

Amylose-Lipid Complex Formation in Acetylated Pea Starch-Lipid Systems

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ABSTRACT

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Starch-lipid interactions involving native and acetylated pea starch were studied by differential scanning calorimetry (DSC) and measurements of iodine affinity. Lipids including lauric acid, monopalmitin, and butterfat were added to aqueous starch dispersions after the starch was gelatinized at 85°C. DSC thermal curves of gelatinized modified pea starch systems containing fatty acid or monoglyceride did not show DSC transitions indicative of amylose complexes with external lipids, whereas a DSC endotherm of amylose-lipid complexes was observed for the corresponding native starch-lipid systems. However, iodine binding studies

revealed that acetylated pea starch amylose complexed with added fatty acid or monoglyceride in the modified pea starch-lipid composites. The failure of DSC detection of the complexes in these systems was attributed to the absence of crystalline structures of acetylated pea starch amylose complexes. Furthermore, acetylation of starch decreased the complexing ability of the pea starch amylose as revealed by a reduction in iodine affinity. Both DSC and iodine affinity studies showed that neither native nor modified pea starch interacted to a significant extent with butterfat that consisted mainly of triglycerides.

Amylose forms helical clathrate inclusion complexes with a variety of organic and inorganic compounds. This complex formation is particularly important to the food industry since many food products contain both starch and lipids, and the interaction between these components influences food quality. In the breadmaking and potato processing industries, for example, monoglycerides and related compounds are used for their antifirming effect or to control stickiness of the product, presumably by the formation of amylose-lipid inclusion complexes (Krog 1971, Hoover and Hadziyev 1981).

Many studies on the structure and properties of amylose-lipid complexes involving native starch have been made over the past years. It is generally believed that during complex formation amylose changes from coil to helix and guest molecules enter the central cavities of amylose helices. This conformational ordering further promotes aggregation of helices, resulting in a partially crystalline amylose structure or V-amylose (Osman et al 1961, Eliasson and Krog 1985, Biliaderis 1991, Biliaderis 1992, Raphaelides and Karkalas 1988). During DSC scans, the semi-crystalline amylose will undergo an order → disorder melting transition, resulting in an endothermic peak. The specific melting temperature of those complexes depends on the type of ligands as well as the crystallization conditions (Biliaderis et al 1985, Eliasson and Krog 1985, Biliaderis and Galloway 1989).

Formation of amylose-lipid complexes has also been reported to be the underlying mechanism of the fat replacers that are based on modified pea starch and lipids (Nickle and Berger 1996). These products are manufactured by Woodstone Foods Corp., Winnipeg, MB. The industrial process involves adding lipid to gelatinized aqueous acetylated pea starch systems. Lipid molecules are thought to be encapsulated in the carbohydrate matrix by complex formation resulting in an improved delivery of fat or oil. The amount of fat or oil necessary for specific food products is therefore reduced. Depending on the end use, various lipids can be incorporated including fatty acids and their mono- and diglycerides. Triglycerides such as butterfat and vegetable oils are used in the manufacture of fat substitutes that have use or potential use in low-fat tortilla, ice cream, and cheese products (Nickle and Berger 1996).

Acetylated starch has improved properties over its native form and has been widely used in many other food applications for its stability and resistance to retrogradation (Wurzburg 1986).

Information on the interactions between modified starch and lipids is, however, very limited in the literature, whereas much research has been done on native starch. Eliasson et al (1988) studied starch-lipid interactions involving modified maize starch by DSC analysis. They found a minimum complexing effect of acetylated high-amylose maize starch (HAMS) with cetyltrimethylammonium bromide (CTAB) and suggested that acetylation of HAMS may decrease the ability of this starch to form the amylose-lipid complex.

The present study was undertaken to investigate the mechanism of amylose-lipid complex formation in acetylated pea starch. Specifically, this research was directed toward understanding starch-lipid interactions in the pea starch-lipid based fat substitutes. DSC was used to study phase transitions of amylose-lipid complexes in pea starch-lipid systems, whereas iodine affinities of starch-lipid systems were determined to probe the underlying molecular event of amylose-lipid reactions.

