

## Enzyme-Resistant Starch. IV. Effect of Endogenous Lipids and Added Sodium Dodecyl Sulfate on Formation of Resistant Starch

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### ABSTRACT

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Wheat starch (lipid content 0.54%) was defatted with different solvents (80% methanol, 80% methanol followed by petroleum ether, water-saturated butanol, water-saturated butanol followed by methanol, or petroleum ether, 70% propanol) and, subsequently, autoclaved and cooled to form resistant starch (RS). Defatting increased RS yields. This effect was much more pronounced than was predicted from calculations of the amount of complexed amylose; thus, the amylose zones complexed with ligands are not the only ones prevented from participating in the retrogradation process. When sodium dodecyl sulfate (SDS) was added

to defatted wheat or amylo maize VII starch, RS yields decreased substantially. X-ray diffraction and differential scanning calorimetry showed that amylose-lipid complexes had formed in the presence of both endogenous and added lipids (SDS). Thus, less amylose was available for interactions leading to the formation of double helices and RS. Adding SDS to the starch also caused a difference in RS quality. Changes in polymorphism (more A-type characteristics) and in the melting enthalpy of the isolated RS could be detected by X-ray diffraction and differential scanning calorimetry, respectively.

Starch, a major component in cereals, tubers, and pulses, is an important component in the diet of man. However, depending on the botanical source of the starch and industrial processing,

its digestibility may not be complete. Apart from factors extrinsic to the starch (differences in chewing the food, transit through the bowel, etc.), incomplete digestion of starch may be caused by different factors intrinsic to the starch. Englyst et al (1992) classified starch as *rapidly digestible*, *slowly digestible*, and *resistant starch* (RS). RS was further divided into three types: type I is physically inaccessible starch; type II is resistant granular starch; and type III is retrograded starch.

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In a previous study (Eerlingen et al 1993a), we demonstrated that the formation of RS type III can be considered a crystal-

lization process of amylose in a partially crystalline polymer system. Accordingly, it consists mainly of crystallized amylose.

Many factors may influence the crystallization process and, thus, the formation of RS type III. Apart from the influence of the water and amylose contents and the autoclaving temperature (Berry 1986, Sievert and Pomeranz 1989), the impact of the storage time and temperature of the starch gels (Eerlingen et al 1993a), and the influence of the amylose chain length (Eerlingen et al 1993b), were demonstrated. In our previous work, however, we did not consider the role of endogenous lipids. In their efforts, Czuchajowska et al (1991) and Szczodrak and Pomeranz (1992) showed that the addition of excess lipids to autoclaved high-amylose maize or barley starch reduced RS yields. Both differential scanning calorimetry (DSC) and X-ray diffraction analysis indicated the existence of amylose-lipid complexes. Amylose crystallization (RS formation) was competitively affected by complexation of the amylose with the lipids. Russell et al (1989) found an increased amount of RS in lipid-depleted starch. The authors, however, did not report whether gelatinization (and retrogradation) had occurred during defatting. In spite of the above-mentioned efforts, no profound study has investigated the significance of the endogenous lipids in RS formation.

According to Morrison (1988), lipids in starch samples can be classified as either true-starch lipids or as starch-surface lipids.

True-starch lipids are lipids inside native starch granules. They are free fatty acids and monoacyl lipids (lysophospholipids) exclusively. Although some investigators (Meredith et al 1978, Galliard and Bowler 1987, Zobel 1988) assumed that starch lipids are complexed with amylose in native starch granules, there are indications that amylose and lipids coexist independently and form complexes only during gelatinization (Kugimiya et al 1980, Stute and Konieczny-Janda 1983, Biliaderis et al 1986, Morrison et al 1993).

Starch-surface lipids are lipids acquired from the surrounding matrix. It is unclear to what extent these have penetrated the starch granules or how they are bound (Morrison 1988). Such lipids can be extracted by different solvents at room temperature. The true-starch lipids, however, are contained in the dense structure of the granule and not easily extracted. The best solvents are generally hot alcohol-water mixtures (Morrison and Coventry 1985). Such defatting procedures can, however, disturb the granule structure.

Although lipids are a minor constituent of cereal starches (up to 1.0%), they have a remarkable influence on the gelatinization and retrogradation of the starch (Lorenz 1976, Eliasson et al 1981, Takahashi and Seib 1988, Hibi et al 1990).

Therefore, the main objective of this work was the study of the influence of endogenous lipids on RS (type III) formation. We also investigated the effect of sodium dodecyl sulfate (SDS) addition on formation of RS (type III).

## MATERIALS AND METHODS

Wheat starch (Meriwit AA) was supplied by Amylum (Aalst, Belgium); amylo maize VII with an amylose content of 75% (Eurylon 7) was supplied by Roquette (Lestrem, France). Enzymes used for isolation of RS were: Termamyl, a thermostable  $\alpha$ -amylase (Novo Nordisk, Bagsvaerd, Denmark); AMG, an amyloglucosidase (Novo Nordisk); and protease (P5147, Sigma Chemical, St. Louis, MO). SDS was obtained from Riedel-de-Haën (Seelze, Germany). In contrast to most complexing lipids, SDS is soluble in cold water, and, for this reason, we chose to work with this compound. Indeed, an excess of the substance can easily be removed in the RS isolation procedure.

