Factors Influencing Corn Starch-Lipid Complexing[1]

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ABSTRACT

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Lipid complexing by starch in aqueous dispersions was measured using equilibrium dialysis. An increase in saturated fatty acid or monoglyceride chain length resulted in higher binding values. Analysis of the cis unsaturated C-18 series of fatty acids showed that binding decreased as the number of double bonds increased. Lipid complexing decreased as temperature increased from 20 to 60° C. Lauric acid was the exception, showing no change. Varying the pH from 3.0 to 7.0 had no effect on binding values for any monoglyceride, C-18 cis unsaturated fatty acid, lauric or myristic acid. Higher levels of palmitic and stearic acid were bound as pH was increased from 3 to 7. Myristic acid and monomyristin incubated at pH 3 and 5 (40° C) showed no change in binding over the buffer molarity range of 0.2–0.8 *M*. In contrast, binding increased for these lipids with an increase in buffer molarity at pH 7. Waxy maize and hydroxypropyl corn starch bound less lipid than unmodified corn starch.

Fatty acids and monoglycerides are known to form inclusion compounds with amylose (Mikus et al 1946, Osman et al 1961), with the hydrocarbon portion of the lipid located within the helical cavity of amylose (Banks and Greenwood 1972). Lipid binding to granular starch results in changes in the physicochemical properties of starch. Hoover and Hadziyev (1981) used swelling power and solubility tests to show that myristic acid was the most effective fatty acid in forming complexes with starch. Similar results were found with monoglycerides. Monomyristin-starch mixtures had the lowest solubility and swelling power, both of which were attributed to increased complex formation. In contrast, Lagendijk and Pennings (1970) found that monoglyceride association with starch increased as chain length increased from monolaurate to monostearate. Ghiasi et al (1982) showed that increasing temperature caused the swelling power and soluble starch values for monoglyceride-starch mixtures to increase. Values for starch alone also increased; therefore, it was difficult to separate the effect of increased granular disruption at higher temperatures from that of complex dissociation. Lagendijk and Pennings (1970) found that complexing decreased as the number of double bonds increased. In contrast, Mercier et al (1980) showed using twin-screw extrusion cooking that values for water-soluble carbohydrate and retrogradation decreased when oleic or linoleic acid were used in place of stearic acid. This latter study suggested that the unsaturated lipids are more effective in complexing amylose.

The purpose of this study was to assess the influence of lipid molecular weight and degree of unsaturation, starch type, and reaction conditions on the starch-lipid complex using the equilibrium dialysis technique.

MATERIALS AND METHODS

The equilibrium dialysis system described previously was used (Hahn and Hood 1986).

Starch (unmodified dent corn, National Starch and Chemical Co., Bridgewater, NJ) in deionized water (0.01%) was autoclaved at 20 psig for 30 min. Lipid solutions (1 μM) were prepared in 0.2 *M* citrate-phosphate buffer and 0.04 NaN_3 at pH 3, 5, or 7 (Gomori 1955) by adding nonradioactive and radioactively labeled compounds to achieve a count rate of 20,000 counts per minute (CPM)/ml. All stock solutions of unsaturated fatty acids were layered with nitrogen gas.

Aliquots of dispersed starch (0.5 ml) and lipid solution (0.5 ml) were added to one cell half, which was designated the starch cell half. Citrate-phosphate buffer (1.0 ml, 0.1 *M*; pH 3, 5, or 7) was added to the opposite side, which was termed the buffer cell half. Separate microsyringes were used to prevent any cross contamination of stock solutions. A 12-hr incubation time and a rotational speed of 20 rpm was used to assure equilibration. Duplicate aliquots (0.4 ml) were removed from both sides of each cell following equilibration. Each aliquot was added to 10 ml of scintillation fluid (Liquiscint, National Diagnostics, Somerville, NJ), and counted in a liquid scintillation counter. All experiments were conducted in duplicate cells.

