utilization, it is necessary to optimize the fermentation process itself (which represents, for sweet potato, a problem due to viscosity of resulting slurries) and to estimate the value of resulting protein-rich byproducts.

Addition of pectinases to sweet potatoes benefits economic recovery of ethanol from varieties of normal and relatively high dry-matter contents. Pectinase also increases the solids content of wet filter cake for all sweet potato varieties and decreases drying costs of wet filter cake since less water needs to be removed for a fixed amount of dry matter. In addition, pectinase increases crude protein contents of sweet potato filter cake and decreases amounts of centrifuged solids to such a degree that centrifugation can be eliminated. Of the varieties examined, HiDry sweet potato, having very high dry-matter content, appears most desirable for ethanol fermentation, since less fresh sweet potato is needed for a fixed amount of ethanol production.

Sweet potato filter cake may have food potential, since it has high crude protein content and an amino acid balance superior to that of cereal grains. The relatively low fat content of sweet potato filter cake, compared with that of corn distillers' grains, may improve storage stability.

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LITERATURE CITED

American Association of Cereal Chemists *Approved Methods*, 8th ed.; AACC: St. Paul, MN, 1983.
Azhar, A.; Hamdy, M. K. *Biotech. Bioeng.* **1981**, *23*, 1297–1307.
Cavins, J. F.; Friedman, M. *Cereal Chem.* **1968**, *45*, 172–176.
Chua, J. W.; Fukui, N.; Wakabayashi, Y.; Yoshida, T.; Taguchi, H. *J. Ferment. Technol.* **1984**, *62*, 123–130.
Garcia, W. J.; Wolf, M. J. *Cereal Chem.* **1972**, *49*, 298–306.
Hamilton, M.; Jones, A., unpublished results, 1984.
Jones, A.; Hamilton, M. G.; Dukes, P. D. Proceedings of the Third Annual Solar Biomass Workshop, Atlanta, GA, April 26–28, 1983; pp 195–198.
Matsuoka, H.; Koba, Y.; Ueda, S. *J. Ferment. Technol.* **1982**, *60*, 599–602.
McQueen, R. E.; Nicholson, J. W. G. *J. Assoc. Off. Anal. Chem.* **1979**, *62*, 676–680.
Moore, S. *J. Biol. Chem.* **1963**, *238*, 235–237.
Morris, C. E. *Food Eng.* **1983**, *55*(6), 107–112.
Picha, D. H. *J. Food Sci.* **1986**, *51*, 239–240.
Purcell, A. E.; Walter, W. M., Jr. *J. Agric. Food Chem.* **1982**, *30*, 443–444.
Wall, J. S.; Bothast, R. J.; Lagoda, A. A.; Sexson, K. R.; Wu, Y. V. *J. Agric. Food Chem.* **1983**, *31*, 770–775.
Walter, W. M., Jr.; Hoover, M. W. *J. Food Sci.* **1984**, *49*, 1258–1261.
Walter, W. M., Jr.; Catignani, G. L.; Yow, L. L.; Porter, D. H. *J. Agric. Food Chem.* **1983**, *31*, 947–949.
Wu, Y. V. *Cereal Chem.* **1986**, *63*, 142–145.
Wu, Y. V.; Sexson, K. R. *Cereal Chem.* **1984**, *61*, 388–391.
Wu, Y. V.; Sexson, K. R.; Lagoda, A. A. *Cereal Chem.* **1984**, *61*, 423–427.

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Mass Spectral Characterization of a Halogenated Azobenzene (3,3′-Dichloroazobenzene) from Potato Peels

Brian L. Worobey,* John C. Pilon, and Wing-Fung Sun

Peels from three potato samples were analyzed for the presence of 3,3′-dichloroazobenzene (3,3′-DCAB, 3,3′-dichlorodiphenyldiazene). Peels were removed, extracted with methanol, and partitioned against methylene chloride/water, and the methylene chloride was exchanged for 2,2,4-trimethylpentane (TMP). The TMP was screened for the presence of 3,3′-DCAB by capillary GC–EC, and a peak was identified in all samples as cochromatographing with a synthesized reference standard of 3,3′-DCAB. 3,3′-DCAB residue levels ranged between 2.1 and 3.9 ppb in potato peels as determined by selective ion monitoring (SIM) by GC–MS of ions at m/z 250, 252, and 254. GC–MS total ion monitoring (TIM) (full scans m/z 25–310) of the peel extracts confirmed the presence of 3,3′-DCAB. The potential sources and toxicological concerns of 3,3′-DCAB in potatoes are discussed.

