# Enzyme-Resistant Starch. II. Influence of Amylose Chain Length on Resistant Starch Formation

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Cereal Chem. 70(3):345-350

ABSTRACT

Potato starch amylose was hydrolyzed to varying degrees by incubation with barley $\beta$-amylase for different periods. Determination of reducing sugars and gel-permeation chromatography showed that amylose fractions with different number average chain lengths ($\bar{DP}_n$ 40–610) were obtained. Enzyme-resistant starch (RS) was formed from the fractions in aqueous solutions (0.83%, w/v) at 4°C. Under the experimental conditions, the yield of RS increased with $\bar{DP}_n$ to plateau values of 23–28% within a region of $\bar{DP}_n$ of 100–610. X-ray diffraction showed a B-pattern for all RS samples obtained. The $\bar{DP}_n$ of the RS varied only between 19 and 26 and, thus, it is independent of the chain length of the amylose from which it was formed ($\bar{DP}_n$ 40–610). The results suggest that RS may be formed by aggregation of amylose helices in a crystalline B-type structure over a particular region of the chain (about 24 glucose units).

During the retrogradation of starch, a fraction may become resistant to amylolytic enzymes. It is generally believed that this resistant starch fraction 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; Sievert et al 1991). In a previous study, we showed that formation of enzyme-resistant starch (RS) in a starch gel can be considered as a crystallization process of amylose in a partially crystalline polymer system (Eerlingen et al 1993).

The objective of this study was to investigate the influence of the amylose chain length on RS formation in an aqueous system as part of a systematic study of the formation and the physicochemical properties of RS. Indeed, it is well documented that aqueous solutions of amylose, even diluted, are very unstable, and that aggregation occurs. It has further been established that polymer chain length has a profound effect on such aggregation processes (Pfannemüller et al 1971, Gidley and Bulpin 1987, Gidley 1989, Clark et al 1989). Thus, the physical form (e.g., precipitate or gel), the turbidity, and the gel strength (if any) all depend on the amylose chain length. Although this is well documented in the relevant literature, insight is lacking into the way the amylose chain length effectively influences the enzymic degradability of the retrograded starch and, hence, the characteristics of the RS. Therefore, it seemed to be of great interest to investigate the degree to which RS formation is influenced by the chain length of amylose.

## MATERIALS AND METHODS

Amylose from potato starch was purchased from Janssen Chimica (Beerse, Belgium). Barley $\beta$-amylase (A7130) was obtained from Sigma Chemical Co., St. Louis, MO. Enzymes used for isolation of RS were Termamyl, a thermostable $\alpha$-amylase from *Bacillus licheniformis* (Novo Nordisk); AMG, an amyloglucosidase from *Aspergillus niger* (Novo Nordisk, Bagsvaerd Denmark); and protease from *Streptomyces griseus* (P5147, Sigma).

### Iodine Potentiometric Titration

The purity of the commercial amylose was determined by an iodine potentiometric titration (Schoch 1964) and was calculated by dividing the iodine affinity value by 20%, the theoretical iodine affinity value of pure amylose (Banks and Greenwood 1975).

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### Formation of Amylose Varying in Average Chain Length

Samples of amylose (15, 1.00 g each) were each dissolved in 15 ml of dimethyl sulfoxide (DMSO). Sodium acetate buffer (110 ml, 200 m*M*, pH 4.8) containing 0.11 mg of $\beta$-amylase was added to eight amylose solutions (A samples), and the same amount of buffer containing 1.10 mg of $\beta$-amylase was added to the other solutions (B samples).

The A samples were incubated at 37°C for 0, 5, 10, 20, 30, 60, 90, and 120 min; the B samples were incubated for 15, 20, 25, 30, 45, 60, and 75 min. After incubation, the samples were placed in a 100°C water bath for 20 min.

After cooling to room temperature, the amylose was precipitated with 375 ml of ethanol. The samples were centrifuged (10 min, 1,000 × *g*), and the sediments were washed with ethanol and filtered through a fritted crucible (no. 4 porosity). The residues were washed with aliquots of ethanol followed by aliquots of diethyl ether (2×). The crucibles with the residues were dried overnight in a desiccator.

### Formation of RS

Fractions of the amylose samples were dissolved in DMSO (100 mg/ml). Phosphate buffer ($KH_2PO_4$/NaOH, pH 6.0, 50 m*M*) was added to obtain a final concentration of amylose and DMSO at 0.83% (w/v) and 8.3% (v/v), respectively.

The solutions were placed in a 100°C water bath for 10 min and cooled at 4°C overnight.

