GEL FORMATION AS RELATED TO CONCENTRATION OF AMYLOSE AND DEGREE OF STARCH SWELLING[1]

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ABSTRACT

The amount of soluble amylose needed for gel structure, in relation to degree of swelling and relative size of hydrated starch granules, has been investigated. Amylose, prepared from defatted corn starch, was added in various amounts to an unmodified waxy (amylopectin) corn and to two waxy corn starches phosphate-bonded to different degrees. The amount of amylose needed for gels of equivalent strength was about three times greater in the absence of amylopectin granules than in the presence of well-hydrated and intact granules. Results confirmed the twofold role of soluble amylose in gel formation: 1) as the chief material that forms the gel network which entraps unabsorbed water; and 2) as a binding material that links together intact starch granules or fragments. The swelling pattern of starch granules appears to determine which role of the linear fraction is most significant.

Pastes of most common cereal starches, in sufficiently high concentration, tend to form gels when cooled. Gelation is believed to occur by the formation of a three-dimensional network in which swollen granules are bound. The network, which is crystalline in nature, probably consists of segments of linear and branched molecules which form a continuous structure; parts of one molecule may be present in several crystalline areas. The linear fraction, amylose, is considered primarily responsible for gel formation and may alone, in concentrations as low as 1.5%, form firm gels (1).

Many factors such as granule composition and structure, degree of polymerization of the starch fractions, chemical modification, addition of other substances, and the rate and extent of heating have been related to the strength of starch gels. Gel formation, as related to particle size and degree of hydration (reflected in the swelling pattern of different starches), has not received particular attention. Experiments reported herein were designed to investigate gel formation as related to: (a) the degree of swelling and fragmentation of granular starch; (b) the concentration of soluble amylose, added after heating the granular starches for 15 min.; and (c) the amount of soluble amylose recovered from the pastes by hot water extraction following an additional 10 min. of heating.

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Materials and Methods

Amylose was prepared from commercial corn starch by selective precipitation with Pentasol. The starch was defatted by three 1-hr. digestions under reflux with 85% methanol, filtered while hot, and washed with 85% methanol after each digestion. The defatted starch was suspended in 40 parts distilled water, phosphate-buffered to pH 6.2–6.3, and then autoclaved for 5 hr. at 18 lb. pressure. One volume of Pentasol was added to 6 volumes of the starch solution, and the mixture was insulated to permit slow cooling and cooled overnight with continuous agitation. It was then stored for 24 hr. at 5°C., and the precipitate was collected by centrifugation at 15,000 r.p.m. in a refrigerated Servall continuous-flow supercentrifuge. The precipitate was dissolved in a 1:10 mixture of butanol and distilled water and heated for 1 hr. with continuous agitation. The hot solution was then centrifuged for 15 min. at 4,000 r.p.m., which was not a sufficiently high speed to remove the impurities successfully. A greater centrifugal force was not used because the superspeed centrifuge available automatically cut off at temperatures above 40°C.; thus, the amylose was recrystallized with butanol only once. The precipitate of crude amylose was dehydrated by stirring for four 3-hr. periods in 10 volumes of cold butanol, filtering, and drying at 70°C. under vacuum.

The iodine affinity of the crude amylose was 13.3%, a value low for pure amylose if 18.7% is used as a reference value. The sample, however, was considered to contain a sufficiently high percentage of the linear fraction for the purpose of this study. The data presented are based on the calculated amount of pure amylose in the crude sample.

The granular starches used were corn, an unmodified waxy corn, and two cross-linked waxy corn starches (sample P-1 containing about one phosphate bridge per 250 anhydroglucose units, and sample P-2 about one bridge per 100 units). Swelling characteristics were observed by changes in paste viscosity while the starch, 5 g./100 g. water, was heated in a Brabender Amylograph to 93°C. and held at that temperature for 15 min. Water absorption or relative swelling power was estimated by the volume occupied by the centrifuged hot pastes following hot water extraction of soluble starch, as subsequently described.