### Defatting of Starch

Defatting with 80% methanol (80% MeOH), water-saturated butanol (WSB), or 70% *n*-propanol (70% PrOH) was performed by the method of Schoch (1964). Starch (400 g) was suspended in 1,200 ml of solvent and refluxed. After 2 hr, the mixture was filtered over a Büchner filter and washed with the same solvent. Refluxing, filtration, and washing were repeated twice.

Part of the starch defatted with 80% MeOH and part of the starch defatted with WSB was defatted with a second solvent (petroleum ether or MeOH) by a Soxhlet extraction (Schoch 1964) for 24 hr. Defatted starches were air-dried at 30°C.

### Fat Content

Fat content in defatted and nondefatted starch samples were determined after acid hydrolysis. About 5.00 g of starch was accurately weighed and suspended in 50.0 ml of water. HCl 8.0*N* (50.0 ml) was added, and the mixture was heated in a vigorously boiling water bath for 25 min. After addition of 100.0 ml of water, the mixture was filtered through paper and washed with water until the filtrate was neutral. The filter paper with the residue was dried overnight at room temperature and transferred to a defatted extraction thimble. A glass-wool plug was placed over the paper. The thimble with the paper was dried at 100°C for 4 hr. The dried samples were Soxhlet-extracted with petroleum ether. The recipients with the extracted fat were then dried at 110°C to constant weight. Determinations were performed in duplicate.

### Moisture Content

Moisture content in defatted and native wheat starches was determined according to AACC method 44-15A (1983).

### Formation of RS

To investigate the influence of defatting, native or defatted wheat starch samples (1.00 g) were autoclaved in excess water for 1 hr at 121°C and stored at 4°C overnight. This heating-cooling cycle was repeated once.

In the study of the influence of SDS on RS formation, wheat starch and amylo maize starch samples that had been defatted with 80% MeOH and petroleum ether were subsequently autoclaved for 1 hr at 121°C in a solution containing different amounts of SDS (0, 0.25, 1, 2, 3, 4, and 5% of starch weight for wheat starch; 0, 1, 2, 5, and 10% of starch weight for amylo maize starch). The samples were then stored at 4°C for 5 hr and at 80°C overnight.

### Isolation of RS

RS was isolated from the above mixtures with Termamyl, amyloglucosidase, and protease as described in Eerlingen et al (1993a). All determinations were performed in duplicate. Yields are given as percent of starch (dmb).

### X-Ray Diffraction

X-ray powder diffraction analysis was performed with a PW 10050/25 diffractometer (Philips, MBL, Brussels, Belgium) with the following operating conditions: 40 kV and 20 mA, CuK  $\alpha$  radiation at 0.154 nm, and a nickel filter. Diffractograms of the samples were obtained from 2–30° 2 $\theta$ .

### DSC

DSC measurements were performed with a DSC-120 (Seiko, Japan). Indium and tin were used as standards.

TABLE I  
Results of Defatting Wheat Starch in Various Solvents

Solvent	Defatting Efficiency, % <sup>a</sup>	Degree of Gelatinization	
		DSC, % <sup>b</sup>	LPCI <sup>c</sup>
None	0.0	0.0	0
80% Methanol (MeOH)	53.9	9.0	0
80% MeOH + Petroleum ether	54.4	10.8	0
Water-saturated butanol (WSB)	71.1	32.4	+
WSB + MeOH	73.0	36.0	+
WSB + petroleum ether	72.2	27.9	+
70% Propanol	88.7	88.3	++

<sup>a</sup> % of fat removed from native starch.

<sup>b</sup> % of loss in gelatinization enthalpy determined by differential scanning calorimetry.

<sup>c</sup> Loss of polarization cross intensity. 0 = no loss, + = some loss, ++ = almost all lost.

TABLE II  
Thermal Transition Temperatures of Native and Defatted Wheat Starch

Solvent	60°C Transition			100°C Transition		
	$T_o^a$	$T_p^b$	$\Delta H^c$	$T_o^a$	$T_o^b$	$\Delta H^c$
None	53.2	59.0	11.1	95.3	101.1	0.7
80% Methanol (MeOH)	54.9	59.3	10.1	nd <sup>d</sup>	nd	nd
80% MeOH + Petroleum ether	54.4	58.6	9.9	nd	nd	nd
Water-saturated butanol (WSB)	59.2	63.8	7.5	nd	nd	nd
WSB + MeOH	58.8	63.5	7.1	nd	nd	nd
WSB + petroleum ether	59.6	63.8	8.0	nd	nd	nd
70% Propanol	62.2	67.7	1.3	nd	nd	nd

<sup>a</sup> Onset temperature (°C).

<sup>b</sup> Peak temperature (°C).

<sup>c</sup> Enthalpy (mJ/mg).

<sup>d</sup> Not detected.

The technique was used to determine the degree of gelatinization of the defatted starches. About 5 mg of sample was weighed into aluminium pans, and water (2× the the sample weight) was added. An empty pan served as reference sample. The starches were heated from 20 to 130°C at a scanning rate of 5°C/min.

DSC was also used to study the autoclaved amylo maize starch preparations and the isolated RS fractions. To that end, about 10 mg of sample was weighed into stainless steel pans, and water (2× the sample weight) was added. In these experiments, 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 ( $\Delta H$ ) of the transition were determined with Seiko software. All analyses were performed in duplicate at least.

