COMPLEXES OF AMYLOSE WITH SURFACTANTS[1]

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ABSTRACT

Eighteen surfactants, fatty acids and their esters with one, two, or more hydrocarbon chains, formed complexes with amylose having the same cell dimensions, as indicated by X-ray diffraction patterns. All surfactants used, with the exception of the diglycerides of hydrogenated soybean oil, greatly reduced the iodine affinity of amylose, but in no case was it reduced to zero. Soybean oil itself showed no indication of complex formation with amylose. For those surfactants containing a single hydrocarbon chain, the percentage of additive calculated for the hypothetical reduction of iodine affinity to zero, indicative of complete complex formation, appeared to be directly related to the percentage of the hydrocarbon portion of the molecule.

The mechanism by which certain surfactants retard some of the changes, particularly the increase in firmness, associated with the staling of bread is not completely understood. The ability of one such surfactant, polyoxyethylene monostearate, to complex with the amylose fraction of starch has been demonstrated quite conclusively (9,21) by its interference with the affinity of the amylose for iodine. Indications that monoglycerides (25) and sucrose monostearate (5) have similar effects have been reported. That the formation of such complexes of amylose in bread is the cause of the bread-softening action of these compounds has been postulated (9). However, surfactants of different chemical structures have been reported (11) to differ widely in their effects on crumb firmness. They likewise have been found (12) to cause different effects on curves obtained with the Brabender Amylograph for corn starch slurries containing soybean oil. Both the temperature at which viscosity increased during heating and the shape of the cooling curve were affected. These differences appeared to be related to the lengths of the hydrocarbon chains and the number of hydrocarbon chains in the molecules, as well as to the structures of the hydrophilic moieties.

The present study was undertaken to determine whether or not these reported differences between surfactants in respect to their effects on amylograph curves and crumb softness might be caused by differences in their ability to form complexes with amylose or differences in the dimensions of the complexes formed.

One method of measuring the extent to which amylose has under-

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gone complex formation with compounds of this type is measurement of the reduction in its iodine affinity. Lord (9) and Schoch (21) reported that a linear relationship existed between the percentage of polyoxyethylene stearate or fatty acid added to starch or amylose and its iodine affinity. It was likewise shown that, on a weight basis, fatty acid was more effective in reducing iodine affinity than was this ester. The difference was attributed to difference in the relative sizes of the two molecules. An explanation proposed for the effect of surfactants or fatty acids on iodine affinity rests on the structure of the complexes involved. Hanes (6), in 1937, postulated a helical configuration for the starch-iodine complex, a hypothesis supported by later studies of Rundle and his co-workers (14,15,17,18,19,24). The latter suggested that the iodine molecules are oriented in a linear arrangement enclosed within and parallel to the axis of an amylose helix. X-ray diffraction studies led Mikus, Hixon, and Rundle (10) to propose a similar structure for complexes of fatty acids with amylose, with fully extended fatty acid molecules forming the core of the helix. Measurements of iodine-binding capacities of complexes containing palmitic, oleic, and lauric acids indicated that the greater the length of the fully extended fatty acid, the more amylose it bound. No similar comparison of the relative effectiveness of surface-active agents of a variety of structures has been made. In fact, the only reports in the literature on the effects of surface-active agents on iodine affinity appear to concern polyoxyethylene monostearate (9,21), monoglycerides (25), and sucrose monostearate (5), all of which have only one hydrocarbon chain.

X-ray diffraction studies of complexes of amylose and surface-active agents have not been reported, but complexes of palmitic, stearic, and oleic acids with amylose have been found to give X-ray patterns with nearly identical spacings (10), very similar to those for the amylose-iodine complex. However, Bear (3) concluded, from patterns obtained with amylose complexes of linear and branched alcohols, that the amylose helix can be enlarged to accommodate the branched-chain alcohols.

In the present study comparison was made of the efficacies of various surface-active agents in reducing the iodine affinity of amylose. X-ray diffraction patterns of purified complexes of amylose and the various surface-active agents were also compared.

Material and Methods

Materials. Amylose was prepared from commercial corn starch by selective precipitation with Pentasol, according to the method described by Lansky, Kooi, and Schoch (8). It was purified by two recrystallizations from water saturated with 1-butanol. After extraction in a

Soxhlet apparatus for 24 hours with 95% ethanol, the amylose was dried 4 hours at 50°C. under vacuum, and pulverized. The iodine affinity was 15.2%, a value low for pure amylose but indicating a sufficiently high percentage of the linear component for the present study.