MATERIALS AND METHODS

Both native and acetylated pea starches were provided by Woodstone Foods Corp., Winnipeg, MB. The degree of substitution (DS) of the acetylated pea starch was ≈0.1 according to the producer.

Aqueous starch suspensions (5%, w/w) were prepared by suspending starch in distilled water. While stirring with a magnetic bar, starch slurries were heated to >85°C to gelatinize the starch and held at the final temperature for 5 min before lipid (0.3%, w/w) was added to the samples. The starch-lipid composites were mixed for 30 min at 85°C. The purpose of a 30-min holding and mixing was to melt the lipid as well as to produce intimate contact between starch and the lipid molecules. The starch-lipid composites were further homogenized for 1 min at the maximum speed with an Omni-Mixer (Ivan Sorvall, Norwalk, CT), and the homogenized samples were cooled to room temperature in a water bath.

Differential scanning calorimetry (DSC) was performed using a thermal analyzer (Dupont 9900, Wilmington, DE) equipped with a DSC cell (Dupont 910). The thermal analyzer was calibrated with indium. Samples of the previously prepared starch-lipid composites (≈15 mg) were sealed in DSC pans. DSC thermal curves were obtained by heating the samples from 20 to 140°C at a rate of 10°C/min with an empty pan as an inert reference. The gelatinization behavior of native and modified pea starch was also studied by DSC. Thermal curves of starch gelatinization were obtained by weighing ≈2 mg of starch into DSC pans, adding distilled water into the pans to make a 30% starch system, and heating the samples in the DSC at 10°C/min from 20 to 140°C. DSC thermal curves were normalized based on starch weight.

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The iodine binding capacities of starch and starch-lipid composites were determined by potentiometric titration following the procedure of Colburn and Schoch (1964). Dimethyl sulphoxide (DMSO) was used in place of calcium chloride to disperse starch to avoid the formation of insoluble calcium soap in the presence of lipids such as fatty acids. The iodine binding capacity of native pea starch amylose was also determined. This linear fraction of native pea starch was fractionated by butanol precipitation following the method of Biliaderis et al (1986a,b). Lauric acid (>98% in purity) was a product of the British Drug House Ltd. (Poole, England). Monopalmitin (1-monopalmitoyl-rac-glycerol, 99% in purity) was from Sigma Chemical Co. (St. Louis, MO). A sample of commercial monoglyceride (>90% α monoester) was obtained from American Ingredients Co. (Kansas City, MO). Butterfat and canola oil were obtained from the local market. All other chemicals were purchased from Sigma Chemical Co.

RESULTS AND DISCUSSION

The DSC thermal curves for gelatinization of acetylated pea starch and its native counterpart are shown in Figure 1. Acetylated pea starch exhibited a lower gelatinization enthalpy (7 ± 0.5 J/g) compared to native pea starch (10 ± 0.7 J/g). Acetylation of pea starch also resulted in a decrease in the DSC gelatinization peak temperature from $65 \pm 0.8^\circ\text{C}$ (for native starch) to $62 \pm 0.4^\circ\text{C}$. These characteristics of starch gelatinization were in broad agreement with previous DSC studies on acetylated starches. Eliasson et al (1988) found that acetylation of high-amylose maize starch caused the gelatinization temperature of starch to decrease from

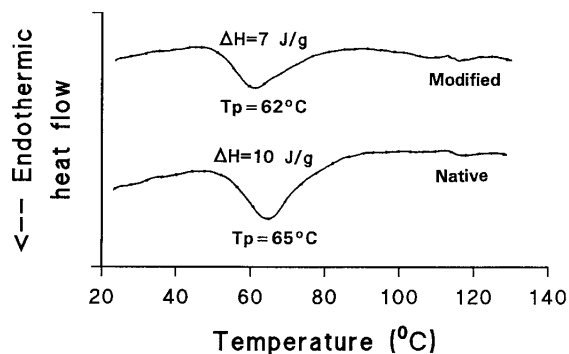


Fig. 1. Differential scanning calorimetry thermal curves of aqueous native and acetylated (modified) pea starch slurries at a starch concentration of 30% (w/w).