#### Polarization Microscopy

Defatted and nondefatted starches were viewed under polarized light (400×) using an Olympus BHS laboratory binocular microscope.

### RESULTS AND DISCUSSION

#### RS

As outlined earlier (Eerlingen et al 1993a), RS determined in the starch gels with the in vitro methodology of the current study is not necessarily the same as starch resisting enzymic hydrolysis under in vivo conditions (with mammalian enzymes at 37°C). Indeed, the in vitro hydrolysis with pancreatin at 37°C of both retrograded amylopectin (R. C. Eerlingen, unpublished data) and amylose-lipid complexes (Holm et al 1983, Seneviratne and Biliaderies 1991), depends on incubation time, enzyme-to-substrate ratio, and the degree of organization of the substrate. It is, however, not clear to what extent retrograded amylopectin and amylose-lipid complexes are digested in vivo. In this study, both a heat-stable  $\alpha$ -amylase (at 100°C) and an amyloglucosidase (at 60°C) were used to isolate RS. These severe conditions have some advantages. Because retrograded amylopectin and amylose-lipid complexes are hydrolyzed under these conditions, the resulting model system is a simplified one without retrograded amylopectin and amylose-lipid complexes in the isolated fraction. In addition, our approach has the definite advantage of easily comparing the results with those of other authors (Björck et al 1987; Siljeström et al 1989; Sievert and Pomeranz 1989, 1990; Czuchajowska et al 1991; Sievert et al 1991).

#### Defatted Wheat Starch

Wheat starch was defatted with different solvents or a combination of solvents. Table I shows the percentage of fat removed from native starch and the degree of gelatinization that occurred during defatting. The degree of gelatinization resulting from the defatting process was calculated as the loss of the  $\Delta H$  determined with DSC (Table II). We, thus, assumed that the difference in the lipid content of the starch had no significant effect on the  $\Delta H$ . In addition to a decrease in  $\Delta H$ , we also noticed an increase in the  $T_o$  and  $T_p$  of the gelatinization endotherm as a result of defatting (Table II). The findings are in agreement with results

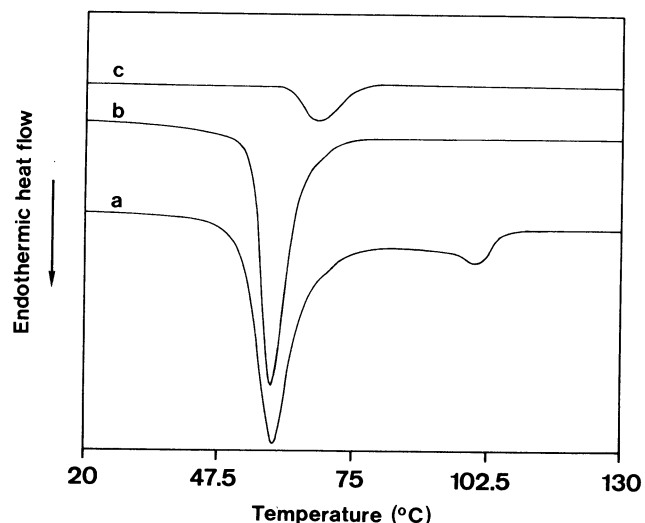


Fig. 1. Differential scanning calorimetry thermograms of native wheat starch (a), wheat starch defatted with 80% methanol followed by petroleum ether (b), and wheat starch defatted with 70% propanol (c).

by Lorenz (1976), who analyzed samples defatted with 80% MeOH (Soxhlet).

Typical DSC thermograms of defatted and native wheat starch heated in water are shown in Figure 1. The gelatinization endotherm was characterized by a  $T_p$  at ~60°C. An additional endotherm was observed at 101°C for native starch and was attributed earlier to the melting of amylose-lipid complexes (Eberstein et al 1980, Kugimiya et al 1980). Indeed, we observed this endotherm only in the native starch and not in the defatted samples (Table II).

Table I further shows that the extent of delipidation of the wheat starch by reflux with the different solvents used (with or without Soxhlet extraction) depended on the solvent used during the reflux step. Defatting with 70% PrOH was the most efficient (about 89% of the lipids were removed after defatting), followed by WSB (about 72%), and finally 80% MeOH (about 54%). An additional defatting step with the Soxhlet method after refluxing did not significantly increase the defatting efficiency.

The degree of gelatinization of the defatted starch followed the same order as the extent of defatting. Starch defatted with 70% PrOH (defatting efficiency 89%) was almost completely gelatinized, while starch defatted with WSB (defatting efficiency 72%) was gelatinized at about 32%, and starch defatted with 80% MeOH (defatting efficiency 54%) was gelatinized at about 10%. It seems reasonable to assume that the extraction of the true-starch lipids contained in the dense granule structure was facilitated when the starch granule was more swollen or disrupted (gelatinized).