Purified Complexes of Amylose with Surfactants. To a suspension of 0.300 g. corn amylose in 5 ml. water, 10.0 ml. of 1*N* potassium hydroxide were added, and the sample was held in a refrigerator with occasional stirring for 30 minutes, or until a clear solution was obtained. The solution was then neutralized to methyl orange with 0.5*N* hydrochloric acid and transferred into a 150-ml., three-necked round-bottom flask provided with a condenser and an efficient stirrer. The flask was placed in a water bath at 65°C. To it was added 0.100 to 0.150 g. of surface-active agent. Water was added until the volume of the mixture was approximately 60 ml., and it was stirred at 65°C. for 3 hours. The heating and stirring were then discontinued and the flask was left in the water bath overnight to cool slowly to room temperature. The precipitate was separated by centrifugation for 30 minutes in a Servall Superspeed Angle Centrifuge at 13,800 r.p.m. (about 24,300 × *g*). The precipitate was washed twice with hot carbon tetrachloride, dried for 1.5 to 2 hours at 65°C. under vacuum, powdered, and stored in a desiccator over sulfuric acid.

An aliquot of the sample was extracted exhaustively in a micro-Soxhlet apparatus with carbon tetrachloride. A second aliquot was extracted exhaustively with absolute methanol.

Infrared absorption spectra were obtained on all extracted samples. The Nujol mull technique was used.

Iodine affinity of samples which had been extracted with methanol was measured by the standard procedure of Schoch (22). The same procedure was used for the samples which had been extracted with carbon tetrachloride, except that the potassium hydroxide was neutralized with hydrochloric acid before the sample was added to it. In this way possibility of saponification of the complexed surfactant was eliminated; dispersion appeared to be as complete as in potassium hydroxide. In neither potassium hydroxide nor the neutralized solution was the complex dissolved, but a fine dispersion was obtained.

X-ray diagrams of samples after extraction with carbon tetrachloride or methanol were made with CuK_α radiation, nickel-filtered, and a cylindrical powder camera of 7-cm. radius. The powder samples were sealed in thin-walled glass capillaries of 0.7-mm. diameter. Exposure time was 4.5 hours at 15 ma. and 40 kv.

Effect of Surfactants on Iodine Affinity of Amylose. A sample of

amylose (37.8 mg., d.b.) was weighed into a 125-ml. Erlenmeyer flask. Approximately 1 ml. of water was added to suspend the sample. Five milliliters of 1*N* potassium hydroxide were added and the sample was immediately dispersed by stirring with a glass rod. It was then held, with occasional stirring, in the refrigerator for 30 minutes. The resulting clear solution was neutralized to methyl orange with 0.5*N* hydrochloric acid. A weighed amount of surfactant was added. Water (25 to 50 ml.) was added and the mixture was stirred in a constant-temperature water bath for 3 to 6 hours. Time and temperature of heating were varied with each surfactant to determine whether or not equilibrium had been reached. The solution was slowly cooled, without agitation, to room temperature and transferred quantitatively to a tared 250-ml. beaker. Ten milliliters of 0.5*N* potassium iodide solution were added. Sufficient water was added to bring the weight of the mixture to 100.9 g.

The potentiometric iodine titration was carried out by the method of Bates, French, and Rundle (1), as modified by Schoch (22), except that readings of the e.m.f. values were taken after intervals of only 1 minute following addition of each milliliter of iodine. Iodine affinity was calculated in the usual way.

Results and Discussion

Interaction between the amylose and the surfactants was indicated by the formation of precipitates in the cooled reaction mixtures after these substances had been heated together in water. Such precipitation of amylose has previously been brought about by addition of fatty acids (23), polyoxyethylene monostearate (5,9), sucrose monostearate, sucrose distearate, and polyoxyethylene sorbitan (5), as well as with a variety of other polar organic compounds. Bourne, Tiffin, and Weigel (5), estimating the amount of precipitation from turbidity measurements, observed that the amount of precipitate was proportional to the amount of surfactant up to a certain point beyond which additional surfactant had no effect. In the present study the amount of surfactant used provided a large excess over that needed to reduce the iodine affinity to a minimum value (cf. Table IV). However, with diglycerides prepared from hydrogenated soybean oil even such a large excess produced only a very little precipitate, although more than appeared possible from impurities in the diglyceride. Soybean oil itself yielded no precipitate with the amylose.

Exhaustive extraction with carbon tetrachloride removed only unbound surfactant from the precipitate, whereas methanol removed the bound surfactant as well. Although the weights of surfactants

extracted were determined only roughly after evaporation of the extracts, it was observed that the methanol extracts yielded larger residues than the corresponding carbon tetrachloride extracts in all cases, except with the precipitate from the diglycerides. That the surfactant remained bound after carbon tetrachloride extraction but not after methanol extraction was shown by the infrared spectra of the extracted products. Those extracted with carbon tetrachloride all showed a characteristic absorption maximum in the region of 1,725 cm.$^{-1}$, indicating the presence of carboxyl groups. Those samples extracted with methanol failed to show such a maximum. Further evidence that the surfactant remained bound after extraction with carbon tetrachloride but not after extraction with methanol was gained by determination of iodine affinity, which was greatly reduced in the carbon tetrachloride-extracted samples, but approximated that of the original amylose after methanol extraction (see Table I).

