# Commercial fumigation of stored products with a propylene oxide, carbon dioxide, and sulfuryl fluoride mixture

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DOI: xx.xxxx/xxx.2014.xxx.xxx.xxx

## Abstract

Each year, the central valley of California produces >2 million metric tons of dried fruit and nuts collectively valued at 5.5 billion dollars. The goal of this research was to provide this sector with a commercially viable postharvest methyl bromide alternative that is effective against insect and microbiological pests. Fumigations at 25°C with mixtures of propylene oxide, carbon dioxide, and sulfuryl fluoride were conducted at normal atmospheric pressure for 24 hours as well as at a pressure of 100 mmHg for 2 hours. We discuss the progression of this research from initial toxicological investigations, through laboratory-scale optimization, to commercial-scale confirmatory testing. We report how to modulate the applied dose of a mixture to ensure control of key insect pests (e.g., dried fruit beetle, red flour beetle, and codling moth) across a variety of commodities, including: dates, figs, prunes, pistachios, almonds and walnuts. We also report on the concomitant reduction of key microbial species following the respective fumigations. Quantifying the residues from fumigation with the mixture was a critical step in assessing commercial viability, as any proposed use must result in residues compliant with domestic (United States) food tolerances as well as international maximum residue level (MRL) regulations. Detailed below is a novel analytical approach, involving solvent extraction with methyl *tert*-butyl ether (MTBE) followed by gas chromatography (GC), which was developed to quantify residues that result from the postharvest fumigation of almonds and walnuts with propylene oxide (PPO).

Keywords: food security, food safety, propylene oxide, sulfuryl fluoride, commercial fumigation

## 1. Introduction

Postharvest chamber fumigation with propylene oxide (PPO) is used for the disinfestation and disinfection (i.e., pasteurization) of tree nuts, including almonds and walnuts. The resultant type and quantity of residues are a function of many factors, including treatment parameters, storage conditions, and time-lapse between treatment and residue analyses. Maximum residue limits (MRLs) associated with PPO fumigation of almonds and walnuts are not currently “harmonized” across the global marketplace. This work addresses a critical need to quantify residues of PPO and its degradation products, propylene chlorohydrin (PCH) and propylene bromohydrin (PBH), following fumigation. As such this research supports the development of MRL-related regulatory and consumer requirements for distribution and trade as well as enables the evaluation of mitigation strategies to ensure compliance.

A variety of analytical approaches have been used to quantify foodstuff residues of PPO, PCH, and PBH. The seminal works of Heuser and Scudamore (1968, 1969) and Scudamore and Heuser (1971) highlight the advantages of using solvent extraction relative to the use of techniques described in Gunther (1965) that accelerate chemical breakdown of residues during the recovery procedure, such as grinding, heating, and distillation. PPO can rapidly convert into propylene glycol, PCH, and PBH via acid-catalyzed hydrolysis in the presence of nucleophiles (i.e., water, hydroxide, chloride, or bromide) (Scudmaore and Heuser, 1971; Whalen and Ross, 1976), with the reaction rate positively correlated to reaction temperature. In fact, a frequently cited method for determining levels of inorganic bromide in foodstuffs following postharvest fumigation with methyl bromide involves the reflux of acidified homogenate with a molar excess of ethylene oxide, a structural analogue of PPO, to form ethylene bromohydrin in nearly quantitative yield (Stijve). Hoffman (1994) further improved analytical methodology for PPO detection in foodstuffs by replacing packed gas chromatography (GC) columns used in ASTA method 23.1 with capillary columns, which enhanced chromatographic resolution between PPO, PCH, and PBH but did not distinguish PCH isomers (1-chloropropen-2-ol (PCH-1) and 2-chloropropen-1-ol (PCH-2)) as well as PBH isomers (1-bromopropen-2-ol (PBH-1) and 2-bromopropen-1-ol (PBH-2)).

