Enzyme-Resistant Starch. VI. Influence of Sugars on Resistant Starch Formation

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ABSTRACT

Solutions of glucose, ribose, maltose, and sucrose were added to autoclaved (1 hr at 121°C in excess water) wheat starch and high-amylose corn starch. After storage of the starch gels for 20- to 320-min intervals, enzyme-resistant starch (RS) yields were determined. Sugars had an influence on RS levels in starch gels only when added in high concentrations (final starch-water-sugar ratio of 1:10:5, w/w). In wheat starch gels, the RS yields decreased from ~3.4% to ~2.8% when sucrose or glucose was present; they decreased to ~2.5% in the presence of ribose or maltose. An increase in RS yield was observed with high-amylose corn starch. The experiments showed that the differences in gelatinization temperature, lipid content, and apparent amylose content of the two starches were not the main causes of the different impact of sugars on the RS yields. RS quality of the isolated RS fractions, determined by X-ray diffraction and differential scanning calorimetry, was not affected by the sugars studied (except for a higher melting enthalpy of isolated RS when it was formed in the presence of ribose).

Previous work (Eerlingen et al 1993a,b; Eerlingen et al 1994a-c), focused on several aspects of enzyme-resistant starch (RS) formation: the impact of amylopectin retrogradation, the influence of amylose chain length, the impact of incubation time and temperature of autoclaved starch, the effect of lipids, and the formation of breads enriched in RS. We here report on the role of sugars in the formation of RS in starch gels (RS type III).

Indeed, when a starch gel retrogrades, a partially crystalline polymer system results that, in part, consists of resistant starch (type III). RS type III consists mainly of retrograded amylose (Berry 1986; Berry et al 1988; Russell et al 1989; Siljeström et al 1989; Sievert and Pomeranz 1989, 1990; Czuchajowska et al 1991).

As the properties of partially crystalline polymer systems are governed by glass transition phenomena (Levine and Slade 1990), it was not to be excluded that the differences in plasticizing effect of water versus sugar-water solutions (Slade and Levine 1991) would exert an influence on the quantity and quality of the RS type III formed.

To the best of our knowledge, no such effort has been reported in the relevant literature, although it is clear that amylopectin retrogradation is influenced by sugars (I'Anson et al 1990, Levine and Slade 1990).

MATERIALS AND METHODS

Materials

Wheat starch (WS, Meriwit AA) was supplied by Amylum (Aalst, Belgium). Amylomaize VII starch (HA) with an apparent amylose content of 75% (Eurylon 7) was supplied by Roquette (Lestrem, France). Enzymes used for isolation of resistant starch were the same as those described in previous studies (Eerlingen et al 1993a,b). Glucose, sucrose, ribose, maltose, and sorbitol were at least of high purity grade.

Defatting of Starch and Determining Fat and Moisture Contents in Starch

Defatting by reflux with 80% methanol and Soxhlet extraction with petroleum ether, and the determinations of fat and moisture in starch, were performed as described previously (Eerlingen et al 1994b).

Formation of RS

Unless indicated otherwise, native or defatted starch samples (1.50 g) were autoclaved in 7.5 ml of water for 1 hr at 121°C. Water (7.5 ml), sugar solution (2.7 g or 7.5 g in 7.5 ml of water), or sorbitol solution (6.0 g in 7.5 ml of water) was added, and the mixtures were stirred with a magnetic stirrer for 15 min. Thus, starch-water-sugar ratios of 1:10:1.8 (w/w) or 1:10:5 (w/w) or a starch-water-sorbitol ratio of 1:10:4 (w/w) were obtained. The homogenized samples were stored at room temperature for 20-320 min.

Isolation of RS

RS was isolated with Termamyl, amyloglucosidase, and protease as described previously (Eerlingen et al 1993a,b). Yields were calculated as percent of starch (dmb).