### Isolation of RS

After cooling the amylose solutions overnight, RS was isolated by an enzymatic-gravimetric method (AOAC 1985) (i.e., a modified method of the procedure for the determination of total dietary fiber). Termamyl (commercial preparation, 0.4 ml per gram of amylose) was added to the samples. After 30 min of incubation at 100°C, each sample was cooled to room temperature, and the pH was adjusted to 4.5 with a 2% phosphoric acid solution. Amyloglucosidase (commercial preparation, 1.0 ml per gram of amylose) was added, and the samples were incubated for 30 min at 60°C. After enzymic digestion, the samples were centrifuged (10 min, 1,000 × *g*), and the sediments were washed (3×) with distilled water. The residues were suspended in 50 ml of phosphate buffer ($KH_2PO_4$/NaOH, pH 7.5, 50 m*M*). Protease was added (1.0 ml of a solution, containing 16 mg of protease in 100 ml of buffer, per gram of amylose), and the residues were incubated for 4 hr at 42°C. Each sample was filtered through a weighed, fritted crucible (no. 4 porosity) and washed (3×) with distilled water. The crucibles with the residue were dried overnight in an oven at 80°C and weighed after cooling to room temperature in a desiccator.

Thus, RS was the insoluble residue after enzymatic digestion of the amylose sample and after removal of the amylolytic enzymes with protease. RS is expressed as the percentage of resistant starch on amylose as is.

### Determination of the Degree of Polymerization

The degree of polymerization (DP) can be determined as:

$$DP = \frac{\text{Total carbohydrate (as } \mu\text{g maltose)}}{\text{Reducing sugar (as } \mu\text{g maltose)}} \times 2$$

Total carbohydrate was determined by the method of Dubois et al (1956). The reducing sugar content was measured with neocuproine hydrochloride, which replaces the molybdate color reagent normally used, and cupric sulfate (Dygert et al 1965). Neocuproine forms a yellow-colored complex by specifically chelating with $Cu^+$ produced by oxidation of reducing sugars. Glucose amounts as low as 5 μg can be detected. In the assay, two reagents are necessary.

Reagent A is made by dissolving 40 g of anhydrous $Na_2CO_3$ in 600 ml of distilled water. Then glycine (16 g) is added and dissolved. Finally $CuSO_4{\cdot}5H_2O$ (0.450 g) and water are added to a final volume of 1,000 ml.

Reagent B is made by dissolving neocuproine hydrochloride (2,9-dimethyl-1,10-phenantroline hydrochloride, 0.120 g) in 100 ml of distilled water and is stored in a brown bottle.

In the procedure, 1.0 ml of sample is pipetted into a graduated test tube, and reagent A (1.0 ml) and reagent B (1.0 ml) are added. The contents are mixed, and the tubes are capped and placed in a vigorously boiling water bath. After 12 min, the samples are cooled under running tap water. The solutions are then diluted to 10 ml, and the absorbance is read at 450 nm.

Maltose was used as the standard in both analyses, and all analyses were performed in duplicate.

### Gel-Permeation Chromatography

Amylose and RS samples were analyzed by gel-permeation chromatography on a column (0.9 × 50 cm) of Sepharose CL-6B. Samples (10 mg) were dissolved in 2.5*N* KOH (0.33 ml). Water (3 ml) was added to make the solution 0.25*N* in KOH. An aliquot of solution containing 1 mg of carbohydrate was eluted with 0.25*N* KOH (0.17 ml/min). Fractions (0.7 ml) were collected and assayed for total carbohydrate by the method of Dubois et al (1956). Calibration of the column was performed with maltose (total volume, $V_t$ = 42.0 ml) and amylopectin of maize (Fluka) (void volume, $V_o$ = 15.5 ml). Each fraction was characterized by its $K_{av}$.

$$K_{av} = (V_e - V_o)/(V_t - V_o)$$

where $V_e$ is the elution volume of the fraction.

### X-Ray Diffraction

X-ray powder diffraction analysis was performed with a Philips diffractometer PW 10050/25 (40 kV and 20 mA, CuK radiation = 0.154 nm, and nickel filter). Diffractograms of the amylose fractions and of the isolated RS samples were obtained from 2° 2θ to 30° 2θ.

**TABLE I**
**Incubation Time of Amylose with β-Amylase and the Number Average Degree of Polymerization ($\bar{DP}_n$) of the Resulting Fractions[a]**

| A | | B | |
|---|---|---|---|
| Time (min) | $\bar{DP}_n$ | Time (min) | $\bar{DP}_n$ |
| 0 | 610 | 15 | 131 |
| 5 | 373 | 20 | 116 |
| 10 | 352 | 25 | 94 |
| 20 | 308 | 30 | 82 |
| 30 | 325 | 45 | 72 |
| 60 | 313 | 60 | 59 |
| 90 | 238 | 75 | 40 |
| 120 | 262 | | |

[a] A and B = 1 and 10 mg of β-amylase per liter of amylose solution, respectively.

## RESULTS AND DISCUSSION

### Amylose Fractions Varying in Average Chain Length

The purity of the commercial potato starch amylose, determined by iodine potentiometric titration, was 96.9%. The amylose, hydrolyzed to varying extents by incubation with β-amylase for different periods, yielded fractions that differ greatly in average chain length. Incubation time and the resulting average chain length of the amylose obtained are shown in Table I. In general, the average degree of polymerization ($\bar{DP}_n$) of the amylose chain decreased with increasing incubation time and increasing concentration of β-amylase.

