Food Chemistry 160 (2014) 255–259

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

A method to detect diphenylamine contamination of apple fruit and storages using headspace solid phase micro-extraction and gas chromatography/mass spectroscopy ☆

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ARTICLE INFO

Article history:
Received 13 November 2013
Received in revised form 5 March 2014
Accepted 19 March 2014
Available online 28 March 2014

Keywords:
DPA
Solid phase micro-extraction (SPME)
Headspace
Apple aroma
Storage materials

ABSTRACT

Analysis of headspace concentrations of diphenylamine using solid phase micro-extraction (SPME) was examined for its suitability to detect DPA contamination and off-gassing in apple (*Malus domestica*) fruit, storage rooms and storage materials. Four SPME fibre coatings including polydimethylsiloxane (PDMS, 100 μm), PDMS/divinylbenzene (PDMS/DVB), Polyacrylate (PA) and PDMS 7 μm were evaluated. The average limits of detection and of quantification for head space DPA ranged from 0.13 to 0.72 μg L^{-1} and 0.42 to 2.35 μg L^{-1}, respectively. Polyacrylate was identified to be the most suitable and compatible fibre for DPA analysis in apple samples, because of its high sensitivity to DPA and low fruit volatile interferences. SPME techniques were further applied to study contamination of DPA in apples, storage rooms and packaging materials. DPA was found in the air of storage rooms containing apples that were not treated with DPA. Wood and plastic bin material, bin liners, and foam insulation all adsorbed and off-gassed DPA and could be potential sources of contamination of untreated apples.

1. Introduction

Diphenylamine is an antioxidant used to treat apples and pears after harvest to prevent superficial scald, a physiological disorder characterised by browning of the skin that develops during storage (Ingle & D'Souza, 1989; Lurie & Watkins, 2012). When scald occurs in apples, the economic value of the fruit for the fresh market is lost. DPA has been widely used in the fruit industry worldwide as the primary protection against scald development (Smock, 1961).

DPA is a crystal solid at ambient temperature with melting point of 53 °C and vapour pressure of 0.03 Pa at room temperature (Scientific Committee on Health & Environmental Risks, 2008). It is only slightly soluble in water, but is soluble in alcohol and other organic solvents. It is applied to apple fruit as an emulsion. Its volatility can result in DPA being released by treated fruit during storage (Robatscher et al., 2012). Volatilised DPA has the potential to be absorbed by the cuticular wax of untreated fruit, by packaging materials such as bins and plastic bin liners and storage facilities, including wall insulation, thus posing the risk of contaminating untreated fruit (Bramlage, Potter, & Ju, 1996; Robatscher et al., 2012). The Food and Drug Administration (FDA) has listed DPA as a pesticide and its residue is monitored in apples and pears (Roy et al., 1997). Although there have been no reports of negative effects of DPA on human health, increasing public awareness of fruit quality, and demand for "chemical-free" and organic produce limits the use of synthetic chemical treatments. DPA residues are not permitted on any type of organic fruit. However, DPA has been detected in organic apple fruit that were never treated with DPA (Robatscher et al., 2012). Therefore, a simple methodology is needed to study DPA contamination of apples and identify possible contamination sources in storage environments.

The analysis of DPA in apples has been conducted using solvent extraction, as well as supercritical fluid extraction followed by gas chromatography/mass spectroscopy (GC/MS) (Kovalczuk, Lacina, Jech, Poustka, & Hajšlová, 2008; Lehotay, 2000; Robatscher et al., 2012; Roy et al., 1997; Yu, Schoen, Dunkin, Firman, & Cushman, 1997). These techniques involve liquid extraction following sample preparation, which are expensive and time-consuming procedures.

Abbreviations: PDMS, poly(dimethylsiloxane); PDMS/DVB, poly(dimethylsiloxane)/divinylbenzene; PA, polyacrylate; SPME, solid phase microextraction; GC, gas chromatography; MS, mass spectrometry; RSD, relative standard deviation; TIC, total ion chromatogram; SIM, selected ion monitoring.

☆ Contribution No. 2229 of the Atlantic Food and Horticulture Research Centre, Agriculture & Agri-Food Canada.

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http://dx.doi.org/10.1016/j.foodchem.2014.03.099
0308-8146/

Little information about methodologies to identify DPA contamination sources in the storage environment and packaging materials is available. Therefore, it is desirable to develop a simple, portable, and economical analytical methodology to quantify DPA in apple fruit and in the storage environment.

Solid phase microextraction (SPME) is a rapid sampling technique that is well adapted to GC analysis of volatile and semi-volatile compounds (Arthur & Pawliszyn, 1990). It has been used to analyse volatiles in many products including fruit beverages and vegetable oils (Yang & Peppard, 1994), and was shown to be a rapid, nondestructive, quantitative sampling method for apple fruit volatiles (Matich, Rowan, & Banks, 1996; Song, Gardener, Holland, & Beaudry, 1997). SPME technology is based on the partitioning of the analytes between the fibre coating and the sample (Zhang & Pawliszyn, 1993). The higher the partition coefficient, the higher is the fibre's affinity for a compound. SPME provides a linear response to concentration covering four orders of magnitude (Arthur & Pawliszyn, 1990). SPME technique was applied to analyse pesticide residues in extracts of grapes, in which DPA was used as an internal standard (Urruty & Montury, 1996). These findings implied that SPME could be used as a sampling tool to analyse DPA in liquid extracts. However, the application of SPME in this case was only in a liquid phase and not in a gas phase. Analysis using SPME would be preferable because it is solvent free, simple and portable.

