Enzyme-Resistant Starch. IV. Effects of Complexing Lipids[1]

Z. CZUCHAJOWSKA, D. SIEVERT, and Y. POMERANZ[2]

Cereal Chem. 68(5):537-542

ABSTRACT

Amylomaize VII starch, autoclaved at 125°C, was reacted during cooling below 100°C with lysophosphatidyl choline (LPC), sodium stearoyl lactylate (SSL), and hydroxylated lecithin (OHL). Differential scanning calorimetry (DSC) peaks at around 95-110°C indicated formation of lamylose-lipid complexes and at around 155°C indicated the presence of enzyme-resistanc starch (RS). Yields of RS from complexed samples isolated by thermostable bacterial α-amylase or amyloglucosidase were lower than yields of RS from the autoclaved and cooled control. Formation of complexes competes with amylose chains involved in generation of RS. Amylose-lipid complexes are enzyme-degradable, and an increase in complexed amylose reduced yields of RS. Amylose recrystallization in RS formation is competitively affected by complexation of amylose with LPC and SSL. Results of X-ray diffraction powder crystallography were in agreement with DSC measurements. Complexes of amylose with LPC, SSL, and OHL gave rise to V-type patterns; enzymatic hydrolysis of the complexes yielded B-type RS structures.

Retrogradation of amylose-containing starches was shown to include the formation of an enzyme-resistant starch (RS) fraction of relatively low molecular weight α-glucan chains (Berry et al 1988, Russell et al 1989, Siljeström et al 1989). The presence of interchain amylose associations in RS fractions was indicated by an endothermic transition at ~155°C in differential scanning calorimetry thermograms (Sievert and Pomeranz 1989, 1990). The crystallinity of amylose fragments in RS fractions apparently is less than the crystallinity of the structure that occurs in native starch (Sievert et al 1991). Examination by X-ray diffraction of RS fractions gave poor B-type patterns (Berry et al 1988, Russell et al 1989, Sievert et al 1991) indicating the presence of small and/or less perfect crystallites. It was suggested that RS comprises crystalline regions and less ordered domains, the proportions of which depend on the heat treatment of the starch sample. Apart from short-chain linear α-glucans, crude RS preparations contain native starch lipids and protein originating from added enzymes used in the isolation procedure (Russell et al 1989). It appears that lipids in RS fractions are present in a noncomplexed form and adhere to undigested starchy material (Sievert and Pomeranz 1990). Although it is known that amylose-lipid complexes decrease the susceptibility of amylose to amylolysis (Eliasson and Krog 1985, Holm et al 1983), these complexes could not be detected in RS material (Sievert and Pomeranz 1989 and 1990, Siljeström et al 1989). This can be related to the use of the thermostable bacterial α-amylase Termamyl for enzymatic isolation of RS. This enzyme hydrolyzes amylose-lipid complexes at high temperatures (Holm et al 1983).

The presence of complexing lipids, however, is likely to affect reassociation behavior of amylose upon retrogradation of starch and thus formation of RS. Sarko and Wu (1978) suggested a competitive mechanism between amylose retrogradation and formation of amylose-lipid complexes. Slade and Levine (1987) reported that the crystallization of amylose-lipid complexes is favored over amylose retrogradation. The objective of this study was to investigate the effects of complexing lipids on the formation of RS. Using enzymatic procedures, we determined the amount of RS formed during retrogradation of starch. Recrystallization of amylose was followed by thermoanalysis. X-ray diffraction was used to assess structural characteristics of retrograded starch samples and respective RS residues.

MATERIALS AND METHODS

Materials

Amylomaize VII starch (containing about 70% amylose, according to manufacturer's data) was from American Maize-Products Co., Hammond, IN.

The following lipids were used in the complexing studies: 1) egg yolk lysophosphatidylcholine (LPC) (L-4129, from Sigma Chemical Co., St. Louis, MO); 2) sodium stearoyl lactylate (SSL) (Grindstedt Products, Inc., Industrial Airport, KS); and 3) hydroxylated lecithin (OHL) (Centrolene 6110-00, from Central Soya, Ft. Wayne, IN). The commercial Centrolene A is a water-dispersible fluid at room temperature with a hydrophilic-lipophilic balance (HLB number) of 10 (manufacturer's data). The acetone solubles are 58% (minimum), hexane insolubles 0.3% (maximum), and moisture 1.5% (maximum).

Enzymes used for isolation of RS were 1) Takalite, a heat-stable bacterial α-amylase (from Miles, Inc., Elkart, IN); 2) amyloglucosidase from *Aspergillus niger* (A-3042, Sigma); and 3) α-amylase from porcine pancreas (A-3176, Sigma).