Radioactive and nonradioactive lipid preparations were used to prepare aqueous lipid solutions having a specific activity of 10.10 μCi/μmol. This level was chosen after considering the final concentration of lipid desired in dialysis cells and the minimum level of radioactivity necessary to achieve valid count rates. Counting efficiency, as monitored by the automatic external standard ratio, was found to be independent of pH and ionic strength and was only affected by the sample volume added to the scintillation cocktail. Using 10.0 ml of scintillation fluid and a fixed sample size of 0.4 ml, the counting efficiency was 90%.

Experiments comparing the binding ability at pH 3, 5, and 7 were conducted at 60° C, and those examining the effect of temperature on binding used pH 7 buffer. Starch-lipid binding in the presence of buffer of varying ionic strength (0.2–0.8 *M*) was examined at 40° C using myristic acid and monomyristin at pH 3, 5, and 7.

The ability of unmodified waxy maize starch and hydroxypropyl corn starch (4.3% propylene oxide content, dry basis; National Starch and Chemical Corp., Bridgewater, NJ) to bind lipid was compared to that of unmodified corn. The autoclaved dispersions (0.005%) were equilibrated with stearic acid (0.5 μM) at 40° C and pH 7.

Data were analyzed using two-way factorial analysis of variance (SAS Institute 1982).

Lipid binding was determined by calculating the difference in lipid concentration across the semipermeable membrane and expressed as moles of lipid bound per mole of amylose ($\bar{\nu}$). Unbound lipid was able to equilibrate across the membrane, whereas lipid bound to starch was unable to diffuse, as dispersed starch has a molecular weight above the molecular weight cutoff of the membrane. Consequently any difference in the concentration of lipid between the two cell halves following equilibration resulted from starch-lipid complex formation.

The second part of calculating $\bar{\nu}$ involved determining the molar concentration of the binding macromolecule in the cell. The difficulties were in: 1) deciding whether to base the calculation of binding values solely on the amylose fraction of starch or on the total quantity of starch added to the cell, 2) estimating the molecular weight for amylose, and 3) estimating the percent amylose in the starch.

There is much disagreement in the literature regarding the ability

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of amylopectin to bind lipid. Conflicting statements are in part caused by the variety of techniques used to detect complexing. The consensus is that amylopectin binds very little, if any, lipid. Therefore, the results in this study were expressed on an amylose basis. A molecular weight of 1×10^6 was used for amylose. Estimates for the amylose content in normal corn starch have ranged from 22 to 28% (French 1973, Mercier 1973, Shannon and Creech 1973, Biliaderis et al 1980, Cluskey et al 1980). In this work the amylose content of unmodified and modified corn starch was assumed to be 25%. Using these values, the number of moles of lipid bound per mole of amylose ($\bar{\nu}$) was calculated as

$$\frac{(\text{CPM/ml starch cell} - \text{CPM/ml buffer cell})\left(\frac{\mu\text{Ci}}{2.2 \times 10^6\ \text{DPM}}\right)}{(\text{counting efficiency})\ (\mu\text{mol amylose/ml})\ (\text{specific activity of lipid})}.$$

RESULTS AND DISCUSSION

Temperature and Lipid Type

The effect of temperature on the quantity of lipid bound for saturated fatty acids at pH 7 is shown in Figure 1. Very little lauric acid is bound to starch under these conditions regardless of the temperature of incubation, with the differences among binding values being statistically insignificant. The other three saturated fatty acids show significantly greater binding as incubation temperature is decreased. The only exception is the slight drop in the amount of stearic acid bound to starch at 20° C compared to 40° C. This decrease may be related to a small percentage of the lipid becoming insoluble at this temperature. At 20 and 40° C, 99% of the stearic acid removed following equilibration was found on the starch cell half. However, the total quantity of stearic acid recovered following equilibration was lower at 20° C than in the case of 40° C incubation, indicating adsorption of stearic acid to the cell or membrane, or stearic acid-starch complex precipitation at the lower temperature.

