The Characterization of Triticale Starch and Its Comparison with Starches of Rye, Durum, and HRS Wheat[1]

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ABSTRACT

The starches of Triticale, rye, hard red spring (HRS), and durum wheats were isolated, studied, and compared. The greatest percentage of starch occurred in the Triticale flour sample. Starches isolated from the corresponding flours were examined for protein, fat, phosphorus, and ash contents. Only small differences were recorded. Subsequently, the starches were characterized by various physicochemical properties. The amylose contents varied from 23.0 to 27.0%, the Triticale and durum starches having the low and high values. Intrinsic viscosities of Triticale and durum starches were similar. The HRS, rye, and durum starches had similar water-binding capacities. Gelatinization curves showed rye starch to have the lowest temperature of initial pasting and HRS starch the highest. Triticale and durum starches indicated the same temperature of initial pasting, similar starch granule densities, and similar granule size distribution data. Rye and Triticale starches recorded the lowest amount of starch damage. The absolute density value for Triticale starch was similar to the durum starch. Amylose and amylopectin were isolated from the respective starch samples and characterized by intrinsic viscosity and periodate oxidation. The two polymers of rye starch showed the lowest intrinsic viscosities and the lowest molecular weight for amylose. Methylation of Triticale starch verified data obtained by periodate oxidation on the Triticale starch fractions.

Triticale, a synthetic cereal grain created by man, is the progeny of a successful cross between wheat, *Triticum*, and rye, *Secale*.

Considering the wide variety of starch sources in the plant world, it is of academic and industrial importance to elucidate this facet of starch chemistry.

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Starch is industrially important when it can be obtained in high yields. Its importance has been emphasized in printing, textile sizing and dressing, paper making, adhesive manufacturing, and foodstuffs. A brief review of the historical development for Triticale was discussed by Vaisey and Unrau (1) and Unrau and Jenkins (2).

The milling and baking performance for a number of hexaploid and octoploid Triticale species has been investigated by Unrau and Jenkins (2). Flour yield appeared to be related to kernel weight in the case of tetraploid and octoploid Triticales. However, within the hexaploid Triticales, no indication of a relationship between flour yield and kernel weight was revealed.

Hill (3) recently stated that Triticale starch, when compared to the starch of its parents, had the lowest peak viscosity as measured by the amylograph. No significant variations were reported in either x-ray diffraction patterns or birefringence end-point temperatures of the various starches. The properties examined showed Triticale starch to resemble its rye parent.

The proteins of Triticale and its parents have been examined by Chen and Bushuk (4). Disc electrophoresis showed that Triticale did not contain any proteins uncommon to either parent.

Villegas (5) found that the lysine content in Triticale was intermediate between that of rye and wheat.

A study of Dedio et al. (6) revealed a similarity in phenolics found in Triticale and its respective wheat parent.

The purpose of this work was to characterize the starch of Triticale and to compare its biochemical and physical properties with starches isolated from hard red spring (HRS) and durum wheats, and rye.

MATERIALS AND METHODS

Samples

The HRS flour and durum semolina were obtained from composite field-plot varieties grown in North Dakota and milled on a Buhler mill. The rye flour was obtained from Bay State Milling Company, Winona, Minn. The Triticale (hexaploid) was grown in North Dakota and milled into flour on a Buhler mill.

Cereal Flour Analysis

Pertinent information on the different samples used for the isolation of the starches is shown in Table I. The protein content ranged from 11.0 to 17.5% and the ash content values were between 0.45 and 0.95%. Similar values were obtained

TABLE I. PRELIMINARY ANALYSIS OF CEREAL GRAIN FLOURS

Sample	Protein[a] %	Ash[a] %	Fat[a] %	Starch[a] %	Pentosan[a] %	Starch[a] Damage %
HRS	16.1	0.45	1.64	71.9	4.0	39.3
Durum	17.5	0.78	1.75	70.2	4.3	18.5
Rye	11.0	0.95	1.92	73.4	6.0	14.0
Triticale	12.4	0.61	1.80	74.7	4.3	14.0

[a]Dry basis.

for fat content, which agrees with data for rye and wheat reported by Kent-Jones and Amos (7).