Substituted azobenzene compounds, especially 3,3′,4,4′-tetrachloroazobenzene (TCAB), have been identified as genotoxins in various organisms (Worobey, 1984, references therein). These compounds may form by condensation of substituted anilines, which are formed by hydrolysis of their parent pesticide(s). Several pesticides contain halogenated anilines as part of their structure, e.g. phenylureas, phenylcarbamates, and acylanilides. Substituted azobenzenes may also enter the environment, and hence foods, by direct application as contaminants of the formulation. Levels of TCAB have been reported as high as 2900 ppm in formulations of propanil (Bunce et al., 1979).

Chlorinated azobenzenes such as TCAB and its azoxy analogue have previously been shown to translocate into plants (Worobey, 1984; Freitag et al., 1984; Still, 1969). Halogenated anilines may also translocate into plants as shown for 3-chloroaniline (3-CA) and 3,4-dichloroaniline (3,4-DCA) in rice plants (Still et al., 1981).

Chlorpropham [CIPC, isopropyl (3-chlorophenyl)carbamate] is used as a plant growth regulator to inhibit

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potato sprouting during storage and is applied postharvest before sprouting has initiated. CIPC may also be applied as a spray on table stock potatoes prior to packaging. Canadian Food and Drug Regulations permit its use on potatoes such that a maximum residue limit of 15.0 ppm is not exceeded after completion of the storage period.

Potato residues of CIPC may be metabolized to 3-CA and 3,3′-DCAB (3,3′-dichloroazobenzene), and formulations could potentially contain contaminants such as 3-CA or 3,3′-DCAB. Because of the potential toxicological concerns for halogenated azobenzenes, several market potatoes were analyzed for residues of 3,3′-DCAB. Only peel tissues were analyzed since previous studies had shown that most CIPC residues remain in the outer layers of treated potato tubers (Coxon and Filmer, 1985, and references therein). Emphasis was placed upon mass spectral identification and estimation of suspected residues for the purpose of establishing their presence.

EXPERIMENTAL SECTION

Extraction of Potatoes. Five samples of potatoes were purchased from local stores. Potatoes were washed thoroughly under tap water and blotted dry. Peels were removed (approximately 1-mm thickness) with a household kitchen potato peeler and 20.0 g was weighed for analysis.

A 50-mL aliquot of methanol was added to a beaker containing the peels, and this was blended in a Polytron blender for 4 min. The mascerate was filtered through a layer of Celite 545 on a coarse sintered-glass filter under vacuum. The blender and filter were rinsed with 2 × 10 mL of methanol, and the combined methanol extract was filtered through anhydrous sodium sulfate into a 100-mL round-bottom flask. The sulfate was rinsed with 2 × 10 mL of methanol, and the extract was evaporated on a rotory evaporator under vacuum in a water bath at 40 °C to approximately 1 mL. This was transferred with methanol washes to a 15-mL graduated centrifuge tube, and the final volume was adjusted to 5 mL with methanol for a final concentration of 4 g/mL. This extract was found to contain several interferences by GC–EC analysis, such that further cleanup was necessary.

Cleanup of Methanol Extract. The 5 mL of methanol extract was added to a 60-mL separatory funnel wtih 2 × 5 mL of methanol rinses, and to this was added 10 mL of saturated sodium chloride and 20 mL of methylene chloride. After the separatory funnel was shaken, the methylene chloride layer was removed and the methanol reextracted with another 20 mL of methylene chloride; the methylene chloride was washed three times with 5 mL of saturated sodium chloride and the aqueous layer discarded.