Expanding on the work of King et al. (1981), Heuser and Scudamore (1968, 1969), and Hoffman (1994), we report novel analytical methodology that uses methyl *tert*-butyl ether (MTBE) to facilitate the extraction of alcohols (i.e., PCH and PBH) from aqueous matrices, cool-on column GC injections to minimize thermal decomposition of analytes in injector ports, and a GSQ capillary analytical column that affords, for the first time, chromatographic resolution of PPO, PCH-1, PCH-2, PBH-1, and PBH-2. Moreover, a combination of electron impact ionization mass spectrometry (EIMS), negative ion chemical ionization mass spectrometry (NCIMS), and electron capture detection (ECD) were utilized for spectrometric confirmation and/or to improve limits of detection (LODs) relative to flame ionization detection (FID), previously reported as ~ 30ppm and 80ppm (μg/g - nut) for PPO and PCH, respectively.

## 2. Materials and Methods

### *2.1. Standards, reagents, and solvents*

Barnstead E-pure™ water (18 MΩ-cm) was used for solutions. HPLC-grade methyl *tert*-butyl ether (MTBE) was from EMD Chemicals (Gibbstown, NJ, USA). Propylene oxide (PPO) was obtained from Balchem Corporation® (New Jersey, USA). Methyl bromide was obtained from Chemtura® (Arkansas, USA) and used as an external standard for quantification. Propylene chlorohydrin (PCH) and propylene bromohydrin (PBH) were each purchased from Toyko Chemical Industry Company LTD (Tokoyo, Japan) as isomeric mixtures enriched in 1-halopropan-2-ol (PCH-1; PBH-1) (~80%) relative to the 2-halopropan-1-ol isomer (PCH-2; PBH-2) (~20%) based on GC-ECD analyses (*vide infra*). Stock solutions (1000 $\text{ng}\mu\text{L}^{-1}$ and 100 $\text{ng}\mu\text{L}^{-1}$) of PPO, PCH, and PBH were prepared in MTBE and stored at ~5°C. All other chemicals were obtained from commercial sources unless otherwise noted.

### *2.2. Analytical procedure, calibration standards, and recoveries*

In general, residues of PPO, PCH, and PBH were identified based on chromatographic and spectrometric agreement with purchased standards. Gas chromatographic retention time and/or mass spectrometry were used for chemical verification and the integral of peak area, referenced relative to linear least-squares analysis of a 7-point plot of concentration versus detector response, was used to determine concentration. Detector response and retention indices were determined each day in calibration studies involving serial dilutions of PPO, PCH, and PBH stock solutions in known volumes of MTBE (i.e., calibration standards). Calibrations for the quantification of residue levels were derived from the detector response to laboratory fortified blanks, in which the MTBE homogenate from non-fumigated almonds or walnuts was fortified (i.e., spiked) with known quantities of PPO, PBH, and PCH stocks.

Extraction recoveries were calculated as the fractional percentage, relative to results from laboratory fortified blanks, of residues quantified following application of known quantities of PPO, PBH, and PCH in MTBE (10μL) to the surface of non-fumigated almonds and walnuts prior to homogenization (i.e., surface-spiked). Extraction recoveries for 1, 10, and 100ppm (ppm (μg/g – nut)) residue were from quintuplicate determinations, with and without a 10-fold concentration of MTBE supernatant (10 to 1 mL) prior to analysis (*vide infra*).