Gel-permeation chromatography (GPC) of the amylose fractions on Sepharose CL-6B (Fig. 1) showed that polydisperse amylose fractions were obtained. Nevertheless, the elution patterns clearly shifted to higher elution volumes with lower calculated number average degree of polymerization ($\bar{DP}_n$). The amylose samples with highest (610) and lowest (40) $\bar{DP}_n$ were eluted at totally different elution volumes. These two samples, therefore, must contain amylose with a distribution of chain lengths that overlap only slightly.

Of further importance is that, in the GPCs, we did not find high molecular weight β-limit dextrins such as those found by Takeda et al (1987) for amyloses of other botanical origins. Our results indicate that the potato amylose sample used in our work contained no detectable levels of branched amylose.

X-ray diffraction revealed a V-type pattern for the different amylose fractions obtained (Fig. 2). V-amylose can indeed be formed in the presence of ethanol (Brisson et al 1991, Welland and Donald 1991). The ethanol molecules do not incorporate in the crystal lattice as do some other alcohols, and the structure is stabilized only by water molecules (Buléon et al 1984).

### DP and GPC of Isolated RS

After dissolution of the amylose fractions (0.83%), a precipitate was formed in the aqueous solutions. RS was isolated as the residue that was obtained after enzymatic digestion. The $\bar{DP}_n$ of the isolated RS samples differed between 19 and 26 glucose units (Table II).

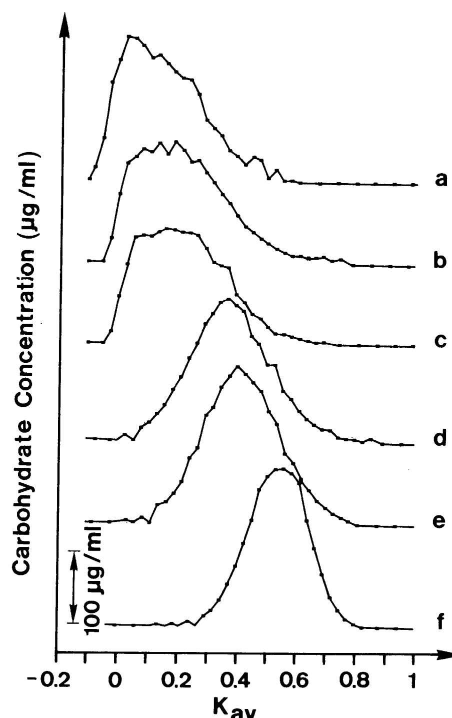


**Fig. 1.** Carbohydrate profiles obtained by gel-permeation chromatography (Sepharose CL-6B) of isolated amylose fractions obtained after incubation with β-amylase for different periods and with average $\bar{DP}_n$ of 610 (**a**), 373 (**b**), 262 (**c**), 131 (**d**), 94 (**e**), and 40 (**f**).

Chain lengths of about $\bar{DP}_n$ 50 (Russell et al 1989) and 65 (Siljeström et al 1989) have been found for RS formed in autoclaved wheat starch gels stored at room temperature. RS formation, generally accepted to be amylose retrogradation, strongly depends on the experimental conditions used. Thus, the chain length of the retrograded amylose in the crystalline zones might be influenced by factors such as concentration, temperature, etc. In our experiments, RS was formed under experimental conditions different from those applied by the cited authors (i.e., RS was formed in an aqueous solution of amylose of 0.83% at 4°C for 13 hr after a 10-min boiling step).

Jane and Robyt (1984) showed that the $\bar{DP}_n$ of resistant amylose (obtained by precipitation in an aqueous amylose solution of 0.35% for one or two weeks at 5°C) depended on the hydrolytic reagent used. Values differed between $\bar{DP}_n$ 32 (16% of sulfuric acid for 20–40 days at 25°C) and $\bar{DP}_n$ 50 (incubation with $\alpha$-amylase from *B. subtilis* during 10 conversion periods).

