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[Received February 21, 1992. Accepted July 30, 1992.]

Assessment of Extrusion-Induced Starch Fragmentation by Gel-Permeation Chromatography and Methylation Analysis

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ABSTRACT

Cereal Chem. 70(2):152-157

Three commercial corn starches (high-amylose, amylopectin-enriched, and corn meal) were extruded under various conditions and analyzed for changes in solubility, molecular size distribution, and linkage content. Each of the extruded starches dissolved in dimethyl sulfoxide at a faster rate than unprocessed material. Gel-permeation chromatography confirmed the occurrence of starch fragmentation. Methylation analysis showed only small changes in linkage distributions, suggesting that under the conditions tested, the total number of fragmentation points is small relative to the the total number of glycosidic linkages in starch. Furthermore, the absence of glucose or maltodextrins indicates that fragmentation sites are largely internal, rather than adjacent to the reducing or nonreducing termini of the amylopectin molecule.

Many physical and chemical changes are known to take place during the extrusion of starch-based materials. One of the most significant and well-documented of these changes is the fragmentation of starch. Starch fragmentation has been observed indirectly, as evidenced by parameters such as decreases in its intrinsic viscosity (Colonna and Mercier 1983, Gomez and Aguilera 1983, Colonna et al 1984, Davidson et al 1984, Diosady et al 1985), and directly, by gel-permeation chromatography (Colonna and Mercier 1983, Colonna et al 1984, Davidson et al 1984, Chinnaswamy and Hanna 1990, Jackson et al 1990, Wen et al 1990, Wasserman and Timpa 1991). Gel-permeation studies uniformly show that extrusion induces the transformation of native (unextruded) starches into low molecular weight fragments. The extent of fragmentation is a function of extrusion conditions and, possibly, of the starch source. Although covalent reassociation of the fragments when starch was subjected to temperatures ranging from 125–213° C has been suggested (Greenwood 1967, Srivastava et al 1970), it has not been confirmed by recent studies (Wen et al 1990, Wasserman and Timpa 1991). Heating starch and other carbohydrates may result in the formation of anhydro linkages (Thompson and Wolfrom 1958, Greenwood 1967, Theander and Westerlund 1988).

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Little is known about changes in the distribution of glycosyl linkages during extrusion. The objective of this study was to analyze native and extruded starch-containing materials using both gel-permeation chromatography and methylation analysis. Methylation analysis is widely used to determine the linkage composition of polysaccharides and glycoproteins (York et al 1985, Carpita and Shea 1989) but has not previously been used as a tool to probe extrusion-induced starch fragmentation.

This article reports the use of methylation analysis to determine relative amounts of terminal (t)-Glc, 4-linked Glc, and 4,6-linked Glc (at branch points). We hypothesized that extensive fragmentation of (1,4)-linkages would be reflected by significant increases in t-Glc and decreases in (1,4)-Glc. On the other hand, lack of a significant change in linkage distribution would suggest that only a small fraction of the total glycosyl bonds was affected by extrusion. The results show that cleavage of only a few glycosidic bonds per starch molecule accounts for extrusion-induced shifts in gel-permeation profiles. Furthermore, the absence of low molecular weight fragmentation products indicates that cleavage sites are more likely to be located internally than near the reducing or nonreducing termini of the starch molecule.

MATERIALS AND METHODS

High-amylose (70% amylose) starches and amylopectin-enriched (98% amylopectin) starches (Hylon-7 and Amioca, respectively) were obtained from National Starch and Chemical (Bridgewater, NJ) and were processed in a Brabender single-screw extruder. The native high-amylose starch (sample UH) was unextruded. Sample XH was high-amylose starch extruded at 120°C, 100 rpm, 31% initial moisture. The native amylopectin-enriched starch (sample UA) was unextruded. Of the extruded amylopectin-enriched starch samples, sample XA1 was extruded at 170°C, 200 rpm, 20% moisture; sample XA2 was extruded at 100°C, 200 rpm, 20% initial moisture; and sample XA3 was extruded at 100°C, 50 rpm, 20% initial moisture.