Almonds and walnuts were prepared for residue analysis via modification to the method of King et al. (1981), Heuser and Scudamore (1968), and Hoffman (1994). A 500-mL air-tight glass blending vessel (Eberbach Corp., No. E8470.00) was filled with (non-fumigated, surface-spiked, or fumigated) shelled walnuts (15 g) or shelled almonds (25 g) as measured gravimetrically. Water (40 mL, pH 7), which was chilled in an ice-water bath for 15 min prior to use, was then added to the blending vessel before introduction of MTBE (160 mL). Polypropylene lids, equipped with rubber gaskets and a LuerLok ® sampling valve, were securely screwed into place. If laboratory fortified blanks were prepared, such as for the residue calibration studies (*vide supra*), injections of PPO, PCH, or PBH stocks were made through the septa covering the sampling port. A laboratory blender (Waring model no. 5BA60VL22) equipped with a General Electric 1/5 horsepower explosion-proof motor was used to macerate the samples for 90 sec. The motor speed was controlled with a Powerstat Variable Transformer, Type 116B, set at 80% power. Blending vessels were opened and ~45 mL of the homogenate was decanted to a 50-mL centrifugation tube that was capped and centrifuged at 80 rpm for 5 min. Aliquots of MTBE supernatant were withdrawn with a 1-mL volumetric pipette and transferred to 2-mL amber glass GC-vials precharged with 10 µL of MTBE containing methyl bromide external standard at 0.8 μL/L MTBE. Vials were clamp-sealed with Teflon-lined caps and three separate gas chromatographic analysis were sequentially conducted with EIMS, NCIMS, and ECD detection.

For each extraction, a 10-mL aliquot of MTBE supernatant was withdrawn with a volumetric pipette and transferred to a volumetrically-graduated glass vial. The contents were concentrated to~0.95 mL with a gentle $N_2$ stream, 10 µL of MTBE containing methyl bromide external standard at 0.8 μL/L MTBE was added, the solution was diluted with MTBE to 1 mL, and a Pasteur pipette was used to transfer the contents to a 2-mL amber glass GC-vial that was subsequently sealed and analyzed as described above.

### *2.3. Gas Chromatography – Electron Impact Ionization Mass Spectrometry (GC-EIMS)*

A Hewlett Packard 6890 gas chromatograph and a 5973N quadrupole mass spectrometer was operated with electron impact ionization. Cool on-column injections (1 μL) were at 140°C with He carrier gas (24.14psi). The oven program was isothermal at 160 °C for 1 min, heated at 30°C/min to 220°C, isothermal for 5 min, heated at 20°C/min to 240°C, and then isothermal for 1min. GlasSeal connectors (Supleco®) fused four columns in series; a deactivated column (L = 8 cm, ID = 0.53 mm) onto which the injection was deposited, a deactivated retention-gap column (L = 2 m, ID = 0.25 mm), a GS-Q analytical column (L = 30 m, ID = 0.53mm, Agilent Technologies, #115-3432), and finally a deactivated column (L = 1.5 m, ID = 0.25 mm) that was directed into the detector. Transfer-line and manifold temperatures were 240 and 230 °C, respectively. Analyte $R_t$, ($\bar{x}$ ± SE): methyl bromide, 1.67 ± 0.04; PPO, 1.92 ± 0.05; PCH-1, 4.46 ± 0.05; PCH-2, 4.73 ± 0.5; PBH-1, 5.66 ± 0.5; and PBH-2 6.04 ± 0.05 min.

Full scan spectra (*m*/z 40 to 150) were acquired at 0.34 s per scan for qualitative verification: methyl bromide, *m/z* (% rel. inten.) 93.9 (49), 94.9 (1), 95.9 (50); PPO, *m/z* 58.0 (97), 59.0 (3); PCH-1, *m/z* 45 (71) 79.0 (21), 81.0 (5), 93.0 (2), 95.0 (<1); PCH-2, *m/z* 58.1 (46), 62.1

(22), 63.1 (18), 65.1 (14), 93.0 (<1), 95.0 (<1); PBH-1, *m/z* 45 (79), 80.1 (<1), 82.1 (<1), 122.8 (10), 124.8 (8); PBH-2, *m/z* 59.0 (60), 106.9 (9 ), 108.9 (9),122.8 (11), 124.8 (11). Three select ion monitoring (SIM) ionization segments were used in series for quantification: *m/z* (+) 26.5-27.5, 43.0-44.0, 57.5-58.5, 79.0-81.0, 93.0-95.0 (0.1-2.27 min); *m/z* (+) 57.0-59.0, 61.5-65.5, 78.5-81.5, 92.5-96.5 (4.45-5.55 min); and m/z (+) 57.0-59.0, 78.0-82.0, 92.5-95.5, 106.5-109.5, 122.0-125.5 (5.55-10.67 min). Respective GC-EIMS limits of quantification (LOQs) for PPO, PCH-1, PCH-2, PBH-1, and PBH-2 in non-concentrated MTBE extracts (ppm (μg/g – nut)) were 0.9, 2.1, 2.5, 30.3, and 50.1for almonds and 0.8, 2.2, 2.0, 41.6, and 45.7 for walnuts.

