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CONSTITUENTS OF CORN

Development of Starch and Phytoglycogen in Golden Sweet Corn

EILEEN MAYWALD, RUTH CHRISTENSEN, and THOMAS JOHN SCHOCH

George M. Moffett Research Laboratories, Corn Products Refining Co., Argo, Ill.

Starch and phytoglycogen have been isolated from golden sweet corn at various stages of development. During ripening, the starch content increases from 8 to 15% (dry basis), with an apparent increase in the linear-chain characteristics of the starch. The phytoglycogen maintains the same molecular size during ripening, but increases from 7 to 30%. Occasional curdling of cream-style canned corn is attributed to coagulation of a heat-sensitive protein. In confirmation, the curd from coagulated canned corn analyzed 71% protein. This protein loses water solubility during field-drying of the corn, thereafter approaching the character of corn gluten. Ripe sweet corn offers a convenient source of pure and undegraded phytoglycogen, suitable for fundamental studies. The soluble protein is readily and completely removed by heat coagulation, and starch-free phytoglycogen has been prepared with a protein content as low as 0.04%. This avoids degradative treatment with trichloroacetic acid or hot alkali.

THE POLYSACCHARIDES OF SWEET CORN have been studied by several investigators in recent years. Morris and Morris (*5*) isolated a soluble polysaccharide which appeared to have properties similar to the animal glycogens of liver tissue and of various shellfish. Hassid and McCready (*2*) further characterized the product as glycogenlike in its chemical structure. Sumner and Somers (*7*) fractionated this soluble polysaccharide and suggested the name "phytoglycogen" to indicate its plant origin. Cameron (*1*) investigated the genetic control over the production of glycogen and starch type in several varieties of sweet corn.

Most of these studies were conducted on the dried corn and provide no evidence of the development of starch and soluble polysaccharide in the maturing corn. Some question may be raised regarding the purity and possible degradation of the phytoglycogen. For example, the phytoglycogen was separated from gelatinized or dissolved starch substance by fractional precipitation with glacial acetic acid, and there is no assurance that the glycogen is free from starchy carbohydrate. Protein was removed either by treatment with 10% trichloroacetic acid or by prolonged digestion in hot 60% caustic soda, with the possible hazards of hydrolytic or oxidative degradation.

It was thought that certain aspects of commercial corn canning might be rationalized by a better understanding of the nature and influence of the phytoglycogen and sweet corn starch. For example, the content of linear fraction in the natural sweet corn starch might be expected to influence the gelatinization and swelling properties of the starch granules and hence the cooking time and consistency of the canned corn. In the canning of cream-style corn, commercial food-grade corn or wheat starch is usually added to impart a smooth creamy consistency. In this type of product, sometimes an undesirable curdling occurs during pressure cooking, accompanied by syneresis of a thin liquid phase. The question has been raised whether this curdling is due to the sweet corn itself or to retrogradation of the added starch.

The purpose of the present investigation was threefold: to isolate and characterize the starch and phytoglycogen fractions, to trace the development of these substances during maturing of the corn, and to determine the cause of curdling during canning.

Materials and Methods

Studies extended over 2 years. During the 1951 growing season, samples of green corn were furnished through the courtesy of the California Packing Co., Rochelle, Ill. During 1952, samples were supplied by the Green Giant Corp., LeSueur, Minn. These sweet corn varieties are special strains developed by the individual canner, but they may be generally described as first-generation single-cross golden hybrids. The corn samples were successively picked at several stages of development ranging from completely immature (white kernels, small and incompletely developed) to overmature (full yellow kernels, somewhat "doughy" in consistency). The term "mature" as here used refers to that stage of ripening which is most suitable for canning purposes. Without husking, the ears were frozen in dry ice immediately after picking and maintained in a frozen state until processed within 2 or 3 days. The ears were thawed out at room temperature, husked, and wiped dry of surface moisture. Each sample consisted of 10 to 30 ears, visually selected for uniform representation of the desired stage of maturity. The total sample was weighed, the kernels were sliced off, the cobs were scraped, and the percentage of kernels was determined as a relative index of maturity. Dry substance on the kernels was likewise determined, though these values are necessarily influenced by the freezing and thawing of the corn.