Methods

The technique of preparing RS by autoclaving starch with excess water and subsequent cooling has been described (Berry 1986, Sievert and Pomeranz 1989). To study the effect of lipids on RS formation, LPC, SSL, or OHL was added during cooling of autoclaved starch according to the following procedure. Ten grams of amylomaize VII starch was suspended in 100 ml of distilled water and autoclaved at 125°C for 1 hr. When the temperature of the autoclave chamber reached 100°C during cooling, the starch sample was taken out of the autoclave, and 25 ml of water (control) or of a solution containing 1 g of LPC, SSL, or OHL was added immediately. The concentration of 1 g of lipid per 10 g of amylomaize starch constituted an excess of lipid and was similar to the concentration used by Eliasson and Krog (1985) for preparation of solution-grown amylose-lipid complexes. Heating the suspension to about 90°C was required to disperse SSL and OHL. The mixture was cooled to room temperature under vigorous stirring, refrigerated at 4°C overnight, and freeze-dried. The dried material was ground on a Udy mill to pass a sieve with 0.5-mm round openings and extracted exhaustively with petroleum ether at a temperature under 60°C. The petroleum ether was allowed to evaporate under a hood at room temperature.

For preparation of RS, 0.5 g of autoclaved starch sample was suspended in 50 ml of phosphate buffer solution (pH was adjusted according to the requirements of the specific enzyme employed) and treated with one of the following: 1) 200 μl of Takalite at 100°C (boiling water bath) for 30 min, at pH 6.0; 2) 500 μl of amyloglucosidase at 60°C for 1 hr, at pH 4.5; or 3) 10 ml of pancreatic α-amylase solution at 37°C overnight, at pH 6.9, under continuous shaking. The pancreatic α-amylase solution was prepared by suspending 5 g of enzyme in 100 ml of phosphate buffer (pH 6.9) under vigorous stirring and subsequent centrifuging and filtering.

The enzymatically treated preparations were filtered, washed with distilled water at the incubation temperature, and freeze-dried. The insoluble, enzyme-resistant starch (RS) residues obtained were weighed and expressed as a percentage (dry matter

[1]Presented at the AACC 75th Annual Meeting, Dallas, TX, October 1990.

[2]Department of Food Science & Human Nutrition, Washington State University, Pullman, WA 99164-6376.

TABLE I
Thermoanalytical Characteristics of Autoclaved Amylomaize VII Starch Cooled in the Presence of Added Complexing Agents[a]

Complexing Agent[b]	Transition Temperatures,[c] T (°C) and Transition Enthalpies,[d] ΔH (J/g)								
	T_O	T_P	ΔH	T_O	T_P	ΔH	T_O	T_P	ΔH
None	...[e]	...	...	88.5	99.9	2.5	145.4	153.6	3.3
LPC	...	...	...	97.0	110.3	14.2	135.6	146.4	2.0
SSL	42.8	51.5	1.6	91.4	104.9	11.0	143.9	153.4	1.6
OHL	...	...	...	77.8	94.7	5.1	148.0	156.1	3.9

[a]Definition of differential scanning calorimetry parameters in text.
[b]LPC = lysophosphatidylcholine, SSL = sodium stearoyl lactylate, and OHL = hydroxylated lecithin.
[c]SD < 1.0°C; $n = 3$.
[d]SD < 10% of the mean; $n = 3$.
[e]None detected.

TABLE II
Effect of Enzyme Preparations on Yields of Enzyme-Resistant Starch (RS) from Amylomaize VII Starch with Complexing Agents

Complexing Agent[a]	Yield of RS (% dm) After Treatment								
	Takalite			Pancreatic α-Amylase			Amyloglucosidase		
	Mean	n	SD	Mean	n	SD	Mean	n	SD
None	17.6	10	0.96	36.9	10	1.05	23.6	14	0.32
LPC	7.0	15	0.25	6.3	10	1.00	12.9	10	1.40
SSL	8.1	10	0.42	11.7	10	2.67	20.4	10	0.48
OHL	14.1	20	0.74	25.5	6	1.18	23.5	10	0.35

[a]LPC = lysophosphatidylcholine, SSL = sodium stearoyl lactylate, and OHL = hydroxylated lecithin.

basis) of the starch used for enzymatic hydrolysis. The dried RS material was ground in a mortar for further use. Differential scanning calorimetry (DSC) measurements were made as described by Sievert and Pomeranz (1989). T_O and T_P denote onset and peak transition temperatures, respectively; ΔH is transition enthalpy computed in joules per gram. X-ray powder diffraction was performed with samples hydrated to 12–13% moisture as described by Sievert et al (1991). All measurements were made at least in triplicate. X-ray patterns were highly reproducible. Standard deviations of DSC measurements were calculated and are indicated in the Results and Discussion.

