, but the crammed amylose of high amylose structure to highly associated forms.

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