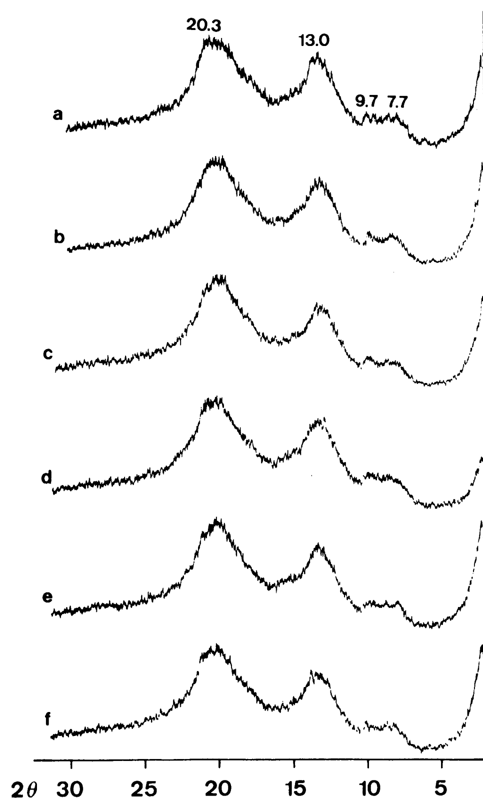


**Fig. 2.** X-ray diffraction patterns (V-type) of isolated amylose fractions of varying average chain length. Numbers above peaks indicate interplanar d-spacings in terms of $2\theta$. Average $\bar{DP}_n$ 610 (**a**), 373 (**b**), 262 (**c**), 131 (**d**), 94 (**e**), and 40 (**f**).

**TABLE II**
**Number Average Degree of Polymerization ($\bar{DP}_n$) of Amylose and of Enzyme-Resistant Starch (RS) Formed**

| $\bar{DP}_n$ Amylose | $\bar{DP}_n$ RS |
|---|---|
| 610 | 23 |
| 373 | 26 |
| 352 | 21 |
| 325 | 25 |
| 313 | 24 |
| 308 | 24 |
| 262 | 23 |
| 238 | 23 |
| 131 | 26 |
| 116 | 24 |
| 94 | 22 |
| 82 | 20 |
| 72 | 23 |
| 59 | 20 |
| 40 | 19 |

Thus, differences in the DP values can be rationalized by invoking differences in experimental conditions used for the RS formation and in hydrolytic reagents (enzymes) employed for the isolation of the resistant starches.

Table II shows that the chain length of the RS ($\bar{DP}_n$ 19–26) is independent of the chain length of the amylose ($\bar{DP}_n$ 610–40) from which it was formed. GPC of the amylose and the RS isolated from it confirmed these findings. From Figure 3 it is obvious that all isolated RS samples eluted at the same elution volume, no matter from which amylose fraction they were formed.

### X-Ray Diffraction of Isolated RS

The isolated RS fractions all showed a B-type X-ray diffraction pattern (Fig. 4). RS formed in starch gel also shows a B-type diffraction pattern when it is formed at such low temperatures (Berry et al 1988, Siljeström et al 1989, Sievert et al 1991, Eerlingen et al 1993).

Obviously, the average chain length of the amylose from which the RS was isolated had no influence on the type of crystals formed under the experimental conditions used. Many factors influence the polymorphic form of $\alpha$-(1→4)-glucans. Higher temperatures, higher concentrations, the presence of salts of high lyotropic number, the presence of water-soluble alcohols and organic acids, and, last, but not least, shorter average chain length of the amylose all favor the formation of the A-type polymorph (Gidley 1987). Gidley and Bulpin (1987) showed that an A-type polymorph can be obtained from debranched glycogen (average chain length 11.2) crystallized from hot aqueous solution of 50% at 30°C. Lower concentrations or lower temperatures yielded a B- or C-type polymorph. Pfannemüller (1987) demonstrated that the X-ray diffraction patterns of precipitated monodisperse amylose (10%) abruptly changed from B to A on going from DP 13 to DP 12. It thereby seems reasonable to assume that an A-type crystalline structure would not be formed in our experimental conditions ($\bar{DP}_n$ 40–610, 0.8% amylose solutions, 4°C).

### Yield of RS Formed from Amylose Fractions with Varying $\bar{DP}_n$

Figure 5 shows the yield of RS (%) as a function of the DP of the amylose from which it was formed. The yield of RS varied

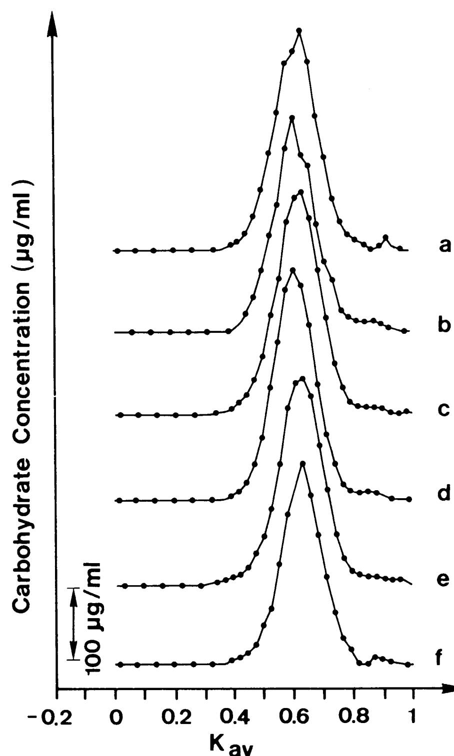


**Fig. 3.** Carbohydrate profiles of some resistant starch fractions by gel-permeation chromatography (Sepharose CL-6B). The resistant starch fractions were isolated from amylose with average $\bar{DP}_n$ of 610 (**a**), 373 (**b**), 262 (**c**), 131 (**d**), 94 (**e**), and 40 (**f**).

with average chain length of the amylose. Under the experimental conditions, a maximum yield of 28% RS was obtained ($\bar{DP}_n$ 260). Amylose with shorter chains obviously gave lower yields, whereas the amount of RS obtained from amylose with higher $\bar{DP}$ was only slightly lower.

