AN EFFECT OF LIPID ON THE ENZYMATIC DEGRADATION OF WHEAT STARCH

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ABSTRACT

The possibility of the lipid component of wheat starch forming a membrane that limits amylolytic degradation of granules has been investigated. Experiments were designed to disrupt any membrane with surfactant and then measure digestion. The results suggest that while lipids do not inhibit degradation by means of a membrane structure, digestion may be limited by the presence of inclusion complexes formed by the interaction of damaged starch with lipid. This was confirmed by investigating the effect of adding lysolecithin, a major lipid component of starch, on amylolytic degradation.

Many investigators have demonstrated the significance of the lipid component of wheat-starch granules to breadmaking. In particular, this constituent has been shown to have a marked effect on gelatinization and retrogradation (1). The question arises as to whether these lipids alter the properties of starch prior to gelatinization. The objective of the present study is to elucidate the influence of the lipid component on one such property, namely, the susceptibility to amylolytic degradation.

Several mechanisms exist by which starch granule lipids could alter digestion. For example, lipids and amylose could complex as discussed by Schmitz and

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Acker (2) and Wren and Merryfield (3). Lipids could also influence digestion by forming a membrane at the periphery of the granule which acts as a barrier to amylolytic enzymes. Bimolecular membranes are formed spontaneously when certain of the lipids present in starch swell in water (4). By assuming that the surface area of the starch grain is 2000 cm^2g^{-1} (5), and that the area per molecule of lipid in the membrane would be 60 Å (6), it can readily be demonstrated that sufficient lipid is present to form such a structure. The possibility of degradation being limited by a membrane was therefore examined. Experiments were designed to disrupt any membrane present with surfactant (7) and then measure amylolytic degradation. In a further series of experiments the influence of the lipid component on digestion was inferred from investigations in which the effect of adding lysolecithin, a major lipid component of starch (2,3), on degradation was examined. This method was adopted as it was not possible to study lipid-free starch without disruption of granule structure (3). Since wheat flour contains damaged and intact granules (8), effects on both types of starch particle were studied.

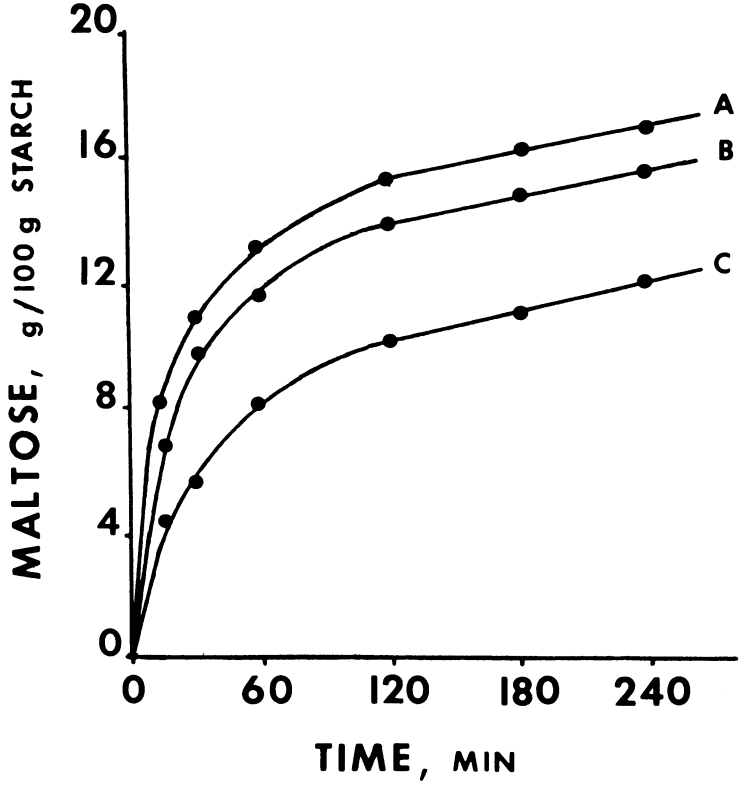

Fig. 1. Effect of sodium lauryl sulfate on the rate of digestion of starch (initial damage level 46 Farrand Units). (A) Control; (B) 0.25 g sodium lauryl sulfate/100 g starch; (C) 1.0 g sodium lauryl sulfate/100 g starch.

MATERIALS AND METHODS

Wheat starch was eluted from flour dough, exhaustively washed with distilled water, collected by centrifugation, and air-dried at room temperature. Damage levels were altered by ball-milling. Chromatographically pure lysolecithin was obtained from Lipid Products Ltd., U.K.; all other reagents were of A.R. grade.

