1982). During cooking of very aromatic rice varieties, even more 2-acetyl-1-pyrroline is formed (Buttery et al., 1986) than in the crust of the wheat breads. The reason for the high concentration of this pyrroline derivative in wheat in comparison to rye bread is yet to be clarified. According to Tressl et al. (1985), it originates from a reaction of proline with monosaccharides.

In rye bread, on the other hand, the pyrazines predominated. However, the 2-acetyl-1-pyrroline may still play a role in the crust flavor of these breads because of its low odor threshold (see above) compared to those of the pyrazines: I = 62 ppb (Teranishi et al., 1975); II = 30 ppb (Fors, 1985); III, between 15 and 25 ppb (Schieberle and Grosch, unpublished results).

The results also indicate that a change in the sourdough process influences the concentrations of the four flavor compounds in the crusts of rye bread. Approximately twice as much pyrazine and also much more pyrroline were formed, when the sourdough was prepared in a three-stage process (Table IV). Obviously the longer fermentation time during this process leads to an increase in the precursors from which these flavor compounds are formed.

This study also shows that differences in the concentrations of important flavor compounds caused by changes in food processing can be accurately quantified by an isotope dilution assay.

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Registry No. I, 22047-25-2; I-*d*, 106162-18-9; II, 15707-23-0; II-*d*, 106191-40-6; III, 65128-99-6; III-*d*, 106191-41-7; IV, 85213-22-5; IV-*d*, 106191-42-8.

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Fractionation and HPLC Determination of Grape Phenolics

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An improved analytical method was developed for the determination of phenolic compounds in grapes. Phenolic compounds were fractionated into acidic and neutral groups by passing deproteinated grape juice through a preconditioned C18 SEP-PAK cartridge, and then the fractions were sequentially injected into a HPLC column. The separation was made on a C18 Radial-PAK column using 5% acetic acid for acidic phenolics and 40% acetonitrile for neutral phenolics. This fractionation technique showed a high recovery and resolution, and it was effective in quantitation of major phenolic compounds in grapes.

Hydroxycinnamic acid tartrates and catechins and procyanidins are important phenolic compounds in grapes and wine. They contribute to the sensory quality and browning of the products (Singleton and Esau, 1969; Lea et al., 1979). One of the major problems involved in separation of these phenolic compounds is their similarity in chemical characteristics. Many traditional separation techniques such as paper, thin-layer, and column chromatography are being replaced by high-performance liquid chromatography (HPLC). Recently, HPLC using reversed-phase columns has been used to separate the various hydroxycinnamic acid tartrates and procyanidins in grapes and wines (Lea et al., 1979; Nagel et al., 1979; Ong and Nagel, 1978; Wulf and Nagel, 1976). However, the quantitation of phenolic compounds by HPLC has not yet been perfected, because many phenolics show similar ultraviolet absorption spectra with maxima in a narrow range

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of 280–320 nm (Ong and Nagel, 1978; Singleton and Trousdale, 1983; Steck, 1967). In order to prevent interferences among phenolic compounds, Salagoity-Auguste and Bertrand (1984) fractionated the phenolic compounds of wine into neutral and acidic groups before injection into the HPLC column. However, their extraction efficiency for procyanidins was very low. The main objective of the present study was to develop a simple and reliable fractionation method for phenolic compounds using C18 SEP-PAK cartridges followed by HPLC.

MATERIALS AND METHODS

Sample Preparation. Grapes used in this study were Niagara harvested in 1985 from vineyards of the New York State Agricultural Experiment Station. To prevent browning during extraction of phenolics, 1 g of ascorbic acid was added to 100 g of fresh or frozen grape berries. The fruits were then quickly crushed in a Waring blender for 1 min for white grapes and 2 min for red grapes to homogenize the flesh and skins, but at low speed so as not to crush the seeds, and then pressed uniformly in a ricer through cheesecloth to remove all liquid. To 10 mL of juice was added 20 mL of 100% ethyl alcohol, and the resultant mixture was left at room temperature for 1 h and then filtered through Whatman No. 4 filter paper to remove protein. Ethanol was removed from the filtrate with a rotary evaporator, and finally the deproteinated sample was diluted to the original volume of 10 mL.

Fractionation of Phenolic Compounds. *Preconditioning of C18 SEP-PAK Cartridges.* The cartridges, obtained from Waters Associates, were preconditioned for neutral phenolics by sequentially passing 2 mL dropwise of methanol and distilled water. For acidic phenolics, SEP-PAKs were preconditioned by passing 2 mL of 0.01 N HCl instead of distilled water.