The influence of the defatting process on the granule order was also investigated by polarization microscopy. No loss of polarization cross could be detected for the starch samples defatted

with 80% MeOH. Some granules of the starch samples defatted with WSB either lost their polarization cross or had crosses that were not as pronounced as those of the native starch. Only a few polarization crosses could be detected in the starch defatted with 70% PrOH. These results were in agreement with the DSC results. Defatting with 80% MeOH caused only minor changes in the gelatinization enthalpy. On the other hand, defatting starch with WSB decreased the gelatinization enthalpy somewhat, and defatting starch with 70% PrOH decreased the gelatinization enthalpy to a great extent.

X-ray diffraction patterns of the native and defatted wheat starch samples are shown in Figure 2. All samples showed an A-type diffraction pattern, except for wheat starch defatted with propanol 70%, which showed no significant peaks. From Table I, it is obvious that this starch sample had been gelatinized during defatting and, thus, had lost its crystallinity. In general, the intensity of the diffraction peak at  $20^\circ$   $2\theta$  was reduced after defatting, except for the starch sample defatted with both WSB and petroleum ether. Indeed, the peak has been interpreted as a V-form due to amylose-lipid complexes (Zobel 1988).

### RS Yield of Native and Defatted Wheat Starch

Table III shows the RS yields of native and defatted starch before and after autoclaving. RS yields increased significantly as a result of autoclaving, as already demonstrated (Berry 1986, Sievert and Pomeranz 1989).

The relatively high RS yield before autoclaving for starch defatted with 70% PrOH can be ascribed to the fact that this starch sample was almost completely gelatinized (Table II). During gelatinization, amylose molecules leach out of the granule, the free amylose molecules may aggregate, and RS (type III) may be formed.

From Table III, it is also obvious that RS yields of autoclaved starch samples were higher as a result of defatting, no matter

the extent of gelatinization occurring during the fat extraction. Therefore, endogenous lipids, although they are a minor constituent of starch, have a significant influence on RS formation. As a result of defatting, fewer lipids are present that can form enzyme-digestible complexes with amylose during gelatinization. Thus, more amylose molecules are free to interact with each other to form double helices and to aggregate, resulting in higher RS yields.

It was not clear why the RS yields of partly defatted starches rose to this extent; indeed, removal of a fraction of the lipids present in the starting material (0.54%) lead to increases in RS yields from 5.9% (nondefatted sample) to anywhere within a 7.0–9.0% interval (see Table III).

From literature data (Karkalas and Raphaelis 1986; Morrison 1988), we can calculate that lipids can bind ~6 their own weight of amylose. Thus, 0.54% lipids can bind ~3.24% of amylose, lowering the amount of amylose theoretically available for RS formation from 25% (amount of amylose in wheat starch) to ~21.76% (25% - 3.24%). The effect of the native lipids on the availability of amylose for RS formation is probably even more pronounced than that described above, because it is highly unlikely that complexed amylose regions alone do not take part in the double helix formation. Complexed regions probably result in steric hindrance for interactions of neighboring segments, thereby preventing association of amylose chains with a certain minimum chain length (Eerlingen et al 1993a).

Accordingly, it seems logical that a removal of lipids has a drastic effect on the quantity of RS formed.

### RS Yield of Autoclaved Nondefatted and Defatted Wheat Starch and Amylo maize Starch in SDS Solution

Defatting wheat starch with 80% MeOH (and petroleum ether) caused little disturbance of the granule structure compared with that observed with WSB and 70% PrOH treatments, so we used 80% MeOH and petroleum ether to defat the amylo maize VII starch. The defatting efficiency was determined to be 75.3%.

RS yield of autoclaved defatted starch decreased significantly when SDS was added (Fig. 3), and this reduction was more sig-

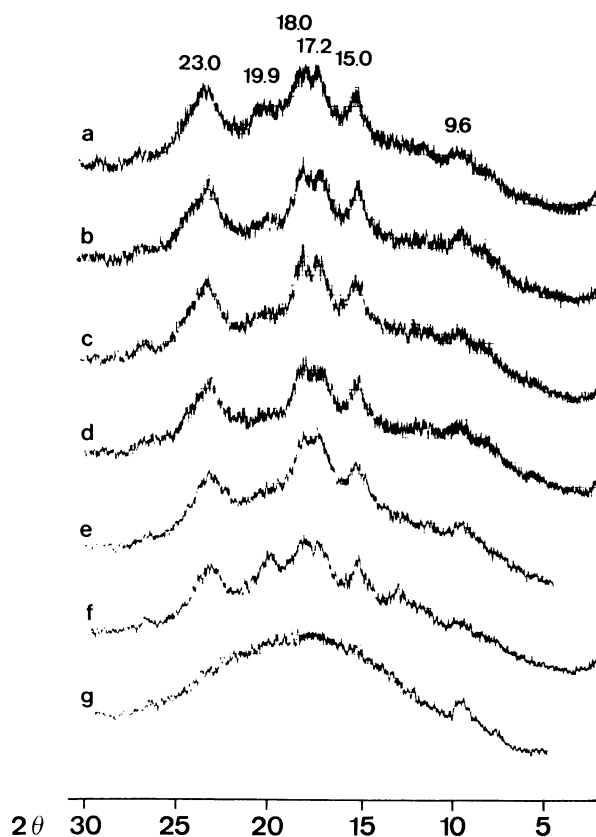


Fig. 2. X-ray diffraction patterns (A-type) of native wheat starch (a) and wheat starch defatted with 80% methanol (b), 80% methanol followed by petroleum ether (c), water-saturated butanol (d), water-saturated butanol followed by methanol (e), water-saturated butanol followed by petroleum ether (f), and 70% propanol (g). Numbers above peaks indicate interplanar  $d$ -spacings in terms of  $2\theta$ .