Amylose with $\bar{DP}_n$ smaller than 100 contains a relatively high number of chains that do not have dimensions critical for incorporation in the crystal structure. It is likely that chains shorter than those of the crystalline zones (about 24 glucose units) will not take part very effectively in the crystallization process. It is, therefore, reasonable to expect that the yields of RS formed in amylose with short chains can be low. On the other hand, amylose samples with high $\bar{DP}$ contain relatively high proportions of long chains. Such chains can easily interact simultaneously over different regions of the chain with other amylose molecules. The data, however, suggest that even the dimensions of the largest amylose molecules used in the study ($\bar{DP}_n$ 610) are not sufficient to impair diffusion of the amylose molecules to the crystallization nuclei.

Even though the amylose fractions have different average chain lengths, most of them overlapped as shown in the gel chromatograms (Fig. 1). As only a maximum of 28% of the amylose fractions became enzyme resistant, the question arose whether amylose molecules with similar chain length or a certain interval of chain lengths are responsible for the enzyme resistance. From the following we could conclude that this is not the case. If amylose molecules with similar chain length or a certain interval of chain lengths were responsible for the enzyme resistance, such amylose molecules would have to occur in all the amylose fractions used to form RS. Because the amylose fraction with $\bar{DP}_n$ 610 yielded about 24% RS and the amylose fraction with $\bar{DP}_n$ 40 yielded about 14% RS, the amylose molecules responsible for the enzyme resistance must have comprised more than 24% of the amylose fraction with $\bar{DP}_n$ 610 and more than 14% of the amylose fraction with $\bar{DP}_n$ 40 (i.e., more than 24 and 14% of the area of the elution profiles of the amylose samples with $\bar{DP}_n$ 610 and $\bar{DP}_n$ 40 respectively, as shown in Figs. 1A and F). However, from Figure 1, it is obvious that such an interval would have had to comprise more than 50% of each of the amylose fractions with $\bar{DP}_n$ between 262 and 94. If this were the case, much higher yields of RS, compared to the other amylose fractions, would be expected. However, the RS yields that we obtained for the amylose fractions with $\bar{DP}_n$ 131 and 94 (fraction d and e in Fig. 1) were only 26 and 25%, respectively, whereas the RS yield of the amylose fraction with $\bar{DP}_n$ 610 (fraction a in Fig. 1) was 24%.

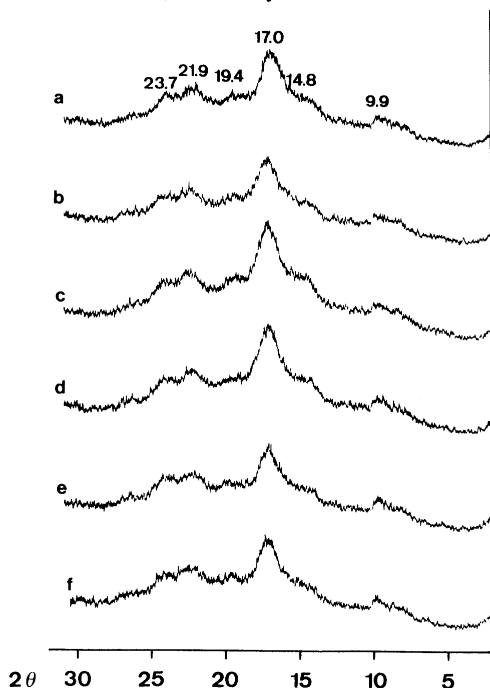


**Fig. 4.** B-type X-ray diffraction patterns of isolated resistant starch from amylose with average $\bar{DP}_n$ of 610 (**a**), 373 (**b**), 262 (**c**), 131 (**d**), 94 (**e**), and 40 (**f**). Numbers above peaks indicate interplanar d-spacings in terms of 2$\theta$.

**Proposed Mechanisms for RS Formation in Amylose Solutions**

The fact that the molecular weight of RS obtained from amylose of varying chain length does not depend on the chain length suggests that the mechanism for the formation of RS in amylose solutions may be the aggregation of amylose helices in a crystalline B-type structure (results of X-ray diffraction) over a particular region of the chain (about 24 glucose units). DP values of 24 glucosyl residues of the resistant fragments correspond to four turns of one of the amylose chains of a double helix. This is equivalent to a length of 84 Å (six glucosyl residues that repeat in 21 Å is typical of one strand of the model double helix [Zobel 1988]). Thus, the chain lengths in the crystalline regions (about 24 glucose units) are certainly only fractions of the total amylose molecule. Indeed, under normal crystallization conditions, the complete alignment of the polymer chains is not possible for reasons of entropy. Thus, the molecules are aligned only over relatively small regions of the chain. Besides, yield data (Fig. 3) (amylose of $\bar{DP}_n > 200$ yielded about 25% of RS with $\bar{DP}_n$ of 24) indicate that amylose molecules pass through more than one of these ordered regions in RS formation. As far as we can judge, micelle formation and chain folding (lamellar structures) both account for these observations.

