NOTE

CARBOHYDRATES

Fractionation of Regular Corn Starch: A Comparison of Aqueous Leaching and Aqueous Dispersion Methods[1]

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Starch is an extraordinarily versatile polymer used in a wide array of products from paper to prepared foods. Despite its current wide usage, its potential for additional applications continues to be explored. Unfortunately, new creative developments are hampered, in part, because of a lack of detailed structure and function studies on all but the basic amylose and amylopectin molecules. To better understand the relationship between starch molecular structure and its functional attributes, pure fractions of amylose and amylopectin with unique molecular weights (MW) or branching patterns would be desirable. Detailed structure and function data would not only partially aid in the development of new uses for starch but would also assist plant breeders and biotechnologists to develop specialty grains with specific molecular structures.

For years, separation and classification of starch into respective amylopectin and amylose components has proven difficult. The most troublesome limitations have been the coexistence of amylopectin and amylose within the starch granule, the existence of an intermediate MW fraction (Whistler and Doane 1961), and the fragile nature of the larger amylopectin molecule. Because granule solvation is a prerequisite for further polymer isolation procedures, these limitations make it practically impossible to yield a sol from which one starch polymer can be precipitated without being contaminated by the other.

Most successful fractionation methods cited in the literature have involved aqueous (aq) dispersion (Adkins and Greenwood 1969) or aq leaching (Kerr and Severson 1943, Meyer et al 1949, Banks et al 1959) of granules, and selective retrogradation (Schoch 1945) or alcohol precipitation (Schoch 1945, Whistler and Doane 1961) of one polymer from a starch dispersion. Studies detailing the sequential combination of these general methods, however, are less numerous. In addition, Lansky et al (1949) noted that the lack of standards or methods by which starch fractions could be measured for purity and extent of degradation has hindered progress. Not until recently has high-performance size-exclusion chromatography (HPSEC) begun to play a pivotal role in the classification of starches based on MW distribution (Takagi and Hizukuri 1984, Kobayashi et al 1985, Jackson et al 1988, Jackson et al 1992). HPSEC can also be an important tool in the assessment of starch fraction purity.

The objective of this study was to compare the effectiveness of different fractionation procedures in obtaining amylose and amylopectin from regular corn starch.

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MATERIALS AND METHODS

Aqueous Dispersion

Using 10% aq butanol (Whistler and Doane 1961) or 90% aq dimethyl sulfoxide (DMSO) (Adkins and Greenwood 1969), starch slurries (4%, w/v) were prepared and heated at either 120°C (autoclave) for 1 hr or at 45°C for 2 hr with gentle stirring. Starch slurries (4% w/v) were also prepared using 14% aq $MgSO_4$ (Bus et al 1958) and heated in an autoclave at 120°C for 1 hr. After dispersion, the various slurries were centrifuged at 3,000 × *g* for 8–10 min. To precipitate amylose, 100% butanol (at a volume equal to one-third the volume of the supernatant) was added to each supernatant. The mixture was swirled and then held at room temperature for 2 hr. The mixture was centrifuged again to obtain the precipitate (amylose). Residues obtained after the first centrifugation were reslurried in methanol (Ward et al 1994) and centrifuged to yield amylopectin. Both amylose and amylopectin fractions were dried at 45°C in a forced-air oven.

Aqueous Leaching

Starch slurries (4%, w/v) were aq leached by gentle stirring at 50, 60, 70, and 80°C for 24, 4, 1, and 0.75 hr, respectively. After centrifugation as above, the supernatant was retained and the residue was again leached (a total of two times at 60, 70, or 80°C and five times at 50°C). Amylose and amylopectin were then collected from the pooled supernatants and the remaining residue, respectively, as outlined above. In addition, two amylose samples were obtained using absolute ethanol (at a volume equal to one-half the volume of the supernatant) or by holding each supernatant at 5°C for two days ("self precipitation" or selective retrogradation without alcohol addition).

HPSEC

Dried starch fractions were solubilized in 90% aq DMSO by boiling for 45 min and holding at 60°C for 12 hr. Each solution was subsequently filtered through a 1.2-μm filter before being injected (25 μl) into an HPSEC system consisting of 4 KS-series Shodex Ionpak columns (Showa Denko, Tokyo, Japan) connected in series to a refractive index detector (Waters 410, Millipore Co., Milford, MA) as described by Jackson (1991). Deionized distilled water at a flow rate of 1.0 ml/min was used as the mobile phase. Apparent MW distributions were determined as outlined by Zhang and Jackson (1992) using pullulan MW standards. MW distributions were calculated as *z*-average MW (M_z), weight-average MW (M_w), and number-average MW (M_n) in decreasing MW order. Purity was calculated by determining the ratio between the area under the amylose curve and that under the entire chromatogram. Fraction yield was calculated (regardless of purity) as the dry weight of fraction divided by the dry weight of original starch.