RESULTS AND DISCUSSION

The DSC data for the autoclaved amylomaize VII starch control sample are given in Table I. Peak temperatures are not as reliable as onset temperatures in thermal transitions for analytical purposes. The trends in onset and peak temperatures in this paper, however, were the same, and the latter are used in discussing transition peaks. The endotherm at ~155°C could be attributed to melting of recrystallized amylose. The small endotherm at ~100°C reflected melting of a complex between amylose and native lipids. High-amylose starches have more lipids than normal starches and there is a positive correlation between amylose content and lipid content (Morrison 1985). High-amylose maize starches contain 385 to 449 mg per 100 g of lysophospholipids (lysophosphatidyl choline + lysophosphatidyl ethanolamine + lysophosphatidyl glycerol) and 543 to 667 mg per 100 g of free fatty acids. These lipids can form complexes with amylose; it was assumed that formation of the complex at the peak temperature of ~100°C observed in this study took place during autoclaving and cooling of the starch sample. Evidence for the existence of amylose-lipid complexes in native starch granules, however, is inconclusive (Morrison 1988a,b).

Addition of LPC, SSL, or OHL upon cooling of autoclaved amylomaize starch induced successful complexation of amylose with the added lipids, as demonstrated by DSC measurements (Table I). In agreement with observations of Evans (1986) and Galloway et al (1989), addition of lipids caused a shift away from the native amylose-lipid properties to those for the added lipids. Amylose-LPC and amylose-SSL complexes gave endothermic transitions at 110.3°C (T_P) and 104.9°C (T_P) with melting enthalpies of 14.2 and 11.0 J/g, respectively. In contrast, the OHL complex had a lower melting temperature (94.7°C [T_P]) and lower melting enthalpy (5.1 J/g). Since all lipids were added in excess, the lower melting enthalpy of the OHL complex indicated OHL had less ability to form complexes with amylose under the conditions used. In interpreting these results, it should be noted that the natural lysophosphatidylcholine from egg yolk and, especially, the synthetic sodium stearoyl lactylate were relatively well defined and pure compounds. Hydroxylated lecithin was a commercial product containing a mixture of several polar and nonpolar compounds.

After amylolytic treatment of the starch samples with the thermostable bacterial α-amylase Takalite, pancreatic α-amylase, or amyloglucosidase, insoluble RS residues were obtained. Yields of RS from complexed starch samples were lower than yields from the control sample (Table II). The data given in Table II indicate that reduction in yields depended on the complexing agent and amylolytic treatment. Among the three enzymes used for isolation of RS, Takalite was most effective in hydrolyzing starchy material of the control sample and complexed starch samples. Noticeably, the exoenzyme amyloglucosidase, in contrast to the endoenzyme pancreatic α-amylase, hydrolyzed more starch of the control sample but was, generally, less effective with regard to the complexed samples.

The combined data of Table I and II indicate that the complexing ability of the added lipid, as assessed by melting enthalpies of the endotherms between 95 and 110°C, was negatively related to the yields of RS. Whereas LPC complexed starch gave the highest melting enthalpy value and lowest yields of RS, OHL complexed starch had the lowest enthalpy value but highest yields. From these findings it can be concluded that good complexing agents such as LPC and SSL effectively compete with amylose chains involved in formation of RS during cooling of autoclaved amylomaize starch. Since amylose-lipid complexes are enzyme-degradable structures (Holm et al 1983), the formation of complexed amylose in autoclaved and cooled starch samples apparently accounted to a large extent for the reduction in yields of RS. Removal of complexes of amylose with LPC, SSL, and OHL as a result of the amylolytic treatment was indicated by the disappearance of corresponding melting transitions (around 100°C) in DSC thermograms of RS residues (compare Tables I and III, Fig. 1). The only exception was the amylose-LPC complex, which had the highest enthalpy value (14.2 J/g in Table I) and still gave a small endotherm (2.9 J/g in Table III) after treatment with pancreatic α-amylase. Factors affecting hydrolysis of amylose-lipid complexes were shown to include conditions used for the amylolytic treatment (e.g., type of enzyme, enzyme concentration, incubation temperature and time) as well as structural characteristics of the complexing agent (Biliaderis and Galloway 1989, Eliasson and Krog 1985, Holm et al 1983, Jane and Robyt 1984).