X-Ray Diffraction Analysis

X-ray powder diffraction analysis was performed with a PW 10050/25 diffractometer equipped with a proportional detector PW 1965/20 (Philips, MBLE, Brussels, Belgium). Operating conditions were: 30 kV and 20 mA with Co radiation = 0.179 nm. Diffractograms of the samples were obtained from 3° 2-θ to 30° 2-θ.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measurements were performed with a Seiko DSC-120 instrument. Indium and tin were used as standards. About 10 mg of isolated RS sample was accurately weighed into stainless steel pans, and two times the sample weight of water was added. A pan with water served as the reference. The DSC run was performed from 20 to 180°C at a heating rate of 2°C/min. The onset temperature (T_o), the peak temperature (T_p), and the enthalpy (ΔH) of the transition were determined with Seiko software. All analyses were performed in triplicate.

Hot-Stage Polarization Microscopy

Suspensions of WS and HA (starch-water ratio of 1:5, w/w) were viewed under polarized light and heated (2°C/min). The equipment used was an Olympus BHS laboratory binocular microscope with a Mettler hot-stage apparatus (FP 82HT and FP 90 central processor).

Statistical Evaluation

The statistical analyses were performed using the general linear model procedure of SAS (1987), including Tukey's studentized range test for pairwise comparisons (5% significance level).

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RESULTS AND DISCUSSION

Influence of Sugars on RS Yield in WS Gels

When glucose was present in a low concentration (starch-water-sugar ratio of 1:10:1.8, w/w) during the retrogradation of starch, no significant influence on RS yields could be detected (Fig. 1). However, when a higher glucose concentration was used (starch-water-sugar ratio of 1:10:5, w/w), RS contents were reduced to a significant degree. It has been suggested (Slade and Levine 1991) that the effect of sugar on the mobility of the aqueous solution environment only becomes obvious at sugar concentrations exceeding 30% (water-sugar ratios of 10:4.3, w/w). Therefore, it would follow that the impact of sugars on RS formation would be insignificant at the lower sugar concentration (Fig. 1). Addition of other sugar solutions (sucrose, ribose, and maltose) at the same concentration (starch-water-sugar ratio of 1:10:5) also decreased RS yields significantly. In WS gels, RS yields decreased from ~3.4% to ~2.5% when ribose or maltose was added. They decreased to ~2.8% in the presence of sucrose or glucose (Fig. 2).

Influence of Sugars on RS Yield in HA Gels

Figure 3 shows the RS yields in HA gels as a function of storage time, with and without the addition of solutions of glucose, ribose, and maltose. Much higher RS yields were obtained in the HA gels (~13.2%) than in the WS gels (~3.4%) (Fig. 1). This is in agreement with previous findings, where RS yields increased with the amylose content of the starch (Berry 1986, Sievert and Pomeranz 1989). In contrast to WS gels, RS yields in HA gels increased when sugars were present.

Influence of Sugars on RS Yield in WS Gels in Terms of Crystallization

As outlined in a previous study (Eerlingen et al 1993a), RS formation in starch gels can be considered a crystallization process of amylose in a partially crystalline polymer system within the temperature range between the glass transition temperature (T_g) and the melting temperature of the amylose crystallites. Addition of most sugar solutions results in a higher T_g of the starch gel matrix (antiplasticizing effect) (Levine and Slade 1990). The elevated T_g exerts a retarding influence on the rate of propagation at storage temperatures $>T_g$. A higher T_g results in smaller differences between the storage temperature and T_g and, consequently, in lower rates of propagation. From this, it follows that sugars can retard crystallization of amylose and, therefore, also formation of RS (type III).

Similar results were obtained earlier for amylopectin retrogradation in the presence of sugars. I'Anson et al (1990) observed that the crystallinity (determined by X-ray diffraction analysis) of WS gels in the presence of sugars (ribose, sucrose, and glucose) decreased when the gels were stored for several days. Levine and Slade (1990) also found that amylopectin retrogradation (determined by DSC) was reduced in WS gels when sugars (glucose, maltose, and sucrose) were added.

