NOTE

Sitostanyl Ferulate as an Indicator of Mechanical Damage to Corn Kernels

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Mechanical kernel damage is an important problem associated with the harvesting, handling, storing, and marketing of corn (Watson and Ramstad 1987). Generally, damaged corn deteriorates faster than corn with little or no damage (Seitz et al 1982, Tuite et al 1985). For research purposes, such as in studies of fungal invasion, it is sometimes necessary to document that the grain is not damaged, or, if it is damaged, to measure the degree of damage.

Determinations of mechanical damage in corn have been based mainly on visual inspections (sometimes with the aid of a microscope and/or staining with Fast Green FCF dye) and on colorimetric tests that measure dye absorbed on damaged portions of kernels (Chowdhury and Buchele 1976a,b; Chowdhury et al 1976). Various other methods have been tried, as summarized by Chowdhury and Buchele (1976b).

Seitz (1989) reported the identification of sitostanyl and campestanyl ferulates in corn, wheat, rye, and triticale grains. Analyses of dissected tissues from corn and wheat indicated that the ferulates were associated mostly with inner pericarp tissues. This, plus their good stability, suggested that an assay for the ferulates in surface extracts of whole kernels could be used to measure degree of pericarp damage. Reported here are results with corn that show the potential usefulness of such an assay.

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MATERIALS AND METHODS

Samples

Nondamaged dent corn samples were obtained by harvesting and shelling the grain by hand. Two yellow corn samples (yel-ds from Riley County, KS, and yel-rm from Rush County, KS) and one white corn sample from Riley County, KS, were used. The white and yellow corn samples were harvested in 1982 and 1983, respectively, and kept in excellent condition in a cold room at 4°C. All nondamaged samples were carefully inspected with the aid of a dissecting microscope to exclude kernels with visual damage (Table I).

Dissected fractions were obtained as described by Seitz (1989). The separation between outer and inner pericarp apparently occurred at the cross cells such that the outer pericarp consisted mostly of epidermal and mesocarp cells (Wolf et al 1952). The inner pericarp fraction consisted of some tube cells, the seed coat, and, probably, most of the aleurone layer. Samples with specific types of damage were prepared as described in Table II by using a dissecting needle, a small abrasive wheel, and a Wisconsin breakage tester (WBT, Watson and Herum 1986). Each kernel of each sample (50 g) was damaged in the prescribed manner.

The corn hybrid Bojac X603 (Table III) was grown near Wamego, KS, in 1986. Some of the corn was harvested and shelled by hand, then stored at ambient temperatures in the laboratory. Two other samples with slightly different degrees of mechanical

damage (Table III) were from different lots collected during combine harvesting. The hand-shelled sample was inadvertently damaged by insects (type unknown) during storage.

Analytical

Surface extractions of damaged and nondamaged whole corn were conducted as follows: 50 g of corn was wrapped in cheesecloth and steeped in 150 ml of chloroform or hexanes (mixture of isomers) for 1 min at ambient temperature. The sample was moved up and down in the solvent three or four times during the steeping period. The extract was filtered through coarse paper, evaporated to about 5 ml, transferred to a 2-dram vial, and evaporated to dryness. All evaporations were done with the aid of nitrogen flow into the flask or vial. The residue was redissolved in 0.5 ml of chloroform and filtered (0.45 μm) into a sample vial (with aluminum seal) for direct analysis by high-performance liquid chromatography (HPLC). The equipment and general methodology for the HPLC analyses were as described previously (Seitz 1989). Sample injection volume was 10 μl, the column was C-18 (Dupont Co.) maintained at 45°C, and mobile phase was methanol at 1.2 ml/min. The campestanyl and sitostanyl ferulates, which were eluted at 8.6 and 9.2 min, respectively, were detected by monitoring ultraviolet light in an 8-nm band width centered at 325 nm. Spectra were recorded to confirm that ferulates were in fact being measured. Quantitative calibrations were obtained by injecting known concentrations of sitostanyl ferulate, the predominant ferulate (Seitz 1989). Therefore, only concentrations of sitostanyl ferulate are reported in this paper.

Dissected fractions (1–4 g) were homogenized for several minutes in chloroform (1–2 ml) by using a Virtis model 45 homogenizer with a "micro ultra shear" assembly. The mixture was filtered, the filtrate evaporated, and the residue redissolved and analyzed as described above.

TABLE I
Sitostanyl Ferulate Extracted from Dissected Fractions of Yellow Corn[a] with Chloroform

Sample	Sitostanyl Ferulate (μg/g)
Nondamaged whole kernels	ND[b]
Whole kernels less outer pericarp	64
Outer pericarp[c]	22
Inner pericarp[c]	270
Endosperm[c]	2
Germ[c]	14

[a]Same corn identified as yel-ds in Table II.
[b]Not detected.
[c]Homogenized in chloroform.