TABLE III  
Resistant Starch (%) in Native and Defatted Wheat Starch

Solvent	Resistant Starch (%)	
	Before Autoclavation	After Autoclavation
None	1.0	5.9
80% Methanol (MeOH)	0.7	7.4
80% MeOH + Petroleum ether	0.3	8.8
Water-saturated butanol (WSB)	1.2	8.3
WSB + MeOH	1.1	9.0
WSB + petroleum ether	0.3	8.0
70% Propanol	2.4	7.0

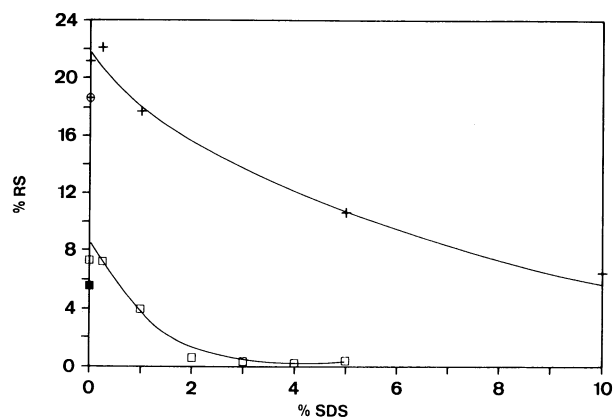


Fig. 3. Resistant starch yield (% RS) of autoclaved nondefatted wheat starch (■) and amylo maize starch (⊕), as well as defatted wheat starch (□) and amylo maize starch (+) autoclaved in the presence of increasing levels of sodium dodecyl sulfate.

nificant when more SDS was present. Addition of 3% SDS, or more, could reduce the RS yield of autoclaved wheat starch to almost negligible readings. These results are in agreement with the findings of Sievert, Pomeranz, and coworkers (Czuchajowska et al 1991, Szczodrak and Pomeranz 1992), who also observed a decrease in RS yield after addition of 10% complexing lipids to autoclaved high-amylose corn starch.

SDS is a known complexing agent for amylose (Eliasson 1985, Biliaderis and Tonogai 1991). It can interact with the amylose leaching out of the granule during gelatinization, thereby forming amylose-lipid complexes and reducing the availability of amylose for retrogradation. Furthermore, although amylose-lipid complexes are quite resistant to enzymatic attack, incubation with a thermostable  $\alpha$ -amylase (Termamyl) at 100°C, as in our procedure, leads to hydrolysis of the amylose-lipid complexes (Holm et al 1983). The resulting free SDS in this isolation procedure is then removed by washing and filtration. Consequently, adding SDS before starch autoclaving leads to lower RS yields.

Another factor needs to be considered, however. Biliaderis and Tonogai (1991) demonstrated that SDS decreases the gelatinization temperature. This was ascribed to the fact that SDS facilitates granule disruption during heating. As a result of a facilitated granule disruption, one would expect higher RS yields, rather than lower yields. This factor, therefore, is probably overruled by the reduced availability of amylose for retrogradation.

The RS yield of autoclaved nondefatted starch is also shown in Figure 3 (0% SDS). Again, we notice that defatting wheat starch or amylo maize starch with 80% MeOH and petroleum ether increases the RS yield after autoclaving: from 5.6 to 7.3% RS in wheat starch and from 12.7 to 21.7% RS in amylo maize starch.

From the SDS-C<sub>12</sub> chain length (1.86 nm), one can calculate that it is contained in 2.3 turns of the amylose helix; therefore, one mole of SDS (272 g) can theoretically (at full saturation) interact with ~2,268 g of amylose. It follows that, when 4 and 5% SDS were added to wheat starch (amylose content 25%) or 10% was added to amylo maize VII starch (amylose content 75%), there is an excess of SDS. In theory, when such quantities of SDS were used, all amylose could be complexed and, thus, no amylose would have been available for retrogradation and RS

formation. In the case of wheat starch, we observed that the RS yields were indeed negligible when 3% (saturation) or more (excess) SDS was present. This is in line with the suggestion by Slade and Levine (1987) that formation of amylose-lipid complexes is favored over amylose retrogradation. On the other hand, with amylo maize starch, significant RS yields were observed in spite of the use of an excess of SDS (10%). This would seem to indicate that, in the latter case, amylose-lipid complex formation is not favored over the retrogradation process, although a competition between the two phenomena may still exist, as recently suggested by Czuchajowska et al (1991). In addition, the difference in gelatinization behavior of the two starches may have contributed to different conditions for interaction with SDS and, accordingly, to different impacts on RS yields.

#### Influence of SDS on the X-Ray Diffraction Patterns of Autoclaved Defatted Amylo maize Starch and Its Isolated RS Fractions

Figure 4 shows the X-ray diffraction patterns of autoclaved defatted amylo maize starch. With no SDS added before autoclaving the starch, a B-type diffraction pattern was obtained with a strong reflection at  $2\theta$  16.9°, and weaker reflections were obtained at  $2\theta$  8.3, 9.5, 19.4, and 22.0°. It is obvious that the reflections around  $2\theta$  17 and 22° gradually disappeared, while the reflection around 20° continuously increased. A new peak around 13° appeared when increasing concentrations of SDS were added. Thus emerged a V-type of pattern, typical for amylose-lipid complexes (Zobel 1964, Kugimiya et al 1980, Zobel 1988).