Triticale and durum contained similar amounts of pentosans; however, they were lower than rye, which contained the highest pentosan content of the materials studied.

While a variation was observed in the percentage of starch present in the flour samples, Triticale and rye had the greatest concentration of starch in the endosperm.

With the exception of a high value for HRS wheat, the starch damage results were similar and in agreement with the literature for wheat flour (8,9).

Starch Isolation

Starch was isolated from HRS flour, durum semolina, and Triticale flour by the dough-kneading procedure (10). The liquid slurry obtained from hand washing of the dough ball was centrifuged (2,000 X g) for 15 min. The most dense layer, starch, was at the bottom of the cup (11). It was removed, reslurried in water, and centrifuged; the prime starch was recovered, air dried, and sieved prior to anlaysis.

Since rye flour does not form a gluten, the method of dough kneading was unsuccessful. The procedure used was as follows: a 400-g. rye-flour sample was mixed with 2,400 ml. of water for three min. at a low speed in a Waring Blendor, after which the procedure of centrifugation, as given above, was performed. This process produced a successful isolation of the rye starch.

Starch Fractionation

The cereal starches were fractionated into amylose and amylopectin according to the procedure of Montgomery and Senti (12). Amyloses were recrystallized three times from n-butanol, isolated by precipitation with acetone, and vacuum dried at 40°C. Amylopectin was isolated from the gel after amylose extraction. The gel was mixed with methanol in a Waring Blendor and the resulting precipitate of amylopectin was collected and vacuum dried at 40°C.

Amylose Determination

The determination of amylose was performed using a colorimetric procedure described by McCready and Hassid (13). Standard curves were made for each cereal starch examined.

Intrinsic Viscosity

The various cereal starches and their respective fractions were dissolved in 1N potassium hydroxide as described by Lansky et al. (14). Intrinsic viscosity was determined at 25°C. with an Ubbelohde viscometer (15).

Water-Binding Capacity

The procedure followed was that of Yamazaki (16), with modifications described by Medcalf and Gilles (17).

Gelatinization Curves

Starch gelatinization curves were obtained following the procedure described by Sandstedt and Abbott (18) utilizing the carboxymethyl cellulose

(CMC)[5]-amylograph technique. A complete description of the procedure used has been given by Medcalf and Gilles (17).

Starch Determination

The percentage of starch present in the milled samples was determined by the polarimetric procedure (19).

Starch Granule Density

The absolute density of the cereal starches was determined by the xylene displacement technique described by Schoch and Leach (20).

Total Phosphorus Determination

The procedure described by Allen (21) for total phosphorus was used with modifications owing to the increased amount of organic material. Starch samples of 500 mg. (d.b.) were placed in 100-ml. micro-Kjeldahl digesting flasks. To each flask, 11 ml. perchloric acid (70 to 72%) and 1.0 ml. hydrogen peroxide (30%) were added. The flasks were heated until the solutions became clear, and then were allowed to cool. The contents were poured into 25-ml. volumetric flasks, and 2 ml. of 2,4-diaminophenol dihydrochloride reagent added, followed by the addition of 1.0 ml. of the ammonium molybdate solution. The contents of the volumetric flask were diluted with deionized water. The color produced was read 10 min. later at 660 nm. A standard curve was made using potassium dihydrogen phosphate.

Starch Damage Determination

Starch damage in the different samples was determined according to the method described by Williams and Fegol (22). An independent evaluation of the Farrand (8) and Williams and Fegol (22) methods provided data for a regression equation (r = 0.95) from which starch damage data were derived indirectly.