The samples were heated in a different manner when amylose was added. A weighed amount of starch was suspended in 200 ml. distilled water in a beaker fitted with a cover. The suspension was continuously agitated and heated at a rate to produce boiling in 15 min. Amylose, in various amounts, was dispersed with vigorous stirring in an additional 50 ml. boiling water. The amylose solution was added to the granular starch paste after the 15-min. heat-treatment, and heating was continued for 10 min. with continuous stirring.

A portion of each hot paste was poured into a glass jar, approximately 60 mm. high and 50 mm. diameter, to a depth of 45 mm. A disk, 19 mm. in diameter and 1.5 mm. thick, attached to a rod was suspended through a hole in the center of the jar cover, and held in place by a small pin. The gels were stored 24 hr. at 28°C., after which the breaking strength was measured by a method essentially the same as that described by Bechtel (2). The breaking strength of a corn starch gel having a concentration of 5 g./100 g. water was used as a standard for comparison. Gels of the corn starch which had been defatted were also included for comparison.

Soluble starch was extracted from 100 g. of each paste, mixed with 60 ml. hot distilled water, and then centrifuged. The supernatant was removed by suction and the washing process repeated four times. The soluble starch was precipitated in 6 vols. of methanol, filtered, and dried under vacuum. Iodine affinity was determined by potentiometric titration according to the method of Bates, French, and Rundle (3) as modified by Schoch (4).

All determinations were made on at least duplicate starch pastes.

Results and Discussion

Marked differences in the swelling patterns of the starches were observed (Table I). The initial rise in viscosity occurred at 70° to 72°C. for the three waxy starches and at 84°C. for corn starch. The swelling of the unmodified waxy corn starch was rapid and unrestricted as shown by the high viscosity at 77°C. The highly swollen granules were fragile, severe fragmentation occurred, and the paste viscosity decreased markedly at 93°C. with a further drop after 15 min. at 93°C. The two cross-linked starches showed gradual and restricted swelling; the paste viscosity had not reached maximum when heating was terminated. The granules of the least-modified starch, when examined under the microscope, appeared highly swollen and no fragmentation was evident. The highly cross-linked starch, one phosphate bridge per 100 glucose units, showed the most restricted swelling; the highest viscosity reached was 70 B.U. Corn starch reached maximum viscosity after 1 min. of holding at 93°C. and the stability of the paste was good.

Four consecutive centrifugations of 100 g. of hot paste, with 60 ml. of hot water added each time, resulted in a volume of packed, swollen

TABLE I

SWELLING CHARACTERISTICS OF CORN, WAXY CORN, AND TWO CROSS-LINKED (PHOSPHATE ESTER) WAXY CORN STARCHES

STARCH[a]	VISCOSITY				
	Temperature of Initial Rise	Maximum		At 93°C.	After 15 Minutes at 93°C.
	°C.	*B.U.*	*°C.*	*B.U.*	*B.U.*
Corn	84	110	93	105	100
Waxy corn	70	490	77	280	200
Waxy corn P-1	71	No maximum		155	175
Waxy corn P-2	72	No maximum		50	70

[a] 5 g. per 100 g. water.

granules of approximately 140 ml. for the unmodified waxy corn, 100 ml. for the distarch phosphate P-1, and 80 ml. for the distarch phosphate P-2. Although the particle size of the unmodified waxy starch was greatly reduced because of granule fragmentation, the amount of water held by the starch appeared to be greater than in the starches cross-linked with phosphorus. Additional swelling and solubilization of the starch probably occurred through the use of hot water for extracting the solubles; thus, the results indicate relative rather than actual swelling power of granules in the freshly cooked pastes which were used for gels.

TABLE II

BREAKING STRENGTH OF STARCH GELS[a]

STARCH[b]	ADDED AMYLOSE[c] PER 100 G. WATER				
	0.35 g.	0.53 g.	0.71 g.	1.06 g.	1.42 g.
	g./cm.²	*g./cm.²*	*g./cm.²*	*g./cm.²*	*g./cm.²*
(Amylose only)			4	15	32
Waxy corn P-1	18	33	46		
Waxy corn P-2	9		30		
Waxy corn			8	30	48

[a] Breaking strength of reference corn starch gels = 29 g./cm.²
[b] 5 g. per 100 g. water.
[c] Pure amylose calculated from iodine affinity of crude amylose.