Corn meal was obtained from Lauhoff Grain (Danville, IL). Native samples were unextruded. Extruded samples were prepared using the specifications outlined in Wen et al (1990). We used the sample numbers 8, 12, and 15, as designated in Wen et al (1990), in this study to permit ready comparison of results. Sample 8 was extruded at 100°C, 100 rpm, 25% moisture; sample 12 at 150°C, 300 rpm, 30% moisture; and sample 15 at 150°C, 300 rpm, 20% moisture.

Solubilization and Gel-Permeation Chromatography

Samples were dissolved by suspending them in 100% dimethyl sulfoxide (DMSO) at a concentration of approximately 6 mg/ml with continuous stirring. Starches were fully dissolved after 24 hr. Percent solubilization of carbohydrate was monitored by removing aliquots at various times, centrifuging at 1,300 × *g*, and analyzing the supernatants by the phenol-sulfuric acid assay (Dubois et al 1956).

Samples of high-amylose and amylopectin-enriched starches were chromatographed on a gel-permeation column (0.9 mm × 50 cm) packed with Sepharose CL-2B. The columns were eluted with 90% DMSO containing 0.6*M* urea, at a flow rate of 7 ml/hr. Corn meal was chromatographed on a 0.9-mm × 55-cm column packed with Sephacryl S-1000 and eluted with 50% DMSO at a flow rate of 5 ml/hr. Fractions (1.4 ml) were collected. Carbohydrate was quantified by analyzing each 3-ml fraction by the phenol-sulfuric acid assay (Dubois et al 1956).

Isolation of Starch from Corn Meal

Samples (300 mg) were defatted by extraction with 25 ml of $CHCl_3$/methanol (2:1, v/v) under magnetic stirring for 5 hr; the suspensions were filtered (Whatman no. 40 filter paper); and the residues were air-dried overnight under a hood. The defatted samples were suspended in 100 ml of 100% DMSO and stirred at room temperature for 72 hr. Alternatively, incubation at 60°C for 8 hr yielded equivalent solubilization. The supernatants were centrifuged at 10,000 × *g* for 30 min to remove any residual particulate matter. Starch was recovered by adding ethanol (75% v/v). The suspensions were held for 30 min, centrifuged at 10,000 × *g* for 30 min, and lyophilized. The extent of purification was monitored by measuring the carbohydrate (Dubois et al 1956) and protein (Brown et al 1989) content before and after starch isolation. The efficacy of the procedure was assessed by conducting gel-permeation chromatography on the samples, before and after purification, to ensure that profiles were not changed by the purification procedure.

Methylation Analysis

This procedure was conducted essentially as it was by Hakomori (1964), with modifications added by Harris et al (1984), Mort et al (1983), and Blakeney and Stone (1985). Use of *n*-butyllithium in DMSO was adapted for the rapid and safe generation of the methyl sulphinyl anion (Blakeney and Stone 1985, Parente et al 1985), thus bypassing the need for separately synthesizing anion. Starch was dissolved in DMSO by stirring overnight. The reaction with butyllithium was for 1 hr at 40°C. Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA) were used for purification of methylated high-amylose and amylopectin-enriched starches (Mort et al 1983); dialysis (molecular weight cut-off: 1,000) was used for corn meal (Harris et al 1984). Ultra-high purity nitrogen was used in place of argon to exclude moisture. Methylated starch was hydrolyzed using 2*N* trifluoroacetic acid and reduced/acetylated as described by Blakeney et al (1983). Myo-inositol was added as an internal standard. Permethylated-peracetylated alditols were separated by gas chromatography with a glass fused-capillary column (60 m × 0.25 mm i.d.; DB-5, 5% phenylmethylsilicone, 0.25 μm film thickness) in a Varian 3400 gas chromatograph (Varian, San Francisco, CA) equipped with a flame ionization detector. Carrier gas (He) flow rate was 1 ml/min. Samples (1 μl) were injected in splitless mode. Two temperature programs were used: 1) isothermal at 140°C for 1 min followed by a 2°C per min increase in temperature to 220°C; and 2) 10°C per min increase to 320°C and isothermal at 320°C for 10 min. The injector was at 275°C and the detector was at 300°C. Identities of the alditols were confirmed by comparing their relative mobilities with known standards and by mass spectrometry (York et al 1985).

