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Xylenes in Oregon Hazelnuts

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Reported here is a sensitive, selective, and rapid headspace SPME-GC/MS method for the analysis of xylenes in hazelnuts. Conventional methods to analyze pesticides and other trace organics in nuts involve a lengthy solvent extraction followed by Florisil cleanup and GC (gas chromatograph) analysis (Pesticide Analytical Manual 1999). VOCs (volatile organic compounds), such as xylenes, are often analyzed by headspace or purge and trap techniques. Headspace analysis is suited to high concentration samples, and purge and trap is subject to contaminated traps and leaks, and uses large quantities of liquid nitrogen (Arthur et al. 1992). Headspace Solid Phase Micro Extraction (SPME) is a promising alternative for the analysis of VOCs in a variety of matrices. It is rapid, sensitive, and easily automated (Arthur et al. 1992; Otson and Kamarathansan 1995; Zhang et al. 1994). The SPME device consists of an adsorbent-coated fiber (e.g., polydimethylsiloxane) attached to a stainless steel plunger mounted to a holder. The SPME fiber is exposed to the headspace or aqueous sample. After reaching dynamic equilibrium between the sample and fiber, the fiber is introduced into the GC injection port for analyte desorption, followed by separation and detection (Zhang and Pawliszyn 1993; Pawliszyn 2000).

The procedure described was developed because of the need for a sensitive method of xylene detection in hazelnuts. Xylene may be applied to the soil in hazelnut orchards as a 5% aqueous solution at a rate of 200 gal per acre to flush earthworms for use as bait. This practice, although not widespread, has recently raised concerns among growers that xylene could be absorbed by tree roots and translocated to the nut, resulting in the potential for human dietary exposure. Xylene (*Dimethylbenzene*), first isolated from a crude wood distillate, is a mixture of three isomers: *o*-, *m*-, and *p*-xylene, with the *m*-isomer predominating (Figure 1). Xylenes are found in petroleum products and used as solvents and thinners. Xylenes were once a major component of many pesticide formulations. The Environmental Protection Agency (EPA) has established a reference dose (RfD) for xylene of 2 mg/kg/day (Fay et al. 1995).

MATERIALS AND METHODS

Four hazelnut orchards in the northern Willamette Valley, Oregon, previously

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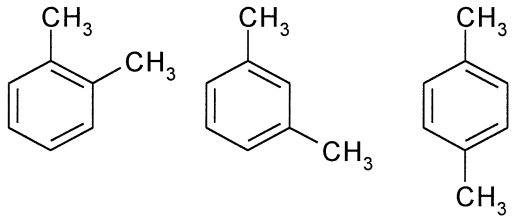

Figure 1. *o*-, *m*-, and *p*-xylene.

treated with xylene, were selected for sampling. Sites 1 and 3 are located near Wilsonville, site 2 near Newberg, and site 4 near Canby. Control hazelnuts were collected from Oregon State University Horticultural Farm near Corvallis, which never received xylene treatment. Three replicate samples from each site (six replicates from the control site) were collected in 2-quart mason jars, sealed airtight, and stored at -10 °C until analysis.

In a 4 °C cold room, 10 g of hazelnuts were shelled and ground for 30 sec with 18 g of dry ice in a coffee grinder. Following a 20-min CO_2 sublimation period, a 2.6–2.8 g subsample was placed in a 20-mL amber glass vial with a Teflon stir bar and sealed with a Teflon septum. Samples were re-weighed and stored overnight at -10 °C. To form a slurry, just prior to SPME adsorption, 8 mL of double deionized (DDI) water were added to the sample vial.

Analytical standards were prepared by adding 1, 10, and 25 ng of xylene, respectively, to vials containing 8 mL of DDI water and a Teflon stir bar. Vials were sealed immediately with a Teflon septum.

Sealed vials containing samples and standards were placed in a 50 °C water bath and stirred for 5 min to reach headspace equilibrium prior to introduction of the SPME needle.

The SPME needle was heated to 200 °C in a GC injection port for 15 min to desorb contaminants. After cooling, it was inserted into the sample or standard vial headspace and left for a 2-min absorption period. The SPME needle was removed and introduced into the Hewlett Packard 6890 GC/MS injection port for a 2-min desorption period under splitless conditions at 180 °C. Separation was achieved on a HP-5MS (30M, 250 μm i.d., 0.25 μm film thickness). Using helium as the carrier gas the column flow rate was 1.6 ml/min. The initial oven temperature of 30 °C was held for 3 min and then ramped to 35 °C at 2 °C/min followed by a second ramp at 30 °C/min to 70 °C for 2 min. Detection was by selective ion monitoring (SIM) for (m/z): 91.05, 106.05, and 105.15 using a Hewlett Packard 5972 Mass Selective Detector.