The endotherm at about 50°C (T_P), consistently observed in RS residues from SSL-complexed starch (Table III) most likely derived from melting of "free" SSL that was released from amylose-SSL complexes during the enzymatic treatment. This is in agreement with previous findings (Sievert and Pomeranz

TABLE III
Thermoanalytical Characteristics of Enzyme-Resistant Starch Residues Isolated by Enzyme Preparations from Amylomaize VII Starch with Complexing Agents[a]

Enzyme Preparation/ Complexing Agent[b]	Transition Temperatures,[c] T (°C) and Transition Enthalpies,[d] ΔH (J/g)								
	T_O	T_P	ΔH	T_O	T_P	ΔH	T_O	T_P	ΔH
Takalite									
none	...[e]	...	...	...	...	...	138.3	155.4	15.5
LPC	...	...	...	...	...	...	134.9	154.0	30.6
SSL	44.9	48.8	6.4	...	...	...	136.4	155.9	32.3
OHL	...	...	...	...	...	...	133.9	155.6	32.0
Pancreatic α-amylase									
none	...	...	...	...	...	...	145.2	157.5	9.2
LPC	...	...	...	90.8	100.0	2.9	134.5	154.7	34.9
SSL	44.4	49.2	4.4	...	...	...	138.2	155.3	26.4
OHL	...	...	...	...	...	...	140.6	159.5	16.9
Amyloglucosidase									
none	...	...	...	...	...	...	136.7	153.1	12.8
LPC	51.0	56.6	1.1	...	...	...	135.8	153.1	16.5
SSL	39.0	48.3	28.8	...	...	...	141.1	151.1	18.9
OHL	...	...	...	...	...	...	129.1	150.8	17.2

[a]Definition of differential scanning calorimetry parameters in text.
[b]LPC = lysophosphatidylcholine, SSL = sodium steroyl lactylate, and OHL = hydroxylated lecithin.
[c]SD < 1.0°C; $n = 3$.
[d]SD < 10% of the mean; $n = 3$.
[e]None detected.

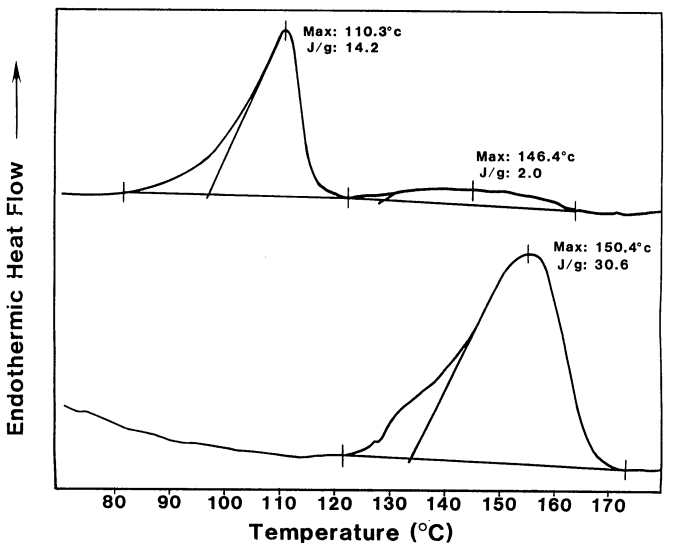

Fig. 1. Differential scanning calorimetric thermograms of autoclaved amylomaize VII starch, cooled in the presence of lysophosphatidylcholine, before (top) and after (bottom) treatment with bacterial α-amylase.

1990) suggesting the presence of noncomplexed lipids that adhere to resistant starch structures in certain RS residues.

The 155°C endotherm of RS residues was considered to represent recrystallized amylose fragments in RS material. When comparing yield data of RS listed in Table II and enthalpy values of the 155°C endotherm of RS residues given in Table III, it became apparent that the substantial decrease in yields of RS from complexed starch samples was accompanied by an increase in melting enthalpies of the 155°C endotherm. Whereas RS residues isolated by Takalite were lowest in yield and highest in enthalpy, RS residues isolated by amyloglucosidase gave the highest yields but lowest enthalpies. Figure 1, which shows the DSC thermograms of LPC-complexed starch before and after treatment with Takalite, illustrates the increase in melting enthalpy of the 155°C endotherm to 30.6 from 2.0 J/g as a result of the amylolytic treatment.