One milliliter of TMP (2,2,4-trimethylpentane) was added and the methylene chloride evaporated on a rotory evaporator under vacuum, in a water bath at 40 °C, to appproximately 1 mL. The TMP was transferred to a 15-mL graduated centrifuge tube and the round-bottom flask rinsed with 3 × 1 mL of TMP; the final volume was adjusted to 5 mL with TMP for gas chromatography–electron capture (GC–EC) and gas chromatography–mass spectrometry (GC–MS) (SIM, selective ion monitor) analysis. For full-spectra scans by GC–MS (TIM, total ion monitor) 4 mL (of 5 mL) was concentrated to 1 mL under nitrogen and injected. All solvents were "distilled in glass" quality from Caledon Chemical Co., Georgetown, Ontario, Canada.

Synthesis of 3,3′-DCAB. 3,3′-DCAB was synthesized by adaptation of a procedure by Lee et al. (1977) for synthesis of TCAB from 3,4-DCA. A 1-mL aliquot of 7.97 mg/mL (in hexane) 3-CA (Aldrich Chemical Co., Milwaukee, WI) was transferred to a 5-mL Reacti-vial (Pierce Chemical Co., Rockford, IL), the hexane was evaporated to dryness under nitrogen, 25 mg of cuprous chloride (CuCl) and 1.5 mL of pyridine (dry) were added, and an air condenser was attached. This was placed in an oil bath at 60 °C for 16 h with constant stirring on a magnetic stirrer hot plate and shielded from light by aluminum foil. After cooling to room temperature, the solution was evaporated to dryness under a stream of nitrogen in the fume hood. The residue was transferred to a 30-mL separatory funnel with 20 mL of distilled water, acidified to pH 1.3 with HCl, and extracted 2× with 5 mL of hexane/benzene (7/3, v/v). The extract was evaporated under nitrogen to 0.5 mL. The aqueous portion was evaporated to dryness and the reaction repeated as above.

The reaction products were streaked on a 20 × 20 cm (0.25-mm thick) precoated silica gel G-25 thin-layer plate. 3-CA and 3,3′-DCAB were spotted as standards on each side of the streaked sample area, and the plate was developed in hexane/ethyl acetate (98/2, v/v) for 16.3 cm. Plates were sprayed with a 0.01% fluorescein (Aldrich) solution in ethanol, dried, and visualized under near- and far-UV lamps. 3-CA standard chromatographed at R_f 0.07; the 3,3′-DCAB streak at 0.61 was removed, and the silica was eluted with ethanol. The silica was filtered through a sintered-glass funnel under vacuum and evaporated to dryness under nitrogen (in the fume hood) for analysis. The yield of 3,3′-DCAB was approximately 26% and the purity, as determined by GC–EC and direct-inlet probe–MS (DIP–MS), was greater than 98% [mp 101 °C (lit. mp 101–102 °C, UV λ_{max} 316 nm (lit. λ_{max} 317 nm); Linke and Pramer, 1969a,b].

Quantitation. Quantitation was performed by an external standard method. Pure solvent was injected between sample extract injections on GC–MS and GC–EC to assure absence of column or syringe carry-over. A separate syringe was used for standards and samples to prevent elution of adsorbed standard into the sample injected; syringes were rinsed with sample and standard solvents and heated in a Hamilton syringe cleaner (300 °C) for 5 min prior to use. 3,3′-DCAB was prepared as a 1 mg/mL (weighing was done on a Cahn Model 26 microbalance, 0.1 μg ± 0.005%; Cahn Instruments, Cerritos, CA) standard in hexane, and dilutions were made in hexane for GC–MS and GC–EC analysis.

Mass Spectrometry. Identification of 3,3′-DCAB was achieved by mass spectrometric analysis performed on a VG ZAB-2F mass spectrometer (VG Analytical Ltd., Altrincham, Cheshire, England) interfaced to a PDP-8a data system and a Varian 3700 GC (Varian Canada Ltd., Georgetown, Ontario, Canada); SIM and DIP–MS analyses were performed on the same instrument. Total ion monitor (TIM) peaks were analyzed via GC–MS. Ion source operating temperature was maintained at 250 °C with 70-eV electron energy during electron-impact (EI) ionization. Low-resolution 1500 (10% valley) mass spectra were collected at a 1 s/decade rate and were normalized after background subtraction.