Free SDS could not be detected in the autoclaved starch samples. X-ray diffraction of SDS treated in the same way as a control (in the absence of starch) yielded a pattern with very strong reflections at  $2\theta$  2.2 and 2.7° (data not shown). These peaks could not be detected in the patterns of autoclaved starch, even when 10% (w/w) SDS was added.

When RS was isolated from the autoclaved amylo maize starch samples by enzymatic hydrolysis, the X-ray diffraction patterns differed from those of the autoclaved samples before hydrolysis (Fig. 5). When no SDS was added before autoclaving, RS isolated from the defatted amylo maize starch sample showed a B-type pattern (Fig. 5a) that was slightly more pronounced than was

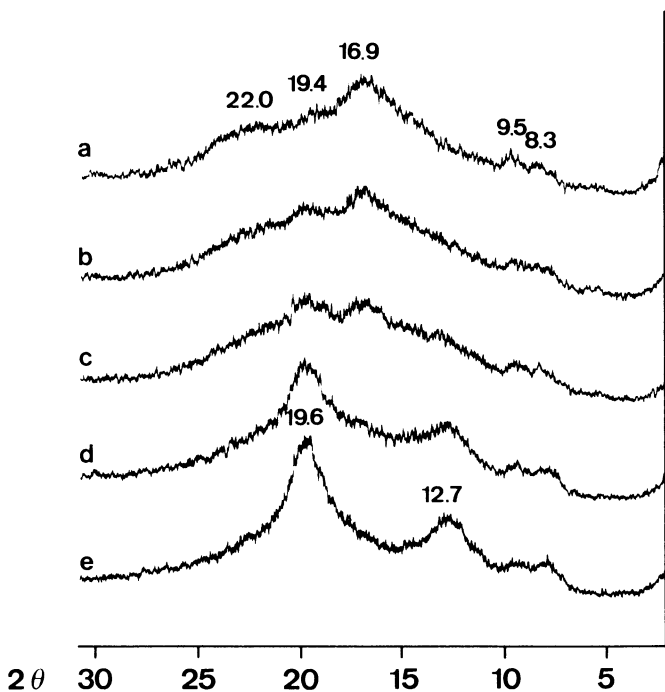


Fig. 4. X-ray diffraction patterns of amylo maize starch, defatted with 80% methanol and petroleum ether and autoclaved in solution containing different amounts of sodium dodecyl sulfate (a-e = 0, 1, 2, 5, and 10%, respectively). Pattern gradually changed from B-type to V-type. Numbers above peaks indicate interplanar  $d$ -spacings in terms of  $2\theta$ .

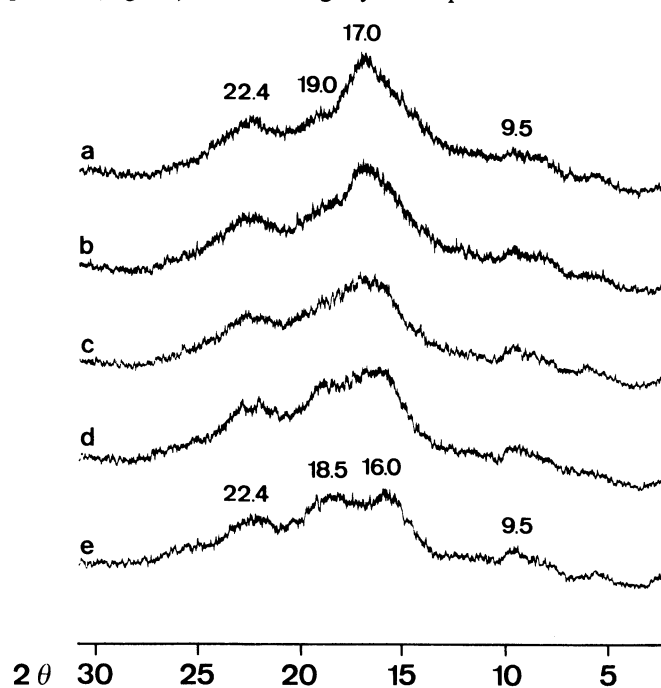


Fig. 5. X-ray diffraction patterns of resistant starch isolated from amylo maize starch, defatted with 80% methanol and petroleum ether and autoclaved in solution containing different amounts of sodium dodecyl sulfate (a-e = 0, 1, 2, 5, and 10%, respectively). Pattern gradually changed from B-type to one with more A-type characteristics. Numbers above peaks indicate interplanar  $d$ -spacings in terms of  $2\theta$ .

the pattern of the autoclaved starch from which it was isolated (Fig. 4a). Apart from the difference in moisture content of the autoclaved, freeze-dried, starch samples and the isolated RS residues, a difference in degree of crystallinity was expected. Enzymes used for the isolation of RS preferably hydrolyzed the amorphous parts in the autoclaved starch, so the residue (RS) we obtained had a higher degree in crystallinity and a more pronounced X-ray diffraction pattern. A B-type pattern is characteristic for RS material formed at low temperature (Berry et al 1988; Siljeström et al 1989; Czuchajowska et al 1991; Sievert et al 1991; Eerlingen et al 1993a,b).