Micelles can be formed by aggregation of a number of different molecules over a particular region of the chain in an ordered structure interspersed with amorphous regions (Jolley 1970, Levine and Slade 1990). In the case of retrograded B-type amylose, these ordered regions must be composed of double helices (Fig. 6) in a hexagonal structure to give a B-type of X-ray diffraction pattern.

Folding of the polymer chains leads to two-dimensional structures or lamellar shapes (Fig. 7). Molecular chains have a perpendicular orientation to the two-dimensional plane. The regions of the foldings are amorphous: the center of the lamella is crystalline. It seems logical that hydrolysis with amylolytic enzymes can remove these folding regions, and that molecules with short chains are obtained. In the case of retrograded B-type amylose, the crystalline center of the lamella would be composed of double helices ordered in a hexagonal structure.

It is well established that crystallization of amylose by complexation with alcohols gives rise to such lamellar structures (Yamashita and Hirai 1966, Yamashita and Monobe 1971, Whittam et al 1989, Buléon et al 1990, Brisson et al 1991, Welland and Donald 1991). Lamellar thickness in these experiments were determined to be about 100 Å. Thus, complexed amylose in dilute

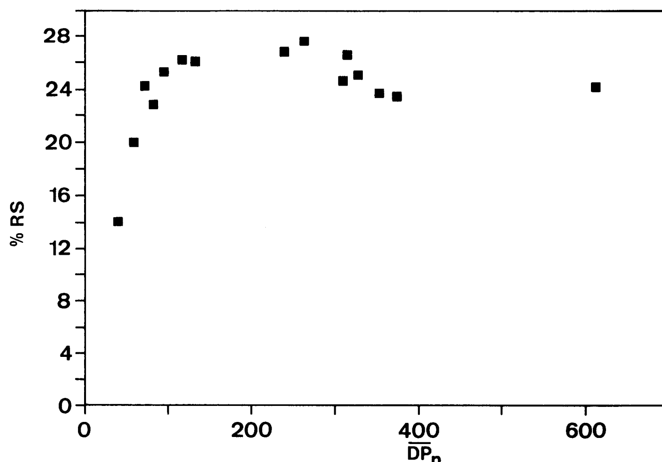


**Fig. 5.** Influence of the average chain length of amylose on the yield of resistant starch (RS) formed in an amylose solution (0.83%) after incubation (4°C) overnight. $\bar{DP}_n$ = number average degree of polymerization.

solution crystallizes in a manner similar to that of synthetic polymers in dilute solution. Hoffman and Lauritzen (Lauritzen and Hoffman 1973, Hoffman et al 1976, Hoffman et al 1979) proposed a kinetic theory for this kind of polymer crystallization that accounts for the experimentally observed dependence of the crystal (or lamellar) thickness and the crystal growth rate on temperature.

In contrast, very little is known about the mechanism of crystallization or the morphology of the crystals formed in aqueous amylose solution without complexation. Pfannemüller and Bauer-Carnap (1977) reported the formation of long fibrils and fibrillar aggregates from retrograded synthetic amylose in aqueous solution (0.1–1.0%) after ultrasonic treatment. The length of the fibrils, with a preferred diameter of about 100 Å, was largely independent of the molecular weight of the amylose. Jane and Robyt (1984) studied the hydrolysis of retrograded amylose with different reagents and proposed a structure for retrograded amylose (aqueous solution of 0.35%) as crystalline micelles (with a length of about 100 Å) interspersed with amorphous regions.

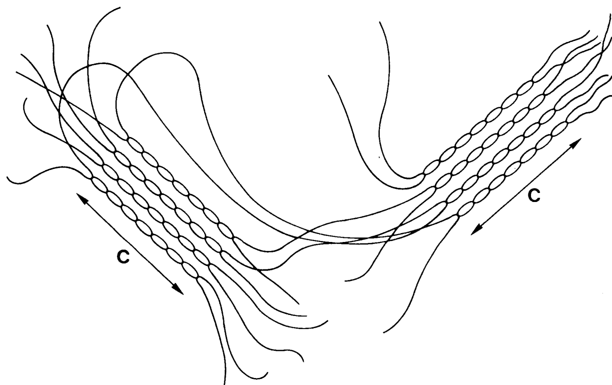


**Fig. 6.** Micelle model (schematic) for the formation of resistant starch in amylose solutions. Aggregation occurs over a particular region of the chain in an ordered structure (double helices in a hexagonal structure, **C**) interspersed with amorphous regions. Different micelles can be linked to each other by the amylose chains.