X-ray powder patterns of all complexes, as well as the amylose remaining after methanol extraction of complexes, had the pattern designated by Katz (7) as the "V" (Verkleisterung) pattern (Table II). The spacings corresponded closely to those previously reported for complexes of fatty acids with amylose (11). The minor differences between the spacings found in the various samples were probably caused by the differences in the structures of the complexing agents, differences in the amounts of the surfactants present in the complexes, and differences in the conditions under which the complexes were prepared, purified, and stored. Samec, Katz, and Derksen (20) early pointed out that slight differences between the diameters of corresponding interference rings can occur with starch preparations having the same pattern, and that apparent trifles in preparation of the materials will result in differences in the sharpness of the lines. Bear (2) has discussed some of the factors influencing the "V" pattern in detail.

The close agreement of the X-ray diagrams of the amylose-surfactant complexes with those of the fatty acid complexes indicated a similar structure, presumably the helical structure proposed for the fatty acid-amylose complexes (10). Although the surfactants with two hydrocarbon chains assume a wedge shape when present at the interface between water and another immiscible liquid, the spacings of their amylose complexes indicated that the diameter of amylose helix was only large enough to accommodate a single hydrocarbon chain. Lecithin, with its third substituent on the glycerol moiety, also formed a complex of similar dimensions, although the triglycerides of soybean oil failed even to yield a precipitate. The cause of the low degree of

TABLE I

IODINE AFFINITY OF AMYLOSE-SURFACTANT COMPLEXES

SURFACTANT	DESCRIPTION	AFTER EXTRACTION WITH CCl_4	AFTER EXTRACTION WITH CH_3OH
		%	%
Monostearins	Minimum monoglyceride content, 90%; composition, 90% stearate, 8% palmitate, 2% other fatty acid residues	1.2 3.2 5.1	17.9 17.8 18.0
Monopalmitins	Minimum monoglyceride content, 95%; composition, 95% palmitate, 5% stearate	1.6	15.7
1-Monopalmitin	90% pure	1.8	15.3
2-Monopalmitin	95% pure	2.0	14.5
D-Glucose 3-stearate	White crystals; research product[a]	0.6	13.6
D-Glucose 3-palmitate	White crystals; research product	0.8	15.3
Methyl α-D-glucoside 6-stearate	White powder; research product	0.9 0.6	14.6 18.3
Methyl α-D-glucoside 6-palmitate	White powder, m.p. 83°–86°C.; research product	1.1	14.4
Methyl α-D-glucoside 6-laurate	White powder, m.p. 68°–71°C.; research product	3.4	15.5
Sucrose monostearate	Monoester, 88%; esterification probably largely in position 6 of glucose moiety	0.6	14.5
Polyoxyethylene (8) monostearate	MYRJ 45; 37.8 – 47.2% monoester, average polymer lengths 7.25–7.50 oxyethylene units[4b]	2.3 1.7	14.9 15.8
Oleic acid	U.S.P.	2.8	17.3
Palmitic acid		8.8	16.2
Diglycerides from hydrogenated soybean oil	Diglyceride content, 95%	5.8	12.4
Methyl α-D-glucoside distearate	Esterification predominantly on carbons 2 and 6; not homogeneous	1.3	12.3
Sucrose distearate	Diester 91.3%; esterification probably largely on carbons 6 of glucose and fructose moieties	2.3 4.3	13.0 13.8
Lecithin	Soybean phosphatides, 95+%, containing true lecithin, cephalin, and lipositol	4.8 5.5 3.3	13.6 13.5 13.4
Sorbitan tetrastearate	Technical grade	2.8	16.2

[a] See reference 13.
[b] See reference 4.

complexing by the diglycerides of hydrogenated soybean oil, indicated by the small yield of precipitate and also by their small effect on iodine affinity of amylose, is not readily apparent.

Exhaustive methanol extraction of the amylose-surfactant complexes resulted in amylose which retained the "V"-type X-ray diffraction pattern (Table II). However, the *d*-spacings were slightly smaller than those found for the corresponding complexes, although Rundle and Edwards (16) had reported the dry precipitate from the butanol complex (presumably butanol-free) to have a somewhat larger

TABLE II

X-RAY DIFFRACTION PATTERNS OF AMYLOSE-SURFACTANT COMPLEXES[a]