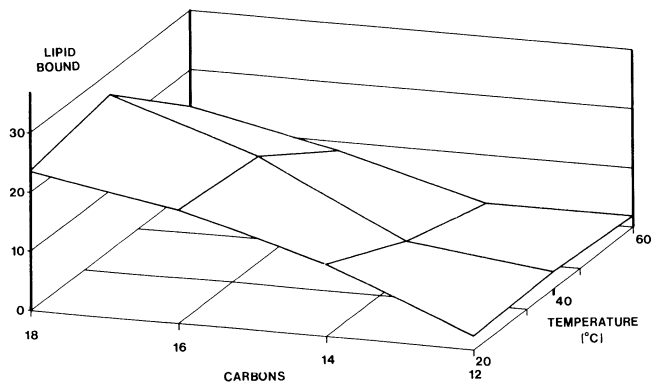

Fig. 1. Effect of temperature and chain length on saturated fatty acid binding at pH 7.

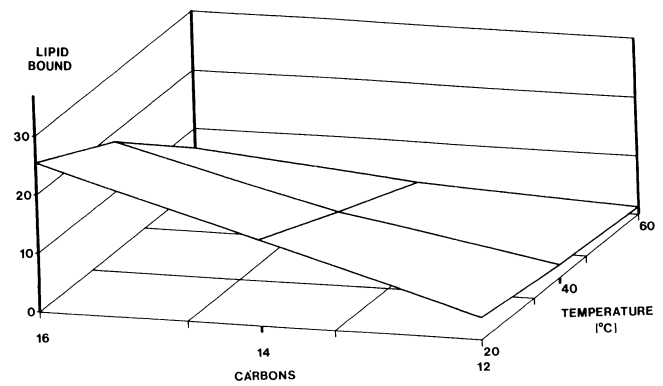

Fig. 2. Effect of temperature and chain length on monoglyceride binding at pH 7.

It is obvious from these data that an increase in the molecular weight of saturated fatty acid resulted in greater binding affinity to starch. Gray and Schoch (1962) and Hoover and Hadziyev (1981) showed that lipid complexing, as measured by a decrease in soluble starch and swelling power, increased as chain length increased from C-8 to C-14. A further increase in chain length caused a slight decrease in complexing. This discrepancy from data presented in this study can be explained by considering fatty acid concentration. Gray and Schoch used a lipid concentration of 1%, and Hoover and Hadziyev utilized 1 m*M* fatty acid. Both of these values are well above the solubility of fatty acid in water, particularly in the case of long chain lipid, such as stearic and palmitic acid. In their reaction systems, the concentration of reactive monomeric fatty acid may have been greater for the shorter chain lipids, which are relatively more soluble. This would result in the longer chain length lipids appearing to be less effective complexing agents than short chain lipids.

Earlier temperature studies on fatty acid-starch complexing have shown that an increase in temperature results in decreased swelling power, soluble starch, and Brabender peak viscosity, all of which have been attributed to increased complex formation (Gray and Schoch 1962, Ishii et al 1976). In their studies, granular corn starch was used as the experimental material. Prior to gelatinization, starch has limited binding capacity for lipid because most of the lipid in the system is unable to come in contact with starch. As the starch is dispersed, the amylose becomes available for complexation. In our work, all starch was autoclaved and thus the granule was destroyed. This treatment results in binding values that are independent of the extent of starch gelantinization.

The decrease in lipid binding as a function of temperature could be related to the increased thermal stress on the weak interactions holding the complex together. Higher temperatures have a disorganizing effect on the helical form of the amylose molecule, resulting in conformational structures with greater free energy (Von Hipple and Schleich 1969).

Decreases in complexing as the temperature is raised are also evident with compounds other than lipids that are known to complex with amylose. The helical complex formed between butanol and amylose in water was reported to dissolve on heating (Maywald et al 1968); these authors claimed the helical amylose structure opens up when heat is applied.

The amylose-iodine complex is also temperature sensitive (Knutson et al 1982). A gradual disappearance of blue color corresponding to complex dissociation occurs between 40 and 80° C. The complex can be reformed by cooling, with the overall phenomena being analogous to the helix-coil-helix transition that occurs on warming and cooling solutions of polypeptides (Banks and Greenwood 1975).