Statistical Analysis

Statistical analysis was performed using SAS software (SAS 1987). Means, standard deviations, and least significant differ-

ences (LSD) at ($P < 0.05$) were calculated for MW and purities determined by HPSEC. Each analysis was performed in triplicate.

RESULTS AND DISCUSSION

After drying, starch fractions obtained by 14% aq $MgSO_4$ dispersion followed by butanol precipitation could not be dissolved in 90% aq DMSO for HPSEC analysis. Granular particles could not be ground into a powder as could those from aq-dispersed or leached counterparts. This grinding resistance suggests that drying may have had an additional firming effect on a polymer-salt-alcohol complex formed during precipitation. Hence, the MW distributions of 14% aq $MgSO_4$ fractionated amylose was analyzed without drying. Yield was calculated on the basis of the dry fraction.

Generally, aq dispersion produced higher yields of amylose (26–32 vs. 5–26%) with lower purity (79–100 vs. 94–100%) than did aq leaching (Table I). These findings are consistent with those of Kerr and Severson (1943) and Meyer et al (1945) in that aq-butanol dispersions gave higher yielding and lower purity amy-

TABLE I
High-Performance Size-Exclusion Chromatography Molecular Weight Distribution, Purity, and Yield of Amylose Fractions Isolated from Regular Corn Starch[a]

	Amylose				Purity[e]		Yield[g]	
Starch Treatment	M_z[b]	M_w[c]	M_n[d]	M_w/M_n	(%)	STD[f]	(%)	STD
Aqueous dispersion[h]								
14% Mg_2SO_4	2.1×10^6	4.4×10^5	9.9×10^4	4.4	100	0.00	26.1	0.71
10% Butanol	3.9×10^6	1.1×10^6	9.0×10^4	12.5	89	0.57	27.4	0.35
90% DMSO[i]	13.5×10^6	1.3×10^6	1.4×10^5	9.6	79	7.64	31.7	0.71
Aqueous leaching[j]								
50°C/Ethanol	6.0×10^5	2.1×10^5	6.3×10^4	3.3	95	0.28	8.3	0.71
50°C/Butanol	3.9×10^5	1.9×10^5	9.7×10^4	2.0	99	0.42	5.0	0.71
60°C/Self	4.3×10^5	1.5×10^5	5.6×10^4	2.7	95	1.41	21.1	0.42
60°C/Ethanol	6.9×10^5	1.9×10^5	5.8×10^4	3.3	94	1.41	20.4	0.57
60°C/Butanol	1.2×10^5	6.1×10^4	2.0×10^4	3.1	100	0.00	15.1	0.28
70°C/Self	1.2×10^6	3.3×10^5	6.2×10^4	5.4	93	0.00	22.7	0.21
70°C/Ethanol	1.6×10^6	3.8×10^5	6.7×10^4	4.5	97	1.27	22.6	0.21
70°C/Butanol	2.0×10^6	2.8×10^5	6.4×10^4	5.7	100	0.00	14.6	0.35
80°C/Self	3.6×10^6	8.4×10^5	1.2×10^5	7.0	94	1.20	26.1	0.35
80°C/Ethanol	3.8×10^6	8.1×10^5	1.2×10^5	6.8	95	0.56	25.4	0.71
80°C/Butanol	3.6×10^6	7.9×10^5	1.2×10^5	6.6	97	0.21	21.4	0.21
LSD[k]	6.6×10^5	2.7×10^4	2.2×10^4	2.2	4.6		1.34	

[a] Values are means of three analyses.
[b] Molecular weight *z*-average.
[c] Molecular weight weight-average.
[d] Molecular weight number-average.
[e] Calculated by determining the ratio between the area under the amylose curve and that under the entire chromatogram.
[f] Standard deviation.
[g] Calculated (regardless of purity) as the dry weight of fraction divided by the dry weight of original starch.
[h] Aqueous dispersion followed by centrifugation and subsequent amylose precipitation from supernatants using butanol.
[i] Dimethyl sulfoxide.
[j] Temperature/precipitation solvent or method. Self = selective retrogradation at 5°C for two days (no alcohol used).
[k] Least significant difference.

