Comparison of Starch from Triticale and its Parental Species[1]

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ABSTRACT

Chemical and physical properties of a triticale starch were compared with starch of the parental cultivars of durum wheat and rye, and with a cultivar of hard red spring wheat. Triticale starch granules were similar in appearance to those of the rye parent. Mean particle diameter was intermediate between those of the two parents. The particle-size distribution was bimodal, with peaks corresponding to the single peaks of each of the parent species. Triticale, like its rye parent, had high amylase activity as measured by amylograph viscosity. The amylose content of the triticale line was considerably lower than that of its parents. Triticale amylose had a higher sedimentation coefficient than the amylose of the parental species, suggesting a higher molecular weight for this component.

While considerable information is available on the inheritance of such chemical components as free sugars, amylose, and amylopectin in maize hybrids, similar information is lacking for such cereal species as wheat and rye. The development of the synthetic cereal species triticale, a genomic combination of wheat (*Triticum*) and rye (*Secale*), offered an opportunity to investigate inheritance of carbohydrate factors from two widely different parents.

Several workers have investigated the inheritance of protein components in triticale, with some reporting the presence of new protein species (1,2), whereas others found no new protein species (3,4). In an extensive study of triticale variety 6A190, Chen and Bushuk (5) found that both the quantitative distribution of the soluble protein fraction and the amino acid composition of triticale proteins were intermediate between analogous properties of its durum wheat and rye parents. From this, and an investigation of the gel-filtration and disc-electrophoresis patterns (5), they concluded (6) that the proteins of triticale were simply inherited from its parents.

Genetic control of carbohydrate components in the plant is indirect and dependent upon control of the particular enzymes synthesizing the component. Vaisey and Unrau (7) reported greater quantities of 80% alcohol-soluble sugars in triticale than in durum and in spring wheats. We have undertaken the present study to determine if genomic combination of a durum wheat (*Triticum turgidum* cv. Stewart 63) and a rye (*Secale cereale* cv. Prolific) produced modifications of physical and chemical characteristics of the starch in the derived triticale (6A190).

MATERIALS AND METHODS

The main seed samples used in this study were as follows: one line of triticale (6A190), a durum wheat (*Triticum turgidum* cv. Stewart 63), rye (*Secale cereale* cv.

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Prolific), and a hard red spring wheat (*Triticum aestivum* cv. Manitou). The samples were grown together on experimental plots at The University of Manitoba in 1966. They were milled on a Buhler experimental mill by the same milling procedure after tempering overnight at 15.5% moisture.

Except where indicated, starch was extracted from the flour, by the methods of Wolf (8). Cheesecloth was used in place of nylon mesh for gluten washing. With rye and triticale, the insoluble protein and cell-wall material was removed by differential centrifugation because of the difficulty in forming a gluten ball. Starch samples with minimum damage were obtained by steeping the grain for 24 hr. at 4°C. in a 0.02M $NaHSO_3$ solution, homogenizing the sample in a Waring Blendor, and extracting the starch as described for flour samples.

Determination of Chemical and Physical Properties

Amylograms were obtained with a Brabender Visco Amylograph, using a final dry flour or starch-to-water ratio of 43 g.:450 ml. and, in most instances, the 350 cm. per g. cartridge. Birefringence end point temperatures were measured with a Mettler microfurnace at a linear temperature program rate of 0.2°C. per min. Particle-size distributions were measured as described by Williams (9) with a Model B Coulter counter equipped with a Model M data-reduction accessory. Protein was determined by the Kjeldahl procedure; and starch, by a polarimetric procedure (10).

Amylose was estimated in the isolated starch by the amperometric procedure described by Williams et al. (11). Amylose was precipitated from starch dispersions in saline solutions with 1-butanol, as described by Gilbert et al. (12). Sedimentation coefficients for the purified amyloses were obtained by sedimentation velocity centrifugation with a Beckman Model E analytical ultracentrifuge. Runs were performed at 20°C. with a rotor speed of 60,000 r.p.m. Schlieren patterns were photographed every 16 min.

RESULTS

Pertinent properties of the flours used in the studies described here are shown in Table I. The mill had been set for optimal flour extraction from Manitou wheat, which accounts, in part, for variations in the flour yield. The protein content of triticale was slightly higher than either of the parent species but less than the reference sample of Manitou. The starch content of triticale was not appreciably different from the parent flours. The most striking characteristic of the triticale

TABLE I. PROPERTIES OF FLOUR DERIVED FROM TRITICALE, ITS PARENTS, AND A COMMON BREAD WHEAT

Sample	Flour Yield	Protein % (N × 5.7)	Ash %	Starch %	Amylograph Viscosity B.U.
Manitou	73.3	13.7	0.39	72.0	390
Stewart 63	73.8	10.9	0.58	73.3	290
Prolific rye	50.0	7.4	0.53	72.3	70
Triticale	61.1	11.8	0.43	73.8	30