Statistical Analysis of Data from Experiments on Purified Starches

Using the 1987 SAS program, (SAS, 1987) data analysis was first performed between date and group (with groups 1 and 2 representing high-amylose and amylopectin-enriched starches, respectively). Because the effect of date was not significant, it was removed and the comparison was made between the treatments (native and extruded) within each group. Finally, all data were pooled for statistical analysis.

RESULTS

Fragmentation and Methylation Analysis of Purified Starches

Solubilization of samples. Extrudates of high-amylose and amylopectin-enriched starches were prepared in a Brabender single-screw extruder. Solvents evaluated for effectivity of solubilizing high-amylose and amylopectin-enriched starches were: boiling water (Hood 1978); alkali (Colonna and Mercier 1983); and DMSO (Killion and Foster 1960, Leach and Schoch 1962, Carpita and Kanabus 1987, Jackson 1991). In accordance with our previous findings with corn meal (Wen et al 1990), DMSO was effective. The solubility of starches in DMSO is enhanced by extrusion. Table I summarizes the relative solubility of native and extruded high-amylose and amylopectin-enriched starches in DMSO. Native high-amylose starch could only be partially solubilized in 100% DMSO. However, complete solubilization was obtained in 90% DMSO containing 0.6*M* urea. In 100% DMSO, the solubility of extruded high-amylose starch was 95% as compared to 68% for the native starch. When the DMSO level was lowered to 75%, a marked decline in the solubility of both native and extruded high-amylose starch was evident.

Amylopectin-enriched starch behaved somewhat differently

than high-amylose starch (Table I). Whether extruded or not, amylopectin-enriched starch was completely soluble in 100% DMSO. However, as DMSO levels were lowered, native material was largely insoluble, while the extrudate, for the most part, dissolved within 2 hr. In addition to overall enhancement of DMSO solubility, these results demonstrate that highly branched starches are more readily solubilized than amylose-rich starches. Because of the closer packing of hydrogen bonds in amylose, these results were consistent with expectations.

Gel-permeation chromatography of purified starches. Figures 1 and 2 show chromatograms for high-amylose and amylopectin-enriched starches, respectively. In each case, the appearance of lower molecular weight material confirmed the occurrence of fragmentation. In high-amylose starch (Fig. 1), most of the fragmentation appeared to be derived from the void-volume amylopectin peak.

TABLE I
Relative Solubility of Starch Samples[a]

Type of Starch in Percentage of Dimethyl Sulfoxide	Percent of Starch Solubilized Depending on Treatment	
	Native	Extruded
High-amylose		
100	68	95
75	21	32
50	5	24
25	4.5	16
Amylopectin-enriched		
100	100	100
75	7	100
50	1.5	100
25	10	100[b]

[a] Samples (6 mg/ml) were stirred at room temperature for 24 hr and then centrifuged at 1,300 × *g* for 30 min.
[b] Supernatant cloudy, but no visible sediment formed.