Gas Chromatography. GC–MS conditions were as follows: column A, 15-m length × 0.259-mm i.d., DB-210 (0.5-μm film thickness), programmed column temperature 50 °C (1-min hold) to 140 °C at 10 °C/min; column B, 30-m length × 0.250-mm i.d., DB-5 (0.25-μm film thickness), column temperature 80 °C (1-min hold) to 200 °C at 10 °C/min; injector temperature 200 °C, transfer lines 160 °C, carrier gas helium at 6 psi (column A) and 18 psi (column B), splitless injection mode. GC–EC analysis was performed on a Varian 3700 GC with a ^{63}Ni electron-capture detector interfaced to a Varian Vista 401 data system.

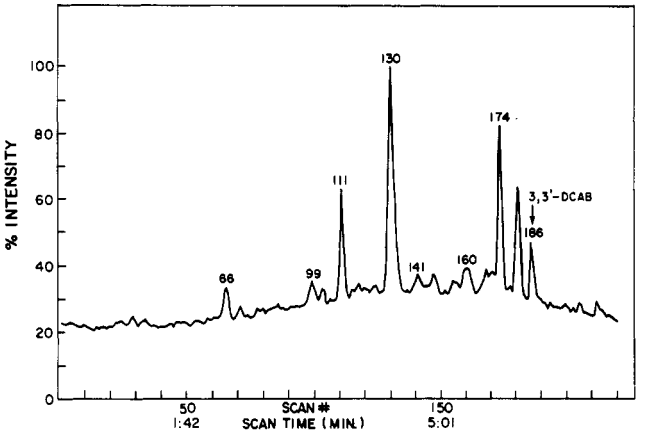

Figure 1. TIC chromatogram of potato peel extract A from GC–MS analysis (1 μL of a 16 g/mL solution) using GC–MS column A.

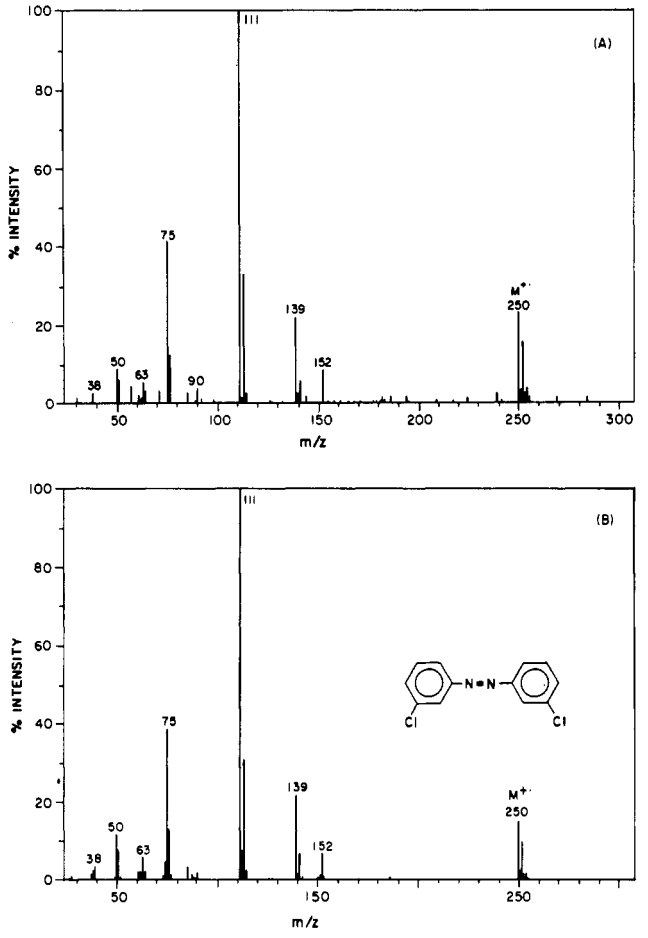

Figure 2. EI spectra: A, peak in peel extract A (1 μL of a 16 g/mL solution) corresponding in R_T to 3,3′-DCAB standard; B, 3,3′-DCAB standard (60 ng injected on GC–MS column A).

GC–EC conditions were as follows: column A, 15-m length × 0.32-mm i.d., OV-17 fused silica (0.25-μm film thickness), column temperature 120 °C, injector temperature 225 °C, detector temperature 300 °C; column B, DB-210 as in GC–MS A above with a column temperature program of 120 °C (3-min hold) to 200 °C/min, 1.5 mL/min (8 psi) nitrogen column flow rate, 27 mL/min nitrogen total flow rate at the detector. All GC–EC analyses were done in the splitless mode.