The binding of saturated monoglycerides to corn starch at 60, 40, and 20° C is illustrated in Figure 2. Similar trends were found by Lagendijk and Pennings (1970) when using a monoglyceride/starch system. Differences among monoglycerides were significant at each temperature. Whereas monoglycerides showed the same binding trends as the saturated fatty acids, values for the monoglycerides were slightly lower than for the corresponding fatty acid incubated under identical conditions. The glyceryl group esterified to the fatty acid may introduce a steric hindrance factor causing decreased binding values.

Another factor which may cause less monoglyceride binding compared to fatty acid is the relative solubility of these compounds in water. Under the experimental conditions used, an equilibrium exists between the free and bound forms of the lipid. Assuming that the starch molecule recognizes the hydrocarbon chain alone, the relatively greater solubility of monoglyceride in water compared to the corresponding fatty acid would shift the equilibrium in the direction of more free ligand.

The temperature effect on monoglyceride binding to starch was similar to that exhibited by the fatty acids. Hoover and Hadziyev (1981) followed the starch-monoglyceride complexing reaction by measuring viscosity over a temperature range of 50–80° C. At any given temperature, the highest viscosity was recorded for starch without added monoglyceride. Viscosity decreased as the size of

the added monoglyceride increased from C-8 to C-16. When the temperature was increased, viscosity increased. Part of the reason for this effect may have been a reduction in the amount of complex at higher temperatures. A compounding factor that would also have raised viscosity in their studies was the increased amount of amylose leached from the starch granules.

The effect of unsaturation on the C-18 series of fatty acids at 20, 40, and 60° C and pH 7 is shown in Figure 3. Differences between lipids varying by one double bond were significant with the exception of 18:2 versus 18:3 at 60° C and 18:0 versus 18:1 at 20° C. From these data, it is apparent that increasing the number of cis unsaturated bonds in the fat acid resulted in decreased complexing with starch.

It is reasonable to assume that the different molecular shape of unsaturated lipids may be responsible for decreased complexing. The saturated hydrocarbon chain is relatively straight and has considerable freedom of motion. When a cis double bond is present, the chain develops a 30° bend at the point of the double bond (Lehninger 1975). In addition, chain rotation about the double bond is prevented. This kinked chain may not fit as well within the amylose helix.

Another reason why unsaturated lipids are bound less to starch than saturated lipid is because of their greater solubility in water. A double bond is more hydrophilic than a saturated bond (Singleton 1960). Under the equilibrium conditions established in these experiments, a compound having greater affinity for solvent would exist in higher amounts in the unbound state.

Saturated and unsaturated fatty acid binding to starch is affected by temperature (Figs. 1 and 3). The quantity of oleic acid (18:1) bound at various temperatures is similar to the values obtained when using palmitic acid. Likewise, comparing linolenic (18:3) and lauric acids resulted in good agreement in binding values. From these data, it can stated that the effect on binding of incorporating a cis double bond into the fatty acid chain is equivalent to the removal of an ethylene group from the hydrocarbon chain.

There is lack of agreement in the literature regarding the effect of unsaturation on binding. Ohashi and co-workers (1980) presented Brabender viscoamylograph data, whereas Lagendijk and Pennings (1970) utilized a lipid extraction technique to suggest that complexing decreased as the number of unsaturated double bonds increased. In contrast, Mercier et al (1980) found that values for water-soluble carbohydrate and retrogradation decreased when oleic or linoleic acid were used in place of stearic acid. Riisom et al (1982) found that the complexing ability of C-18 cis unsaturated lipids can be better or worse than their saturated counterparts depending on the physical state of the lipid when added to the system. This illustrates the difficulties involved in comparing results from various indirect methods for measuring lipid-starch complexing.

pH

The extent of binding for the saturated fatty acid series at pH 3, 5, and 7 is shown in Figure 4. There were no significant differences in the extent of binding when comparing lauric to myristic acid at any pH or myristic to palmitic acid at pH 3. Differences between other fatty acids varying in chain length by two or more carbons were significant at each pH. Palmitic and stearic acids had much higher levels of bound lipid as pH was increased, whereas the two lower molecular weight lipids showed no such change.