Granule Size Distribution of Starch

Starch granule size distribution was determined with a Mine Safety Appliance (MSA) Particle Size Analyzer (23). It was necessary to develop a procedure before the cereal starches could be analyzed. Because the starch granule density had been determined previously, it became necessary to develop a time schedule for each cereal starch (23).

One gram of starch was weighed accurately, placed in a Waring Blendor, and 100 ml. of distilled water added. After 1-min. mixing at low speed, the suspension was swirled occasionally for 3 min. and then centrifuged. The supernatant was decanted, 100 ml. feeding solution added, and the remaining procedure conducted according to the MSA Particle Size Analyzer technique (23).

Methylation

A successful methylation of Triticale starch was accomplished by the technique of Cheetham and McIlroy (24). To obtain a methylated starch, additions of sodium hydride (2.0 g.) and dimethyl sulfate (10 ml.) were made on 20 successive days. After 10 days an additional 100 ml. of methyl sulfoxide was added to the flask.

[5]The CMC obtained from Hercules, Inc., Harbor Beach, Mich., was type 7HF and had a degree of substitution of 0.76.

When the reaction was complete, chloroform (500 ml.) was added to the mixture in an effort to remove the methylated starch.

Methoxyl group determination was performed using a Zeisel apparatus (25). The procedure followed was according to OAC Methods of Analysis (26). Infrared spectra were examined to check for the absence of hydroxyl groups in the 2.8 to 2.9 μ region, which would be indicative of complete methylation.

Although potassium bromide pellets were used, best results were obtained with a mull (27), using fluorolube oil.

Methanolysis of the methylated starch was performed according to Ingle and Whistler (28). A slight modification was incorporated after hydrolysis. The hydrolysate was neutralized with barium carbonate, filtered, and concentrated on a rotary evaporator under reduced pressure, after which it was ready for gas-liquid chromatographic analysis on a column of 10% XE-60 on chromosorb W temperature-programmed from 120° to 240°C. The other gas-liquid chromatographic conditions were as follows: column length, 6 ft.; inside diameter, 3.5 mm.; mesh of solid phase, 80/100; rear cell bath, 280°C.; injection temperature, 270°C.; carrier gas, nitrogen; rate of flow, 35 ml. per min.; inlet pressure, 50 p.s.i.; chart speed, 1 in. per 2 min.

The preparation of 2,3,4,6-tetra-O-methyl glucose was performed according to Walker et al. (29). After methylation, the methyl 2,3,4,6-tetra-O-methyl glucoside was hydrolyzed by refluxing with 1N sulfuric acid for 10 hr. After neutralization with a weak ion-exchange resin, the product was crystallized from dry petroleum ether (60° to 90°C., b.p.).

Synthesis of 2,3-di-O-methyl glucose was achieved by the method of Irvine and Scott (30).

Preparation of 2,3,6-tri-O-methyl glucose was accomplished by complete methylation of the amylose fraction. Methanolysis was performed according to the procedure of Ingle and Whistler (28).

Periodate Oxidation

Periodate oxidation was performed on the starch fractions of the respective cereal starches according to Shasha and Whistler (31). The procedure was modified somewhat. To dry amylose or amylopectin (approximately 200 mg.) in a 250-ml. glass-stoppered bottle, 90 ml. of a 5% potassium chloride solution and 10 ml. of a 0.3M sodium periodate solution were added. The blank contained reagents but no sample. The bottles were shaken slowly at 1° to 3°C. in the dark. At 24-hr. intervals, a 10-ml. aliquot was pipetted and mixed with 1 ml. ethylene glycol, and this mixture allowed to stand for a minimum of 10 min. in the absence of light. The formic acid liberated was titrated with standard 0.01N sodium hydroxide. The calculations were performed as described by Shasha and Whistler (31).

Other Analyses

AACC official methods were utilized for protein, fat, ash, pentosan, and diastatic activity determinations (19).