Influence of Sugars on RS Yield in HA Gels in Terms of Crystallization

The data obtained for HA starch gels with added sugars could not be interpreted in terms of the impact of these sugars on the T_g of the polymer system and their subsequent role in the crystallization process. Therefore, we designed some experiments to explain the differences in response to sugars of WS and HA gels.

Differences in Response to Sugars of WS and HA Gels

The differences in degree of gelatinization, lipid content, and amylose-amylopectin ratio of HA and WS were considered to be potentially responsible for the differences in response to sugars of WS and HA gels.

Degree of gelatinization. HA gelatinizes at much higher temperatures than does WS, so the response to autoclavation of the two starches was quite different. With hot-stage microscopy, we could observe that the temperature range of gelatinization of WS was between 53 and 60°C, while the T_o of gelatinization of HA

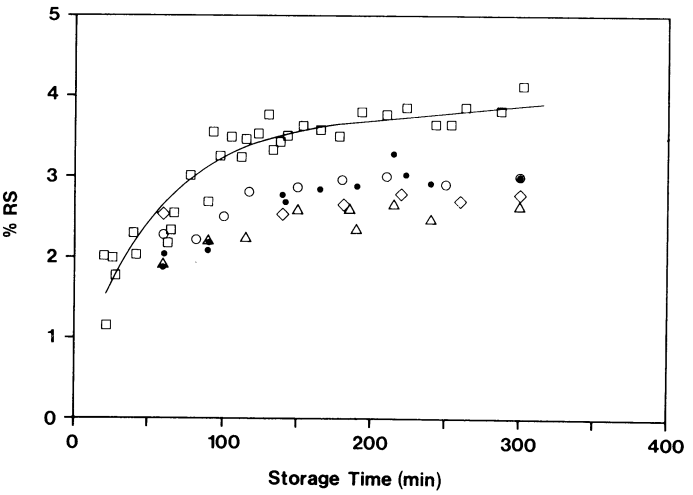

Fig. 2. Enzyme-resistant starch (RS) yields in wheat starch gels as a function of storage time, with or without different sugars. No sugar (□), glucose (○), maltose (◇), sucrose (●), ribose (△). Starch-water-sugar weight ratios were 1:10:0 for water and 1:10:5 for sugar solutions.

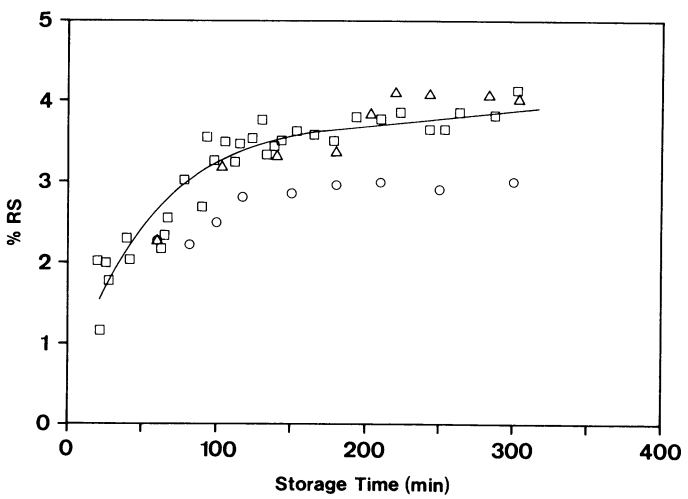

Fig. 1. Enzyme-resistant starch (RS) yields in wheat starch gels as a function of storage time, with or without glucose. Starch-water-sugar weight ratios: 1:10:0 (□), 1:10:1.8 (△), 1:10:5 (○).