Significant differences in binding existed between monoglycerides of different chain lengths at each pH (Fig. 5). As with lauric and myristic acids, levels of monolaurin and monomyristin bound to starch were unaffected by pH. In addition, pH had no effect on the extent of monopalmitin binding, in contrast to the results obtained for palmitic acid.

Increasing the degree of unsaturation resulted in lower fatty acid binding values at all pH levels evaluated (Fig. 6). On the other hand, C-18 cis unsaturated fatty acids showed no significant change in binding values from pH 3 to 7.

Binding values for all lipids investigated, except stearic and palmitic acids, were uniform over the pH range of 3–7. A change in starch molecular conformation within this pH range could alter complex-forming ability. If this were so, binding values for all lipids would be expected to change at the same pH value. Because this was not found, it was concluded that the ability of starch to form or maintain a helical conformation was not prevented at pH values between 3 and 7.

A possible explanation of why only stearic and palmitic acids had binding values that varied according to pH relates to their pK_a and behavior in solution. It is generally agreed that the pK_a for fatty acids is in the range of 4.7–5.0 (Spector 1975, Taylor and Princen 1979). At pH values in or below this range, intermolecular hydrogen bonds could form between the carboxylic acid groups of two fatty acids. However this cyclic dimerization of fatty acids has not been found in water (Pimentel and McClellan 1960). An arrangement whereby hydrocarbon chains of the two lipids are parallel while permitting intermolecular hydrogen bonding is feasible (Schrier et al 1964). This structure, which would be formed when the carbonyl group is protonated, would result in a hydrocarbon tail having a diameter twice as large as the monomeric lipid. This larger diameter hydrophobic tail would

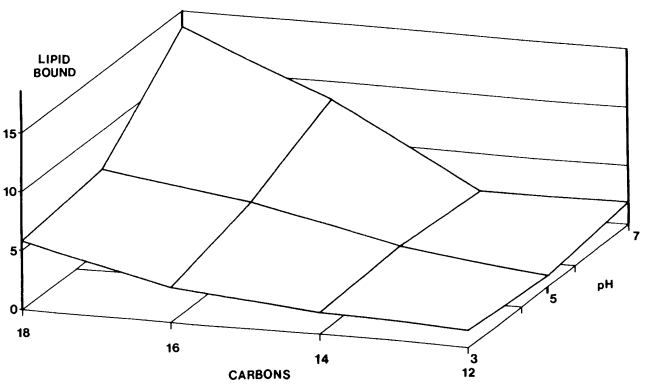

Fig. 4. Effect of pH and chain length on saturated fatty acid binding at 60° C.

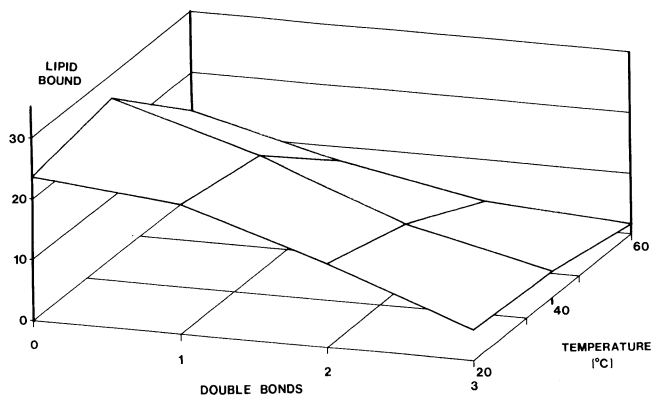

Fig. 3. Effect of temperature and cis unsaturation on C-18 fatty acid binding at pH 7.