RESULTS AND DISCUSSION

The purity of the synthesized 3,3′-DCAB was checked by analysis of the stock solution using GC–EC, DIP–MS, and GC–MS (TIM); the presence of extraneous GC peaks

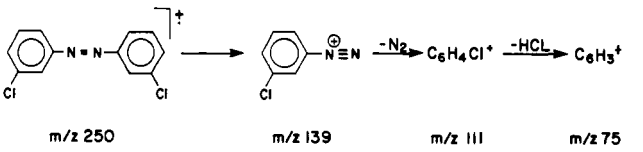

Figure 3. Fragmentation pattern for 3,3′-DCAB by 70-eV EI–mass spectrometry.

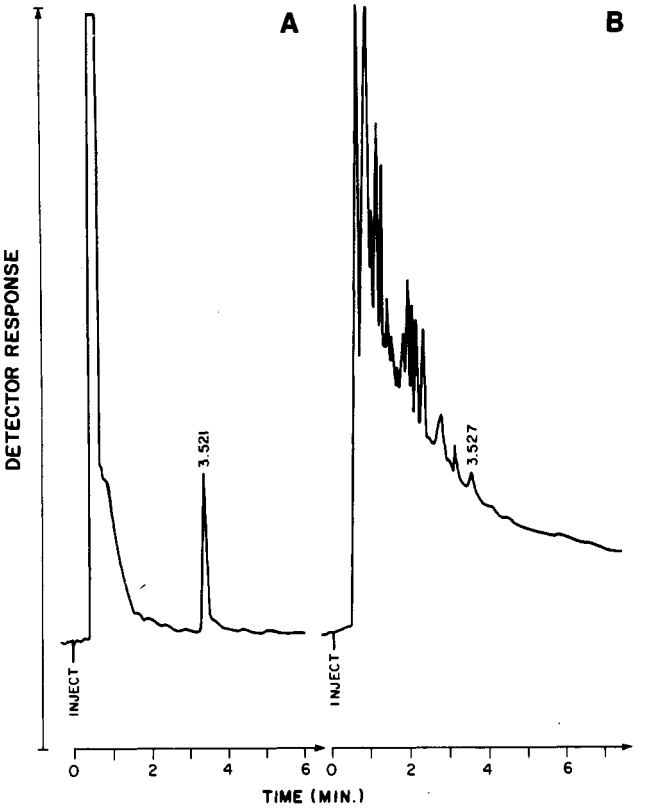

Figure 4. GC–EC chromatograms (GC–EC column A): A, 3,3′-DCAB standard (2 ng); B, potato peel extract A (0.2 μL of a 4 g/mL solution).

or ion fragments accounted for less than 2% of the total peak areas or of the base fragment ion normalized to 100%. GC analysis (EC or MS), using the various columns described, resulted in only one peak being detected. The cochromatography of the sample 3,3′-DCAB peak with synthetic 3,3′-DCAB by GC (EC and MS) on three different columns was further evidence confirming its presence.

For GC–MS (TIM) analysis the extracts were concentrated four times prior to analysis (using column A). Two of the five potato extracts were analyzed by producing a chromatogram of the total ion current (TIC) and scanning between m/z 25 and 310. A typical TIC chromatogram is shown in Figure 1, and the full-scan spectrum for the sample peak (scan number 186) chromatographing with the same R_T as 3,3′-DCAB is shown in Figure 2A. The spectrum is virtually identical with that obtained by GC–MS for the synthesized standard in Figure 2B. GC–MS (TIM or SIM) (M + 2)/M and (M + 4)/M ratios for chlorine isotope peaks agreed with theoretical values (100/65/10). The proposed fragmentation pattern for 3,3′-DCAB is illustrated in Figure 3.