### *2.4. Gas Chromatography – Negative Chemical Ionization Mass Spectrometry (GC-NCIMS)*

A Hewlett Packard 7890 gas chromatograph and a 5975 quadrupole mass spectrometer operated with negative (-) chemical ionization and methane reagent gas. Raw scan spectra were acquired over *m/z* 50 to 190 xxxxxx Chromatographic conditions and relative retentions were as above. GC-NCIMS spectra were acquired for qualitative verification: PCH-1, *m/z* (% rel. inten.) 58.0 (18), 63.0 (7), 65.0 (7), 78.8 (42), 80.8 (17), 93.0 $[M-H]^-$ (5), 95.0 (4); PCH-2, *m/z* 58.0 (40), 63.0 (18), 65.0 (18), 79.0(12), 81.0 (5), 93.0 $[M-H]^-$ (4), 95.0 (3); PBH-1, *m/z* 59.0 (18), 107.0 (19), 109.0 (12), 123.0 (19), 125.0 (12), 137.0 (11) $[M-H]^-$,139.0 (9); PBH-2, *m/z* 59.0 (23), 107.0 (19), 109.0 (13), 123.0 (18), 125.0 (9), 137.0 (10) $[M-H]^-$ ,139.0 (8). Respective GC-NCIMS LOQs for PPO, PCH-1, PCH-2, PBH-1, and PBH-2 in non-concentrated MTBE extracts (ppm (μg/g – nut)) were 18.3, 10.0, 20.1, 32.0, and 31.1 for almonds and 11.1, 24.1, 35.2, and 29.6 for walnuts.

### *2.5. Gas Chromatography –Electron Capture Detection (GC-ECD)*

A Hewlett Packard 7890 gas chromatograph was operated with an μECD detector at 275°C with 60mL $min^{-1}$ $N_2$ make-up flow. Chromatographic conditions and relative retentions of PCH-1, PCH-2, PBH-1, and PBH-2 were as above. Respective GC-ECD LOQs for PCH-1, PCH-2, PBH-1, and PBH-2 in non-concentrated MTBE extracts (ppm (μg/g – nut)) were 2.1, 2.2, 0.7, and 0.7 for almonds and 2.3, 2.0, 0.7, and 0.7 for walnuts. PPO was not amenable to analysis with GC-ECD detection.

### *2.6. Treatment of error*

For each detector type, with and without a 10-fold concentration of the MTBE extract, a limit of blank (LoB), a LOD, and a LOQ were calculated as in Armbruster and Pry (2008) for PPO, PCH-1, PCH-2, PBH-1, and PBH-2. General procedures for data treatment, including the calculation of grand means, standard deviations, pooled standard deviations, were as reported in Skoog and Leary (1992).

## 3. Results and Discussion

### *3.1. Residue recoveries*

Average extraction recoveries ($\bar{x}$ ± rsd) from five replicates were calculated for almonds as well as walnuts at 1, 10, and 100ppm (μg/g-nuts) analyte with and without 10-fold concentration of the extracts, followed by quantitative analysis with GC-EIMS (Table 1). PPO recoveries exceeded 90% when MTBE extract was not concentrated. However, when the MTBE extract was concentrated the recoveries were below 50%, likely due to the similar vapor pressure of MTBE (~250 mm Hg at 25°C) and PPO (~532 mm Hg at 25°C) (12). PCH-1 and PCH-2 have respective vapor pressures of ~ 3.4 and ~4.8 mm Hg at 25°C (12), approximately 100-fold lower than MTBE; Recoveries of PCH-1 and PCH-2 ranged from 51 to 72% when the MTBE extract was concentrated and >90% with no concentration of

MTBE prior to quantification. PBH-1 and PBH-2 recoveries ranged from 80 to 105% regardless of whether or not the MTBE extract was concentrated prior to GC-EIMS analyses; PBH-1 and PBH-2 have respective vapor pressures estimated to be ~1.6 and ~1.3 mm Hg at 25°C (12).