The breaking strengths of the gels are presented in Table II. The concentration of the granular starches was 5 g./100 g. water; the added amylose is given as a calculated amount of pure amylose. Amylose alone provided gel formation in a concentration as low as 1.06 g./100 g. water, but the gel structure was very weak. With 1.42 g. pure amylose (2 g. of the crude preparation), the gel strength was equivalent to that of the reference corn starch, but the amylose gel showed severe syneresis. Less amylose was required for gel formation in the presence of the granular starches, but differences among these starches were marked. For gels of equal breaking strength the less highly cross-linked waxy starch required only half as much amylose as its unmodified counterpart; the former had plump and intact granules, whereas the latter showed severe granule fragmentation. An intermediate amount of amylose was needed by the starch containing more phosphate bridges. This starch had undergone the least swelling of all of the samples, but the intact granules were larger than the fragments of the unmodified waxy corn starch. There is also the possibility that the presence of more soluble branched material in the unmodified waxy corn starch pastes may have interfered with gel formation by amylose, thus increasing the amount of the linear fraction required for a given gel strength in the presence of this granular starch.

The straight-line relationship between the concentration of amylose and the strength of the starch gels is shown in Fig. 1. In these experiments the total solids (calculated linear and branched fractions) in each gel were 5 g./100 g. water rather than the additive amounts of amylose given in Table II. The straight-line relationship existed in both experiments. Once a sufficiently high percentage of the linear fraction was present for gel formation, the slope of the line was similar for each of the waxy starches. For any given gel strength,

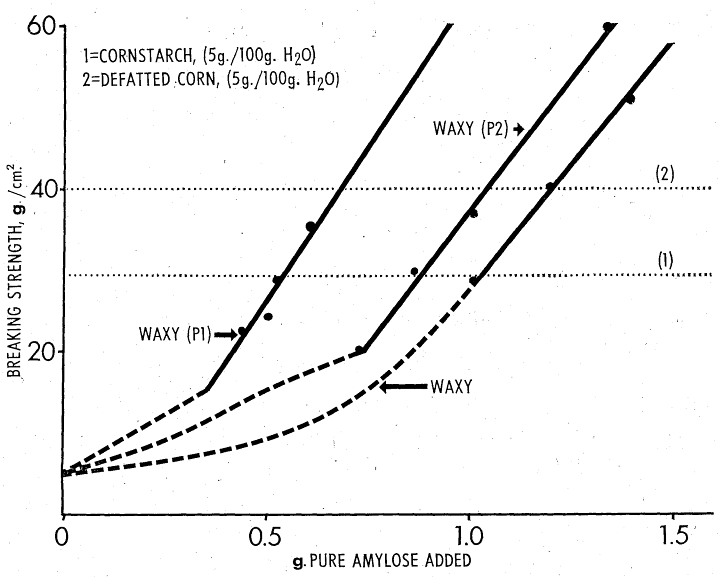

Fig. 1. Breaking strength of gels containing waxy starch and increasing amounts of amylose. Each gel contained 5 g. total starch solids per 100 g. water. Solid lines are calculated by the method of least squares; broken lines are estimated.

however, the system containing the largest particles required the least amount of amylose; as particle size decreased, the requirements for amylose increased.

To determine the possible fate of the added amylose, the amount and iodine affinity of the soluble starch extracted from the hot pastes were measured and the recovery of soluble amylose was calculated (see Table III). The largest amount of soluble starch was extracted from the unmodified waxy corn, the granules of which had disintegrated upon cooking. Very small amounts of solubles were obtained from the cross-linked starches which had shown restricted and gradual swelling. As the concentration of added amylose increased, the amount of soluble starch extracted from the unmodified waxy corn starch paste decreased; whereas that from the pastes of cross-linked starches increased. The added amylose was completely soluble; thus changes in recovery of soluble amylose could be attributed to interactions between the linear and branched fractions.