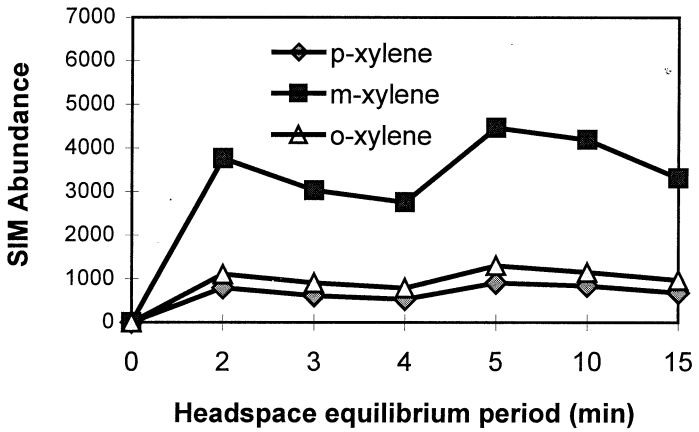

Figure 2. Headspace equilibrium period. Vials containing 2.0 g hazelnut samples, 1 μg xylene, and 8 mL DDI were placed in a 50 °C water bath and stirred for 2, 3, 4, 5, 10, or 15 min prior to a 2-min SPME needle absorption period.

RESULTS AND DISCUSSION

Figure 2 shows the results of experiments to determine optimal headspace equilibrium period prior SPME needle absorption period. Vials containing 2.0 g hazelnut samples, 1 μg xylene, and 8 mL DDI were placed in a 50 °C water bath and stirred for 2, 3, 4, 5, 10, or 15 min prior to a 2-min SPME needle absorption period. Results indicate that all equilibrium periods tested gave similar results.

Based on these data, a 5-min headspace equilibrium period was chosen. Results of the analysis of triplicate samples collected at four locations in the northern Willamette Valley, Oregon, and six control samples from the OSU Horticultural Farm near Corvallis are shown in Table 1. Total xylenes in each sample were determined from the sum of *o*-, *m*-, and *p*-xylene isomers quantitated separately.

The data show little difference between the xylene treated sites and the control; mean values for Sites 1, 2, and 4 were roughly one half the control, and the mean value for Site 3 was roughly equal to the control. Low residue levels in nuts from both treated and untreated sites may result from naturally-occurring xylenes or ambient air concentrations (EPA 1983). All xylene residue levels were at, or near, the lowest analytical standard. Standard curve r^2 values were always > 0.98 and intercepts were at or near zero. As xylene residues were not expected to be found in the hazelnut samples, a low limit of detection was a primary objective in method development. It is unlikely that a procedure based on traditional methods for the analysis of organic contaminants (i.e., pesticides) in hazelnuts, which rely on solvent extraction and cleanup of co-extractants, could achieve the limits of quantitation (~0.3 ng/g) observed here. However, special requirements for preservation of samples containing VOCs was a concern in method development,

since processing the nuts (shelling and grinding) required exposure of the matrix to the atmosphere prior to sampling the headspace. Xylene loss during shelling and grinding was minimized by processing samples in a 4 °C cold room and grinding with dry ice. A method-recovery study was not conducted because it was deemed that no practical method of fortification was possible.

SPME with GC/MS is a selective, sensitive, and rapid method for the analysis of semi-volatile organics in hazelnuts. The method yields reproducible results, and SPME eliminates solvent use. SPME in combination with GC/MS allows the detection of xylenes in hazelnuts at parts per trillion (pg/g) levels.

Table 1. Total xylenes in Oregon hazelnuts.

Location	Total Xylene SIM response[1] (mean ± SD)	Total Xylenes ng/sample (mean ± SD)	Total Xylenes ng/g (mean ± SD)
Site 1 (n=3)	85 ± 42	0.66 ± 0.33	0.32 ± 0.17
Site 2 (n=3)	96 ± 11	0.75 ± 0.08	0.36 ± 0.05
Site 3 (n=3)	185 ± 40	1.43 ± 0.31	0.65 ± 0.18
Site 4 (n=3)	84 ± 21	0.65 ± 0.16	0.26 ± 0.04
Control (n=6)	192 ± 76	1.49 ± 0.58	0.62 ± 0.24

[1] Sum of *o*-, *m*-, and *p*-xylene SIM response.

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