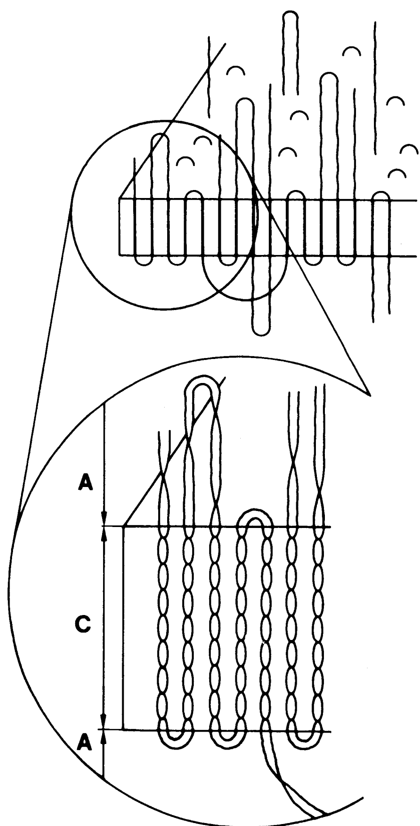


**Fig. 7.** Lamella (schematic) model for the formation of resistant starch in amylose solutions. Two-dimensional structures are formed by folding of the polymer chains. The fold zones are amorphous (**A**). The center of the lamella is crystalline (double helices in a hexagonal structure, **C**). Amylose chains connecting different lamellae can also occur.

## CONCLUSIONS

RS from amylose fractions with different average chain lengths ($\bar{DP}_n$ 40–610) formed by precipitation in aqueous solution, differed in yield but not in quality. Under the experimental conditions, the yield of RS increased with $\bar{DP}_n$ to plateau values of 23–28% RS. The isolated RS samples had B-type patterns and consisted of short chains ($\bar{DP}_n$ between 19 and 26). Because of the similarity in chain length of the RS, two plausible mechanisms are suggested for RS formation in aqueous amylose solutions. Besides micelle formation by aggregation of different amylose molecules over a short region of the chains, formation of lamellar structures by chain folding is also a possibility. More work is needed to differentiate between these two mechanisms.

### ACKNOWLEDGMENTS

R. C. Eerlingen thanks the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek for a research position as Aspirant. We thank H. J. Bosmans (KU Leuven) for making available the X-ray diffraction equipment. Technical assistance by L. Van den Ende is gratefully acknowledged.

### LITERATURE CITED

AMERICAN ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1985. Changes in methods: Total dietary fiber in foods. Enzymatic-gravimetric method. First action 43. A-14-43. A-20. J. Assoc. Off. Anal. Chem. 68:399.

BANKS, W., and GREENWOOD, C. T. 1975. Pages 5-66 in: Starch and Its Components. University Press: UK.

BERRY, C. S. 1986. Resistant starch: Formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fibre. J. Cereal Sci. 4:301-314.

BERRY, C. S., I'ANSON, I., MILES, M. J., MORRIS, V. J., and RUSSELL, P. L. 1988. Physical-chemical characterization of resistant starch from wheat. J. Cereal Sci. 8:203-206.

BRISSON, J., CHANZY, H., and WINTER, W. T. 1991. The crystal and molecular structure of $V_h$ amylose by electron diffraction analysis. Int. J. Biol. Macromol. 13:31-39.

BULEON, A., DUPRAT, F., BOOY, F. P., and CHANZY, H. 1984. Single crystals of amylose with a low degree of polymerization. Carbohydr. Polym. 4:161-173.

BULEON, A., DELAGE, M. M., BRISSON, J., and CHANZY, H. 1990. Single crystals of V amylose complexed with isopopanol and acetone. Int. J. Biol. Macromol. 12:25-33.

CLARK, A. J., GIDLEY, M. J., RICHARDSON, R. K., and ROSS-MURPHY, S. B. 1989. Rheological studies of aqueous amylose gels: The effect of chain length and concentration on gel modulus. Macromolecules 22:346-351.

CZUCHAJOWSKA, Z., SIEVERT, D., and POMERANZ, Y. 1991. Enzyme-resistant starch. IV. Effects of complexing lipids. Cereal Chem. 68:537-542.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

DYGERT, S., LI, L. G., FLORIDA, D., and THOMA, J. A. 1965. Determination of reducing sugar with improved precision. Anal. Biochem. 13:367-374.

EERLINGEN, R. C., CROMBEZ, M., and DELCOUR, J. A. 1993. Enzyme-resistant starch I. Quantitative and qualitative influence of incubation time and temperature of autoclaved starch resistant starch formation. Cereal Chem. 70:339-344.

GIDLEY, M. J. 1987. Factors affecting the crystalline type (A-C) of native starches and model compounds: A rationalisation of observed effects in terms of polymorphic structures. Carbohydr. Res. 161:301-304.