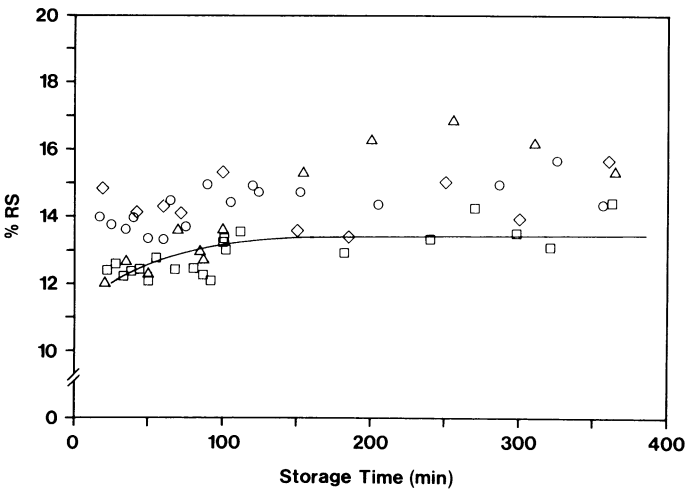

Fig. 3. Enzyme-resistant starch (RS) yields in amylomaize VII starch gels as a function of storage time, with and without different sugars. No sugar (□), glucose (○), maltose (◇), ribose (△). Starch-water-sugar weight ratios were 1:10:0 for water and 1:10:5 for sugar solutions.

was 70°C. Even at 125°C, birefringent regions were still present in the starch. Thus, native crystalline fractions were still present in HA after autoclavation. To simulate incomplete gelatinization conditions for WS also, this starch was heated at 58°C for 1 hr in an excess of water (2°C below the hot-stage end-temperature of gelatinization). Here also, incomplete gelatinization was evident. We observed that sugars (maltose in a starch-water-sugar ratio of 1:10:5, w/w) still decreased RS levels significantly (from 1.6 to 1.3% RS). This suggests that an incomplete gelatinization of starch did not influence the relative effect of sugars on RS yields in WS gels. We further noticed that when wheat starch was heated at 58°C, RS yields were much lower than what was observed upon autoclavation at 121°C. Our data are therefore in agreement with those of Berry (1986), who found an increase in RS yield of WS gels as the autoclaving temperature was increased from 100 to 134°C.

Lipid content. We examined whether the difference in lipid content could be the reason for the different behavior of the two starches. Indeed, endogenous lipids can interact with amylose to form amylose-lipid complexes that can crystallize (Biliaderis and Galloway 1989). Theoretically, we can speculate that sugars may retard the crystallization of amylose-lipid complexes by increasing the T_g of the system (much in the same way as they influence the crystallization of amylose or amylopectin). Experimental evidence in support of this speculation has been reported by Biliaderis and Seneviratne (1990). Amylose molecules in amylose-lipid complexes are prevented from double-helix formation and consequently from RS formation (Czuchajowska et al 1991, Eerlingen et al 1994b). In view of this, it seems logical to assume that sugars may potentially increase RS yields indirectly by interfering with the complexation of amylose by lipids. On the other hand, sugars may decrease RS yields by retarding amylose crystallization.

Depending upon the lipid content, the net result may be that sugars either increase or decrease RS levels. To investigate this hypothesis, HA was defatted by refluxing with 80% methanol and Soxhlet extraction with petroleum ether. Lipid content of HA decreased from 1.17 to 0.29%. This is even less than the lipid content of WS (0.54%). Even after defatting, and after 150 min of storage, RS yields in HA gels increased from 19.7 to 24.5% when sugar (maltose in a starch-water-sugar ratio of 1:10:5, w/w) was present. Thus, the differences in lipid content of the two starches could not be the reason for the different responses to sugars in RS formation. We further observed that defatting the starch increased the RS yield (from 13.2 to 19.7%), as already demonstrated in previous work (Eerlingen et al 1994b).

Amylose-amylopectin ratio. HA (genotype *ae*) contains a starch fraction with structural properties intermediate between those of amylose and amylopectin as found in WS or normal maize starch. The molecules of this fraction show molecular weight values between those of amylose and amylopectin of WS. It is poorly branched and has very long, loosely clustered chains (Shannon and Garwood 1984, Takeda et al 1989, Takeda et al 1993).