TABLE II
Sitostanyl Ferulate (μg/g) Extracted from Surfaces of Corn Kernels with Chloroform

	Sample		
Type of Damage	yel-ds	yel-rm	White
None	0.005[a]	ND[a]	0.005[a]
Five punctures (back)[b]	···	0.78	0.40
Five punctures (germ)[c]	···	0.11	0.09
Five scratches (back and top)[d]	4.13	···	···
Germ scraped[e]	0.80	···	···
Tip broken[f]	···	0.48	0.27
WBT, >50% of each kernel intact[g]	2.70	···	···
WBT, <50% of each kernel intact[g]	4.90	···	···
WBT, through 5.84 mm, over 4.76 mm[g]	8.00	···	···
WBT, through 4.76 mm[g]	18.30	···	···

[a]Near the detection limit; ND = not detected.
[b]Small punctures were made in the side opposite the germ with a sharp dissecting needle.
[c]A sharp dissecting needle was used to inflict minor punctures in the pericarp over the germ with little or no penetration into the germ.
[d]An abrasive cutoff wheel (Dremel no. 409) was used to make four scratches (3–4 mm × 1 mm) on the side opposite the germ and one across the top (dent end) of the kernel. Most of the scratches were deep enough to expose endosperm.
[e]Pericarp over the germ was mostly removed by using the cutoff wheel described above.
[f]Tweezers were used to break the tip and expose the black layer.
[g]Hand selected and sieved fractions after damage by a Wisconsin breakage tester (WBT); 4.76 mm = 12/64 in. and 5.84 mm = 15/64 in.

RESULTS AND DISCUSSION

Results in Tables I, II, and III represent a single observation. Results from analyses of dissected fractions clearly showed that sitostanyl ferulate was associated mostly with inner pericarp (Table I). Some sitostanyl ferulate was in the outer pericarp fraction and was probably related to the difficulty encountered in separating the fractions cleanly (Seitz 1989). Nondamaged whole kernels yielded little or no sitostanyl ferulate (Tables I and II), which is consistent with the absence of sitostanyl ferulate in the outer pericarp (or at least the outer part of the outer pericarp). When the inner pericarp was exposed by peeling off the outer pericarp, the sitostanyl ferulate became readily extractable (Table I). All fractions containing sitostanyl ferulate also contained lower concentrations of campestanyl ferulate (Seitz 1989).

Very slight pericarp damage, such as small punctures over the germ or on the side opposite the germ (backside) was detected by an assay for sitostanyl ferulate in an extract of kernel surfaces (Table II). Punctures on the backside of the kernel yielded more extractable sitostanyl ferulate than punctures over the germ. Broken tips also yielded extractable sitostanyl ferulate. Kernels with pericarp scraped away from the germ yielded extractable sitostanyl ferulate, apparently due to exposed edges of inner pericarp around the germ. As expected, corn with relatively severe scratches in the pericarp and corn damaged by the WBT yielded high levels of sitostanyl ferulate (Table II). Also, with the fractions from the WBT, ferulate level increased as severity of damage increased. The least amount of sitostanyl ferulate was extracted from the fraction with more than half of each kernel intact, whereas the most was extracted from the fraction that passed through the 4.76-mm (12/64-in.) sieve.

Damage from combine harvesting yielded nearly as much sitostanyl ferulate as the sample of hand-harvested corn damaged by the WBT and having >50% of each kernel intact (Tables II and III). Corn with a relatively low percentage of kernels damaged by insects released sitostanyl ferulate at a level that was easily detected (Table III). The hexanes solvent was slightly less effective than chloroform for extracting the ferulates from the surfaces of damaged kernels (Table III).

At present, this method will probably be most useful as a research tool. It could also be used in conjunction with other methods, i.e., visual inspection, dye staining, sieving, etc. Its best application might be to verify that a sample has little or no damage. With further simplification and development, it may have potential for routine assessment of damage in commercial samples.

TABLE III
Effect of Solvent, Insect Damage, and Combine Damage on Sitostanyl Ferulate (μg/g) Extracted from Surfaces of Corn Kernels (Bojac X603)

	Solvent	
Sample	Chloroform	Hexanes
Hand shelled with insect damage (2.6%)[a]	0.32	0.17
Combine damage (9.8%)[b]	1.67	1.34
Combine damage (12.5%)[b]	2.06	1.58

[a]Percent by weight having slight visible insect damage to various parts of the kernel surface.
[b]Broken corn and foreign material was removed with a 4.76-mm (12/64-in.) sieve; percent by weight having various types and degrees of damage visible without magnification or staining.

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