RESULTS AND DISCUSSION

Comparisons of Triticale Starch with Other Cereal Starches

Physicochemical Properties. Table II shows chemical and physicochemical data

TABLE II. CHEMICAL AND PHYSICOCHEMICAL ANALYSIS OF CEREAL STARCHES

Sample	Starch Recovery %	Nitrogen %	Ash %	Fat %	Phosphorus %	Absolute Density[a] 30°C.	Water-Binding Capacity[b] %	Intrinsic Viscosity[b]	Amylose[b] %
HRS	55	0.04	0.38	0.75	0.047	1.4832	85.0	1.88	24.5
Durum	54	0.05	0.49	0.70	0.047	1.4460	85.5	2.10	27.0
Rye	42	0.04	0.37	0.98	0.025	1.4209	86.5	1.96	24.0
Triticale	53	0.04	0.39	0.78	0.042	1.4465	c	2.13	23.0

[a]As-is basis.

[b]Dry basis.

[c]No sample available.

for the different starches investigated. Little variation in nitrogen content was observed The ash content of Triticale starch was similar to the other cereal starches, except durum starch, which had the highest value. The starch isolated from rye flour had the highest content of fatty material.

The absolute densities of Triticale and durum starch were similar. The absolute densities suggest that HRS wheat-starch granules may have a more compact granule structure than the other cereal starches (17). Rye starch exhibited the lowest value of all cereal starches.

The water-binding capacity determinations revealed small differences between HRS wheat starch and the remaining starches. However, the reported values were in agreement with previously recorded results (16,17).

The data in Table II for viscosities of the cereal starches show few differences. Values ranged from 1.88 to 2.13. Medcalf and Gilles (17) examined numerous wheat samples, but reported no significant differences among wheat classes. Relatively large differences occurred among samples of the same class.

Amylose contents ranged from 23 to 27%. Triticale and durum starches reported the respective extremes and agree well with reported values (9,17).

Gelatinization. Results of the flour and starch paste viscosities are given in Table III. Figure I illustrates the type of curves observed from the various cereal flours

TABLE III. SUMMARY OF CEREAL GRAIN AMYLOGRAMS

Sample	Temperature of Initial Pasting °C.	Peak Height B.U.	15 min. Hold Height B.U.	50° Height B.U.
Flour[a]				
HRS	64	1000	760	910
Durum	79	920	900	990
Rye	58	140	50	50
Triticale	58	110	0	0
Starch[b]				
HRS	58	700	650	670
Durum	55	695	660	665
Rye	52	635	605	625
Triticale	55	800	705	695

[a]Cereal flour amylograms with pH 5.35 buffer.

[b]Cereal starch amylograms incorporating CMC.

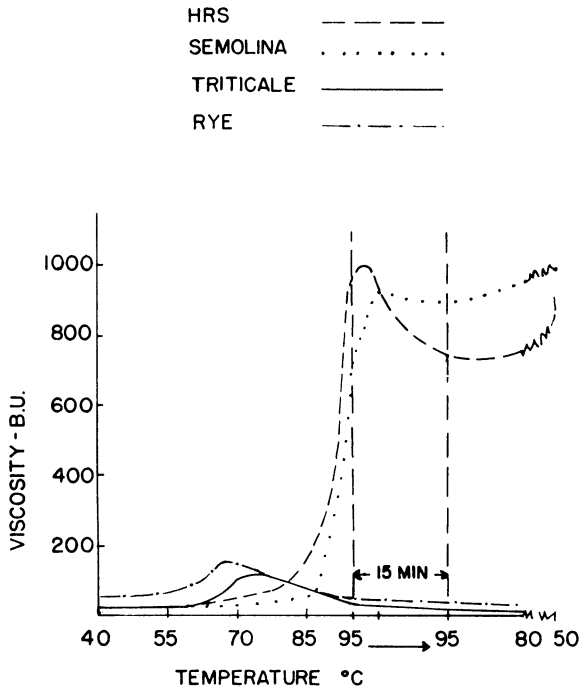

Fig. 1. Cereal flour amylographs with pH 5.35 buffer.

using a buffer with a pH of 5.35. The HRS and durum wheats had higher peak heights than rye and Triticale. The amylase activity of rye and Triticale is considerable at pH 5.35, as indicated by the low peak height, whereas the enzymatic activity in the wheat samples is not so great. The effect of pH on amylograph peak height in rye has been observed previously (32).