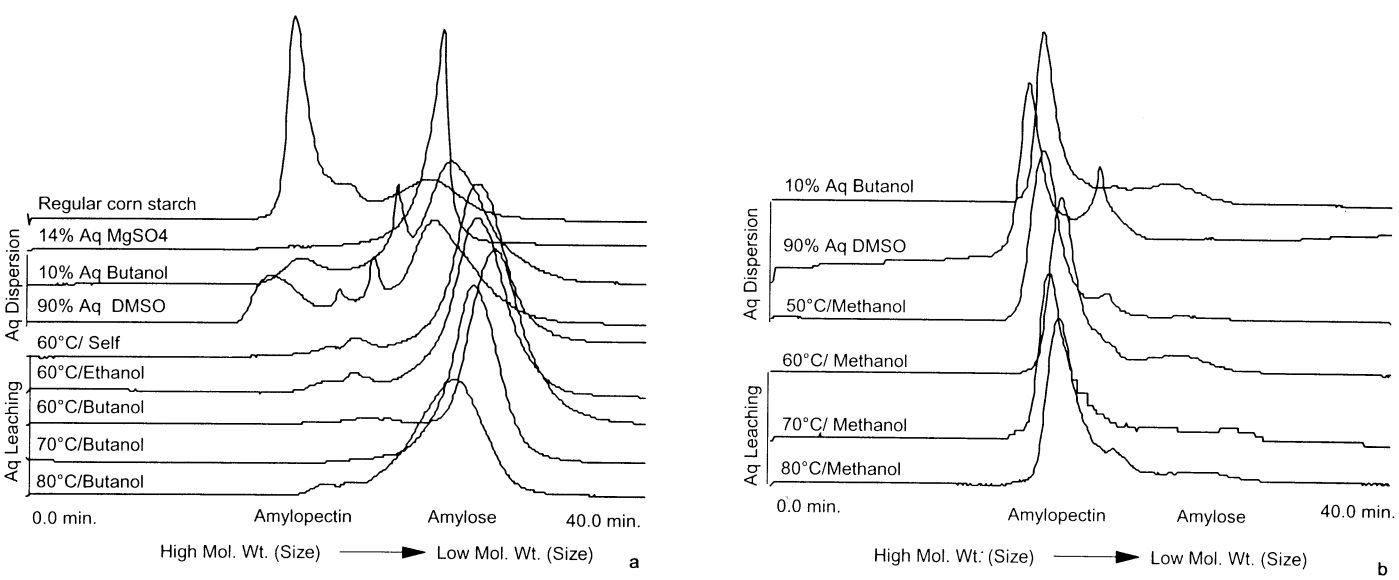

Fig. 1a, High-performance size-exclusion chromatography (HPSEC) profiles of regular corn starch and amylose fractions obtained from aqueous (aq) dispersion or aq leaching from regular corn starch. Aq dispersion was followed by centrifugation and subsequent amylose precipitation from supernatant using butanol. Aq leaching temperatures are followed by precipitation solvent or self method (selective retrogradation without alcohol at 5°C for two days). **b,** HPSEC profiles of amylopectin fractions obtained from aq dispersion or aq leaching from regular corn starch. Aq dispersion or aq leaching was followed by centrifugation and subsequent reslurrying of residue in methanol before drying.

loses than did aq leaching at 70–80°C. Studies conducting aq DMSO dispersion (Adkins and Greenwood 1969) and aq-butanol dispersion (Schoch 1945, Lanky et al 1945) reported similar amylose yield trends, but reported higher purities than that obtained by aq leaching (70–80°C). Different starch sources and isolation conditions, pretreatments before fractionation (defatting), and multiple purification and recrystallization steps performed on aq-dispersed (butanol and DMSO) amyloses may account for dissimilarities in reported purity values among various studies. The inverse proportion between yield and purity suggests that aq leaching solubilized mostly amylose from corn starch granules, while aq dispersion solubilized amylose and some (>10%) amylopectin.

Figure 1a illustrates the MW profiles of amylose fractions. Amylose fractionated by 10% aq-butanol dispersion had the widest MW range, while the wet 14% aq $MgSO_4$ dispersed fraction had the narrowest range. Generally, the wider the MW range, the less pure the amylose fraction. Overall, aq leaching followed by ethanol or butanol precipitation yielded amylose of high purity.