The iodine uptake of soluble starch from the unmodified waxy corn-amylose pastes was low; only 22 to 26% of the added amylose was recovered in a soluble form. Loss of amylose solubility, coupled with the decreasing amounts of total solubles as the amylose concentration was increased, suggests the formation of an insoluble complex between amylose and soluble amylopectin or amylopectin present in the granule fragments. Such a complex would appear to form rapidly in the hot paste, preventing recovery of part of both fractions as soluble starch. In the distarch phosphate pastes, both the amount and iodine uptake of soluble starch increased with increasing amounts of added amylose. The relatively large recovery of soluble amylose, 42 to 82%, indicates more limited association of amylose with these amylopectin starches, either by means of penetration of the linear fraction into the

TABLE III

EFFECT OF ADDED AMYLOSE ON THE AMOUNT OF SOLUBLE STARCH AND RECOVERY OF SOLUBLE AMYLOSE FROM HOT WAXY CORN STARCH PASTES

Pure Amylose in 100 g. Paste	Waxy Corn [a]			Waxy Corn P-1 [a]			Waxy Corn P-2 [a]		
	Soluble Starch	Soluble Amylose [b]	Recovery of Sol. Amylose	Soluble Starch	Soluble Amylose [b]	Recovery of Sol. Amylose	Soluble Starch	Soluble Amylose [b]	Recovery of Sol. Amylose
g.	*g.*	*g.*	%	*g.*	*g.*	%	*g.*	*g.*	%
0.00	1.30	0.00	0	0.11	0.00	0	0.01	0.00	0
0.33	...	...	..	0.39	0.14	42	0.42	0.27	82
0.50	...	...	..	0.74	0.32	64	...	...	..
0.67	1.17	0.16	24	0.75	0.36	54	0.72	0.50	75
1.00	0.98	0.26	26	...	...	..	...	...	..
1.34	0.95	0.30	22	...	...	..	...	...	..

[a] 4.76 g./100 g. paste (5.0 g./100 g. water).
[b] Calculated on basis of iodine uptake of 18.7 for pure amylose.

intact granules or by bonding at the granule surface. As might be predicted, a larger amount of soluble amylose was recovered from the more highly phosphate-bonded sample which showed the most restricted swelling.

Microscopic observations of iodine-stained samples were not conclusive, but they suggest that a more amorphous granule structure favors greater intermingling of the linear and branched fractions. The cross-linked waxy starches stained a reddish brown, and in the presence of amylose a blue cloudiness appeared to surround the granules. The unmodified waxy starch stained a deeper violet color in the presence than in the absence of amylose; blue cloudiness was not observed around the granule fragments.

In another series of gels, sufficient crude amylose was added to the waxy starches to provide samples of the same composition as the reference corn starch, 27.8% pure amylose and 72.2% amylopectin. All gels contained 4.76 g. solids/100 g. paste (5 g./100 g. water). The breaking strength of the gels, and the amount and iodine affinity of the soluble starch extracted from the hot pastes, are shown in Table IV. The results indicate the efficiency of soluble amylose as opposed

TABLE IV

Breaking Strength of Gels, and Amount of Soluble Starch and Recovery of Soluble Amylose from Hot Pastes. Constant Ratio of Amylose to Amylopectin (Total solids, 4.76 g./100 g. paste)

Parent Starch	Gel Breaking Strength	Soluble Starch	Soluble Amylose	Recovery of Soluble Amylose
	g./cm.^{2}	*g.*	*g.*[a]	%
Corn	29	0.59	0.28	21
Defatted corn	39	0.86	0.52	40
Waxy corn[b]	52	1.29	0.48	37
Waxy corn P-1[b]	93	1.39	0.98	75
Waxy corn P-2[b]	67	1.51	1.11	85

[a] Calculated on basis of iodine uptake of 18.7 for pure amylose.
[b] Pure amylose, 1.31 g., plus 3.45 g. waxy starch and impurities of crude amylose/100 g. paste.

to granular amylose in providing gel structure. The breaking strength of the gels was from 2 to 3 times greater when the total amylose was in a soluble form. The iodine affinity of the soluble starch extracted from these samples again showed the binding of amylose by the regular waxy starch and the lack of amylose binding by the starch phosphate esters in hot pastes.