Digestion of starch by a mixture of α- and β-amylases was investigated by measuring the produced maltose as a function of time (9). The enzyme system and experimental conditions were similar to those described by Farrand (10); this assured the reaction rate to be substrate limited. One of the surfactants employed, either sodium lauryl sulfate or cetyl trimethyl ammonium bromide, was added to the starch sample and degradation was then examined. Measurements were made at a number of surfactant concentrations and with samples at different damage levels. Similar experiments were performed with lysolecithin. Appropriate blanks were incorporated to show that the presence of surfactant or lysolecithin did not interfere with the determination of maltose. The influence of these compounds on damaged starch was also measured directly by estimating damage levels according to the method of Farrand (10). This technique entails measurement of the reducing sugars produced by a high concentration of α-amylase under specified conditions. Experiments were done on both flour and starch samples.

X-ray diffraction was used to establish whether or not lipids and starch interacted to form inclusion compounds under the conditions that amylolytic degradation was examined. Diffractometer traces were obtained as described elsewhere (11). The digestion of lipid-amylose complexes by the amylase enzyme system, employed to measure damage levels, was also studied.

RESULTS AND DISCUSSION

Figure 1 illustrates the effect of sodium lauryl sulfate on the rate of starch digestion. Similar results were obtained with cetyl trimethyl ammonium bromide. Clearly, surfactant does not cause an increase in enzymatic digestion as might be expected if a membrane were present. It has been concluded from data such as that in the control curve in Fig. 1 that the amylolytic breakdown of starch is essentially a two-phase process (9). The initial stage is characterized by a high rate of production of reducing sugars, corresponding to the digestion of the damaged component, although there is limited digestion of intact granules. Once the more reactive damaged starch is degraded, the rate decreases to constant level arising from the breakdown of the undamaged fraction. In the presence of surfactants, the curves in Fig. 1 are the same shape as the control curve; hence, the enzyme system is not being appreciably altered by these compounds. This result may possibly be attributed to both the protective effect starch has against the denaturation of amylase by surfactant (12) and to the fact that a relatively high concentration of enzyme was employed. The shape of the curves in Fig. 1 further indicates that the digestion of intact starch grains appears to be unaffected.

Surfactants have been shown to adsorb onto the surface of intact granules (13); however, if this occurred in the present study, it would seem that the adsorbed layer did not constitute a barrier to enzyme activity. From Fig. 1 it is also evident

that while the degradation of intact starch grains appears unchanged, there is a decrease in the amount of material that is relatively susceptible to digestion. This alteration in the damaged component is consistent with the data in Table I which show that as the concentration of sodium lauryl sulfate increases, the apparent starch-damage level decreases.

A possible explanation of the above results is that surfactant and damaged starch interact to form an inclusion complex which is not subject to amylolytic degradation. As such complexes are known to contain about 90% polymer (2,3), it would be possible for the relatively small quantities of surfactant employed to have the observed effect. The 'V' type X-ray diffraction pattern (14), obtained of the compound formed by the interaction of extensively milled starch and sodium lauryl sulfate, confirms the presence of inclusion complexes. A similar interaction did not take place in the case of intact grains. This may be because unmodified granules swell by comparatively small amounts in aqueous solution at room temperature (8); hence, penetration of surfactant into such particles is hindered. Moreover, the polymer chains in intact granules are in a more ordered state than in damaged particles (11), and transition to the helical configuration required for complex formation (3) may be energetically less favorable. When the inclusion compound was reacted with the amylase enzyme system, a relatively small quantity of reducing sugar was produced. Therefore, it appears that the helical arrangement of the polymer molecule precludes rapid digestion by these enzymes. A similar result has been reported for other amylose inclusion complexes (15,16).

TABLE I
Effect of Sodium Lauryl Sulfate on the Damaged Levels of a Series of Starches

Sodium Lauryl Sulfate Added g/100 g starch	Starch Damage Level %			
0.00	26	33	46	50
0.25	18	25	38	43
0.50	4	18	31	38
1.00	0	5	18	26
1.25	0	0	6	14

TABLE II
Effect of Lysolecithin on Damaged Level Values Determined in Flour and Starch

Lysolecithin Added g/100 g starch	Flour Damage Level %	Starch Damage Level %
0.0	26	26
0.5	14	4
1.0	5	0
1.5	0	0

Addition of lysolecithin had a similar influence on digestion to that of surfactant. This suggests that any lipid capable of forming a polymer inclusion complex may alter the amylolytic degradation of damaged starch. However, when the experiments were performed on flour rather than starch, the effect was less marked (Table II). This is possibly because the lysolecithin became associated with the noncarbohydrate components of flour. Hence, although the results of the present study indicate that lipids influence the course of the amylolytic degradation of starch in dough, the overall magnitude of the effect may be relatively small.

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