When SDS (1, 2, 5, and 10%) was added to the amylo maize starch, the B-type X-ray diffraction pattern of the RS residue gradually gained more of the characteristics of an A-type pattern (Fig. 5). The reflection around  $17^\circ 2\theta$  gradually decreased, and peaks at  $\sim 16$  and  $18.5^\circ 2\theta$  appeared. Hizukuri and coworkers have demonstrated that both inorganic ions and aliphatic hydrocarbon groups can influence the polymorphic form of amylo-dextrin crystals (Hizukuri et al 1960, 1980). The authors demonstrated that aliphatic alcohols and fatty acids influenced the type of crystallinity (more A-type characteristics), and that this effect increased with the size of the hydrocarbon group. They observed the same phenomenon when inorganic salts (e.g., sodium salts) were added in increasing concentration. It seems reasonable that SDS with a chain of 12 carbons and a sodium ion may, in the same way, influence the polymorphic form of isolated RS residues.

Thus, the presence of lipids not only has an impact on RS yield but can also have an influence on RS quality (polymorphism).

#### Thermoanalytical Characteristics of Autoclaved Amylo maize Starch and Its Isolated RS Fractions

Table IV lists DSC data for defatted amylo maize starch autoclaved with and without addition of SDS. All samples showed an endotherm with a  $T_p$  temperature at  $\sim 150^\circ\text{C}$  (Fig. 6). This has been attributed to the melting of amylose crystals (Ring et al 1987). Our observations are in good agreement with earlier results on autoclaved starch and RS (Sievert and Pomeranz 1989, Czuchajowska et al 1991, Szczodrak and Pomeranz 1991).

The transition endotherm at  $90^\circ\text{C}$ , which could be detected when sufficient SDS (5 or 10%, w/w) had been added to the amylo maize starch before autoclaving (Fig. 6), is due to the melting of amylose-SDS complexes (Biliaderis and Tonogai 1991). The melting enthalpy increased from 5.1 mJ/mg to 8.2 mJ/mg when the SDS concentration was increased from 5 to 10% (w/w). The concentration of the amylose-SDS complexes was probably too low to be detected by DSC when only 1 or 2% SDS had been added. Indeed, from the X-ray diffraction analysis (Fig. 3), it was already clear that the pattern gradually changed from the B-type to the V-type, the latter being characteristic of amylose-lipid complexes. The typical V reflections at  $2\theta 20$  and  $13^\circ$  were still weak for autoclaved defatted amylo maize starch with 1 and 2% SDS; they were very evident for the defatted amylo maize starch autoclaved in 5 and 10% SDS.

Comparison of the enthalpy values of the endotherm at  $150^\circ\text{C}$  (Table IV) and RS yields (Fig. 2), shows that the transition

enthalpy did not decrease in the same way as the RS yield. This could be explained by examining the thermal characteristics of the isolated RS residues. The thermograms of the RS residues isolated from the autoclaved defatted amylo maize starch samples showed only one endotherm at  $150^\circ\text{C}$  due to the melting of amylose crystals (Fig. 6). No amylose-lipid complexes could be detected. Indeed, in the isolation procedure for RS, Termamyl (a thermostable  $\alpha$ -amylase) was used at  $100^\circ\text{C}$ . Under such conditions, amylose-lipid complexes are hydrolyzed (Holm et al 1983) as explained above. By incubating the autoclaved starches with amylases (amyloglucosidase and Termamyl), the degradable structures are removed. Thus, RS present in the autoclaved starch was concentrated, and higher enthalpy values of the transition at  $150^\circ\text{C}$  were obtained (Tables IV and V).

The data in Table V show that, while the  $T_o$  decreased with increasing concentrations of SDS ( $140.0$ – $132.6^\circ\text{C}$ ), the  $T_p$  of the endotherm remained almost the same ( $151.3$ – $149.0^\circ\text{C}$ ). The enthalpy values of the transition increased substantially with increasing levels of SDS (from 11.7 to 26.6 mJ/mg). This shows that a decrease in RS yield (Fig. 3) was accompanied by an increase in melting enthalpy, and, thus, by a change in quality of the isolated RS when more SDS was added to the starch. The increase in melting enthalpy of RS in the presence of SDS may result from the change in polymorphic form from the B-type to the A-type (Fig. 5), or from an increase in the proportion of ordered domains in the RS. A higher proportion of ordered domains requires more energy for dissociation, but it does not necessarily