To evaluate the effect of lipids on recrystallization of amylose, as assessed by enthalpies of the 155°C endotherm, we used data obtained from starch samples before and after amylolytic treatment. In the following discussion, enthalpy values (155°C endotherm) of autoclaved starch samples before amylolysis are referred to as "measured enthalpies." Based on data obtained after amylolysis we introduce the term "yield of RS (% dm) × enthalpy (J/g) of the 155°C endotherm of RS" and refer to it as "calculated enthalpy." This theoretical value takes into account the effectiveness of the different enzymes to isolate recrystallized amylose from a starch sample and allows one to deduce the amount of energy required to melt recrystallized amylose in 1 g of starch sample before amylolysis when the following simplifying assumptions are made: 1) amylose associations that contribute to the 155°C endotherm are not removed by enzymatic isolation of RS, and 2) amylolytic removal of degradable starch structures that do not contribute to the 155°C endotherm is accompanied by a corresponding (on percentage basis) increase in melting enthalpy of the 155°C endotherm in RS residues.

TABLE IV
Measured and Calculated Enthalpies of Amylomaize VII Starch with Complexing Agents

Complexing Agent[a]	Calculated Enthalpies (J/g) After Enzymic Treatment			Measured Enthalpies (J/g)
	Takalite	Pancreatic α-Amylase	Amyloglu-cosidase	
None	2.73	3.36	3.01	3.3
LPC	2.14	2.20	2.13	2.0
SSL	2.62	3.09	3.86	1.6
OHL	4.51	4.31	4.04	3.9

[a]LPC = lysophosphatidylcholine, SSL = sodium stearoyl lactylate, OHL = hydroxylated lecithin.

Compared to measured enthalpy values of the control sample:

1) Lower calculated enthalpy values of a complexed sample indicate that the relative decrease in yield was not accompanied by a relative increase in enthalpy. This suggests that amylose association was hindered by the addition of lipids and a competitive mechanism of amylose association and amylose-lipid complex formation would apply.

2) Higher calculated enthalpies indicate that the relative decrease in yield was accompanied by a relatively higher increase in enthalpy. This could be interpreted as amylose association being favored by addition of lipids.

3) Equal calculated enthalpies indicate that a relative decrease in yield was accompanied by a corresponding relative increase in enthalpy; therefore, it would appear that amylose association was not affected by addition of lipids.

As shown in Table IV, the calculated enthalpy values of the

control sample are in fairly good agreement with the actual enthalpy measured. Calculated values for LPC and OHL complexed starch samples are slightly higher than measured enthalpies but are still in agreement. Major differences between calculated and measured enthalpies existed with regard to SSL-complexed starch. This might be related to the structures responsible for the peak around 50°C in the RS residues from SSL complexed starch. As discussed above, free SSL, released from amylose-lipid complexes during the enzymatic treatment, was assumed to account for this transition.

The largest difference in calculated and measured enthalpy was observed when calculated value was based on the RS residue from SSL-complexed starch isolated by amyloglucosidase treatment. This residue gave the highest enthalpy value around 50°C (28.8 J/g) (Table III). The presence of free SSL in RS might increase the yield, thus resulting in higher calculated enthalpy values.

LPC-Complexed Starch

Compared with the measured enthalpy (3.3 J/g) (Table IV) of the autoclaved amylomaize starch control, the lower measured enthalpy (2.0 J/g) of LPC-complexed starch suggested a competitive mechanism between amylose chain association and amylose-LPC complex formation during cooling of amylomaize starch. This was confirmed by calculated enthalpy values. Regardless of the enzyme used for isolation of RS, the calculated values were consistently lower for LPC-complexed starch than for the control sample (Table IV). This indicates that decreases in yields of RS were not accompanied by corresponding relative increases in melting enthalpies. It appears, therefore, that added LPC interacted with amylose chains that were involved in the recrystallization process in the control sample. Formation of recrystallized amylose upon addition of LPC was thereby reduced. Consequently, lower measured and calculated enthalpy values were obtained for the 155°C endotherm of LPC-complexed starch.

SSL-Complexed Starch

As in the case of LPC, a lower measured enthalpy (1.6 J/g) (Table IV) of SSL-complexed starch compared with the control sample (3.3 J/g) was recorded. Hence, the data strongly suggested a competitive mechanism between amylose association and SSL-complex formation. Compared with the calculated enthalpy of the control sample (3.36 J/g), as based on the RS residue from pancreatic α-amylase treatment, the slightly lower value calculated for the complexed sample (3.09 J/g) provided a supporting indication that complexing of amylose with SSL interfered with recrystallization of amylose. In contrast, similar values calculated for the control and SSL-complexed sample were obtained when RS from Takalite incubation was taken as a reference (Table IV). Based on these data, it appears that addition of SSL had no effect on recrystallization of amylose. Takalite seemed to be more effective in isolating and concentrating recrystallized amylose from SSL-complexed starch than from the control. The higher value calculated (3.86 J/g), based on the RS residue from amyloglucosidase treatment, even suggested that amylose association was favored by addition of SSL. However, if structures responsible for the peak at 50°C of amyloglucosidase-isolated RS residues also contributed to a higher yield and therefore a higher calculated enthalpy value, the actual yield of RS would tend to be lower. Hence, the possibility of an SSL-induced mechanism that favors recrystallization of amylose can most likely be rejected.