Amylose M_w and yield increased ($P < 0.05$) with aq leaching temperatures at 60–80°C, but purity tended to decrease with increasing temperatures for butanol-precipitated amylose (Table I, Fig. 1a). A similar pattern in M_w increase and decrease in purity due to higher leaching temperatures had been reported (Schoch 1945, Meyer et al 1949, Banks et al 1959). Purity and M_w also depended on precipitation solvent or method. Self-precipitation or precipitation using ethanol resulted in amylose with similar M_w and purity (Fig. 1a), suggesting that both procedures initiate amylose complex formation in a similar manner. However, ethanol precipitated amylose more rapidly (2 hr vs. two days) than self-precipitation. Amylose precipitated using ethanol, however, was less pure ($P < 0.05$) and showed a higher M_w than that precipitated using butanol (Table I, Fig. 1a). This suggests that butanol selectively complexes with molecules of a lower MW range than does ethanol, and this may be the reason why yields by butanol precipitation were only 65–85% of those of the ethanol-treated counterparts.

Figure 1b exhibits the HPSEC profiles of regular corn starch amylopectin fractions obtained by aq dispersion or aq leaching. Aq 90% DMSO fractionated amylopectin had a higher M_w (similar to that of native starch) than those fractionated by aq leaching or aq-butanol dispersion. The lower M_w for aq leaching (two to five times that of the same sample) and aq-butanol dispersion (120°C) suggests that these treatments may have caused slight amylopectin depolymerization. The elution of an intermediate MW fraction on the chromatogram of the 90% aq DMSO dispersed amylopectin (Fig. 1b) suggests that DMSO solubilized the entire granule. Hence, the chances of precipitating one polymer from a DMSO dispersion without it being contaminated by another is unlikely. The amylopectin purity range was 79–89% and 84–96% for aq dispersion and aq leaching, respectively (Table II).

Amylopectin M_w decreased with leaching temperatures at 50–60°C but remained constant at 60–80°C. Leaching temperatures of 60, 70, and 80°C fell within the inherent differential scanning calorimetry gelatinization temperature range (60–84°C) of the native starch (data not shown). This probably resulted in residues with similar MW polymers and may have contributed to the similarities in M_w. Jackson et al (1989) have previously shown that starch solubility, as measured by HPSEC, is directly related to differential scanning calorimetry gelatinization temperature.

CONCLUSION

Aq leaching at temperatures that fall within the inherent gelatinization temperature range of regular corn starch resulted in pure (94–100%) amylose fractions with narrow but different MW distributions. The most efficient fractionation procedure based on yield, purity, and preparation time was aq leaching at 70–80°C for 1 hr, followed by centrifugation and amylose precipitation from the supernatant using butanol. Leaching at 50°C (five times) resulted in a residue containing 96% amylopectin. This suggests that more leaching would be required for the samples treated at 60–80°C to remove small amounts of amylose remaining in the swollen granule.

Aq dispersion involves the solvation of almost the entire granule. Thus, about 10% amylopectin or partially depolymerized amylopectin is precipitated along with amylose, which is why these "amyloses" have a wide MW distribution and low purity. On the other hand, aq leaching involves the discrete leaching of mostly amylose into solution. Although its yield is low, the amylose precipitated from aq leaching has a narrow MW range and high purity.

TABLE II
High-Performance Size-Exclusion Chromatography Molecular Weight Distribution and Purity of Amylopectin Fractions Isolated from Regular Corn Starch[a]

	Amylopectin[b]			Purity[f]		
Starch Treatment	M_z[c]	M_w[d]	M_n[e]	M_w/M_n	(%)	STD[g]
Aqueous dispersion[h]						
10% Butanol	6.1×10^6	4.9×10^6	4.6×10^6	1.1	89	1.34
90% DMSO[i]	7.2×10^6	5.8×10^6	3.6×10^6	1.7	79	0.42
Aqueous leaching[j]						
50°C/Methanol	6.7×10^6	5.5×10^6	3.9×10^6	1.4	96	0.78
60°C/Methanol	5.8×10^6	4.9×10^6	3.6×10^6	1.3	84	0.42
70°C/Methanol	6.4×10^6	5.1×10^6	3.8×10^6	1.5	85	1.48
80°C/Methanol	6.9×10^6	4.8×10^6	4.1×10^6	1.1	89	0.21
LSD[k]	7.8×10^5	8.1×10^5	8.6×10^5	0.1	1.8	

[a] Values are means of three analyses.
[b] Amylopectin was obtained by reslurrying each residue in methanol before drying.
[c] Molecular weight z-average.
[d] Molecular weight weight-average.
[e] Molecular weight number-average.
[f] Calculated by determining the ratio between the area under the amylopectin curve and that under the entire chromatogram.
[g] Standard deviation.
[h] Aqueous dispersion followed by centrifugation and residual treatment with methanol.
[i] Dimethyl sulfoxide.
[j] Temperature/ solvent used for residue treatment.
[k] Least significant difference.

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