

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 μ m) 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 μ l, the column was C-18 (Dupont Co.) maintained at 45°C, and mobile phase was methanol at 1.2 ml/min. The campestanol 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.

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 campestanol 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 I
Sitostanyl Ferulate Extracted from Dissected Fractions of Yellow Corn^a with Chloroform

Sample	Sitostanyl Ferulate (μ g/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

^aSame corn identified as yel-ds in Table II.

^bNot detected.

^cHomogenized in chloroform.

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

Type of Damage	Sample		
	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

^aNear the detection limit; ND = not detected.

^bSmall punctures were made in the side opposite the germ with a sharp dissecting needle.

^cA sharp dissecting needle was used to inflict minor punctures in the pericarp over the germ with little or no penetration into the germ.

^dAn abrasive cutoff wheel (Dremel no. 409) was used to make four scratches (3–4 mm \times 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.

^ePericarp over the germ was mostly removed by using the cutoff wheel described above.

^fTweezers were used to break the tip and expose the black layer.

^gHand 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.

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

Sample	Solvent	
	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

^aPercent by weight having slight visible insect damage to various parts of the kernel surface.

^bBroken 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.