GIDLEY, M. J. 1989. Molecular mechanisms underlying amylose aggregation and gelation. Macromolecules 22:351-358.

GIDLEY, M. J., and BULPIN, P. V. 1987. Crystallization of malto-oligosaccharides as models of the crystalline forms of starch: Minimum

chain-length requirement for the formation of double helices. Carbohydr. Res. 161:291-300.
HOFFMAN, J. D., DAVIS, G. T., and LAURITZEN, J. I. 1976. The rate of crystallization of linear polymers with chain folding. Pages 497–614 in: Treatise on Solid State Chemistry, Vol. 3. X Hannay, ed. Plenum Press: New York.
HOFFMAN, J. D., GUTTMAN, C. M., and DIMARZIO, E. A. 1979. On the problem of crystallization of polymers from the melt with chain folding. Faraday Discuss. Chem. Soc. 68:177-197.
JANE, J. L., and ROBYT, J. F. 1984. Structure studies of amylose-V complexes and retrograded amylose by action of alpha amylases, and a new method for preparing amylodextrins. Carbohydr. Res. 132:105-118.
JOLLEY, J. E. 1970. The microstructure of photographic gelatin binders. Photogr. Sci. Eng. 14:169-177.
LAURITZEN, J. I., and HOFFMAN, J. D. 1973. Extension of theory of growth of chain-folded polymer crystals to large undercoolings. J. Appl. Phys. 44:4340-4353.
LEVINE, H., and SLADE, L. 1990. Influences of the glassy and rubbery states on the thermal, mechanical and structural properties of doughs and baked products. Pages 157–330 in: Dough Rheology and Baked Product Texture. H. Faridi and J. M. Faubion, eds. Van Nostrand Reinhold: New York.
PFANNEMÜLLER, B., and BAUER-CARNAP, A. 1977. Electron microscopic studies on fibrils formed from retrograded synthetic amyloses. Colloid & Polymer Sci. 255:844-848.
PFANNEMÜLLER, B. 1987. Influence of chain length of short monodisperse amyloses on the formation of A- and B-type X-ray diffraction patterns. Int. J. Biol. Macromol. 9:105-108.
PFANNEMÜLLER, B., MAYERHÖFER, H., and SCHULZ, R. C. 1971. Conformation of amylose in aqueous solution: Optical rotatory dispersion and circular dichroism of amylose-iodine complexes and dependence on chain length of retrogradation of amylose. Biopolymers 10:243-261.
RUSSELL, P. L., BERRY, C. S., and GREENWELL, P. 1989. Characterization of resistant starch from wheat and maize. J. Cereal Sci. 9:1-15.
SCHOCH, T. J. 1964. Iodimetric determination of amylose potentiometric titrations: Standard methods. Pages 157–160 in: Methods in Carbohydrate Chemistry, Vol 4. R. L. Whistler, ed. Academic Press: Orlando, FL.
SIEVERT, C., and POMERANZ, Y. 1989. Enzyme-resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical, and microscopic methods. Cereal Chem. 66:342-347.
SIEVERT, C., and POMERANZ, Y. 1990. Enzyme-resistant starch. II. Differential scanning calorimetry studies on heat-treated starches and enzyme-resistant starch residues. Cereal Chem. 67:217-221.
SIEVERT, C., CZUCHAJOWSKA, Z., and POMERANZ, Y. 1991. Enzyme-resistant starch. III. X-ray diffraction of autoclaved amylomaize VII starch and enzyme-resistant starch residues. Cereal Chem. 68:86-91.
SILJESTRÖM, M., ELIASSON, A.-C., and BJÖRCK, I. 1989. Characterization of resistant starch from autoclaved wheat starch. Starch/Staerke 41:147-151.
TAKEDA, Y., HIZKURI, S., TAKEDA, C., and SUZUKI, A. 1987. Structures of branched molecules of amyloses of various origins, and molar fractions of branched and unbranched molecules. Carbohydr. Res. 165:139-145.
WELLAND, E. L., and DONALD, A. M. 1991. Single crystals of V amylose. Int. J. Biol. Macromol. 13:69-72.
WHITTAM, M. A., ORFORD, P. D., RING, S. G., CLARK, S. A., PARKER, M. L., CAIRNS, P., and MILES, M. J. 1989. Aqueous dissolution of crystalline and amorphous amylose-alcohol complexes. Int. J. Biol. Macromol. 11:339-344.
YAMASHITA, Y., and HIRAI, N. 1966. Single crystals of amylose V complexes. II. Crystals with $7_1$ helical configuration. J. Polym. Sci. Part A-2. 4:161-171.
YAMASHITA, Y., and MONOBE, K. 1971. Single crystals of amylose V complexes. III. Crystals with $8_1$ helical configuration. J. Polym. Sci. Part A-2. 9:1471-1481.
ZOBEL, H. F. 1988. Molecules to granules: A comprehensive starch review. Starch/Staerke 40:44-50.

[Received September 24, 1992. Accepted December 30, 1992.]