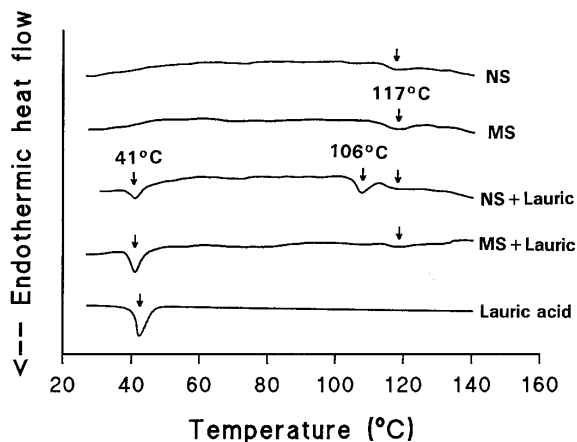


Fig. 2. Differential scanning calorimetry thermal curves of native pea starch (NS) gel, acetylated (modified) pea starch (MS) gel, and native pea starch-lauric acid (NS-Lauric), modified pea starch-lauric acid (MS-Lauric), and lauric acid alone. Starch and lipid concentrations were 5 and 0.3% (w/w), respectively.

74.6°C in native starch to 72.1°C in the acetylated derivative. Wootton and Bamunuarachchi (1979) also observed a decrease in gelatinization peak temperatures for various substituted wheat starches including acetylated wheat starch. The size of the gelatinization endotherm for acetylated starch has been reported to be both increased and decreased. Eliasson et al (1988) observed an increase in the gelatinization enthalpy for acetylated high-amylose maize starch, whereas Wootton and Bamunuarachchi (1979) found a decrease for acetylated wheat starch. The origin of enthalpic transition during starch gelatinization is controversial and presently under debate (Slade and Levine 1988, Biliaderis 1991, Liu and Lelievre 1992, Cooke and Gidley 1992). Glass transition or melting of starch crystallites has been suggested to be the major cause of starch gelatinization. It appears that introduction of acetyl groups into the polymer chains would destabilize the granular structure as indicated by increased swelling tendency of substituted starches (Wootton and Bamunuarachchi 1979). Therefore, a decrease in both the gelatinization temperature and enthalpy would be expected.

Figure 2 shows DSC thermal curves for pea starch-lauric acid systems and the corresponding starch gels without the fatty acid. Because starch had been gelatinized before the DSC scans, peaks were not observed on the thermograms over the temperature range of $50\text{--}80^\circ\text{C}$ where endothermic transitions for starch gelatinization occurred (Fig. 1). This was not due to the starch concentration because the 5% starch concentration had been shown previously to produce a gelatinization peak with DSC (Liu and Lelievre 1993). The high temperature transition at $117 \pm 0.5^\circ\text{C}$ was assigned to the melting of amylose-native lipid complexes (Biliaderis 1991). The presence of amylose-lipid complexes with noncereal starches has been implied in previous studies (Vasanthan and Hoover 1992). As seen in Figure 2, this transition was observed in both native and modified pea starch, with or without the added fatty acid. This supported the notion that this transition was associated with the order \rightarrow disorder processes of amylose complexes with natural starch lipids.