Fractionation. Five milliliters of the deproteinated juice sample was adjusted to pH 7.0 with NaOH and passed through the preconditioned neutral SEP-PAK to absorb the neutral phenolic compounds. The effluent portion was then adjusted to pH 2.5 with HCl and passed through the second acidic SEP-PAK to absorb acidic phenolics. The absorbed fractions were eluted with methanol from their respective cartridges, and the first 2 mL was collected for the HPLC analysis.

Standards. Catechin and epicatechin were purchased from Sigma Chemical Co. Procyanidin B2 was furnished by Dr. A. Lea, Long Ashton Research Station, University of Bristol, England. Procyanidin B3 was synthesized and purified in this laboratory by a method of Delcour et al. (1983). Esters of caffeoyl tartaric acid, *cis-p*-coumaroyl tartaric acid, and *trans-p*-coumaroyl tartaric acid were isolated and purified in this laboratory according to the method of Singleton et al. (1978).

HPLC Analysis. A high-pressure liquid chromatography apparatus (Hewlett-Packard, Model 1090M) equipped with a diode array detector and 98580A series 300 computer was used. Separation of phenolics was carried out on an 8 mm × 10 cm C18 Radial-PAK column (Waters) at room temperature. The acidic phenolic compounds were eluted with 5% acetic acid in water, and the neutral phenolics were separated with a linear solvent system of 40% acetonitrile in water from 0 to 100%, at a flow rate of 1 mL/min.

RESULTS AND DISCUSSION

Typical chromatographs of acidic and neutral phenolics of Niagara grapes are shown in Figures 1 and 2, respectively. Each compound was identified by its retention time and/or by spiking with the standards under the same conditions. Analysis of the acidic fraction was completed within 30 min, and that of the neutral fraction required 70 min, including column equlibration time. All major phenolic compounds were separated with good resolution due to the effective fractionation technique. Some portions of anthocyanin pigments were excluded during fractionation with SEP-PAKs, and the residual pigments in the sample injected into the HPLC column were eluted later (longer retention time) and did not interfere with the analysis. The phenolic content of Niagara grape juice and the recovery of phenolic compounds added to the juice before fractionation are shown in Table I. Reproducibility of seven phenolics in the juice was very good with a low standard deviation. The overall recovery of phenolics added to the juice was remarkably high; over 90% recovery was obtained for acidic phenolics and for catechin and epicatechin, which are common phenolic constituents in grapes. The recoveries of procyanidins B2 and B3 were 104% and 118%, respectively. It is a simple fractionation method and easy to use. Since this technique improved

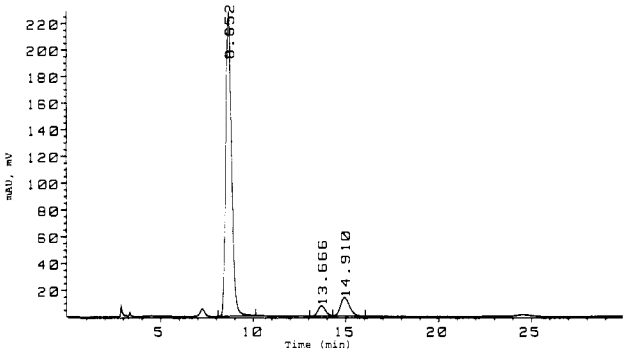

Figure 1. High-performance liquid chromatogram of phenolic compounds in Niagara grapes (acidic fraction). Retention times: 8.6, *trans*-caffeoyl tartrate; 13.6, *cis-p*-coumaroyl tartrate; 14.9, *trans-p*-coumaroyl tartrate.

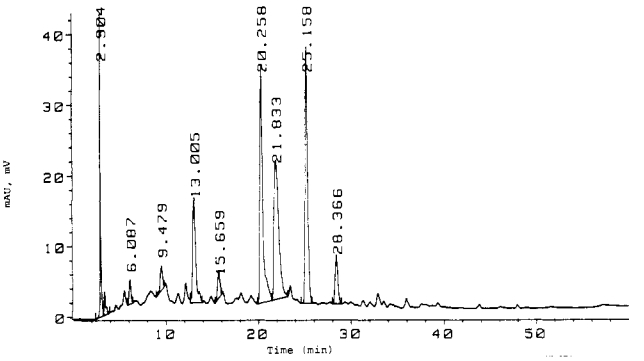

Figure 2. High-performance liquid chromatogram of phenolic compounds in Niagara grapes (neutral fraction). Retention times: 9.4, procyanidin B3; 13.0, catechin; 15.6, procyanidin B2; 20.2, epicatechin.