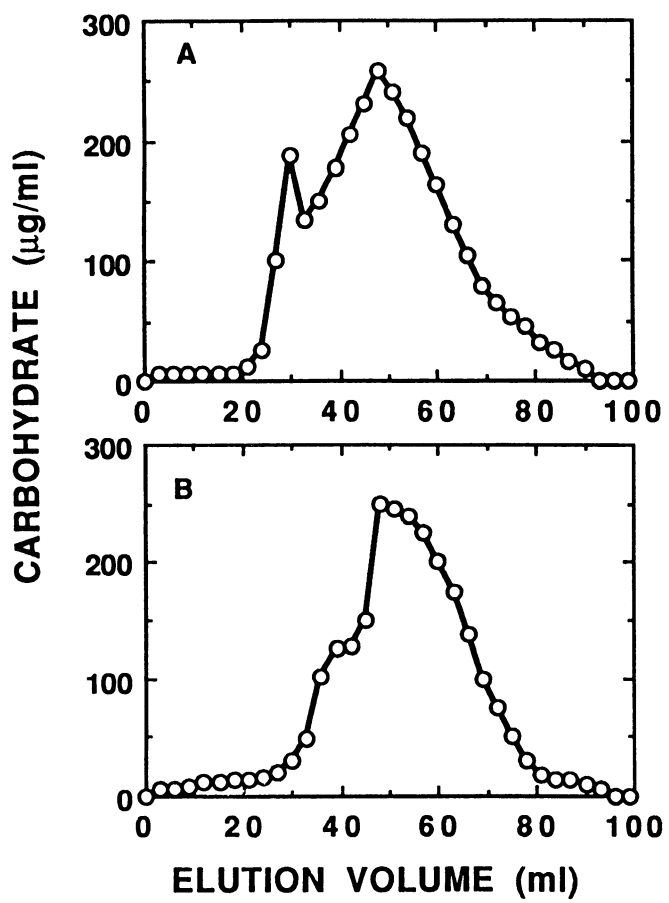

Fig. 1. Molecular size profiles of high-amylose starch (duplicate runs). **A,** Native sample. **B,** Extruded sample.

Figure 2 compares the native and three extruded samples of amylopectin-enriched starch. Carbohydrate levels in the tailing fractions of the main peak were expressed as a percentage of total carbohydrate recovered from all fractions. In the native sample, only 8% of the carbohydrate trailed the void-volume peak. The relative proportion of material in the lower molecular weight fractions varied with extrusion conditions; i.e., at 100°C and 200 rpm (sample XA2), 20% of the carbohydrate had lower molecular weight compared with 13% of the carbohydrate extruded at at 170°C (sample XA1). This pattern is consistent with that of corn meal, where the extent of fragmentation increased as extrusion temperatures are lowered (Wen et al 1990). This is attributable to increased viscosity of the material as extrusion temperatures are lowered.

Methylation analysis of purified starches. The methylation analysis procedure determines the extent of extrusion-induced changes in linkage profiles. This experiment was based on the hypothesis that extensive fragmentation of (1,4)-linkages would be reflected by significant increases in t-Glc and decreases in (1,4)-Glc. Molar percentages of partially methylated glucitol acetates were based on analyses conducted on 10 different dates. These data were corrected using the response factors of Sweet et al (1975) and then analyzed statistically.

The first sets of statistical tests were designed to determine whether data could be pooled. Differences among dates were tested at the $P = 0.05$ level. None were significant, except for t-Glc of amylopectin-enriched starch (samples UA, XA1, XA2, and XA3). With 4-Glc and 4,6-Glc, little day-to-day variability was observed. Next, the differences between the treatments were tested: the native and extruded high-amylose (samples UH vs. XH), and the native and extruded amylopectin-enriched starch (samples UA vs. XA1, XA2, and XA3). At the $P = 0.1$ level, there was no statistically significant difference in linkage patterns as the result of extrusion.

After the effect of dates was removed and the treatments of the high-amylose and amylopectin-enriched starches were compared, it was found that unextruded high-amylose starch (sample UH) contained statistically higher t-Glc levels. No significant differences were observed between treatments of amylopectin-enriched starch. (Table II)

Pooling all of these data, we found no significant differences in linkage distribution at the $P = 0.05$ level between native and extruded high-amylose samples (UH vs. XH) or between native