When lauric acid was added to native starch, an additional high temperature transition at $106 \pm 0.6^\circ\text{C}$ was observed on the DSC thermal curve (Fig. 2, curve labeled NS+Lauric). This endotherm appeared significant and must be due to the melting transition of the amylose complexes that formed with the added fatty acid. DSC studies on potato and faba bean amylose by other workers showed DSC peaks at $95\text{--}114^\circ\text{C}$ for amylose-lauric acid complexes (Biliaderis et al 1986a, Raphaelides and Karkalas 1988). In addition to the high temperature transition, a low temperature DSC peak at $41 \pm 0.7^\circ\text{C}$ was also observed when lauric acid was present. This was due to the melting of uncomplexed fatty acid as confirmed by comparison with the DSC curve of the fatty acid alone.

In the modified pea starch-lauric acid system, the thermal event at 106°C attributed to the amylose-lauric acid complexes in the corresponding native pea starch system was not found (Fig. 2, curve labeled MS+Lauric). If the lipid interacted with modified starch as with native starch, an endothermic phase transition of lipid-amylose complexes should be apparent because the thermal event is detectable by calorimetry. The absence of the transition may suggest that the interactions between amylose and lipids in acetylated pea starch may derive from different mechanisms.

The DSC thermal curves of starch-monoglyceride systems are given in Figure 3 for monopalmitin. As with lauric acid, the 117°C transition was attributed to the melting of amylose complexes with native lipids, whereas the low temperature peak at $49 \pm 0.3^\circ\text{C}$ was due to the melting of uncomplexed monoglyceride as confirmed by comparison with the starch and monopalmitin controls. As in the native starch-fatty acid system, a high temperature transition at $111 \pm 0.8^\circ\text{C}$ was detected in the native pea starch-monopalmitin system (Fig. 3, curve labeled NS+MP), which was attributed to the melting transition of amylose-monopalmitin complexes. This melting temperature was within the temperature range ($95\text{--}115^\circ\text{C}$) reported for phase transition of potato amylose

and faba bean amylose-monopalmitin complexes (Hoover and Hadziyev 1981, Eliasson and Krog 1985, Biliaderis et al 1986a,b). This additional endotherm observed for the native pea starch-monopalmitin system indicated an interaction between starch and the added monoglyceride. Again, DSC did not suggest the existence of amylose-monopalmitin complexes in the modified pea starch-monopalmitin system (Fig. 3, curve labeled MS+MP).

The DSC thermal curves of the starch-butterfat systems revealed only phase transitions associated with butterfat and amylose-native lipid complexes (data not shown). Transitions characteristic of amylose complexes with added lipids were not observed in either native or acetylated pea starches. This may suggest that amylose did not complex with butterfat that consisted mainly of triglycerides. These results were in agreement with previous findings on triglycerides. Osman et al (1961) found that soybean oil did not form complexes with amylose although lecithin, also with three substituents on the glycerol moiety, would form complexes with amylose.

Starch-lipid composites consisting of modified starch (6%) and lipids (12% butterfat or canola oil with or without 12% commercial monoglyceride) were also subjected to DSC studies. These systems closely resembled commercial fat-replacing products (Nickle and Berger 1996). A stable hydrogel was obtained, irrespective as to whether monoglyceride was used, indicating interactions between modified starch and the lipids. However, no calorimetric evidence was found suggesting a phase transition of amylose complexes with the external lipids, although the lipid content was in excess of that required for complex reaction (Krog 1971) so that the formation of complexes should have been maximal in these products.

To determine whether complex reaction occurred when lipid was added to modified pea starch, and to study the mechanism of modified starch-lipid interactions, further experiments were conducted. One method used to study amylose-lipid complex formation was the measurement of iodine affinity by amylose. It is postulated that when lipid molecules have occupied the central cavities of the amylose helices, the cavities will not be available for amylose-iodine complex formation, because experiments showed that both complexes have similar inclusion structures and that the dissociation constants of amylose-lipid complex are much smaller than that of amylose-iodine complex (Krog 1971, Rutschmann and Solms 1990). This is also implied by the fact that native lipid present in a trace amount in starch will result in a lower iodine binding capacity and defatting of lipid-containing starch is necessary to accurately determine the iodine binding capacity of amylose (Vasanthan and Hoover 1992). By measuring the reduction in iodine affinity of amylose, the amylose complexing capacities for a number of emulsifiers and surfactants have been determined (Osman et al 1961, Krog 1971).