GC–EC analysis of the peel extracts gave chromatograms (Figure 4) containing a peak with the same retention time as the 3,3′-DCAB standard: R_T = 3.52 (standard), R_T = 3.52 (sample). Analysis of the same extracts using temperature programming resulted in sample peaks at R_T 10.50, which corresponded to that for the 3,3′-DCAB

Table I. GC-MS (SIM) Analysis of Three Potato Peel Extracts for 3,3′-DCAB

	m/z							
	250		252		254			
sample[a]	height	concn[b]	height	concn	height	concn	av concn	concn, ppb
standard	74.09		42.19		9.83			
solvent blank	ND[c]		ND		ND			
potato A peel extract	63.31	88.5	48.18 (41.15)[d]	114.2	7.89 (6.71)	80.3	93.8	23.45 [35.0][e]
solvent blank	ND		ND		ND			
potato B peel extract	106.47	143.7	63.56 (69.20)	150.7	17.32 (11.29)	176.2	156.9	39.22 [50.1]
potato C peel extract	3.28	8.9	2.02 (2.13)	9.6	0.36 (0.35)	7.3	8.6	2.15 [5.0]
reagent blank	ND		ND		ND			

[a] Standard = 1.0 μL of a 100 pg/μL solution; sample extracts and blanks = 0.5–2.0 μL of a 4-mL final volume. [b] Concentration is recorded in picograms/microliter. GC–MS (SIM) single analysis was done with GC–MS column B. [c] ND = not detected. [d] Parentheses indicate calculated peak height based on normalization of m/z 250 peak height to 100%. [e] Brackets indicate GC–EC quantitation, average of duplicate injections.

standard. GC–EC analysis of the peak at the same R_T as 3,3′-DCAB, by an external standard method, resulted in tentative 3,3′-DCAB levels of 3.4–45 ppb for the five potato peel samples.

GC–MS (SIM) of the three peel extracts (Table I) resulted in approximate residue levels of 2.1, 23, and 39 ppb for three potato peel samples from potatoes grown in different geographic locations. The isolation method (extraction) used herein was not validated in terms of recoveries. Since our purpose was to identify and give an indication of 3,3′-DCAB residues in potato peels, absolute residue levels may be greater than reported. Solvent blanks were injected between standards and samples during GC–EC and GC–MS analysis—no carry-over was detected in either case. A reagent blank did contain a peak corresponding to 3,3′-DCAB as determined by GC–EC, but GC–MS (SIM) analysis was negative for m/z 250, 252, and 254. This emphasizes the importance of confirming the residues by GC–MS, since our initial levels of 3,3′-DCAB, as determined by GC–EC, were greater than GC–MS (SIM) results; that is, an EC-sensitive peak eluted at the same R_T as 3,3′-DCAB.

The presence of 3,3′-DCAB in market potatoes may arise from one or more of several mechanisms previously reported (Parris, 1980) involving enzymatic, nonenzymatic, or both reactions affecting hydrolysis of CIPC followed by azo bond formation via 3-CA (Clark and Wright, 1970; Menzie, 1969; Kearney et al., 1969, Bartha et al., 1968). CIPC is applied about 30 days after harvest or just before packaging for market. Thus, the use of CIPC in a closed-type system, with few if any additional routes of dissipation other than the potato and enclosure, could result in formation of 3-CA (via hydrolysis), which may condense chemically or biochemically to 3,3′-DCAB. The presence of chloropropham or its 3-CA metabolite in foods has previously been described (Still and Mansanger, 1969; Heikes, 1985; Podrebarac, 1984; Johnson et al., 1981).

Since the market potatoes used in our study were purchased in April, they would have had several months in storage during which CIPC degraded, resulting in traces of 3,3′-DCAB. Coxon and Filmer (1985) reported treating potato tubers with various concentrations of [^{14}C]- or [^{36}Cl]CIPC, and after various storage periods the peel was analyzed for CIPC and several of its metabolites. 3,3′-DCAB was not identified in any of the peel extracts. However, TLC radiochromatogram scanning may not have been sensitive enough to detect the low levels reported in our study.

In addition, 3-CA [3-CA reacted with isopropyl chloroformate is used to synthesize CIPC commercially (Sittig, 1977)] and 3,3′-DCAB may occur as contaminants of the formulation, although this has not been demonstrated. We were unable to detect 3,3′-DCAB in analytical- or technical-grade CIPC (500 μg/mL) by GC–EC. Both cis and trans isomers could be present for 3,3′-DCAB; however, it was shown previously that the trans forms of chloroazobenzenes (including 3,3′-DCAB) are the most stable and are formed exclusively when prepared by oxidative methods (Linke and Pramer, 1969b).