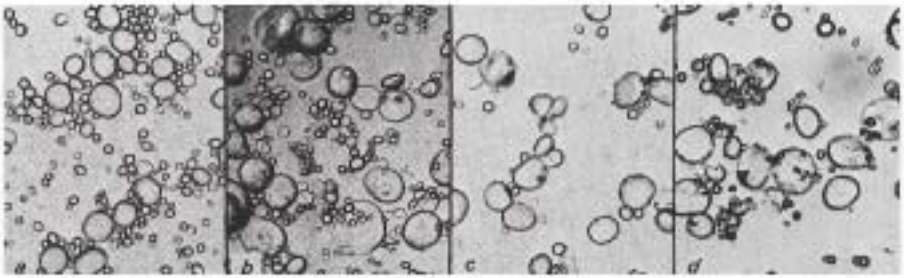

Fig. 1. Microscopic appearance of starch granules of a) Manitou, b) Stewart 63, c) Prolific, and d) Triticale.

flour is its low amylograph viscosity, a factor also present in the rye parent.

The appearance of the triticale starch granules under normal light is shown in Fig. 1. There was evidence of considerable disintegration of Stewart 63 starch granules as a result of milling that was not evident in either the Prolific or the triticale sample. The shape of the granules and the type of fracturing were comparable for Prolific and triticale samples. The starch hilum characteristics of rye starch can be seen in triticale starch, although at a lower frequency. Similar observations were obtained when the granules were viewed under plane-polarized light. The mean particle diameter and particle-size distribution of starches extracted by wet milling are shown in Table II and Fig. 2, respectively. In addition to the data for the four species used in this study, data for five other triticale lines are included for comparison. There was considerable variation in particle diameter among the triticale lines that may be a reflection of the parentage of these lines. For example, lines that have relatively low particle diameters (6517, 6A250, and Rosner) originated from crosses involving *Triticum persicum*. Unfortunately these crosses are complex, involving a number of backcrosses; and to determine whether there is any inheritance of this characteristic from *Triticum persicum* is difficult. It is interesting that the mean particle diameter of line 6A190 is intermediate between the particle diameters of its two parents. The particle-size distribution in 6A190 is peculiar (Fig. 2), exhibiting a major peak at a diameter of approximately 18 μ, corresponding to Stewart 63 and the Manitou control. A definite shoulder is apparent in the profile at a larger particle diameter which best coincides with the

TABLE II. MEAN PARTICLE DIAMETER OF SOME TRITICALE VARIETIES, PROLIFIC RYE, STEWART 63 DURUM WHEAT, AND MANITOU WHEAT

Sample	Mean Particle Diameter (μ)	Sample	Mean Particle Diameter (μ)
Manitou	18.7	6A250	19.1
Stewart 63	21.8	Rosner	19.7
Prolific	28.2	6211.2	23.5
6A190	25.1	8A92	25.6
6517	17.8		

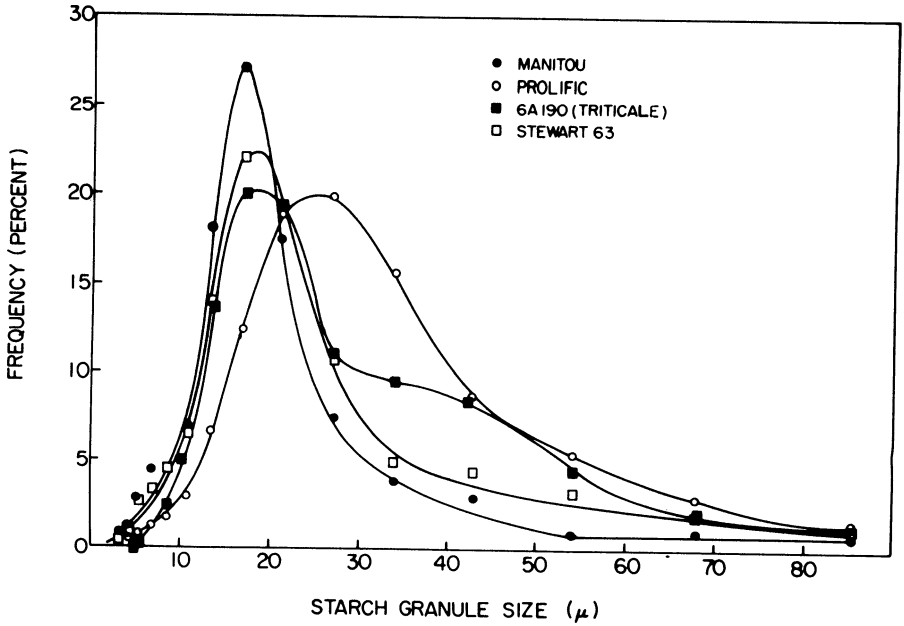

Fig. 2. Starch particle size distributions in samples of Manitou, Prolific, Stewart 63, and Triticale.

profile of Prolific starch granules. This unusual size distribution is not a general characteristic of all triticale lines, however. In fact, of the eight lines tested, only 8A92, an octaploid, gave a similar profile.