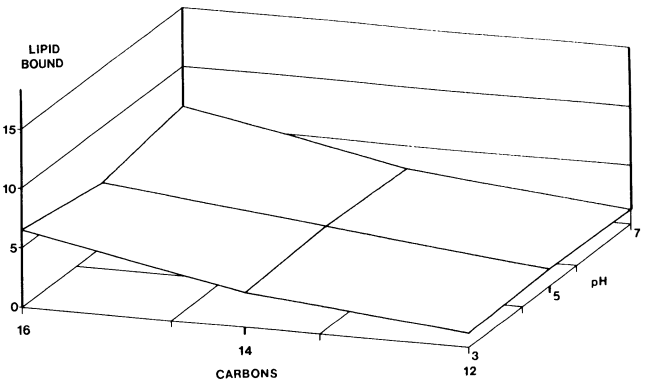

Fig. 5. Effect of pH and chain length on monoglyceride binding at 60° C.

have an effective diameter greater than the space available within the V_6 helical conformation of amylose. Smaller molecular weight fatty acids do not show a trend similar to that of palmitic and stearic acids because dimer stability increases with increasing length of the nonpolar portion of the acid (Kauzmann 1959, Nemethy and Scheraga 1962, Hand and Cohen 1965). The binding affinity of lauric and myristic acid were not affected by pH changes, suggesting that these lipids do not dimerize under these experimental conditions.

Additional evidence to support the fact that hydrogen bond formation is important in reducing binding is seen when comparing the pH binding data of palmitic acid to monopalmitin (Figs. 4 and 5). If the hydrocarbon tail was the only part of the lipid responsible for changes in binding with pH, one would expect to find a similar pH effect for these two compounds.

Ionic Strength

At pH 7, both myristic acid and monomyristin showed increased binding to starch as buffer molarity increased from 0.2 to 0.8*M* (Table I). At pH 5 there was also a trend toward higher $\bar{\nu}$ at higher ionic strength; however, in this case the change was not significant ($P < 0.05$). The binding values at pH 3 remained relatively constant, ranging from 5.8 to 7.9.

It was shown earlier (Figs. 4 and 5) that $\bar{\nu}$ for myristic acid and monomyristin is independent of pH when the reaction is carried out in 0.1*M* citrate-phosphate buffer. In contrast, the use of 0.6 and 0.8*M* buffer resulted in significant increases in binding values as the pH was raised from 3 to 7.

TABLE I
Effect of Buffer Molarity on Moles of Lipid Bound[a]

pH	Lipid	Buffer Molarity 0.2	0.4	0.6	0.8
3	Myristic acid	7.30 ± 0.57	7.05 ± 0.49	6.70 ± 0.28	6.80 ± 0.42
	Monomyristin	5.83 ± 2.30	7.90 ± 1.80	6.90 ± 0.42	5.75 ± 0.64
5	Myristic acid	8.30 ± 0.28	9.65 ± 0.78	9.90 ± 2.40	9.10 ± 0.85
	Monomyristin	8.13 ± 1.20	7.20 ± 0.01	9.60 ± 1.70	11.90 ± 0.71
7	Myristic acid	6.33 ± 0.40	9.77 ± 1.62	10.20 ± 0.28	12.63 ± 1.30
	Monomyristin	7.95 ± 0.35	9.20 ± 0.42	10.05 ± 0.49	13.05 ± 0.07

[a]Per mole amylose, 40° C, mean ± SD, $n = 4$.

TABLE II
Stearic Acid Binding to Various Starches[a]

Starch	Moles Stearic Acid Bound I[b]	II[c]	III[d]
Unmodified corn	20.9 ± 1.9 e[f]	···	5.25 ± 0.47 e
Hydroxypropyl corn[e]	5.80 ± 1.24 f	···	1.46 ± 0.31 f
Waxy maize	···	11.4 ± 1.6	0.76 ± 0.12 f

[a]0.005% Starch, 0.5 μM stearic acid, 40° C, pH 7; mean ± SD, $n = 4$.
[b]Per mole amylose.
[c]Per mole amylopectin, molecular weight of 1.5×10^7 used in calculation.
[d]Per gram starch.
[e]4.3% Propylene oxide content, dry basis.
[f]Within each column, means followed by different letters are significantly different ($P < 0.05$).