Several publications have reported the genotoxicity of halogenated azobenzenes, including the dichloroazobenzene 4,4′-DCAB (Mercier et al., 1978; Gilbert et al., 1980; Worobey, 1984, references therein). The fact that 3,3′-DCAB is a congener of some very toxic halogenated azobenzenes and an isomer related to 4,4′-DCAB, which is a proven mutagen, suggests that its presence even at the low levels identified in our study may be undesirable. Therefore, further research seems prudent in light of these potential concerns and the isolation and identification of 3,3′-DCAB in potato peels.

Registry No. 3,3′-DCAB, 15426-14-9; 3-chloroaniline, 108-42-9.

LITERATURE CITED

Bartha, R.; Linke, H. A.; Pramer, D. *Science (Washington, D.C.)* **1968**, *161*, 582.

Bunce, N. J.; Corke, C. T.; Merrick, R. L.; Bright, J. H. *Chemosphere* **1979**, *8*(5), 283–284.

Clark, C. G.; Wright, S. J. L. *Soil Biol. Biochem.* **1970**, *2*, 217.

Coxon, D. T. Filmer, A. E. *Pestic. Sci.* **1985**, *16*, 355.

Freitag, D.; Scheunert, I.; Klein, W.; Korte, F. *J. Agric. Food Chem.* **1984**, *32*, 203.

Gilbert, P.; Saint-Ruf, G.; Poncelet, F.; Mercier, M. *Arch. Environ. Contam. Toxicol.* **1980**, *9*, 533.

Heikes, D. L. *J. Agric. Food Chem.* **1985**, *33*, 246.

Johnson, R. D.; Manske, D. D.; Podrebarac, D. S. *Pestic. Monit. J.* **1981**, *15*(1) 54.

Kearney, P. C.; Plimmer, J. R.; Guardia, F. B. *J. Agric. Food Chem.* **1969**, *17*, 1418.

Lee, J.-K.; Fournier, J.-C.; Catroux, G. *J. Korean Agric. Chem. Soc.* **1977**, *20*(1), 109; *Chem. Abstr.* **1977**, *87*, 200966c.

Linke, H. A.; Pramer, D. *Z. Naturforsch., B: Anorg. Chem., Org. Chem., Biochem., Biophys., Biol.* **1969a**, *24B*, 994.

Linke, H. A.; Pramer, D. *Z. Naturforsch., B: Anorg. Chem., Org. Chem., Biochem., Biophys., Biol.* **1969b**, *24B*, 977.

Menzie, C. M. *Metabolism of Pesticides*, Special Scientific Report, Wildlife No. 127; Bureau of Sport Fisheries and Wildlife Department of the Interior: Washington, DC, July 1969; pp 81–82 and references therein.

Mercier, M.; Gilbert, P.; Roberfroid, M.; Poncelet, F. *Arch. Int. Physiol. Biochim.* **1978**, *86*(4), 950.

Parris, G. E. *Residue Rev.* **1980**, *76*, 1.

Podrebarac, D. S. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 166.

Sittig, M. *Pesticides Process Encyclopedia*; Noyes Data: Park Ridge, NJ, 1977; p 114.
Still, G. G. *Weed Res.* **1969**, *9*, 224.
Still, G. G.; Mansanger, E. R. *Weed Res.* **1969**, *9*, 218.
Still, G. G.; Balba, H. M.; Mansanger, E. R. *J. Agric. Food Chem.* **1981**, *29*, 739.
Worobey, B. L. *Chemosphere* **1984**, *13*(10), 1103.

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High-Performance Liquid Chromatographic Determination of Ascorbic Acid in Aseptically Packaged Orange Juice Using Ultraviolet and Electrochemical Detectors

Charles W. Wilson, III,* and Philip E. Shaw

Three different methods were used to measure ascorbic acid values in aseptically packaged orange juice from commercial sources. Values using ultraviolet (UV) and electrochemical (EC) detectors were not significantly different from those obtained by a potentiometric titration method. The EC detector was 100 times more sensitive to ascorbic acid than was the UV detector. Ascorbic acid values generally declined slowly and steadily in samples stored under normal marketing conditions.