The objectives of this investigation were (1) to examine the potential use of SPME to sample head-space DPA by determining DPA absorption characteristics of commercially available SPME fibre coatings; (2) to select a suitable fibre for DPA headspace analysis; and (3) to investigate the applications of head-space SPME to determine DPA in apple fruit, packing materials and storage environments.

2. Materials and methods

2.1. Chemicals

Authenticated, high purity DPA (>99%) was obtained from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada) for use as a standard. A stock solution was prepared by dissolving pure DPA into 1-propanol. Solutions with concentrations ranging from 1.5 to 30 μg L^{-1} were prepared by diluting aliquots of the stock solution in 1-propanol. 1-Propanol without DPA was used as a control. Diluted solutions were placed in a glass volumetric flask (6.23-L) fitted with a specially-made ground glass stopper containing a gas-tight Minert valve (Alltech Assoc., Inc., Deerfield, IL, USA). Standards at concentrations of 0.24, 0.48, 2.47 or 4.81 μg L^{-1} were made and held at 22 °C until the standard was fully volatilised. A commercial DPA solution with a purity of 31% was supplied by Noggins Corner Farm (Greenwich, NS), from which a DPA solution of 1 g L^{-1} was prepared.

2.2. Apples

Apple fruit (*Malus domestica* Borkh. cv. 'Red Delicious') were obtained from a local orchard at the commercial harvest stage. Healthy fruit without physical damage of regular shape and with uniform size and colour were selected.

2.3. Application of SPME

SPME fibres coated with polydimethylsiloxane (PDMS, 100 μm), PDMS/divinylbenzene (PDMS/DVB), Polyacrylate (PA) or PDMS 7 μm (Supelco Co., Bellefonte, PA) were conditioned at 250–320 °C for 2–3 h prior to use depending on the manufacture's recommendation. Sampling of standards was accomplished by placing the SPME fibre through the Mininert valve and into the 6.23-L flask. Sampling temperature was 22 °C.

Adsorbed DPA and volatiles were desorbed from the fibre coating by inserting the SPME fibre through a pre-drilled septum (Thermogreen™ LB-2, Supelco Co., Bellefonte, PA) and into a glass-lined, splitless injector port (200 °C) of a gas chromatograph (Varian 3400, Varian Inc. Walnut Creek, CA, USA). The desorption time was 120 s. DPA was analysed on a 30 m × 0.25 mm ID capillary column (DB-5, J&W Scientific, Folsom, CA, USA) with a film thickness of 0.25 μm. Ultra purified helium (99.999%) was used as the carrier gas at a flow rate of 0.022 mL s^{-1}. Linear velocity was 44 cm s^{-1}. The initial temperature of the column was 75 °C and was increased at 20 °C min^{-1} to a final temperature of 250 °C, which was maintained for 1 min. Between each injection, the SPME fibre was inserted into the injection port of the GC for 5 min to prevent carry over. Detection and identification of DPA was made using a Magnum GC/MS system (Finnigan MAT, San Jose, Calif.) with a 70 eV electron ionisation source, the GC/MS transfer line temperature was kept at 250 °C. Mass spectra of total ion chromatograms were generated at a rate of 3 spectra/s over the range of *m/z* 30–300. The ion at *m/z* 169 was selected for single ion monitoring (SIM) analysis. Identification of volatile components was confirmed by comparison of collected mass spectra with those of authentic reference standards and those in the National Institute of Standards and Technology (NIST) mass spectra library, Search Version 1.6.

2.4. SPME saturation kinetics

To determine the equilibrium of DPA in the headspace, fibres were exposed to the head-space in jars containing the DPA standard at a concentration of 0.48 μg L^{-1} (prepared from the stock solution) for different lengths of time up to 120 min at 22 °C. This experiment was repeated three times.

2.5. Fruit headspace sampling

Apple fruit (20) were dipped in a commercial DPA solution with a concentration of 1 g L^{-1} for 30 s. After treatment fruit were placed on filter paper to prevent dripping, allowed to dry and then stored for future use. Untreated apple fruit were also sealed in a 128 L chambers containing 50 mL solutions of DPA (1 g L^{-1}) at 22 °C for 24 h. For DPA analysis of apples, apple fruit (ca. 2.0 kg) were placed in three 4.0 L glass jars and sealed with insert lids composed of Teflon-PFE (Cole-Parmer Instrument Co., Vernon Hill, IL). The head-space in the jar was allowed to equilibrate for 180 min. SPME fibres, previously cleaned by heating were placed through the septum of the jars and exposed to the headspace for 30 min. fibres were then immediately transferred to the GC injection port and desorbed for 3.0 min. The parameters of the GC and MS protocols were the same as those described previously. Measurements were taken three times from each jar. Non treated apples were used as the control.

2.6. DPA in the air of commercial storages

Air in four commercial CA storage rooms, each containing 800 bins of apples, was monitored monthly for DPA during the storage season. The storage room included two rooms that contained apples treated with DPA (Room 1 – 'Cortland' and Room 2 – 'Delicious' and 'Golden Delicious') and two rooms that contained untreated apples (Room 3 – 'Idared' and Room 4 – 'Northern Spy'). DPA in the atmosphere of each room was sampled using a portable pump at a flow rate of 1 L min^{-1}. A SPME fibre was placed in the air stream of the pump outlet for volatile sampling for 30 min.