The iodine affinities of pea starch and its acetylated derivative were $6.35 \pm 0.27\%$ and $4.41 \pm 0.18\%$, respectively. This suggested that the acetyl groups in modified amylose interfered with complex formation, resulting in a lower iodine binding capacity in the modified starch. In a study of starch-CTAB interaction, Eliasson et al (1988) observed decreased complexing ability in acetylated high-amylose maize starch. The iodine affinity of native pea starch amylose was determined to be $19.3 \pm 0.12\%$, which is similar to that reported for various other starches (Schoch 1964). By dividing the iodine affinity of starch by that of amylose, the amylose content of the pea starch used in this study was $\approx 33\%$.

Complex formation in pea starch-lipid systems was followed by measurements of iodine affinity. Figure 4 shows some typical potentiometric titration curves for various systems. In contrast to pure starch, the titration curves for composites of starch (both native and acetylated) and monopalmitin did not show the normal sigmoid-shaped characteristics where the reflection point in the curves is taken as the end-point of complex reaction. Similar results were obtained with the presence of lauric acid. The

absence of such a reflection point is a feature of titration curves for amylopectin, which is known for its inability to complex with lipids to a significant extent (Bates et al 1943). Thus, the iodine affinities of amylose in these systems were taken as zero. These results were also supported by visual observation of color change of the solutions during titration. When amylose complexes with iodine, an indicative blue color of amylose-iodine complexes will appear. However, solutions of these samples did not turn blue but purple upon addition of the iodine solution, indicating amylose-iodine complexes did not form and therefore suggesting that the amylose had been occupied by lipids. The failure of appearance of the blue color in these samples could not be due to the interaction of iodine-lipids because uncomplexed amylose could react with iodine to form blue complexes even in the presence of free lipids (Krog 1971). Furthermore, titration of starch-butterfat systems showed normal sigmoid characteristics, and the solution became blue upon addition of the iodine solution.

The amount of amylose that complexes with various lipids, as determined by a loss of iodine affinity, was calculated by the method of Krog (1971) and the results are summarized in Table I. Amylose in acetylated pea starch reacted with lauric acid or monopalmitin as in native pea starch by the formation of amylose-lipid inclusion complexes. In starch-butterfat systems, however, most amylose was not reacted with lipids as shown by the nearly unchanged iodine binding capacities of both native and acetylated starch. A small reduction in the complexing capacity of starch amylose in the presence of butterfat may be due to fatty acids or monoglycerides present in the fat rather than to triglycerides. The difference in the amount of complexed amylose was indicative of different complexing abilities of amylose in native and acetylated starch.

Because iodine binding studies showed that amylose-lipid com-

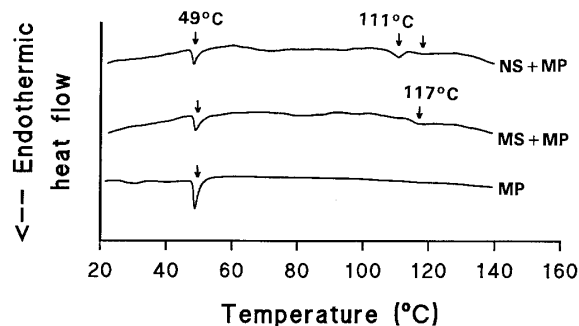


Fig. 3. Differential scanning calorimetry thermal curves of native pea starch-monopalmitin (NS-MP), acetylated (modified) pea starch-monopalmitin (MS-MP), and monopalmitin (MP) alone. Starch and lipid concentrations were 5 and 0.3% (w/w), respectively.