TABLE V
Interplanar *d*-Spacings (nm) as Determined by X-ray Crystallography[a] for Starch and Enzyme-Resistant Starch (RS) Residues

Complexing Agent[b]	Amylomaize VII Starch		RS Isolation Enzyme		
	Native	Autoclaved[c]	Takalite	Pancreatic α-Amylase	Amyloglucosidase
None	0.521s[d]	0.527s	0.534s	0.531s	0.532s
	0.451s	0.454b	0.396b	0.395b	0.472b
	0.404b	0.401b	0.752sh	1.627b	0.399b
LPC	1.577b	0.448s	0.543s	0.538s	0.532s
	···	0.697s	0.395s	0.458s	0.464b
	···	1.143b	1.723b	0.398s	0.417b
SSL	···	0.449s	0.536s	0.534s	0.468s
	···	0.697s	0.419b	0.418b	0.524s
	···	1.145b	0.476sh	0.464sh	1.645b
OHL	···	0.451s	0.532s	0.531s	0.526s
	···	0.527s	0.396b	0.396b	0.469s
	···	0.685sh	0.350sh	1.663b	0.407b

[a] In order of decreasing intensity.
[b] LPC = lysophosphatidylcholine, SSL = sodium stearoyl lactylate, and OHL = hydroxylated lecithin.
[c] Autoclaved and cooled in the presence of added complexing agents.
[d] s = Sharp, b = broad, sh = shoulder.

OHL-Complexed Starch

From the low melting enthalpy of the amylose-OHL complex (Table I), the relatively high enthalpy value (3.9 J/g) measured for the 155°C endotherm (Table I), and the high yields of RS from OHL-complexed starch (Table II) it could be concluded that OHL interacted in a different manner with amylose than LPC or SSL. Tables II and III show that treatment of OHL complexed starch with Takalite and amyloglucosidase resulted in almost similar yields but much higher melting enthalpies of the 155°C endotherm than corresponding control samples. As indicated by DSC thermograms, "free" OHL that in the presence of water gives a very small melting peak around 97°C (data not shown) did not contribute to yields of RS. Therefore, the high values for calculated enthalpies given in Table IV, regardless of the enzyme used, suggested a higher concentration of recrystallized amylose in OHL-complexed starch than in the control sample. In light of these observations, it seemed that addition of OHL upon cooling of autoclaved starch not only did not compete with formation of recrystallized amylose but actually favored the recrystallization process.

X-Ray Crystallography

In addition to differential scanning calorimetry, we used X-ray crystallography to follow structural changes of amylomaize starch induced by added complexing agents and subsequent amylolytic treatment. As indicated in Materials and Methods, X-ray diffraction studies were performed with samples hydrated to about 13% moisture. Attaining higher moisture levels required prolonged exposure to very high humidities; the increases in moisture were difficult to control and reproduce. Increasing the moisture content to about 18% had marginal effects on resolution of X-ray patterns and did not affect the interpretation of the data (results not shown). The results in Table V are arranged in order of decreasing intensity. In agreement with previous reports (Sievert et al 1991), the profile of the control autoclaved amylomaize starch showed the appearance of a poor B-type pattern, as indicated by a single peak at 1.651 nm, a strong reflection at 0.527 nm, and a poorly resolved doublet (0.401–0.378 nm) (Table V, Fig. 2A). The peak at 0.454 nm likely reflected the presence of a V-type amylose structure.

Addition of complexing agents to solubilized amylose is known to give rise to the V-type diffraction pattern, representing complexed amylose structures (Kugimiya et al 1980, Mercier et al 1980, Takeo et al 1973, Zobel 1988). In accord with this view, addition of LPC during cooling of autoclaved amylomaize starch induced a typical V-type pattern including three reflections at 1.143, 0.697, and 0.448 nm (Table V, Fig. 2B). A similar strong V-type profile was recorded for SSL-complexed starch (Table V). In contrast, addition of OHL resulted in reduced intensities of the V-type reflections (1.113, 0.685, and 0.451 nm) and still yielded a peak at 0.527 nm (Table V, Fig. 2C). The latter might reflect a B-type structure, although no peak was observed at 1.6 nm.