Disposable cartons of shelf-stored aseptically packaged citrus juices provide the consumer with a convenient source of individual servings. This new product is the fastest growing segment of the fruit beverage industry (Tillotson, 1984). The single-strength 100% juice product is generally prepared from concentrate and packaged in 250-mL flexible, multilayered cartons. It is reported to be shelf stable for periods of 3–6 months (Kryger, 1985), but there is relatively little information in the literature on ascorbic acid retention in this product. Since citrus juice is considered one of the most reliable sources of dietary ascorbic acid (Rouseff, 1979), there is a need for information on ascorbic acid retention in stored aseptically packaged citrus juices produced commercially.

The standard method for measuring ascorbic acid in foods is by titration with 2,6-dichlorophenol–indophenol (AOAC, 1980). For orange juice, the method can be limited by substances in the juice matrix that obscure end point determination; use of potentiometric titration eliminates this problem in orange juice (Nagy and Smoot, 1977). Toledo (1984) studied the effect of residual hydrogen peroxide on the stability of experimental samples of aseptically packed orange juice and found results similar to those reported by Nagy and Smoot (1977) for stored single-strength juice. High-performance liquid chromatography (HPLC) using strong anion-exchange or reversed-phase columns and ultraviolet (UV) detection has been reported to be a rapid and sensitive method for analyzing citrus juices and other food products for ascorbic acid (Sood et al., 1976; Rouseff, 1979; Carnevale, 1980; Shaw and Wilson, 1982; Haddad and Lau, 1984; Wills et al., 1984). Recoveries of added ascorbic acid determined by HPLC were generally quite good as were comparisons of HPLC values with those obtained by a standard titration method (Haddad and Lau, 1984; Augustin et al., 1981; Carnevale, 1980; Rouseff, 1979).

Detection and quantification of ascorbic acid has been carried out primarily with a UV detector at 254 nm, but there are disadvantages to the method. Other UV-absorbing compounds can either coelute or elute close to ascorbic acid, thereby causing interference in the method (Rouseff, 1979; Wills et al., 1984). Detection of ascorbic acid with an electrochemical (EC) detector, which reduces interference from coeluting compounds because of its specificity and increased sensitivity, has been reported in animal tissue, blood plasma, urine, multivitamins, and foods (Tsao and Salami, 1982; Pachla and Kissinger, 1976). Values for citrus juice concentrate and for whole fruit were within the expected range and were comparable to values determined by titration (Pachla and Kissinger, 1976).

In the current study the abscorbic acid content of aseptically packaged orange juice was determined with EC and UV detectors, and the results were compared to those determined by a potentiometric titration method.

EXPERIMENTAL SECTION

Juice Samples. Samples of aseptically packaged orange juice were obtained from local markets and stored at 5 °C until use.

Sample Preparation. Juice samples (ca. 25 mL) were centrifuged at maximum switch setting for 5 min in an International clinical centrifuge, Model CL (International Equipment Co., Boston, MA), to remove suspended solids. A 2-mL sample of juice was mixed with 2 mL of 6% metaphosphoric acid and then filtered successively through 1.2- and 0.45-μm Acrodisc filters (Gelman Sciences, Inc., Ann Arbor, MI). Filtered samples were stored at 0 °C.

Analytical Methods. The instrument used was a Perkin-Elmer Series 2 pump and Model LC-85B variable-wavelength detector (245-nm maximum sensitivity for ascorbic acid) connected to a Hewlett-Packard Model 3390A recording integrator. Electrochemical detection was accomplished at +0.6 V with an LDC-Milton Roy Chromatronix CMX-20 amperometric detector fitted with a glassy carbon working electrode, an Ag/AgCl reference electrode, and a stainless-steel auxiliary electrode. A Rheodyne Model 7120 injector fitted with a 5-μL loop was used with either a 4.6 mm × 22 cm or a 4.6 mm × 10 cm, 5-μm C18 Brownlee column connected to a 4.6 mm × 3 cm, 5-μm, C18 Brownlee guard column. The mobile phase was aqueous 2% ammonium dihydrogen phosphate adjusted to pH 2.8 with phosphoric acid at flow rates of 0.5 mL/min (10-cm column) or 1.0 mL/min (22-cm column). Ascorbic

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