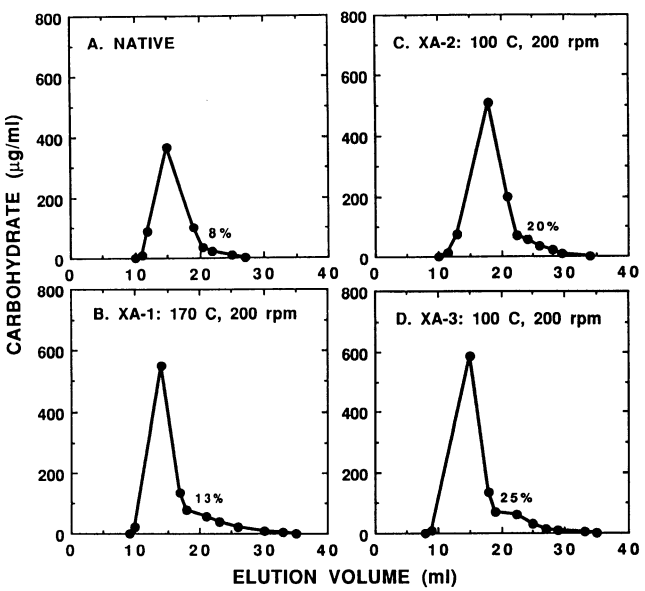

Fig. 2. Molecular size profiles of amylopectin-enriched starch (duplicate runs). **A,** Native sample. **B,** Sample XA1. **C,** Sample XA2. **D,** Sample XA3. Areas under the "tail regions" determined by extrapolating right-hand line of main peak to zero. Size of this area, relative to the total area of the chromatogram, used to calculate the proportional quantity of low molecular weight material.

TABLE II
Glycosidic Linkage Profiles (%) of Native and Extruded Starch Samples as Assessed by Methylation Analysis

	High-Amylose		Amylopectin-Enriched			
Linkage Type	UH[a]	XH[b]	UA[c]	XA1[d]	XA2[e]	XA3[f]
Terminal-Glc	3.0 ± 0.5	2.6 ± 0.5	9.9 ± 1.6	9.1 ± 1.8	8.7 ± 0.9	9.2 ± 0.9
4-Glc	91.9 ± 3.8	90.5 ± 3.8	83.1 ± 1.8	81.8 ± 2.6	84.1 ± 3.2	83.1 ± 4.1
4,6-Glc	5.05 ± 4.1	7.8 ± 4.5	7.4 ± 2.0	8.6 ± 2.5	7.2 ± 3.2	7.7 ± 3.7

[a] UH = native, unextruded sample, six replicates analyzed.
[b] XH = extruded sample, five replicates analyzed.
[c] UA = native, unextruded sample, 11 replicates analyzed.
[d] XA1 = extruded at 170°C, eight replicates analyzed.
[e] XA2 = extruded at 100°C, 200 rpm; 11 replicates analyzed.
[f] XA3 = extruded at 100°C, 50 rpm; 11 replicates analyzed.

and extruded amylopectin-enriched samples (UA vs. XA1, XA2, and XA3). Thus, while gel-permeation chromatography demonstrated that a small amount of fragmentation occurs during single-screw extrusion, methylation analysis was insufficiently sensitive in detecting which specific linkages were affected in these samples.

Fragmentation and Methylation Analysis of Corn Meal

For this part of the study, four of the corn meal samples (native, and samples 8, 12, and 15), as described in Wen et al (1990), that exhibited significant levels of starch fragmentation were analyzed. For all analyses, native corn meal served as a control.

Corn meal sample preparation and size fractionation. During initial attempts to conduct methylation analysis directly on native and extruded corn meal, we found that proteins and lipids induced undermethylation, and were, therefore, a source of variability. To circumvent this problem, starch was purified by a delipidation procedure consisting of extraction in $CHCl_3$-methanol, followed by solubilization of protein and starch in DMSO, and precipitation of the starch in 75% ethanol. Under these conditions, starch precipitates and zein remains soluble. Chromatograms showing the distribution of protein and carbohydrate before and after starch purification are shown in Figure 3. Although carbohydrate profiles are largely unchanged, the two protein peaks in the parent materials were completely removed by the purification procedure. The isolated starch was also analyzed by the bicinchoninic acid protein assay (Brown et al 1989), and tested negative for protein by this method. Recovery of purified starch ranged from 70 to 90%. The similarity of starch profiles, before and after the isolation procedure, indicated that starch molecular size was not adversely affected by this procedure. The ratio of high to low molecular weight polysaccharide (68:32) remained constant, indicating that neither was selectively removed during the isolation procedure.