SURFACTANT	AFTER EXTRACTION WITH CCl_4[b]				AFTER EXTRACTION WITH CH_3OH[b]					
Monostearins	12.33m	7.06m	4.51s	3.17m	11.18m	6.99m	5.01vw	4.28s		
	12.26m	6.99m	4.48s	3.16s	11.42m	6.99m	5.08vw	4.26s	3.35vw	2.92vw
	12.00m[c]	6.97m	4.49s[c]	3.17s						
Monopalmitins	11.86s	6.95s	4.46vs	3.17m	11.13s	6.59s		4.27vs		
1-Monopalmitin	11.73m	6.93m	4.48s	3.17m						
2-Monopalmitin	11.42m	7.02m	4.48s	3.17s						
D-Glucose 3-stearate	11.79m	6.90m	4.47s	3.17m	11.24m	6.59m		4.31s		
D-Glucose 3-palmitate	11.79m	6.95m	4.49s	3.16m	11.42m	6.65m		4.31s		
Methyl α-D-glucoside 6-stearate	11.79m	7.02m	4.49s	3.16s						
	11.42m	7.02m	4.49s		11.24m	6.57m		4.29s		
Methyl α-D-glucoside 6-palmitate	12.06s	6.86s	4.49s	3.16s	11.36s	6.63s	5.02vw	4.31s		
Methyl α-D-glucoside 6-laurate	12.19s	7.02s	4.49vs	3.16m	11.48s	6.63s	5.08vw	4.27vs		
Sucrose monostearate	11.18m	6.99m	4.49s	3.16vw						
Polyoxyethylene (8) monostearate	11.79s	6.95s	4.45s	3.16s	11.54s	6.65s		4.33s		
	11.67m	6.99s	4.46vs	3.16s	11.48m	6.73s		4.35vs		
Oleic acid	11.67s	6.97s	4.44s	3.17w	11.42s	6.53s	5.07vw	4.24s	3.41w	2.91w
	12.69m	7.06m[d]	4.43m	3.16s						
Palmitic acid	11.67s	6.97s	4.48s	3.17m	11.61s	6.88s		4.31s		
Diglycerides from hydrogenated soybean oil	11.79i	7.08i	4.50s	3.16s						
Methyl α-D-glucoside distearate	11.67m	6.95s	4.46vs	3.16vw	11.27m	6.65s		4.33vs		
Sucrose distearate	11.67m	6.97m	4.49vw	3.17m						
	11.73m	6.90m	4.47s		11.36m	6.65m		4.33s	3.42vw	2.89vw
Lecithin	11.42m	7.32m	4.51s	3.17s						
	12.41s	6.95s	4.45s		11.61s	6.63s	5.09vw	4.29s		
	12.26m	6.93s	4.38s		11.24m	5.59s		4.29s	3.42vw	
Sorbitan tetrastearate	11.54m	6.97m	4.48s	3.17m						
Butanol	12.12m[e]	6.82m[e]	4.48s[e]							

[a] Intensity notation: vs, very strong; s, strong; m, medium; w, weak; vw, very weak; i, indistinct.
[b] Interplanar spacing, *d*, in angstroms.
[c] An extra diffraction line also appears at 4.09vw.
[d] An extra diffraction line also appears at 4.88m.
[e] Complex not extracted with CCl_4 before X-ray examination.

distance between helices than in either the amylose-butanol or amylose-iodine complex.

Study of the iodine uptake of amylose without isolation of the complex showed that each surfactant reduced the iodine affinity to a minimum value beyond which it could be reduced no further by addition of more surfactant, by different temperature during heating, or by longer heating time. These values are shown in Table III, together with conditions of heating which produced equilibria.

TABLE III

IODINE AFFINITY OF AMYLOSE WITH EXCESS SURFACTANT

SURFACTANT	AMOUNT ADDED [a]	CONDITIONS OF HEATING		IODINE AFFINITY
	%	*hours*	°C	%
Monostearins	54	4	84	1.7
Monopalmitins	60	3	83	1.5
1-Monopalmitin	48	4	81	2.3
2-Monopalmitin	45	4	81	1.4
D-Glucose 3-stearate	50	5	83	0.3
D-Glucose 3-palmitate	51	4	85	0.6
Methyl α-D-glucoside 6-stearate	39	4	84	0.8
Methyl α-D-glucoside 6-palmitate	41	4	83	0.9
Methyl α-D-glucoside 6-laurate	52	4	84	2.1
Sucrose monostearate	47	4	85	0.2
Polyoxyethylene (8) monostearate	37	4	81	4.5
Polyoxyethylene (40) monostearate	64	4	83	4.7
Polyoxyethlene sorbitan mono-oleate	46	4	83	4.8
Oleic acid	46	4	78	3.5
Palmitic acid	44	4	70	3.6
Sodium lauryl sulfate	43	4	84	1.5
Diglycerides from hydrogenated soybean oil	229	4.75	85	11.7
Methyl α-D-glucoside distearate	55	5	85	3.0
Sucrose distearate	46	4	70	3.3
Lecithin	56	4	84	2.1
Sorbitan tetrastearate	50	6	66	4.1

[a] Based on dry weight of amylose.

Temperatures used were influenced by the fact that some of the additives, especially the monoglycerides, became much less soluble near 100°C. The surfactants themselves were found to have no effect on the iodine titration in the absence of amylose. The iodine affinity of the amylose was reduced below 5% by all of the surfactants used except the diglycerides. The latter preparation reduced it only to 11.7%, thus exhibiting the same reluctance to complex that had been found during preparation of samples for X-ray analysis.