The X-ray diffraction data of complexed starch samples were in good accord with the DSC transition characteristics of the

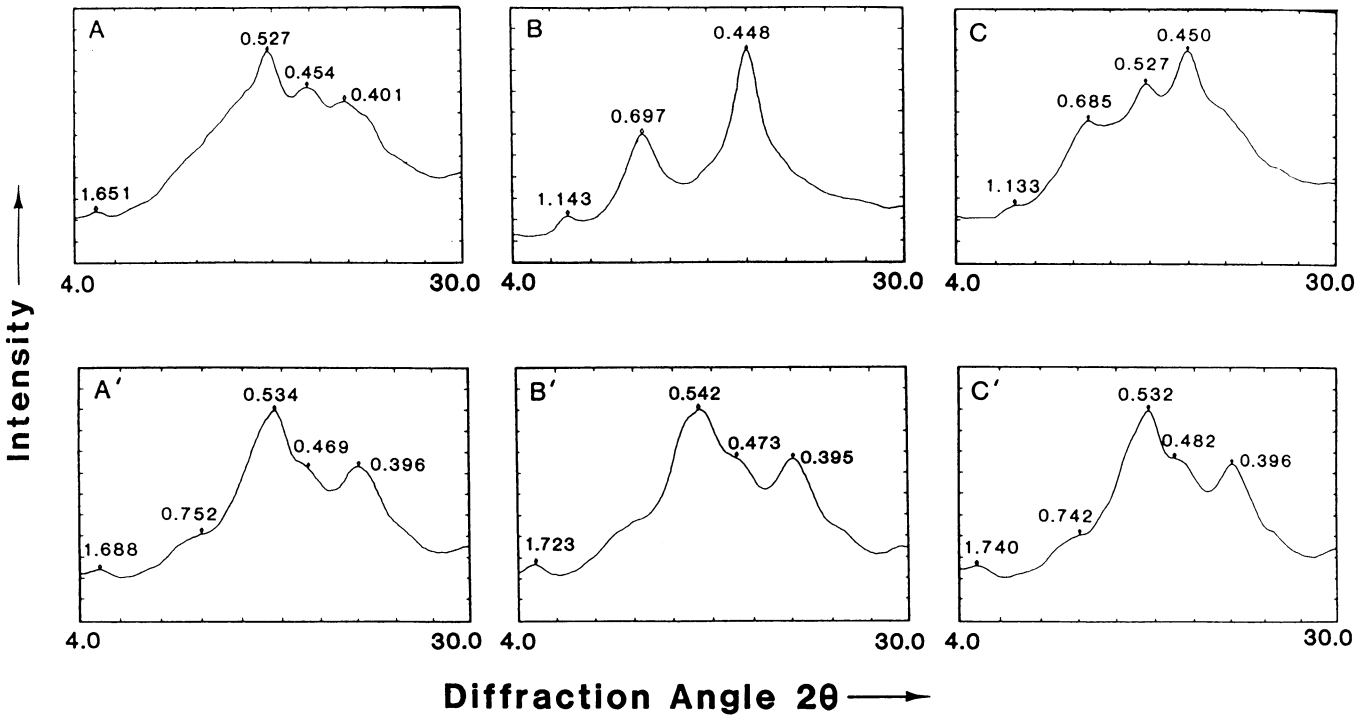

Fig. 2. Smoothed X-ray diffraction patterns of autoclaved amylomaize VII starch: **A,** cooled in the absence of added complexing agents (control sample); **B,** cooled in the presence of lysophosphatidylcholine; and **C,** hydroxylated lecithin. Patterns of corresponding enzyme-resistant starch residues isolated by Takalite treatment are indicated by A′, B′, C′.

amylose-lipid complexes. Amylose-LPC and amylose-SSL complexes showed high melting enthalpies and strong V-type reflection lines, whereas the amylose-OHL complex had a low melting enthalpy and yielded a less intense V-type pattern.

All X-ray profiles of RS residues isolated from the control sample and complexed starches with Takalite showed similar patterns (Table V, Fig. 2A′–C′). The typical V-type reflections disappeared, and poor B-type patterns (as identified by peaks around 1.6, 0.53, and 0.4 nm) emerged. The alterations in the pattern from V to B were due to enzymatic hydrolysis of amylose-lipid complexes and concentration of RS structures. The latter gave rise to the poor B-type patterns that are characteristic for RS material (Berry et al 1988, Russell et al 1989, Sievert et al 1991, Siljeström et al 1989).