### *3.2. Verification of PCH and PBH isomers.*

Isomeric assignment of chromatographic peaks was with GC-EIMS and specifically the mass fragments involving the secondary carbon, C2 (Figure 1). Cleavage of the C1-C2 bond yielded mass fragments with the isotopic signature of the respective halogen for PCH-2 (*m/z* 63, 65) as well as PBH-2 (*m/z* 107, 109) and not for PCH-1 or PBH-1, which both result in a fragment with *m/z* 45. C-X bond cleavage was evident in the spectra of PCH-2 and PBH-2 as *m/z* 58, but was not observed for PCH-1 and PBH-1 likely due to the relative instability of a primary radical cation at C1. Differences in the spectrometric signature of the PCH as well as PBH isomers were evident with GC-NCIMS analysis. Commercial standards analyzed with GC-ECD, a relatively structurally indiscriminate technique, showed an isomeric distribution between PCH-1 and PCH-2 as well as PBH-1 and PBH-2 of ~ 80:20, which supports those reported by the supplier, GC-EIMS analyses, and GC-NCIMS analyses.

### *3.3. Quantification of PPO, PCH, and PBH*

GC-EIMS analysis resulted in a LOQ for PPO ~ 20-fold lower relative to GC-NCIMS. Neither GC-ECD analysis, which is not amenable to PPO detection, nor 10-fold concentration of the MTBE extract, which yielded recoveries below 50%, are recommended for PPO analysis based on the results of this study. GC-ECD and GC-EIMS analyses resulted in similar detector responses to PCH isomers, which were ~ 10-fold higher than observed using NCIMS detection (Figure 2). The percentage recovery of PCH isomers following 10-fold concentration of MTBE extracts ranged from 51 to 72%, which decreases the confidence of the measurement irrespective of detector type. It is important to note critical features of this method that improve PBH detection, relative to referenced methodology, thereby offering novel insight to potential residue levels in almonds and walnuts following postharvest fumigation with PPO. There was a >100-fold relative enhancement of PBH-1 and PBH-2 with GC-ECD as compared to GC-EIMS and GC-NCIMS detection. For both PBH isomers, a 10-fold concentration of the MTBE extract resulted in a concomitant 10-fold decrease in the respective LOQs for EIMS, NCIMS, and ECD detection. Respective GC-ECD LOQs for PBH-1 and PBH-2 in MTBE extracts of nut meat following 10-fold concentration (ppb (ng/g – nut)) were 75.1 and 75.3 for almonds, while 74.8 and 77.3 for walnuts. Respective GC-ECD LODs for PBH-1 and PBH-2 in MTBE extracts of nut meat following 10-fold concentration (ppb (ng/g – nut)) were 61.5 and 42.7 for almonds, while 58.2 and 55.1 for walnuts.

## 4. Conclusions

A novel analytical approach, involving solvent extraction with methyl *tert*-butyl ether (MTBE) followed by gas chromatography (GC), was developed to quantify residues that result from the postharvest fumigation of almonds and walnuts with propylene oxide (PPO). Verification and quantification of PPO, propylene chlorohydrin (PCH) (1-chloropropen-2-ol (PCH-1) and 2-chloropropen-1-ol (PCH-2)), and propylene bromohydrin (PBH) (1-bromopropen-2-ol (PBH-1) and 2-bromopropen-1-ol (PBH-2)) was accomplished with a combination of electron impact ionization mass spectrometry (EIMS), negative ion chemical ionization mass spectrometry (NCIMS), and electron capture detection (ECD). Respective GC-EIMS limits of quantification (LOQs) for PPO, PCH-1, PCH-2, PBH-1, and PBH-2 in MTBE extracts (ppm (µg/g – nut)) were 0.9, 2.1, 2.5, 30.3, and 50.0 for almonds and 0.8, 2.2, 2.02, 41.6, and 45.7 for walnuts. Relative to GC-EIMS, GC-ECD analyses resulted in no detection of PPO, similar detector responses for PCH isomers, and >100-fold more sensitive detection of PBH isomers. NCIMS did not enhance detection of PBH isomers relative to EIMS and was ~10-fold less sensitive to PPO and PCH isomers. MTBE extraction efficiencies were > 90% for all analytes. The 10-fold concentration of MTBE extracts yielded recoveries of 85-105% for the PBH isomers and a concomitant decrease in limits of detection (LODs) and LOQs across detector types. The percent recovery of PCH isomers and PPO in the MTBE concentrate were relatively low (ca. 50 to 75%), which confound improvements in LODs and LOQs regardless of detector type.