More striking than the differences in total iodine uptake were the differences in the slopes of the titration curves for amylose treated with excess amounts of surfactants of various structures. The compounds with one fatty acid moiety (Fig. 1) gave curves completely free

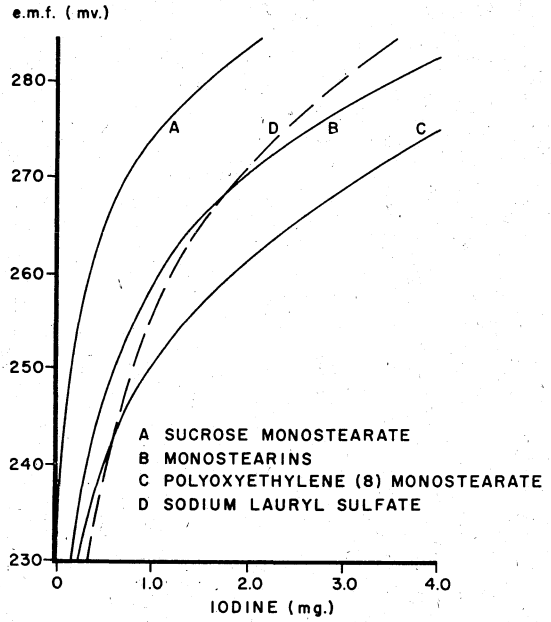

Fig. 1. Titration curves of amylose treated with excesses of some monoesters.

of the sigmoid shape characteristic for free amylose. Sucrose monostearate gave a curve which rose very rapidly; those for D-glucose 3-stearate and D-glucose 3-palmitate were very similar to it. The curves for all the monoglyceride preparations used (Table III) were almost identical, regardless of the position of the ester group in the molecule or whether the fatty acid group was palmitic or stearic, and rose somewhat less sharply than that for sucrose monostearate (Fig. 1). Polyoxyethylene (8) monostearate and the other polyoxyethylene compounds produced curves which rose still more gradually, but all three were alike.

Potentiometric titration curves of amylose to which excess palmitic or oleic acid had been added were nearly identical with that for polyoxyethylene (8) monostearate, but addition of sodium lauryl sulfate caused the curve to rise more rapidly (Fig. 1).

The esters of methyl α-D-glucoside lay between those for sucrose monostearate and the monoglycerides (Fig. 2), but were of special interest because the weight of iodine required to raise the e.m.f. to 280 mv. varied inversely with the length of the fatty acid residue.

Lecithin yielded a titration curve similar to those for the monoesters, but all the other additives examined which had more than one fatty acid moiety caused the titration curves to be sigmoid in shape

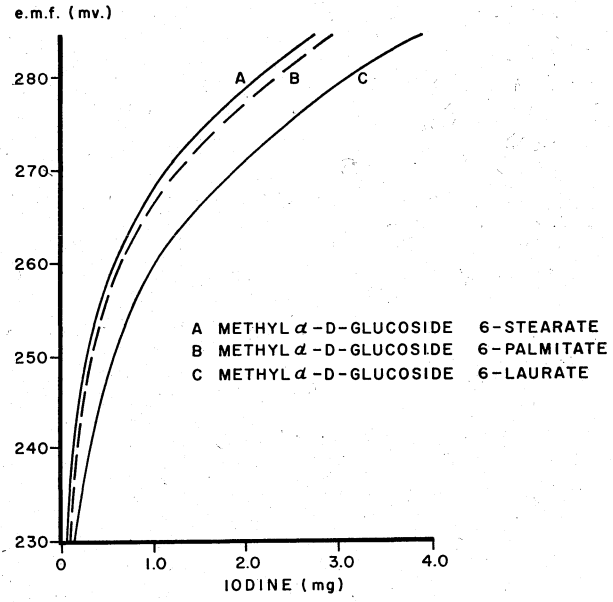

Fig. 2. Titration curves of amylose treated with excesses of methyl α-D-glucoside esters.

(Fig. 3). Sucrose distearate, methyl glucoside distearate, and sorbitan tetrastearate all caused great reductions in iodine affinity (Table III),