The effect of pH on rye and Triticale flours and durum semolina was studied with the amylograph. The following pH values were used: 5.35, 5.70, 6.50, and 7.00. A marked increase in viscosity was observed in the case of rye and Triticale. The increase in viscosity at pH 7 was in excess of three times the viscosity observed at pH 5.35 for rye. Triticale showed an increase in excess of four times the viscosity at pH 7.

Table III also shows data obtained from the amylograph for the various starches. Triticale and durum starch had the same initial gelatinization temperature. The initial temperature of gelatinization varied from 52° to 58°C., with rye and HRS wheat starches recording the low and high temperatures, respectively. The temperature of initial gelatinization of HRS and durum wheat starches has been studied previously (17).

The absolute density of the various cereal starches given in Table II supports the idea of "granule compactness" (17). The Triticale and durum samples recorded intermediate values for the initial temperature of gelatinization and for density. The highest initial temperature of gelatinization was recorded by the HRS wheat starch.

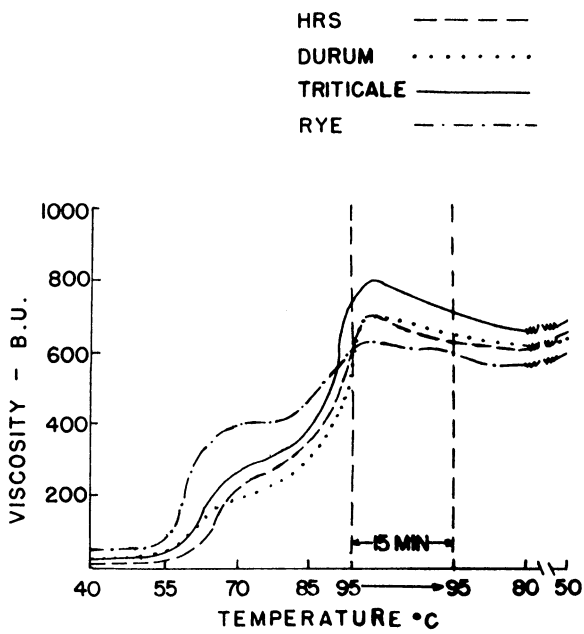

Fig. 2 Starch CMC-amylograph curves (corrected for viscosity of CMC).

Likewise, it had the highest absolute density. At the other extreme was rye, which recorded the lowest absolute density and lowest initial temperature of gelatinization. It would appear that the density of the granules plays an important role in determining the initial temperature of gelatinization of the starch. Once gelatinization has started, granule density does not appear to be afactor in further gelatinization.

Figure 2 illustrates the two-step gelatinization observed for the different starches when employing the CMC-amylograph technique. These curves present the total picture of gelatinization. The first step of gelatinization was very pronounced with the rye starch sample, and the remaining samples recorded similar curves. A relatively broad range in peak viscosity was observed: 635 to 800 B.U. The extremes were recorded by rye and Triticale, respectively. All the starches showed a decrease in viscosity once the maximum had been attained; however, all recovered to form a gel. Triticale had the strongest gel according to the recorded data.

Granule Size and Distribution. The granule size distribution of the cereal starches was examined with the MSA Particle Size Analyzer (23). Preliminary analysis was aided with photomicrographs of the starches. Figure 3 shows photographs of Triticale, durum, rye, and HRS starch granules. The picture represents an average field observation of the respective starches. The photograph was enlarged to preserve actual observation (X 470) under the microscope.