The cut kernels were ground for 5

minutes in the Waring Blendor with an equal weight of water, and the slurry was screened through No. 9 nylon bolting cloth (97-mesh per inch) to remove coarse fiber; the magma was squeezed as dry as possible. The press cake was ground a second time with fresh water in the Blendor, then screened, and the residue was discarded. The combined extracts were washed through No. 17 nylon bolting cloth (163-mesh). This mode of double screening is much more rapid than use of No. 17 cloth on the original ground corn. The starchy milk was then passed twice through a Sharples continuous-flow supercentrifuge fitted with a clarifier bowl and operating at 50,000 r.p.m. The first pass was made at a relatively rapid flow rate (500 ml. per minute), the second pass at a much slower flow rate (200 ml. per minute). This completely removed the starch and any residual fine fiber, together with insoluble gluten and a portion of entrained oil. This fraction was held for subsequent separation of granular starch. The supernate from the centrifuging operation was then heated in the boiling water bath for 30 minutes.

At approximately 75° C., the soluble protein commenced to coagulate as a curdy floc, creamy white in the case of immature corn and golden yellow from the more mature samples. After 30 minutes' heating, the major portion of the protein had coagulated. This was removed by screening through No. 9 bolting cloth and the hot solution was then passed through the supercentrifuge at a slow flow rate. The protein curd was dried to constant weight in the vacuum oven at 70° C., Kjeldahl assays were run, and coagulated protein was calculated to a dry-kernel basis. Methanol (2 to 3 volumes) was added to the centrifugate with vigorous agitation to precipitate the phytoglycogen, the mixture was heated for 15 to 20 minutes in the boiling water bath, then the glycogen was allowed to settle and the supernate decanted. Fresh methanol (3 to 4 volumes) was added to the precipitate, and the mixture was gently refluxed for 30 minutes in the boiling water bath. The glycogen was then filtered in a Büchner funnel and dried to constant weight in the vacuum oven at 70° C. to give a fluffy white product. This procedure of rigorous dehydration with hot alcohol is necessary to avoid gummy precipitates which will not filter or which cake on drying. Yields of glycogen were calculated on the basis of the dried kernels.

As an additional purification, the phytoglycogen was redissolved in hot water, and the solution was heated in the boiling water bath and finally supercentrifuged at a slow flow rate. This removed a small amount of additional protein (apparently insolubilized during drying), and the purified glycogen solution then showed a characteristic blue-white haze. The phytoglycogen was reprecipitated, alcohol-dehydrated, and dried as previously described. In this second precipitation with alcohol, it was necessary to add a pinch of sodium chloride to effect coagulation of the glycogen. This purification reduced the protein content to the range of 0.04 to 0.19%. Chemical and physical characteristics were determined on these purified products.

For isolation of the starch fraction, the original centrifuge cake was dispersed in distilled water and washed through No. 25 (200-mesh) nylon bolting cloth to remove most of the fine fiber. No "break" could be obtained between starch and gluten by sedimenting or tabling; hence a modification of the procedure of Watson (*8*) was employed. Approximately 20% of Pentasol (a mixture of primary amyl alcohols marketed by Sharples Solvents) was added to the starch milk, and the mixture was stirred for 30 minutes and supercentrifuged. The gluten is apparently wetted and swollen by the Pentasol and passes off in the overflow from the supercentrifuge; the starch packs firmly in the centrifuge rotor. The starch cake was again dispersed in distilled water, stirred with Pentasol, and centrifuged. After two or three such treatments, the starch cake was white in color and the centrifuge overflow showed no visible solids. The starch yields from the immature corn samples were low, and these operations were therefore conducted in a bottle centrifuge. The final starch was dehydrated with cold methanol, filtered, and Soxhlet-extracted for 48 hours with 95% ethyl alcohol to remove associated fatty acids. The starches were then dried and yields calculated on a dry-kernel basis.