Chromatograms of native corn meal and samples 8 and 15 are shown in Figure 3. The amount of fragmented starch present in each of the corn meal samples was significantly greater than that of the high-amylose and amylopectin-enriched starches; however, this was more likely caused by using a twin-screw instead of a single-screw extrusion process than by the presence of protein or lipid.

Methylation analysis of corn meal. Quantitative results of methylation analyses of corn meal are summarized in Table III. In starch from native corn meal, levels of 4-Glc, t-Glc, and 4,6-Glc, expressed as percentages of the total amount of Glc recovered, were 86.2, 9.9, and 4.0%, respectively. These percentages are in accordance with the known structural features of amylopectin (Manners 1989). No evidence for the formation of anhydro linkages or maltosan was found. In each of extruded samples, small decreases in 4,6-Glc (1–3%) were observed, suggesting that some cleavage might occur at branch points. A significant increase in t-Glc was observed in sample 12, indicative of the cleavage of α-(1,4)-linkages, and this was accompanied by corresponding increases in t-Glc (2–5%).

Overall, size fractionation techniques, such as gel-permeation chromatography, confirm that significant fragmentation of amylopectin is induced by twin-screw extrusion. However, the relatively small amount of t-Glc formed, as measured using methylation analysis, indicates that the observed size changes result from cleavage of a small percentage of the total number of linkages present in amylopectin.

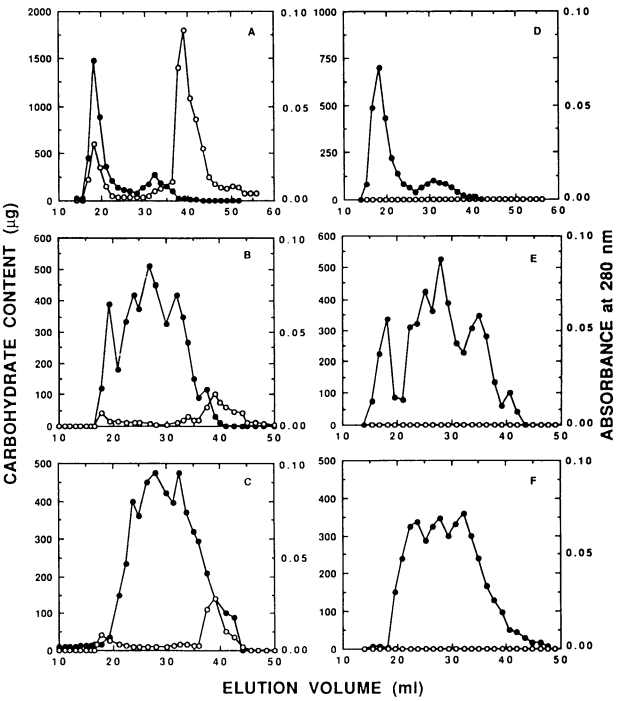

Fig. 3. Gel-permeation profiles demonstrating the purification of starch from corn meal. **A–C**, starting material. **D–F**, purified starch. Purification conducted on native sample (**A** and **D**); on sample 8 (**B** and **E**); and on sample 15 (**C** and **F**). ● = carbohydrate. ○ = protein.

TABLE III
Glycosidic Linkage Profiles (%) of Native and Extruded Corn Meal Samples as Assessed by Methylation Analysis

Linkage Type	Native[a]	Sample 8	Sample 12	Sample 15
Terminal Glc	9.7 ± 0.8	10.8 ± 1.7	14.0 ± 1.2	8.5 ± 2.5
4-Glc	83.5 ± 2.2	83.1 ± 2.9	83.5 ± 1.4	85.5 ± 1.7
4,6-Glc	6.7 ± 2.6	6.1 ± 3.1	2.6 ± 0.4	5.0 ± 1.2

[a] Number of replicates analyzed: Native, five; Sample 8 and Sample 15, five; Sample 12, three.