Triticale starch had a greater abundance of smaller granules when compared with rye starch. The Triticale, durum, and HRS starches were similar in granule size distribution. Rye starch had the largest granules, and generally contained few small granules.

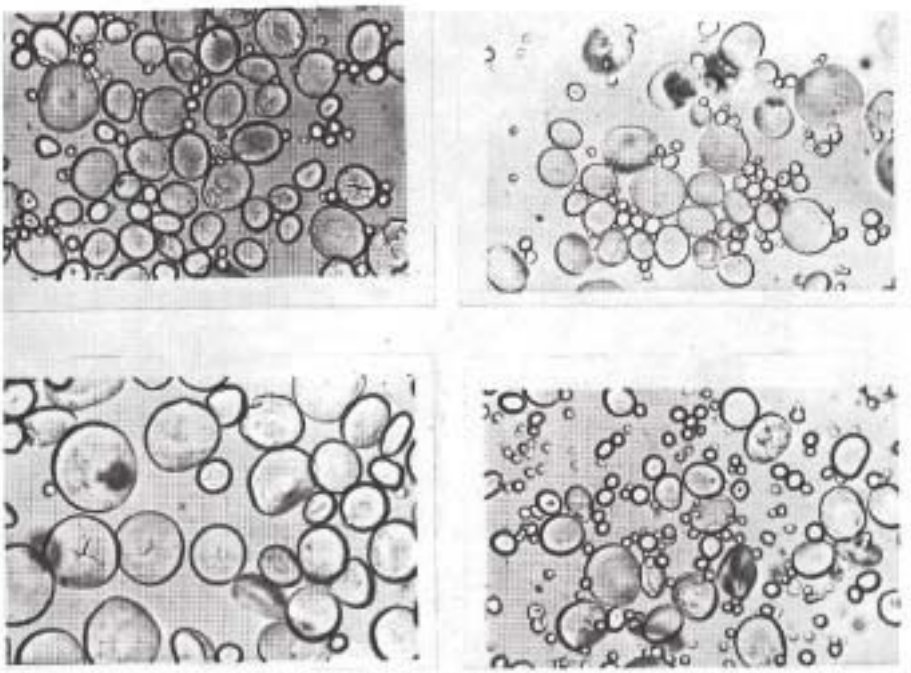

Fig. 3. Photomicrographs of different starch granules. Magnification (X 470). Upper left: Triticale; upper right: durum; lower left: rye; lower right: HRS.

The results of the MSA Particle Size Analyzer are summarized in Table IV. Triticale and durum wheat starch granules are similar in overall granule distribution. The similarity between Triticale and durum wheat starch and the large granule distribution of rye starch is evident.

Structural Studies: Methylation. Methylation of Triticale starch was achieved employing a technique discussed earlier. To determine the degree of starch methylation, methoxyl content and infrared absorption at 2.8 to 2.9 μ were determined. Successful methylation of the Triticale starch was evidenced by the high methoxyl content (found, 44.7%; theoretical, 45.6%) and negligible I.R. absorption. Doubtless some water was present, which may have contributed to the insignificant absorption.

Gas-liquid chromatography was used to analyze the methyl sugars from the

TABLE IV. PARTICLE-SIZE DISTRIBUTION OF CEREAL STARCHES

Sample	SED μ Range Distribution				
	>40 %	30–40 %	20–30 %	10–20 %	<10 %
HRS	0	3	41	46	10
Durum	0	2	31	49	18
Rye	6	17	49	26	2
Triticale	0	7	28	55	10