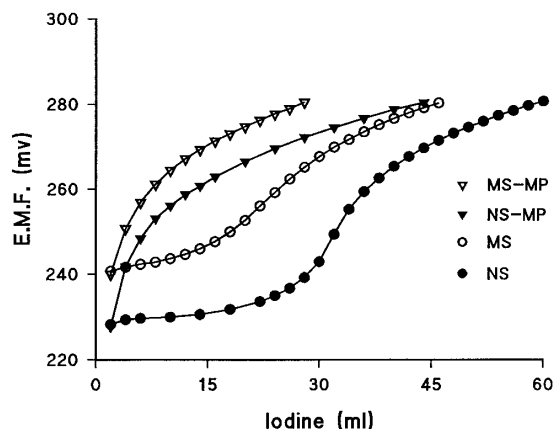


Fig. 4. Potentiometric titration curves of native pea starch (NS), acetylated (modified) pea starch (MS), native pea starch-monopalmitin (NS-MP), and acetylated pea starch-monopalmitin (MS-MP). E.M.F. = electromotive force.

TABLE I
Amount (%) of Amylose Complexed with Lipids in Native and Acetylated Pea Starch-Lipid Systems

	Lauric Acid	Monopalmitin	Butterfat
Native starch	100	100	6.7 ± 1.4
Acetylated starch	100	100	2.3 ± 0.8

plexes had formed in modified starch-fatty acid or modified starch-monopalmitin systems, it was concluded that the absence of transitions of amylose-lipid complexes in the DSC curves of modified starch systems must be due to different supermolecular structures of complexes in these systems. One characteristic of acetylated starch is the introduction of acetyl groups in the polymer chains to prevent starch retrogradation, which involves amylose-amylose chain association. Because of steric hindrance of the acetyl side groups, the aggregation process of amylose-lipid complexes to form crystallites in the modified starch will be inhibited. In other words, amylose-lipid complexes in acetylated starch may exist in an amorphous, nonsolid state, and therefore were not detected by DSC. This conclusion was further supported by studies conducted on a starch-butanol complex. It is well known that amylose complexes with *n*-butanol to form an insoluble crystalline precipitate, and starch can be fractionated by butanol precipitation (Muetgeert 1961). Attempts to isolate amylose from an acetylated pea starch solution by butanol precipitation, however, were unsuccessful even after many weeks of reaction and incubation, although amylose was easily fractionated from native pea starch with butanol. It appeared that acetylation of starch effectively prevented the crystallization process of amylose-butanol complex.

The notion that the thermal event, as detected by DSC, is associated with a phase transition of semicrystalline structures of amylose-lipid complexes, was in accord with the view of Whittam et al (1989), who, based on calorimetry, suggested that a substantial portion of the transition enthalpy of crystalline V-complexes corresponded to the thermal energy needed to overcome intermolecular crystalline lattice forces. However, this appears to be in contrast to the findings of Seneviratne and Biliaderis (1991), who proposed that the enthalpy change on dissociation of amylose-lipid complexes represented mainly the energy required to disrupt individual helices and included very little contribution from intermolecular effects. Their conclusion was mainly based on a polymorphic form of an amylose-monoacylglyceride complex, which exhibited an amorphous X-ray pattern but gave DSC endothermic transitions. However, the fact that the complex material was a solid precipitate, despite being in an amorphous state, suggested that intermolecular interactions must be involved for the phase change from solid to liquid, and should contribute to the DSC transition. It appears that the intermolecular forces involved in both crystalline and amorphous solid complex materials are of a similar order of magnitude as revealed by the similar DSC enthalpies (Seneviratne and Biliaderis 1991).

In conclusion, when a complexing lipid was added to acetylated pea starch, the amylose reacted with the lipid forming complex molecules. However, unlike in native pea starch, these complex molecules did not further aggregate into an order structure because steric hindrance of acetyl groups prevented crystallization. Since DSC could not detect the dissociation process of complex molecules, the thermal event of dissociation of acetylated amylose-lipid complexes could not be observed by the thermal technique.

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