The intermediate fraction may be regarded as (apparent) amylose (e.g., based on iodine affinity) or as amylopectin (e.g., based on precipitation with 1-butanol or gel-permeation chromatography). Indeed, since the average chain length of amylopectin of WS is about 20, no deep blue color with iodine is obtained. On the other hand, the average chain length of the molecules of the intermediate HA fraction is ~40, and the chains are long enough to yield a deep blue color. Accordingly, they are analyzed as amylose, although they are more branched than amylose of WS and the molecular weight is much higher.

Apart from this, the presence of slightly branched or linear, short-chain molecules has been demonstrated in HA (Takeda et al 1993).

Because the intermediate starch fraction contributes to RS formation much in the same way as WS amylose does, the RS formation in the intermediate HA fraction has been regarded as amylose (Berry 1986, Sievert and Pomeranz 1989). Thus, the amylose content of HA is considered to be ~75%, in contrast with 25% in WS. Therefore, the ratio of amylose-sugar solution was not the same for the WS and the HA. This may have been important because it is generally accepted that RS (type III) consists mainly of crystalline amylose. The crystallization process may have been affected by the relative amount of plasticizing sugar solution present. To investigate whether this factor could be the cause of the different responses of WS and HA to the presence of sugars, a mixture of 33.3% HA (amylose content ~75%) and 66.6% WS (amylose content ~0%) was prepared. Because the amylose content in the mixture was the same as that for WS (25%), the same ratio of amylose-sugar solution was present during retrogradation. RS yields of the mixed starch gel, with and without the addition of maltose and ribose, are shown in Figure 4. RS yields were still higher when sugars were present than when no sugars had been added. Thus, we concluded that the difference in amylose-sugar solution ratio between WS and HA was not the reason for the different behavior of the two starches.

Another consequence of the difference in (apparent) amylose content of the two starches is the different crystallization rate of amylose. HA contains a much higher level of amylose, so the crystallization rate of this component most likely was different (higher) from that in WS. Accordingly, crystallization of amylose in the HA gel may have occurred to some extent before the sugar solution was added. This is in contrast with what would be expected to have occurred in the WS gel. To examine whether this was important, a polyol (sorbitol) was added to WS and HA both before and after autoclavation. A polyol was used because the addition of the sugars to the starch before autoclavation resulted in caramelization. An increase in RS content was noted for HA, while a decrease of RS concentrations was observed for WS. Thus, if crystallization had already occurred before the polyol solution was added, it was of little consequence (even if the addition of sorbitol before autoclavation also had influenced the gelatinization temperature of the starch).

This implies that the different behavior of WS and HA cannot be explained by the difference in apparent amylose content. Further research is needed on the impact of the differences in the structure of the starch molecules on crystallization before conclusions can be drawn about the influence of the molecular structure of both starches on their different behavior in RS formation.

X-Ray Diffraction and DSC

X-ray diffraction patterns of the freeze-dried HA gels showed very little crystallinity (Fig. 5b), except for the starch gel with ribose (not shown). The diffraction peaks observed in this pattern, however, could be ascribed to crystalline ribose.

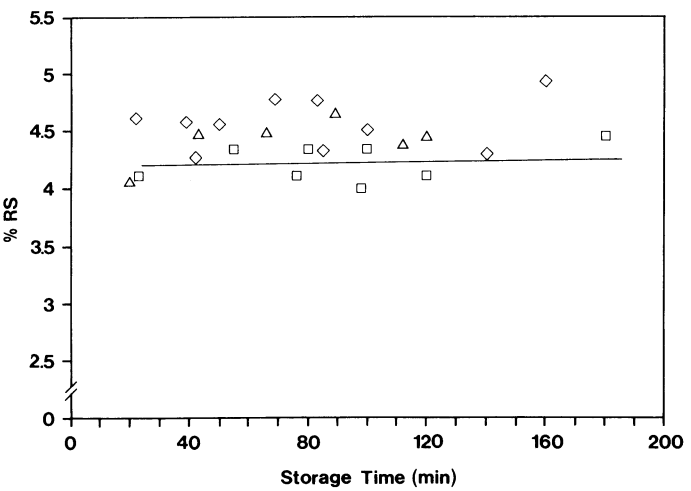

Fig. 4. Enzyme-resistant starch (RS) yields in a mixed starch gel of waxy maize starch and amylomaize VII starch (25% of amylose) as a function of storage time, with no sugar (□) and with maltose (◇) and ribose (△). Starch-water-sugar weight ratios were 1:10:0 for water and 1:10:5 for sugar solutions.