Intrinsic viscosities (*4*) of the various starch and phytoglycogen fractions in 1*N* potassium hydroxide afforded a criterion of molecular dimensions. Alkali numbers (*6*) of the phytoglycogens were determined as a possible index of relative molecular weight. Iodine affinities (*4*) on the various starches indicate the content of linear-chain substance. The phytoglycogen samples all showed iodine affinities less than 0.1%; iodine colorations were red to red-brown with no trace of blue or purple. Hence it appears that the phytoglycogen fractions are completely free from starch. To provide a measure of relative retrogradation tendencies of the various starches, percentage light transmittance at 650 mμ was determined on 1.00% pastes. The pastes were cooked for 30 minutes in the boiling water bath with continuous agitation, then cooled and allowed to stand 24 hours at room temperature. Light transmittance (to the nearest 0.5%) was determined against water in 1.3-cm. cuvettes in the Coleman No. 14 spectrophotometer. This measurement of paste clarity is routinely used in this laboratory for evaluation of various starch products.

Results and Discussion

During the period studied, the starch content in the maturing sweet corn in-

Table I. Properties of Starch and Phytoglycogen from 1951 Corn

	Immature	Mature	Overmature
Starch[a]			
Intrinsic viscosity	...	1.04	1.24
Iodine affinity, %	2.46	3.95	5.03
Paste clarity, % transmittance	3.5	8.5	10.0
Glycogen			
Yield, %	...	17	23
Intrinsic viscosity	0.077	0.087	0.081
Alkali number	4.0	3.7	3.9

[a] In comparison, a typical food-grade corn starch has an intrinsic viscosity of 1.55, iodine affinity of 5.30% and paste clarity of 17%.

Table II. Processing of 1952 Corn

	Completely Immature	Slightly Immature	Mature	Overmature
Appearance of kernels	White, small, incompletely filled out	Light yellow	Full yellow	Full yellow, doughy
Yield of kernels from ears, as-is basis, %	43	54	61	71
Moisture in kernels, %	84	71	68	65
Yield of starch, dry-kernel basis, %	8	10	15	15
Yield of glycogen, dry-kernel basis, %	7	24	31	31
Yield of coagulated protein, dry-kernel basis, %	3.7	3.3	2.2	1.2

Table III. Properties of Starch and Phytoglycogen from 1952 Corn

	Completely Immature	Slightly Immature	Mature	Overmature
Starch				
Intrinsic viscosity	1.13	1.16	1.18	1.27
Iodine affinity, %	3.07	4.51	4.83	5.19
Paste clarity, % transmittance	7.0	3.5	4.5	5.5
Glycogen				
Intrinsic viscosity	0.11[a]	0.084	0.088	0.092
Alkali number	2.0[a]	4.1	3.8	4.7

[a] Completely immature glycogen was not repurified because of insufficient sample.

creased from 8 to 15% of the dry kernel weight (Table II). Starch granules in the immature corn were extremely small, approximately 2 to 3 microns in diameter; the size increased substantially during ripening. Intrinsic viscosities show a slight but possibly significant increase during maturing (Tables I and III), which may indicate a progressive increase in the size of the starch molecules. The iodine affinity increases markedly during maturing, representing an increase in the linearity of the starch substance. Careful fractionation studies would be required to identify the precise nature of this change, whether due to increased content of linear fraction, or to increase in average chain length of the linear molecules. One per cent pastes of the sweet corn starches were all more opaque than commercial dent corn starch, indicating strong retrogradation tendencies. Cooked 5% pastes of the more mature sweet corn starches gave rigid opaque gels which were indistinguishable from similar gels of commercial dent corn starch. The immature starch gave a somewhat weaker gel, perhaps because of incomplete swelling of the very small granules. When the corn is sufficiently mature for canning, the sweet corn starch does not differ in any major respect from commercial dent corn starch. This of course applies only to two golden hybrids studied in the present work, and starches from other sweet corn varieties may conceivably vary from so-called "high-amylose" to waxy types, depending on genetic strain.