Table I. Phenolic Content in Niagara Grapes and Recovery of Phenolic Compounds Added to the Juice

phenolic compd	concentration, μg/mL			
	original	added	found	rec, %
trans-caffeoyl tartrate	213.8 ± 14.6	62.8	270.6 ± 7.3	90.4 ± 11.3
cis-p-coumaroyl tartrate	19.6 ± 3.6	15.2	34.4 ± 2.3	97.3 ± 15.1
trans-p-coumaroyl tartrate	44.3 ± 0.5	28.2	74.3 ± 8.2	106.3 ± 19.0
catechin	46.8 ± 4.0	29.9	74.4 ± 3.3	92.3 ± 12.1
epicatechin	71.3 ± 2.2	39.9	107.8 ± 6.5	91.4 ± 16.4
procyanidin B2	18.3 ± 1.2	40.4	60.4 ± 4.2	104.2 ± 10.5
procyanidin B3	27.5 ± 6.3	40.4	75.2 ± 1.3	118.0 ± 3.4

the resolution of many phenolic peaks on the HPLC column, it facilitates the collection of unknown peaks individually, which makes possible their analysis and identification.

Registry No. *trans*-Caffeoyl tartrate, 67879-58-7; *cis*-*p*-coumaroyl tartrate, 67920-37-0; *trans*-*p*-coumaroyl tartrate, 27174-07-8; catechin, 154-23-4; epicatechin, 490-46-0; procyanidin B2, 29106-49-8; procyanidin B3, 23567-23-9.

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Detoxification of Deoxynivalenol with Sodium Bisulfite and Evaluation of the Effects When Pure Mycotoxin or Contaminated Corn Was Treated and Given to Pigs[1]

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Deoxynivalenol- (DON-) contaminated corn was treated with aqueous sodium bisulfite in order to (a) determine optimum conditions for reduction in free DON levels and (b) evaluate in a feeding trial the effects of the bisulfite treatment on feed intake and weight gains in pigs. The greatest reductions (up to 95% DON) were achieved when the contaminated corn was autoclaved for 1 h at 121 °C in the presence of 8.33% aqueous sodium bisulfite (600 mL/kg of corn, by weight). In the feeding trial, a diet containing 7.2 mg of DON/kg from infected corn caused reduction in feed consumption and weight gains by pigs. When the infected corn was autoclaved with sodium bisulfite, mixed with a basal diet, and fed to pigs for 7 days, feed intake and body weight gain were improved compared with pigs fed untreated inoculated corn and were similar to the cases of the controls. In an additional toxicological trial using pure compounds, no effects were seen when DON sulfonate was administered orally to swine at the same level (molar equivalent) at which nonderivatized DON caused severe emesis.

Cereals contaminated with the fungus *Fusarium graminearum* Schwabe may contain variable amounts of the mycotoxin deoxynivalenol (DON, vomitoxin, 3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one). This mycotoxin is known to affect the feed intake and weight gains of pigs (Friend et al., 1983). However, toxicity studies with DON in swine indicate that pigs may tolerate up to 2 mg of DON/kg before these effects occur (Trenholm et al., 1984).

Previous studies (Young, 1986a,b; Young et al., 1986) have shown that pure DON in aqueous solution or DON in contaminated wheat or corn reacted readily with sodium bisulfite. The product is the 10-sulfonate adduct (DON-S), which is stable in acid but hydrolyzes to DON under alkaline conditions (Young, 1986b).

The present study was done to determine optimum conditions for sodium bisulfite reduction of DON levels in contaminated corn. In addition, as an initial approach to determine whether DON-S is toxic to animals, pure DON-S was given orally and bisulfite-treated contaminated corn was fed to pigs.

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MATERIALS AND METHODS

Materials. DON was prepared biosynthetically from liquid cultures of *Fusarium culmorum* (CMI 14764) (Greenhalgh et al., 1986), and DON-S was prepared as per Young (1986b).

Corn. Two sources of corn were used. One was field corn that had been artificially inoculated with *F. graminearum* strain M69 (Miller et al., 1983) during the growing period. It was harvested, ground, and stored frozen (−18 °C) until treated with sodium bisulfite under a variety of conditions. The other source of corn, used for the feeding study, was inoculated (*F. graminearum* DAOM 180378) and after harvest was stored as the cob in a corn crib. It was ground (3-mm screen) and treated immediately before the preparation of diets fed to pigs. The latter source of corn was chosen because the response of pigs to a diet containing the same inoculated corn appeared similar to that observed with a diet containing the same amount of pure DON (Foster et al., 1986). Consequently, interference by other toxic metabolites was probably low.

Analysis of Deoxynivalenol. DON in corn was extracted by the method of Trenholm et al. (1985) and analyzed by high-performance liquid chromatography (Young, 1986b). DON in diet samples was extracted and analyzed by the method of Trenholm et al. (1985).

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