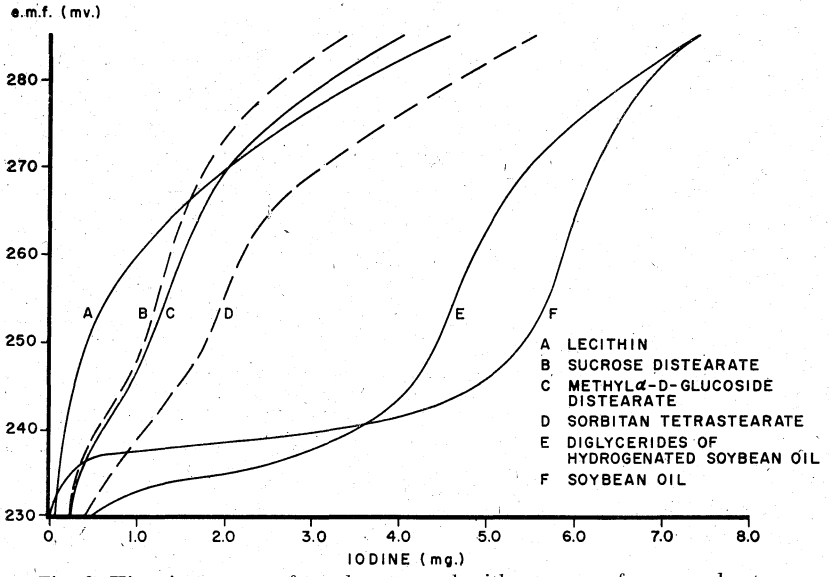

Fig. 3. Titration curves of amylose treated with excesses of some polyesters.

however. The diglyceride compound, on the other hand, was very much less effective in lowering the iodine affinity and yielded a titration curve which was much more like that produced by the addition of fat or with no additive at all.

Curves of sigmoid shape were characteristic of those titration mixtures in which little or no complex formation had occurred (i.e., with fat or diglycerides), as well as those in which the amount of additive present was less than that required to reduce iodine affinity to its minimum value (Fig. 4). It therefore seems probable that the sigmoid

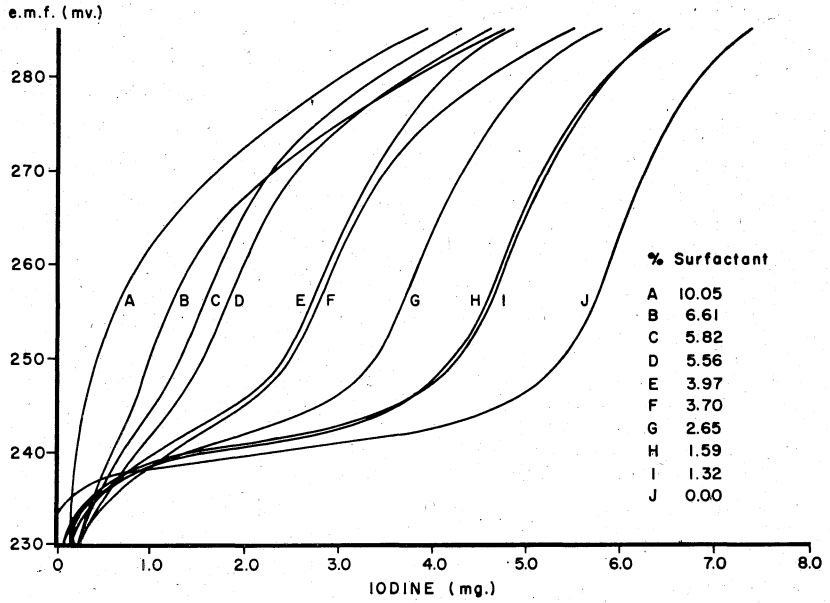

Fig. 4. Titration curves of amylose treated with different amounts of 1-palmitin.

shapes of the curves for mixtures containing sucrose distearate, methyl glucoside distearate, and sorbitan tetrastearate were evidence of the inability of these compounds to complex the amylose completely. Although the mixtures containing large amounts of these surfactants had iodine affinities no higher than those with many of the other surfactants used, they also differed in that addition of a small amount of iodine at the start of the titration immediately produced the blue color typical of the starch-iodine complex.

The slopes of the curves obtained with the surfactants containing only one ester group may be related to the stabilities of the complexes formed, since those with the greatest slopes (Fig. 1) show the greatest reduction in iodine affinity (Table III). The smaller slopes show less

reduction in iodine affinity, possibly because iodine may have replaced some of the surfactant.

Titration curves gradually lost their pronounced sigmoid shape as the amount of surfactant added approached that required for complete complex formation (Fig. 4). Similar series of curves had been obtained by Mikus, Hixon, and Rundle (10) when increasing amounts of oleic and palmitic acids were added to amylose. A linear relationship was observed between the percentage of surfactant added and the resulting iodine affinity (Fig. 5), until the iodine uptake was reduced to nearly