DISCUSSION

Fragmentation of starch is a well-documented consequence of the extrusion process. Amount of fragmentation is highly affected by the chemical nature of the extrudate, design and configuration of the extruder, and extruder operating conditions. Significant effort has been devoted to quantifying the contribution of each

of these parameters to the fragmentation process. Traditionally, the major technique used to assess the relative amount of fragmentation has been chromatography based on molecular size fractionation (Chinnaswamy and Hanna 1990, Wen et al 1990). Gel-permeation profiles in this article (Figs. 1–3), and in Wen et al (1990) and Wasserman and Timpa (1991), provide data showing size changes but do not provide any information that might hint as to which specific linkages may have been affected by the extrusion process. Furthermore, one cannot estimate the percentage of the total number of glycosyl linkages present in native starch that are cleaved.

In this study, methylation analysis was used to analyze the linkage content of extrudates prepared from three commercial starches using single- and twin-screw extruders. We hypothesized that extensive fragmentation of (1,4)-linkages would be reflected by significant increases in t-Glc and decreases in (1,4)-Glc. Extensive fragmentation at (1,6)-branch points would result in decreased levels of (1,6)-Glc and, perhaps, in increased levels of (1,4)-Glc. The occurrence of fragmentation was confirmed by gel-permeation chromatography (Figs. 1–3) and measured by methylation analysis. The major finding of this study was that statistically significant alterations in linkage distribution could not be measured because of the limitations in the sensitivity of the methylation analysis technique. No evidence for the formation of anhydro linkages or maltosan (Theander and Westerlund 1988) was observed.

The importance of these experiments is that only a small fraction of the total glycosyl bonds in these samples, perhaps on the order of 1 or 2%, were affected by extrusion. The lack of glucose or maltodextrin formation suggests that cleavage occurs in the internal regions of the amylopectin molecule, rather than adjacent to the reducing or nonreducing termini. We were unable to determine the exact location where cleavage occurred; however, these data are consistent with breakages that might occur in the intercluster regions of the amylopectin molecule (Manners 1989).

Part of the difficulty in the quantitative detection of extrusion-induced differences in linkage profiles may lie in the fact that methylation analysis is complex and involves many derivatization and clean-up steps. This introduces variability, as illustrated in Tables II and III, making it highly possible that small differences are obscured. It should also be noted that the amounts of t-Glc and 4,6-Glc are quite low relative to the amount of 4-Glc. This introduces another source of variability. Day-to-day differences of 4,6-Glc and t-Glc levels, measured in high-amylose starch and corn meal, disappeared once data from all of the analyses were pooled.

We conclude that cleavage of relatively few glycosidic bonds results in fragmentation detectable by size chromatographic techniques, but not by methylation analysis. While methylation analysis adequately predicts the linkage composition of high-amylose and amylopectin-enriched starches, its inherent variablity does not lend sufficient sensitivity to quantitative documentation of extrusion-induced changes of linkage profiles, at least under the conditions and with the material investigated here.

ACKNOWLEDGMENTS

Appreciation is expressed to Thomas Hartman and Robert Rosen of the Center for Advanced Food Technology, and to Kenneth C. Gross of the USDA Beltsville Agricultural Research Center for assistance with methylation analysis. We thank Pamela McPartland for secretarial assistance and Ai-Ping Shao for technical support. This research was funded by by the Center for Advanced Food Technology (CAFT) and the New Jersey Agricultural Experiment Station with State and Hatch Act funds. New Jersey Agricultural Experiment Station publication D-10544-5-92.

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[Received April 21, 1992. Accepted September 30, 1992.]

Genotype and Environment Effects on Tocols of Barley and Oats[1]

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ABSTRACT

Cereal Chem. 70(2):157-162

Grain of 12 oat and 30 barley genotypes, each from three locations, was analyzed for tocols (tocopherols and tocotrienols) by high-performance liquid chromatography with fluorescence detection. The objective was to assess the variation in levels of tocols among genotypes and locations. Significant genotype differences existed for most tocols in both species. Total tocol concentrations for genotypes ranged from 19 to 30 mg kg^{-1} for oats and from 42 to 80 mg kg^{-1} for barley. Location differences were significant for oats but not for barley. Only a small percentage of the variance was associated with the interaction of genotype and location. α-Tocotrienol and α-tocopherol were the predominant tocol isomers in both species; β- and γ-tocotrienol were also present in significant amounts in barley. The major isomers of barley, but not of oats, were generally positively correlated with each other. The data indicate the feasibility of attempting to increase tocol concentration in these crops by hybridization and selection. That could lead to food products that might lower serum low-density-lipoprotein cholesterol, a risk factor for cardiovascular disease.