The birefringence end point temperatures (BEPT) and gelatinization temperature ranges of the four starch samples are given in Table III. The BEPT does not vary appreciably among any of the four samples, but the BEPT for triticale is closest to that of its rye parent and is significantly higher than the Stewart 63 sample. Some authors (13) feel that with constant environmental conditions the BEPT may reflect amylopectin:amylose ratios. However, the values probably reflect physical characteristics of the starch-granule structure (14).

The low amylograph viscosity of triticale flour prompted a more complete study of the amylograph characteristics of the isolated starches. We were interested in establishing that the low amylograph viscosity of triticale starch granules was due to the α-amylase activity associated with this starch. Various testing procedures were investigated and the results of these are shown in Fig. 3. Amylograms labelled a were obtained from starches extracted from flour. Starches for curves b and c were obtained from wet-milled grain; but in amylograms labelled c, 200 μmolar (μM) silver nitrate ($AgNO_3$)was included in the starch-water slurry. The amylograph peak viscosity, and temperature at peak viscosity, of triticale starch are only slightly increased by wet-milling the grain, whereas inclusion of $AgNO_3$ greatly increased both the temperature at peak viscosity and the peak viscosity. The amylograph peak viscosity of Prolific is also increased considerably by addition of 200 μM $AgNO_3$; but the effect of wet-milling of the grain is to decrease the peak viscosity

TABLE III. BIREFRINGENCE END POINT TEMPERATURES (BEPT) OF EXTRACTED STARCHES[a]

Sample	BEPT °C.	Gelatinization Temperature Range
Manitou	62.9	8.6
Stewart 63	56.1	10.2
Prolific	59.6	9.6
Triticale	61.5	6.3

[a]Extraction performed with dry-milled flour.

TABLE IV. EFFECT OF $AgNO_3$ ON AMYLOGRAPH PEAK VISCOSITY OF COMMERCIAL WHEAT STARCH-AMYLASE SLURRIES

Additions to Starch-Water Slurry	Peak Viscosity B.U.
None	705
α-Amylase[a]	360
$AgNO_3$[b]	1010
α-Amylase plus $AgNO_3$[b]	1010

[a]Bacterial enzyme from Mann Research Laboratories, New York.

[b]Final concentration, 200 μM.