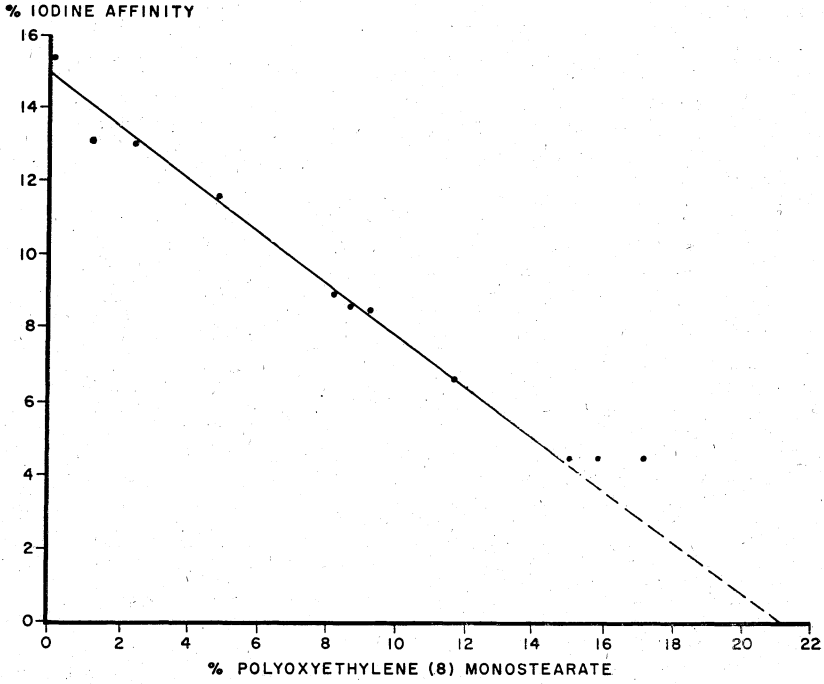

Fig. 5. Relationship between percentage of polyoxyethylene (8) monostearate added and iodine affinity of amylose.

the maximum extent which had been determined previously by use of large excesses of the surfactant. Although none of the surfactants reduced the iodine affinity to zero, the linear portion of the curves could be extrapolated to intercept the X-axis and provide values for comparison of the various surfactants with each other and with values reported by other investigators (9,21,23). The values so obtained represent the percentage of each surfactant which would be needed to reduce the iodine affinity of the amylose to zero if the iodine affinity did not reach a limiting minimum value, as already noted. They varied

from 5.4 to 29.8% of the weight of the amylose and were higher for compounds of greater molecular weight (Table IV). For surfactants

TABLE IV

WEIGHT OF SURFACTANT COMPLEXED COMPARED TO ITS STRUCTURE

SURFACTANT	NEEDED FOR ZERO IODINE AFFINITY [a]	MOLECULAR WEIGHT	HYDROCARBON CHAIN IN MOLECULE
	%		%
Palmitic acid	5.4	256	82
Monostearins	6.4	359	67
Monopalmitins	7.5	331	64
Methyl α-D-glucoside 6-palmitate	8.4	433	49
Sodium lauryl sulfate	9.3	288	59
D-Glucose 3-stearate	10.0	447	54
Methyl α-D-glucoside 6-stearate	10.0	461	52
Methyl α-D-glucoside 6-laurate	11.3	376	41
D-Glucose 3-palmitate	15.4	419	50
Polyoxyethylene (8) monostearate	21.0 (17.6)[b]	679[c]	43 (34)[d]
Lecithin	29.8	790[e]	60 (30)[d]

[a] Calculated by extrapolation of linear portion of curve (cf. Fig. 5); actually iodine affinity reached minimum value (Table III) below which no further reduction could be obtained.
[b] Estimated weight of esters in sample, without free polyglycols (4).
[c] Estimated molecular weight of mono- and di-esters in sample (4).
[d] Estimated percentage of molecule composed of a single hydrocarbon chain (assuming the additional hydrocarbon chains in molecule are unable to form complex).
[e] Calculated for lecithin molecule containing two stearoyl groups.

with a single hydrocarbon chain, the amount of surfactant required appeared to be still more closely related to the percentage of the molecule composed of the hydrocarbon chain, although the amounts of D-glucose 3-palmitate and polyoxyethylene (8) monostearate needed were appreciably higher than would be anticipated from such a relationship. The former, however, has previously been shown to give results different from those which might be expected in its action on both crumb firmness (10) and Brabender Amylograph curves of starch pastes (12).

The action of the polyoxyethylene (8) monostearate preparation in reducing iodine affinity may have been lessened considerably by the presence of a large proportion of the diester (4). As mentioned above, diglycerides are very inefficient in reducing iodine affinity, and lecithin, although much more effective, is far less so than the majority of monoesters studied.

Effects of surfactants on crumb firmness in bread (11) and on the Brabender Amylograph curves of corn starch pastes (12) do not appear to be directly related to their complex formation with amylose under the conditions of these experiments or to the cell dimensions of the complexes. However, the possibility remains that they may differ in their abilities to form complexes under the conditions present in starch pastes or in bread.