Vitamin E activity results from the complex of tocols found in various foodstuffs. Tocols include tocopherols and tocotrienols, the difference being a saturated side-chain in tocopherols and a triunsaturated side-chain in tocotrienols. Each class of tocols consists of at least four isomers, differing in the number and position of methyl substituents on the benzene ring (Pennock et al 1964).

Many biological activities of vitamin E are believed to result from its antioxidant action, specifically the inhibition of lipid peroxidation in biological membranes (Burton and Traber 1990). Although α-tocopherol is considered to have the greatest biological activity (Taylor and Barnes 1981), recent evidence suggests that α-tocotrienol may have 40–60 times higher antioxidant activity (Serbinova et al 1991).

Recently, a cholesterol biosynthesis inhibitor identified as α-tocotrienol was found in barley (Qureshi et al 1986). Further research has shown that tocols from barley, oats, palm oil, and other sources lower the cholesterol levels in chickens, swine, and humans (Qureshi et al 1991a–c). γ-Tocotrienol and δ-tocotrienol appeared to be more effective than the more abundant α-tocotrienol (Weber et al 1990, Qureshi et al 1991c). Also, α-tocopherol and α-tocotrienol had opposite effects on cholesterol metabolism in chicks, indicating that a higher ratio of tocotrienols to tocopherols in the diet may be important in metabolic regulation (Qureshi et al 1989).

Cereal grains are rich sources of tocols. The quantities of tocols in various cereal grains (Barnes 1983, Cort et al 1983) and cereal products (Piironen et al 1986) have been reported. The lack of a standardized method for extraction and analysis until recently (AOCS 1989) has led to a wide variation in quantities of the various tocols reported, especially in the earlier literature. In barley, all eight tocol isomers have been detected; α-tocotrienol is highest in concentration, followed by γ-tocotrienol, α-tocopherol, and β-tocotrienol (Barnes 1983). In oats, α-tocotrienol is also highest, followed by α-tocopherol (Barnes 1983). Most reports on oats have identified only the α and β isomers, but Lásztity et al (1980) found quantities of all eight.

We have found no reports indicating that variation in tocol concentrations may exist among barley cultivars and only a single report for oats (Lásztity et al 1980). Nor have there been any reports indicating that variation may exist due to different growing conditions. One study reported effects of air and moisture on tocols of stored barley grain (Hakkarainen et al 1983).

As more research results on the benefits of tocols in the human diet become available, there will be an interest among plant breeders in developing new cultivars of cereals having higher levels of total tocols and more favorable ratios of the isomers, i.e., more γ- and δ-tocotrienols. For that reason, we have measured the concentration of tocol isomers in a number of barley cultivars and experimental lines and oat cultivars grown in diverse environments. Our objective was to determine the variation in concentrations of tocols among diverse genotypes, the effects of location, the presence or absence of a location-genotype interaction, and the relationships among the isomers.

MATERIALS AND METHODS

Samples

Barley samples grown in 1990 were obtained from the USDA Western Regional Spring Barley Nursery. The nursery contained 30 entries, consisting of advanced breeding selections of feed and malting barleys and check cultivars. Samples from three replications at each of three locations (Ontario, OR; Aberdeen, ID; and Fargo, ND) were chosen for analysis. Oat samples were 12 popular cultivars that were grown in replicated trials at a number of university experiment stations in 1989. As with barley, oat seed supplied to each location for planting was from a common lot. Seed lots from each of three replications from Carrington, ND; W. Lafayette, IN; and Ithaca, NY, were chosen for analysis.

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