The X-ray measurements presented in Table V revealed structural differences among RS isolated by Takalite, pancreatic α-amylase, and amyloglucosidase, respectively. RS from pancreatic α-amylase treatment showed slightly sharper peaks at 0.53 nm than RS from Takalite incubation. RS from LPC-complexed starch obtained by pancreatic α-amylase treatment still yielded a reflection at 0.458 nm, which likely reflected the presence of residual amylose-LPC-complexed structures. This is consistent with the endotherm at 100°C observed in the corresponding DSC thermogram (Table III).

X-ray patterns of RS isolated by amyloglucosidase treatment showed broader peaks at 0.53 nm than the pattern of RS from Takalite or pancreatic α-amylase treatment. Furthermore, all RS residues isolated from complexed starch by amyloglucosidase gave weak peaks at 0.46 nm (Table V) that might indicate the presence of residual amylose-lipid complexes. However, no melting of amylose-lipid complexes was detected in corresponding DSC thermograms (Table III).

CONCLUSION

This study showed that amylose-LPC and amylose-SSL complex formation effectively compete with amylose retrogradation and thereby with formation of RS during cooling of autoclaved amylomaize VII starch. Whether this competitive mechanism is also involved in the effects of various starch-complexing food emulsifiers on freshness retention of baked products remains to be determined.

LITERATURE CITED

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.

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

BILIADERIS, C. G., and GALLOWAY, G. 1989. Crystallization behavior of amylose V complexes: Structure-property relationships. Carbohydr. Res. 189:31.

ELIASSON, A.-C., and KROG, N. 1985. Physical properties of amylose-monoglyceride complexes. J. Cereal Sci. 3:239.

EVANS, I. D. 1986. An investigation of starch/surfactant interactions using viscosimetry and differential scanning calorimetry. Starch/Staerke 38:227.

GALLOWAY, G. I., BILIADERIS, C. G., and STANLEY, D. W. 1989. Properties and structure of amylose-glyceryl monostearate complexes formed in solution or on extrusion of wheat flour. J. Food Sci. 54:950.

HOLM, J., BJÖRCK, OSTROUSKA, S., ELIASSON, A.-C., ASP, N.-G., LARSSON, K., and LUNDQUIST, I. 1983. Digestibility of amylose-lipid complexes in-vitro and in-vivo. Starch/Staerke 35:294.

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 of preparing amylodextrins. Carbohydrate Res. 132:105.

KUGIMIYA, M., DONOVAN, J. W., and WONG, R. Y. 1980. Phase transitions of amylose-lipid complexes in starches: A calorimetric study. Starch/Staerke 32:265.

MERCIER, C., CHARBOUNIERE, R., GREBAUT, J., and DE LA GUERIVIERE, J. F. 1980. Formation of amylose-lipid complexes by twin screw extrusion of manioc starch. Cereal Chem. 57:4.

MORRISON, W. R. 1988a. Lipids in cereal starches: A review. J. Cereal Sci. 8:1.

MORRISON, W. R. 1988b. Lipids. Pages 420-426 in: Wheat Chemistry and Technology. Vol. 1. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

RUSSELL, P. L., BERRY, C. S., and GREENWELL, P. 1989. Characterization of resistant starch from wheat and maize. J. Cereal Sci. 9:1.

SARKO, A., and WU, H.-C. H. 1978. The crystal structures of A-, B- and C-polymorphs of amylose and starch. Starch/Staerke 30:73.

SIEVERT, D., and POMERANZ, Y. 1989. Enzyme-resistant starch. I.

Characterization and evaluation by enzymatic, thermoanalytical, and microscopic methods. Cereal Chem. 66:342.
SIEVERT, D., 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.
SIEVERT, D., 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.
SILJESTRÖM, M., ELIASSON, A.-C., and BJÖRCK, I. 1989. Characterization of resistant starch from autoclaved wheat starch. Starch/Staerke 41:147.
SLADE, L., and LEVINE, H. 1987. Recent advances in starch retrogradation. Pages 387-430 in: Industrial Polysaccharides—The Impact of Biotechnology and Advanced Methodologies S. S. Stivala, V. Crescenzi, and I. C. M. Dea, eds. Gordon and Breach Science: New York.
TAKEO, K., TOKUMURA, A., and KUGE, T. 1973. Complexes of starch and its related materials with organic compounds. Part X. X-ray diffraction of amylose-fatty acid complexes. Starch/Staerke 25:357.
ZOBEL, H. F. 1988. Starch crystal transformations and their industrial importance. Starch/Staerke 40:1.

[Received October 15, 1990. Accepted March 20, 1991.]