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Literature Cited

1. BATES, F. L., FRENCH, D., and RUNDLE, R. E. Amylose and amylopectin content of starches determined by their iodine complex formation. J. Am. Chem. Soc. **65:** 142–148 (1943).
2. BEAR, R. S. The significance of the "V" X-ray diffraction patterns of starches. J. Am. Chem. Soc. **64:** 1388–1392 (1942).
3. BEAR, R. S. Complex formation between starch and organic molecules. J. Am. Chem. Soc. **66:** 2122–2123 (1944).
4. BIRKMEIER, R. L., and BRANDNER, J. D. Composition of polyoxyethylene (8) stearate. J. Agr. Food Chem. **6:** 471–475 (1958).
5. BOURNE, E. J., TIFFIN, A. I., and WEIGEL, H. Interaction of starch with sucrose stearates and other antistaling agents. J. Sci. Food Agr. **11:** 101–109 (1960).
6. HANES, C. S. The action of amylases in relation to the structure of starch and its metabolism in the plant. Parts IV–VII. New Phytologist **36:** 189–239 (1937).
7. KATZ, J. R. Abhandlungen zur physikalischen Chemie der Stärke und der Brotbereitung. I. Über die Änderungen im Röntgenspektrum der Stärke beim Backen und beim Altbackenwerden des Brotes. Z. physik. Chem. (Leipzig) **150A:** 37–59 (1930).
8. LANSKY, SYLVIA, KOOI, MARY, and SCHOCH, T. J. Properties of the fractions and linear subfractions from various starches. J. Am. Chem. Soc. **71:** 4066–4075 (1949).
9. LORD, D. D. The action of polyoxyethylene monostearate upon starch with reference to its softening action in bread. J. Colloid Sci. **5:** 360–375 (1950).
10. MIKUS, F. F., HIXON, R. M., and RUNDLE, R. E. The complexes of fatty acids with amylose. J. Am. Chem. Soc. **68:** 1115–1123 (1946).
11. OFELT, C. W., MEHLTRETTER, C. L., MACMASTERS, MAJEL M., OTEY, F. H., and SENTI, F. R. Effect on crumb firmness. II. Action of additives in relation to their chemical structure. Cereal Chem. **35:** 142–145 (1958).
12. OSMAN, ELIZABETH M., and DIX, MARION R. Effects of fats and nonionic surface-active agents on starch pastes. Cereal Chem. **37:** 464–474 (1960).
13. OTEY, F. H., and MEHLTRETTER, C. L. Preparation of 3-stearoyl-D-glucose — a bread-softening agent. J. Am. Oil Chemists' Soc. **35:** 455–457 (1958).
14. RUNDLE, R. E. The configuration of starch in the starch-iodine complex. V. Fourier projections from X-ray diagrams. J. Am. Chem. Soc. **69:** 1769–1772 (1947).
15. RUNDLE, R. E., and BALDWIN, R. R. The configuration of starch and the starch-iodine complex. I. The dichroism of flow of starch-iodine solutions. J. Am. Chem. Soc. **65:** 554–558 (1943).
16. RUNDLE, R. E., and EDWARDS, F. C. The configuration of starch in the starch-iodine complex. IV. An X-ray diffraction investigation of butanol-precipitated amylose. J. Am. Chem. Soc. **65:** 2200–2203 (1943).
17. RUNDLE, R. E., FOSTER, J. F., and BALDWIN, R. R. On the nature of the starch-iodine complex. J. Am. Chem. Soc. **66:** 2116–2120 (1944).
18. RUNDLE, R. E., and FRENCH, D. The configuration of starch and the starch-iodine complex. II. Optical properties of crystalline starch fractions. J. Am. Chem. Soc. **65:** 558–561 (1943).
19. RUNDLE, R. E., and FRENCH, D. The configuration of starch in the starch-iodine complex. III. X-ray diffraction studies of the starch-iodine complex. J. Am. Chem. Soc. **65:** 1707–1710 (1943).

20. SAMEC, M., and KATZ, J. R. (with DERKSEN, J. C.) Abhandlungen zur physikalischen Chemie der Stärke und der Brotbereitung. VIII. Inwieweit bestehen Verkleistern und Retrogradieren bei den mit nativer Stärke verwandten Substanzen? Z. physik. Chem. (Leipzig) **158A:** 321–336 (1932).
21. SCHOCH, T. J. The starch fractions. *In* Starch and its derivatives, ed. by J. A. RADLEY, 3rd ed., vol. 1, pp. 123–200. WILEY: New York (1954).
22. SCHOCH, T. J. Preparation of starch and the starch fractions. *In* Methods in enzymology, ed. by S. P. Colowick and N. O. Kaplan, vol. 3, pp. 5–17. Academic Press; New York (1957).
23. SCHOCH, T. J., and WILLIAMS, C. B. Adsorption of fatty acid by the linear component of corn starch. J. Am. Chem. Soc. **66:** 1232–1233 (1944).
24. STEIN, R. S., and RUNDLE, R. E. On the nature of the interaction between starch and iodine. J. Chem. Phys. **16:** 195–207 (1948).
25. STRANDINE, E. J., CARLIN, G. T., WERNER, G. A., and HOPPER, R. P. Effect of monoglycerides on starch, flour, and bread: a microscopic and chemical study. Cereal Chem. **28:** 449–462 (1951).