Starch Type

Substituting hydroxypropyl starch for unmodified starch resulted in a significant decrease in binding (Table II). Whereas little has been published regarding lipid binding to modified starch, iodine binding has been shown to be affected by derivatizing amylose or starch. In one study, iodine absorption progressively decreased from 11.3 to 8.1% as the molar substitution of hydroxyethyl amylose increased from 0.03 to 0.14 (Staverman et al 1961). In that study, 9.1% iodine was bound to 0.12 molar substitution amylose, which was equivalent to the level of modification use in this study (4.3% propylene oxide, dry basis).

Mikus et al (1946) were the first to present evidence suggesting that iodine and fatty acids occupy the same position when complexed to amylose. Both iodine and lipid ligands result in the "V" form of amylose having six anhydroglucose units per helical turn (Zobel 1964, Acker 1977). It is reasonable to assume that the forces destabilizing one of these complexes would have a similar effect on the other. The relatively bulky hydroxypropyl groups etherified to the anhydroglucose unit may limit the extent of helix formation, causing a reduction in lipid binding.

Our results indicate that waxy maize starch binds stearic acid (Table II). Therefore, a portion of the lipid-binding ability of regular corn starch should be attributed to amylopectin. When binding is expressed on the basis of micromoles of stearic acid bound per gram of starch, regular corn starch binds approximately seven times more lipid than does waxy maize. This difference occurs because amylose is a better lipid-complexing agent than amylopectin.

The literature is replete with conflicting information regarding the lipid-binding ability of amylopectin. Lord (1950) and Krog (1971) state that lipids do not result in precipitation of amylopectin, as is the case with amylose. Perhaps both starch fractions do in fact form lipid complexes, and it is the inherent instability of dispersed linear amylose, and not complex formation, that results in precipitation. Goering et al (1975) used the Brabender viscoamylograph to show that waxy maize does not complex to fatty acid. In contrast, Orthoefer (1976) showed increased peak viscosity and setback when glycerol monostearate was added to waxy maize. The presence of the granule complicated the interpretation of results in both these studies.

Others have shown that amylopectin has the ability to bind lipids (Gray and Schoch 1962, DeStefanis et al 1977). Quantitative information on monoglyceride binding to amylopectin has been provided by Lagendijk and Pennings (1970). Binding of lipid increased linearly from monolaurate to monoarachidate, with monostearin resulting in 25 μmol of bound lipid per gram of amylopectin. This value is higher than obtained in this study; however, the use of a higher concentration of monoglyceride in their reaction system may have shifted the equilibrium toward the formation of more complexed lipid.

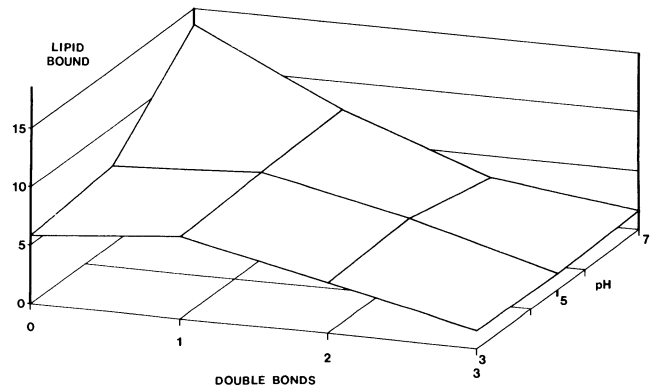

Fig. 6. Effect of pH and cis unsaturation on C-18 fatty acid binding at 60° C.

CONCLUSION

This study was the first to utilize the technique of equilibrium dialysis to investigate starch-lipid complexes. Increasing the chain length of saturated fatty acids resulted in increased $\bar{\nu}$ (moles of lipid bound per mole of amylose). An evaluation of a cis unsaturated C-18 series of fatty acids revealed that increasing unsaturation resulted in less binding between starch and lipid. Addition of a double bond to a C-18 fatty acid had the equivalent effect on binding as a two-carbon reduction in hydrocarbon chain length. The experimental parameters of temperature, pH, and ionic strength were shown to affect complexing.

Modifying starch with propylene oxide caused a reduction in binding values. Waxy maize starch was able to complex stearic acid, although at lower levels than with corn starch. This implied that amylopectin does possess some lipid binding ability.

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