## Acknowledgments

This research was funded by the USDA-Agricultural Research Service, the Almond Board of California, and California Walnut Board. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

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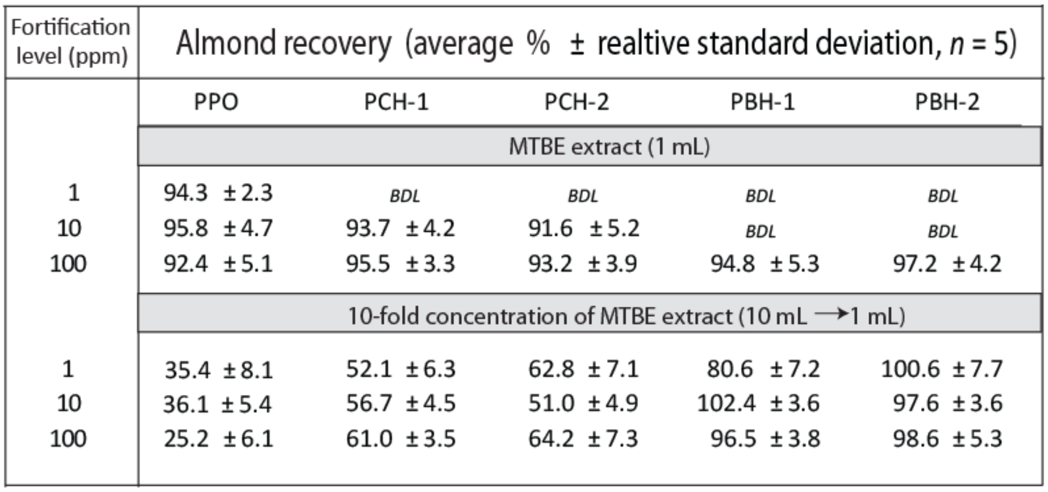
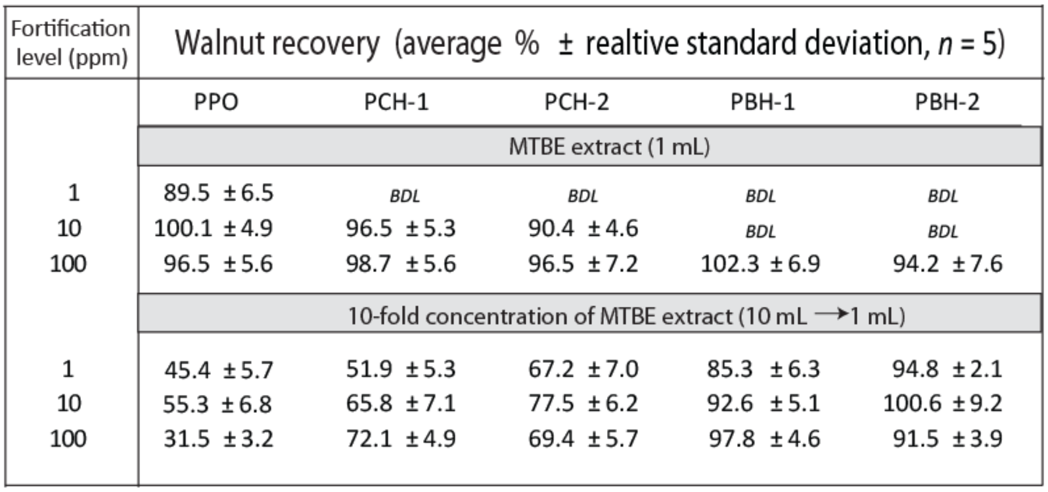