The phytoglycogen increased from 7 to 31% of the dry kernel weight during the period studied. As judged by intrinsic viscosity, there was little change in molecular dimensions of the glycogen during maturation. Indeed, phytoglycogen has been isolated from the dried seed corn of these two golden hybrids in yields of 21 to 24% and with intrinsic viscosities in the range of 0.089 to 0.096, little different from the values during the maturing period. The alkali number of corn phytoglycogen is similar to that of the branched-chain fraction of corn starch, indicating molecular weights of the same order of magnitude. The much higher intrinsic viscosity of the branched corn starch fraction—viz., 1.25 to 1.35—is in accord with the concept of a large treelike structure, as contrasted with the much smaller and more compact bushlike molecule of glycogen. On the identical 1951 phytoglycogen samples here described, Cori (*3*) has found an increase in average chain lengths of the branches with increasing maturity of the corn—viz., 10.8, 11.9, and 13.2 glucose units per average branch length for the immature, mature, and overmature samples, respectively. However, these changes within the structure of the glycogen molecule apparently do not greatly influence the molecular size or shape.

Heat-coagulable protein decreased progressively during ripening of the corn, from about 4% on the immature sample to approximately 1% on the overmature corn (Table II). No such material could be isolated from the dried seed of sweet corn. During the process of drying, the protein is apparently transformed to a product very similar to ordinary corn gluten. Hence it was impossible to obtain a protein-free glycogen from dried sweet corn. Finely ground meal of the seed corn was extracted with water for 2 hours at 50° C. Even after repeated hot digestion and supercentrifugation, these phytoglycogen samples still contained 1.5 to 2.2% protein.

It is therefore believed that the coagulation sometimes encountered in cream-style canned corn is due entirely to this heat-sensitive sweet corn protein. An important function of added corn or wheat starch is to suppress this coagulation, or at least to prevent the formation of visible clots. There is no evidence that curdling is due to retrogradation either of the natural sweet corn starch or of the small amount of added commercial starch. In confirmation of this, a very badly curdled sample of canned corn was obtained in which the curds were sufficiently large ($^1/_{16}$ to $^1/_8$ inch) to permit manual isolation. The separated curds were washed free of adhering starchy paste on a screen, then dried and analyzed. Protein content was 71%, as compared with 9.5% for the total canned corn sample, both on a dry basis. It is possible that those occasional batches which exhibit curdling may contain a larger proportion of slightly immature corn and hence a higher amount of coagulable protein.

Green sweet corn offers a very convenient laboratory source of undegraded and low-protein glycogen, of particular interest to laboratories engaged in the fundamental aspects of polymeric carbohydrates. Animal glycogens are difficult to obtain, and removal of associated protein may well cause considerable molecular deterioration. Sweet corn on the ear (either fresh or frozen) can now be obtained in the larger retail markets on a year-round basis. However, ears which have been blanched with hot water should be used with caution, as some gelatinization of the starch may have occurred. Obviously, canned corn (either cream-style or whole-kernel) is useless as a laboratory source of glycogen, because of the impossibility of separating gelatinized and dissolved starch substance. The pH level throughout the processes here described was 7.1 to 7.3, and hence there is no possibility of acidic or oxidative degradation. Corn reputedly has no α-amylase activity, and storage of the crude phytoglycogen extract for 24 hours in the refrigerator caused no decrease in its intrinsic viscosity. Hence it is believed that the present mode of isolation has not altered in any way the molecular structure of the native phytoglycogen.

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