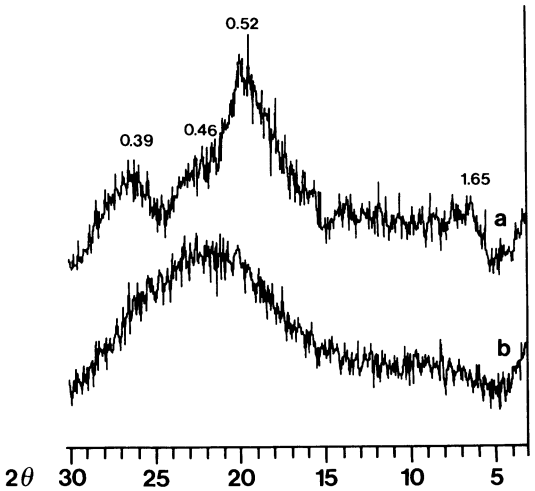

Fig. 5. Typical X-ray diffraction patterns. Enzyme-resistant starch (a) isolated from freeze-dried amylomaize VII starch gel (b). Numbers above peaks indicate *d*-spacings in nanometers.

Peaks in the patterns of the isolated RS (Fig. 5a) were more pronounced than those in the patterns of the freeze-dried gels (Fig. 5b). The difference in degree of crystallinity was to be expected, whether the moisture content differed or not. During isolation of RS, the crystalline fraction is concentrated, so a more pronounced X-ray diffraction pattern was obtained for the isolated RS. Sugars did not affect the pattern significantly. A B-pattern, which is generally found for RS and retrograded starch (Berry et al 1988, Siljeström et al 1989, Sievert et al 1991, Eerlingen et al 1993a), was obtained in all cases.

DSC thermograms of isolated RS fractions all showed a melting endotherm at a peak temperature of ~152°C. Therefore, no influence of the sugars on the peak temperature of the isolated RS was detected. However, from a theoretical standpoint, it is not to be excluded that RS, in the presence of sugars, may have a different melting temperature than in their absence. The presence of sugars in the aqueous environment during the melting process may interfere with the melting of RS, even though the RS is of the same quality as that produced in the absence of sugars. The melting enthalpy of the isolated RS was 16.1 mJ/mg for the control; the enthalpy of the RS formed in presence of sugars was slightly higher. Only in the case of ribose was the average enthalpy value of 20.0 mJ/mg significantly higher than that of the control (16.1 mJ/mg).

CONCLUSIONS

Sugars (glucose, maltose, sucrose, and ribose) have a significant influence on RS yield in starch gels when present in a high concentration (starch-water-sugar ratio of 1:10:5, w/w).

The effect may be positive or negative, depending upon the starch type. A decrease in RS yield was observed for WS, while an increase was noticed for HA. The differences in the response of the RS yields to sugars in HA and WS gels could not be rationalized, although it is clear that the differences in apparent amylose content, lipid content, and gelatinization temperature of the two starches were not the causes of the different behaviors of the starches in the presence of sugars.

Further research is needed to elucidate the different behavior of HA and WS. Studies on the impact of amylopectin and amylose structure on crystallization have to be performed to evaluate whether the difference in molecular structure of WS and HA starch molecules can cause the different impact of sugars on RS formation.

No influence of the sugars on the characteristics (X-ray diffraction, DSC) of the isolated RS could be detected, except for a higher melting enthalpy of isolated RS when it was formed in the presence of ribose.

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