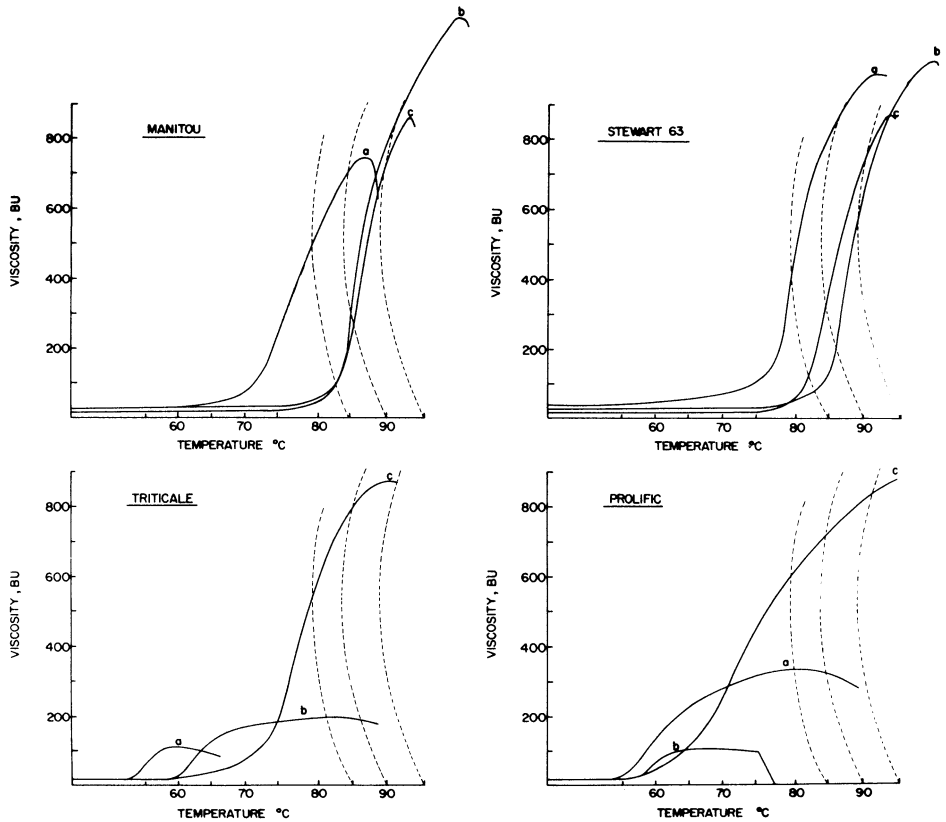

Fig. 3. Amylograph curves of Manitou, Prolific, Stewart 63, and Triticale starches using: a) starch extracted from flour; b) starch extracted by wet-milling of grain; and c) 200 μM $AgNO_3$ in the starch-water slurry.

relative to starch extracted from milled flour. There is little change in the amylograph curves of Stewart 63 starch as the result of the various treatments, whereas wet-milling appears to increase the amylograph viscosity of Manitou starch. The protein content of the starches used for this study varied between 1% for Prolific and 2.25% for triticale. Stewart 63 had 1.2% protein; and Manitou, 1.8%. Similar results were obtained when samples containing 0.5 to 1.0% protein were used.

The effects of $AgNO_3$ on amylograph peak viscosities of a commercial wheat starch-amylase system are shown in Table IV. The amylase concentration was adjusted to give about a 50% decrease in peak viscosity. Addition of $AgNO_3$ to this system completely abolishes the effect of the amylase. Enhancement of the peak viscosity of a slurry not containing added amylase is probably owing to inhibition of amylase in the commercial starch preparation. Yasunaga et al. (15) have demonstrated a similar effect of $AgNO_3$ on amylograph peak viscosities and attributed this effect to an inhibition of amylase activity.

Amylose contents of the extracted starches are given in Table V together with sedimentation coefficients of purified amylose from each sample. The triticale line has a substantially lower percent linear fraction than either the Stewart 63 or Prolific samples, both of which have identical amylose contents. Manitou has only a slightly lower amylose content than these two samples. Triticale amylose has a larger sedimentation coefficient in the ultracentrifuge than either of its parents, the value being closer to that of its rye parent. The amylose of triticale would therefore have a larger average molecular size than the durum or rye amylose. The Schlieren patterns of all the samples gave symmetrical peaks, with no indications of additional components.

DISCUSSION

The starch of the triticale line used in this study has some characteristics of the starch of the parent species. None of the characteristics differ to such an extent that they cannot be accounted for by variability within the parental species. Starch particle size and appearance are undoubtedly determined by the traits of the

TABLE V. PERCENT AMYLOSE AND ITS SEDIMENTATION COEFFICIENT IN STARCHES OF PROLIFIC RYE, TRITICALE (6A190), STEWART 63, AND MANITOU WHEAT

	Amylose in Starch %	Sedimentation Coefficient of Purified Amylose $S_{20,w}$
Triticale (6A190)	23.7	3.63
Stewart 63 wheat	30.1	3.17
Prolific rye	30.1	3.49
Manitou wheat	28.9	3.42

endosperm cells. The bimodal size distribution and the appearance of the granules suggest that cells characteristic of both parents exist in kernels of the amphiploid line. This could come about in one of two ways: There could be variability from seed to seed in the genetic information dictating cell type, or there could be cells of both types within a single seed. Since this line is formed directly from an interspecies cross and has not been backcrossed, seed variability is the most likely cause.

The high amylase activity of the triticale line examined is probably not a general characteristic of all triticale lines but rather is peculiar to variety 6A190. This line has shrunken kernels, low dormancy, and about 20 times the amylase activity at maturity of other lines tested (16). There was, however, no visible evidence of sprouting in the sample used for these trials. As evidenced by the amylograph viscosity curves, the high amylase appears to be related more to the rye parent than the durum wheat parent. This, along with the bimodal distribution demonstrated for the triticale, leads one to speculate that air classification of this starch would result in most of the amylase activity's being associated with the fraction having the largest granule size.

The lower amylose content in triticale could be the result of an enhanced production of branching enzyme, owing to the introduction from both parents of loci controlling this factor. Since the difference is not large, however, there is probably sufficient variability in the amylose content of the parental species to account for the lower amylose in triticale. It is also likely that the large molecular size of the amylose from triticale can be accounted for by variability in the parental species.

The chemical and physical properties of starch in a new variety produced by a cross within a species are limited by the variability of these properties in the parent material. Our data suggest that this is also true of the interspecies crosses producing triticale varieties. The data on granule size variations and amylase activity in triticale starch indicate that there is a possibility of determining the genetic control and inheritance of these factors in this new cereal species.

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