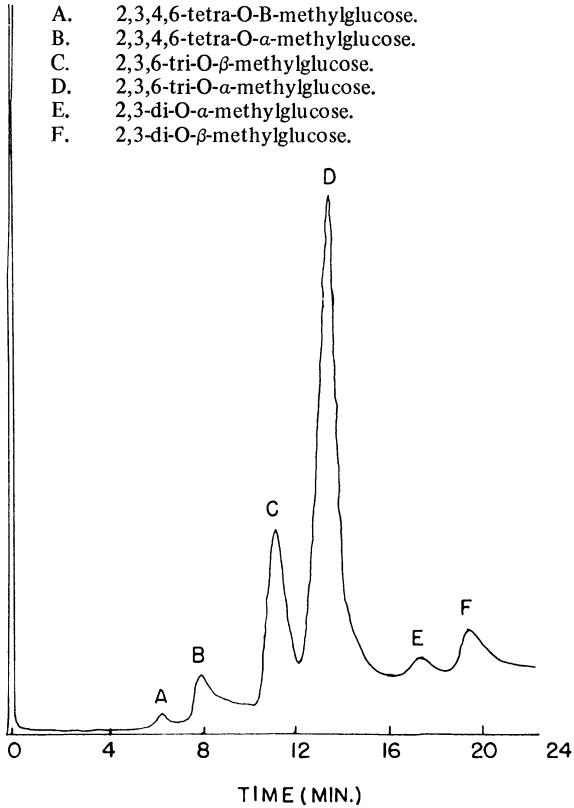

Fig. 4. A gas-liquid chromatogram illustrating separation of the methanolysis products of methylated Triticale starch.

methylated starch hydrolysate. A gas chromatogram illustrating separation of the methyl sugars and their respective anomers is shown in Fig. 4. Standard methyl sugars were used to identify the sugars; however, establishment of anomers was aided with previous publications (24,33). The relative percentage of each methylated sugar was calculated by the triangulation method. The percentages obtained were 4.2% for 2,3,4,6-tetra-O-methylglucose, 87.7% for 2,3,6-tri-O-methylglucose, and 8.1% for 2,3-di-O-methylglucose. The value obtained for 2,3,4,6-tetra-O-methylglucose agrees with previously reported results (34). Values above 5% for 2,3-di-O-methylglucose have also been reported (34,35). The methylation experiment indicates that one terminal group exists for about every 22 to 24 glucose units in starch. This is in agreement with the results summarized in Table V for periodate oxidation.

The results of the methylation studies would indicate that no structural abnormalities appear to exist in Triticale starch.

Structural Studies: Periodate Oxidation and Intrinsic Viscosities of Amylose and Amylopectin Fractions. Table V summarizes the periodate oxidation data accumulated for the various cereal starch fractions. The only difference noted was

TABLE V. PERIODATE OXIDATION AND INTRINSIC VISCOSITY

Sample	Fraction	Branching %	Glucose Units in a Segment	Molecular Weight	Intrinsic Viscosity
HRS	Amylose	...	...	284,840	2.78
Durum	Amylose	...	...	272,829	2.87
Rye	Amylose	...	...	218,420	2.60
Triticale	Amylose	...	...	261,713	2.86
HRS	Amylopectin	4.4	23	...	1.83
Durum	Amylopectin	4.8	21	...	1.96
Rye	Amylopectin	4.8	21	...	1.33
Triticale	Amylopectin	4.5	22	...	1.78

the lower molecular-weight value obtained for the amylose fraction of rye. The other cereal grains were somewhat higher. Similar values have been recorded for wheat (36,37,38). Values for other starch types have been summarized elsewhere (34).

The intrinsic viscosities for the respective amylose fractions of Triticale, HRS, and durum wheats were similar in value and likewise in molecular weight. The lowest molecular-weight amylose, that of the rye sample, also showed the lowest viscosity.

The two wheat samples and Triticale showed the highest intrinsic viscosity values for the amylopectin fraction of starch. As observed with amylose, rye showed the lowest viscosity of the amylopectin fraction.

The amylose and amylopectin values for the two wheat samples are in agreement with previously reported data (17).

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