| Fortification level (ppm) | Almond recovery (average % ± realtive standard deviation, $n$ = 5) | | | | |
|---|---|---|---|---|---|
| | PPO | PCH-1 | PCH-2 | PBH-1 | PBH-2 |
| | MTBE extract (1 mL) | | | | |
| 1 | 94.3 ± 2.3 | *BDL* | *BDL* | *BDL* | *BDL* |
| 10 | 95.8 ± 4.7 | 93.7 ± 4.2 | 91.6 ± 5.2 | *BDL* | *BDL* |
| 100 | 92.4 ± 5.1 | 95.5 ± 3.3 | 93.2 ± 3.9 | 94.8 ± 5.3 | 97.2 ± 4.2 |
| | 10-fold concentration of MTBE extract (10 mL → 1 mL) | | | | |
| 1 | 35.4 ± 8.1 | 52.1 ± 6.3 | 62.8 ± 7.1 | 80.6 ± 7.2 | 100.6 ± 7.7 |
| 10 | 36.1 ± 5.4 | 56.7 ± 4.5 | 51.0 ± 4.9 | 102.4 ± 3.6 | 97.6 ± 3.6 |
| 100 | 25.2 ± 6.1 | 61.0 ± 3.5 | 64.2 ± 7.3 | 96.5 ± 3.8 | 98.6 ± 5.3 |



| Fortification level (ppm) | Walnut recovery (average % ± realtive standard deviation, $n$ = 5) | | | | |
|---|---|---|---|---|---|
| | PPO | PCH-1 | PCH-2 | PBH-1 | PBH-2 |
| | MTBE extract (1 mL) | | | | |
| 1 | 89.5 ± 6.5 | *BDL* | *BDL* | *BDL* | *BDL* |
| 10 | 100.1 ± 4.9 | 96.5 ± 5.3 | 90.4 ± 4.6 | *BDL* | *BDL* |
| 100 | 96.5 ± 5.6 | 98.7 ± 5.6 | 96.5 ± 7.2 | 102.3 ± 6.9 | 94.2 ± 7.6 |
| | 10-fold concentration of MTBE extract (10 mL → 1 mL) | | | | |
| 1 | 45.4 ± 5.7 | 51.9 ± 5.3 | 67.2 ± 7.0 | 85.3 ± 6.3 | 94.8 ± 2.1 |
| 10 | 55.3 ± 6.8 | 65.8 ± 7.1 | 77.5 ± 6.2 | 92.6 ± 5.1 | 100.6 ± 9.2 |
| 100 | 31.5 ± 3.2 | 72.1 ± 4.9 | 69.4 ± 5.7 | 97.8 ± 4.6 | 91.5 ± 3.9 |

**Table 1** Average recoveries ± relative standard deviation ($n$ = 5) from five replicate almond (*top*) and walnut (*bottom*) samples for PPO, PCH-1, PCH-2, PBH-1, and PBH-2 at 1, 10, and 100ppm (μg/g-nut) levels. MTBE solvent extraction, with and without 10-fold concentration of the extracts, was followed by quantitative analysis with GC-EIMS. Note that several of the fortifications yielded residues below the method limit of detection (*BDL*).