General Discussion of Gel Structure

On the basis of conditions of this study, soluble amylose appears to have a twofold role in starch gel formation: 1) as the chief material that forms a network which binds and entraps unabsorbed water, and 2) as a material that links together intact starch granules or fragments, thus providing additional structure in the network. Other factors being equal, the degree of hydration of starch granules and the size of the swollen particles appear to determine: 1) the amount of amylose needed for a firm gel structure, and 2) the predominant role of amylose in the gel system.

Postulated functions of amylose in starch gel systems are illustrated in Fig. 2. In a gel system containing only amylose and water, it is generally accepted that the linear molecules form a three-dimensional network in which the water is bound and entrapped. This is illus-

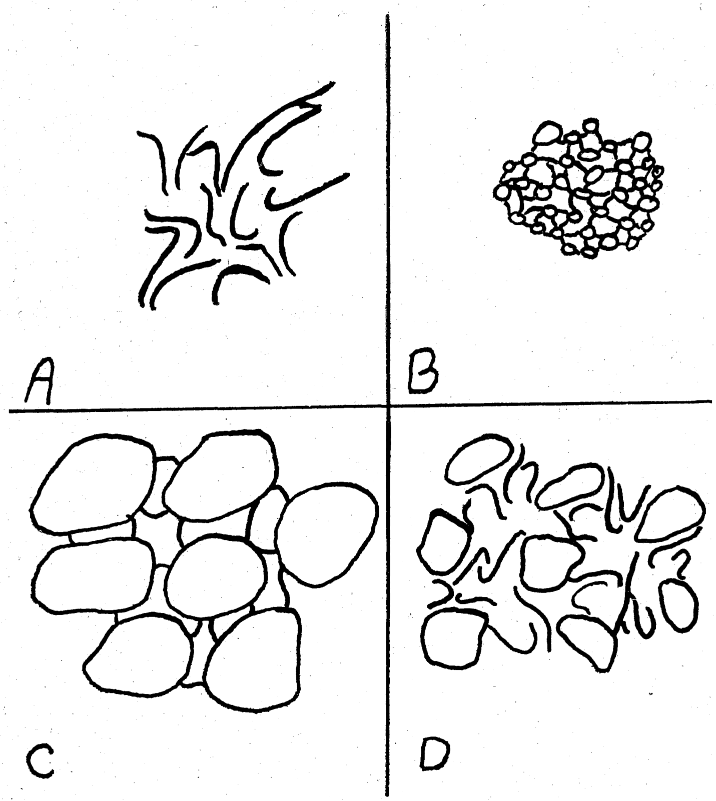

Fig. 2. Postulated functions of amylose in starch gel systems. A, amylose gel with water bound or entrapped in a three-dimensional network. B, gel with highly swollen and fragmented starch granules. High proportion of water held by fragments; amylose necessary to join them in a continuous network. C, gel with highly swollen and intact starch granules. High proportion of water held by granules; only limited amylose necessary to join the large particles in a continuous network. D, gel with intact starch granules which are not highly swollen. Amylose necessary to bind or entrap free water, and to join granules in a continuous network.

trated in Fig. 2, A, and is the familiar structure generally proposed for amylose gels. In the presence of granular starch to provide some structure, however, a smaller amount of amylose is needed to form a sufficiently strong network to hold a given amount of water.

Figure 2, B, illustrates a gel structure containing highly swollen granules but ones that have fragmented into many small particles. In this case, as was true for the regular waxy corn starch, a high proportion of the water is held by the granule fragments. The chief function of the amylose is to link together the closely packed fragments in a continuous structure. Since there are many small particles to be joined, a relatively large amount of amylose is needed.

Figure 2, C, illustrates a gel structure in which the starch granules are also highly swollen, but in this case the granules are intact and large. Again a high proportion of the water is held by the granules; only a limited amount of amylose is needed to join the large particles in a continuous network.

Figure 2, D, illustrates a gel system in which both roles of amylose may be of equal significance. A starch which has undergone limited swelling may leave much unabsorbed water. Amylose serves both to bind or entrap the free water and to join the granules in a continuous network. Less amylose would be needed in this type of system than in a system containing larger amounts of free water, as in Fig. 2, A, or in one containing little free water but many small particles to be linked in a continuous structure, as in Fig. 2, B.

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