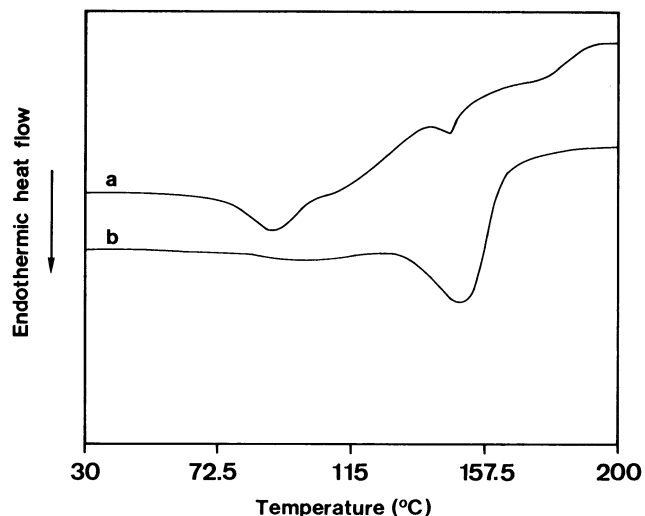


Fig. 6. Typical differential scanning calorimetry thermograms of defatted amylo maize starch autoclaved in solution containing 5% sodium dodecyl sulfate with an endotherm at  $\sim 90^\circ\text{C}$  (peak temperature) of the melting of the amylose-sodium dodecyl sulfate complexes and an endotherm at  $\sim 150^\circ\text{C}$  (peak temperature) attributed to the melting of the amylose crystals (a); and of resistant starch isolated from defatted amylo maize starch autoclaved in solution containing 5% sodium dodecyl sulfate with an endotherm at  $\sim 150^\circ\text{C}$  (peak temperature) (b).

TABLE IV  
Thermal Transition Characteristics of Autoclaved Defatted Amylo maize Starch in Different Concentrations of Sodium Dodecyl Sulfate (SDS) (% w/w)

% SDS	90°C Transition			150°C Transition		
	$T_o^a$	$T_p^b$	$\Delta H^c$	$T_o^a$	$T_o^b$	$\Delta H^c$
0	nd <sup>d</sup>	nd	nd	144.9	148.7	3.3
1	nd	nd	nd	146.9	149.8	3.1
2	nd	nd	nd	142.7	148.7	3.9
5	79.2	89.3	5.1	144.4	148.1	3.6
10	82.2	91.4	8.2	141.4	143.4	2.4

<sup>a</sup> Onset temperature ( $^\circ\text{C}$ ).

<sup>b</sup> Peak temperature ( $^\circ\text{C}$ ).

<sup>c</sup> Enthalpy (mJ/mg).

<sup>d</sup> Not detected.

TABLE V  
Thermal Transition Characteristics of Autoclaved Defatted Amylo maize Starch in Different Concentrations of Sodium Dodecyl Sulfate (SDS) (% w/w)

% SDS	150°C Transition		
	$T_o^a$	$T_p^b$	$\Delta H^c$
0	140.0	151.3	11.7
1	138.4	149.2	14.5
2	135.1	149.3	16.2
5	133.5	149.0	21.5
10	132.6	149.2	26.6

<sup>a</sup> Onset temperature ( $^\circ\text{C}$ ).

<sup>b</sup> Peak temperature ( $^\circ\text{C}$ ).

<sup>c</sup> Enthalpy (mJ/mg).

**TABLE VI**  
**Measured and Calculated Enthalpies (mJ/mg)**  
**of Amylo maize Starch Autoclaved in Different Concentrations**  
**of Sodium Dodecyl Sulfate (SDS) (% w/w)**

% SDS	Measured Enthalpy	% RS <sup>a</sup> Yield	×	Enthalpy of RS	=	Calculated Enthalpy
0	3.3	21.2		11.7		2.5
1	3.1	22.1		14.5		3.2
2	3.9	17.7		16.2		2.9
5	3.6	10.7		21.5		2.3
10	2.4	6.4		26.6		1.7

<sup>a</sup> Resistant starch.

contribute to X-ray crystallinity. No increase in crystallinity was detected by X-ray diffraction analysis (Fig. 5).

The interplay of a decrease in RS yield and an increase in melting enthalpy of the isolated RS per weight unit, accounted for the observed enthalpy values of the transition at 150°C of the autoclaved defatted amylo maize starch samples, with increasing concentrations of SDS (Table IV). Table VI shows that when the enthalpy values of the transition at 150°C of the autoclaved starch samples were calculated as the product of the RS yield, and the enthalpy value of the RS isolated from the specific autoclaved starch sample, the same tendency could be found. Absolute values were only slightly lower.

## CONCLUSIONS

Both added and endogenous lipids, although present in low quantities, have an influence on RS (type III) formation in starch. Endogenous and added lipids can form enzyme-digestible inclusion complexes with amylose. Less amylose is then available to form enzyme-resistant double helices. Thus, RS yields of autoclaved starch increase by defatting starch and decrease by addition of exogenous lipids. Minor quantities of lipids may cause significant changes in RS yield by occupying segments of the amylose chain (amylose-lipid complexation) and by causing steric hindrance for formation of double helices in the uncomplexed segments of the chain.

When SDS was added, we observed not only a reduction in RS quantity, but also an influence on RS quality as demonstrated by the change in polymorphism observed with X-ray diffraction analysis and by the increase in enthalpy of the melting of the isolated RS determined by DSC. Although the endothermic transition at 150°C has been attributed to the melting of crystalline amylose and is a measure for RS type-III, the enthalpy values of the transition will not necessarily be in line with the RS yields determined by enzymatic isolation. Indeed, differences in crystal quantity, as well as differences in crystal quality of the RS, can cause variation in the melting enthalpy (expressed as mJ/mg starch sample).

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