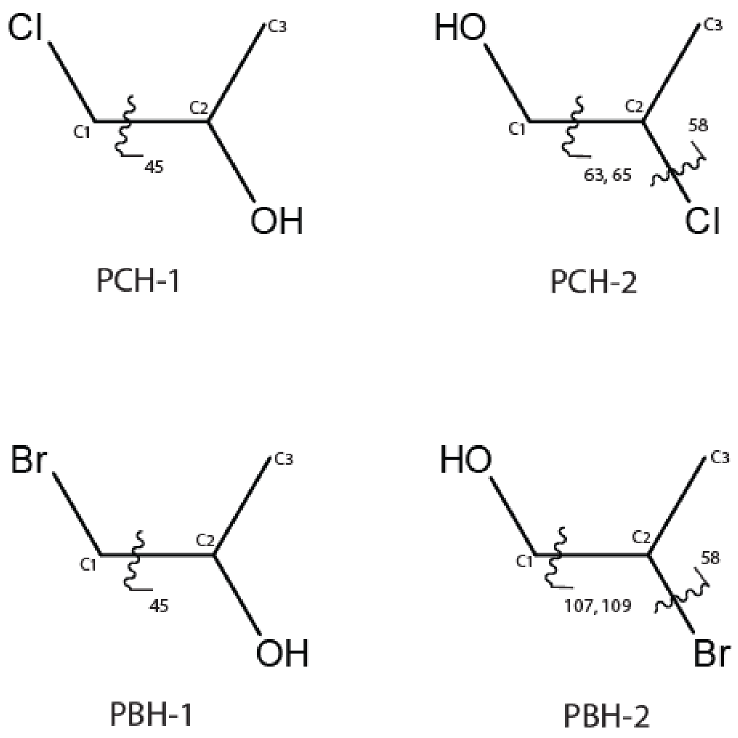


**Figure 1** Schematic of the prominent mass fragments in GC-EIMS spectra that were used to assign PCH isomers as well as PBH isomers to respective chromatographic peaks.

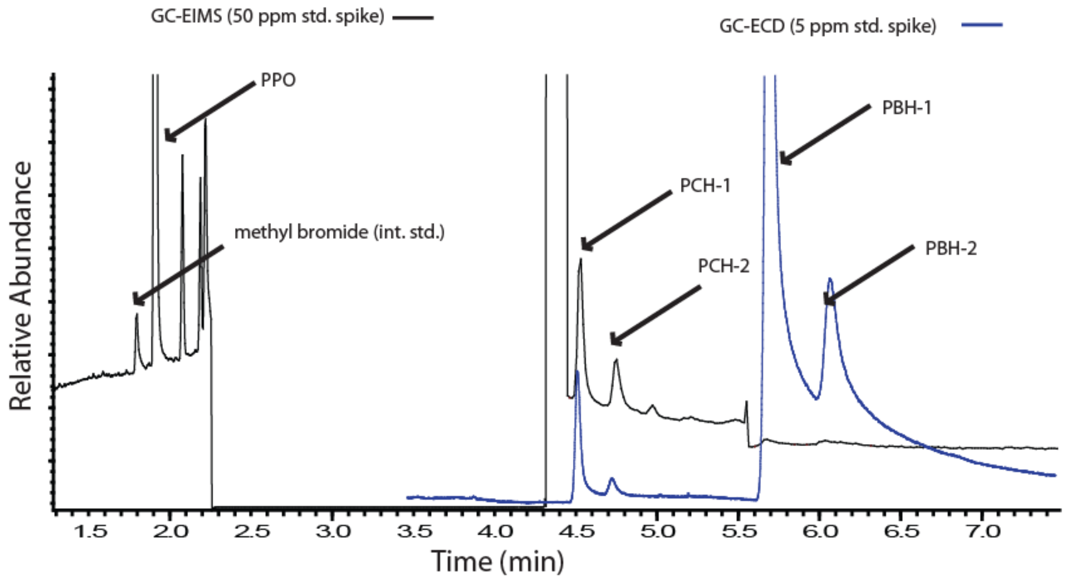


**Figure 2** GC-EIMS (*top trace*) and GC-ECD (*bottom trace*) detector responses to almond samples fortified (i.e., spiked) with analytical standards show chromatographic resolution between PPO, PCH-1, PCH-2, PBH-1, and PBH-2; GC-ECD was markedly more sensitive than GC-EIMS to the isomers of PBH.