Purge and Trap/Gas Chromatography/Mass Spectrometry Method for Determining Smoke Contamination of Foods and Packaging Materials

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Food and packaging materials from three separate fires were analyzed for volatiles by purge and trap/gas chromatography/mass spectrometry (PT/GC/MS) and compared to unexposed control samples. Concentrations of naphthalene and small alkyl-substituted (methyl, ethyl, dimethyl) naphthalene residues were consistently higher in the smoke odor containing foods than in the controls. Naphthalene and methylnaphthalene residues were used as indicators of smoke exposure in food and packaging materials. By using this method to analyze foods that had been exposed to smoke yet contained no organoleptically detectable smoke residues, it was shown that this PT/GC/MS naphthalene method is a more sensitive indicator of smoke exposure than is organoleptic evaluation.

Keywords: *Smoke; purge and trap; mass spectrometry; food safety*

INTRODUCTION

As a result of a fire in a food storage facility in December 1991, millions of pounds of food were potentially contaminated by smoke and/or volatilized chemicals. The U.S. Department of Agriculture (USDA) was concerned that volatilized chemicals may have contaminated food products and rendered them unfit for human consumption. Food products in this facility were embargoed by the Kansas Department of Health and Environment. All food products under the jurisdiction of the Food Safety Inspection Service were held for subsequent reinspection by the USDA. As there is a desire in the agency to supplement organoleptic inspection with scientifically valid analytical methods, the Food Safety Inspection Service, USDA, initiated studies to develop analytical methodologies that are more sensitive and precise than organoleptic screening to determine smoke contamination in food and food packaging materials.

Smoke contaminants such as phenols and polyaromatic compounds are usually extracted from foods and other products with organic solvents and subsequently separated chromatographically. However, coextracted fats often interfere with chromatography and must be removed via a cleanup step prior to GC/MS analysis (Lo and Sandi, 1978).

Excellent results were obtained in our laboratory with minimal sample preparation when contaminated samples were analyzed by purge and trap/gas chromatography/mass spectrometry (PT/GC/MS) and the resulting total ion chromatograms compared to those generated from the analyses of retail control samples. While there were elevated levels of many small alkyl and aromatic hydrocarbons in the smoke-exposed foods, naphthalene and methylnaphthalenes proved to be the residues that were consistently higher in the smoke-exposed foods. Also, the naphthalenes were more persistent during storage than were the more volatile, smaller hydrocarbons. Furthermore, even though naphthalenes are not a natural component of raw beef (MacLeod and Seyyedain-Ardebili, 1981), naphthalene has been shown to be a major constituent of smoke in meat and fish smoking plants (Hansen et al., 1992; Nordholm et al., 1986; Anderson et al., 1983) and naphthalene and methylnaphthalene residues have been used for forensic analyses of arson cases (Nowicki, 1990). To prove the ruggedness and widespread applicability of this method, samples of various smoke-exposed foods and packaging materials from the aforementioned food storage facility as well as two other food storage mishaps were subsequently analyzed by PT/GC/MS.

MATERIALS AND METHODS

Chemicals. The internal standard (deuterium labeled) chlorobenzene-d_5 and the analytical standards naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene were purchased from Aldrich Chemical Co. Pesticide grade methanol was obtained from Fisher Scientific. The internal standard stock solution was prepared to give a final concentration of 1 μg of chlorobenzene-d_5/μL of methanol. Naphthalene stock solution was prepared to give a final concentration of 1 mg of each naphthalene, 1-methylnaphthalene and 2-methylnaphthalene/μL of methanol. The internal standard working solution was prepared by diluting an aliquot of the internal standard stock solution to give a final concentration of 10 ng of chlorobenzene-d_5/μL of methanol. The naphthalene working solution was prepared by diluting an aliquot of the internal standard stock solution to give 10 ng of chlorobenzene-d_5 and 2 ng of each naphthalene/μL of methanol. Standard curve working solutions contained 10 ng of chlorobenzene-d_5 and 0.1, 1, or 10 ng of naphthalenes/μL of methanol. All four working solutions were used for the generation of the standard curve. All standard solutions were stored at <0 °C. Standards were permitted to warm to room temperature prior to use.

Sample Preparation. Samples were packed in glass jars with aluminum foil lined lids or double bagged in plastic bags and shipped frozen via overnight carrier. Positive controls, suspect samples, and negative controls were shipped in separate boxes. Positive controls were samples that had failed organoleptic screening; they possessed a smoky odor and/or

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visual smoke contamination. Suspect samples had been potentially exposed to smoke but passed organoleptic screening. Negative controls were never exposed to smoke and were frequently purchased at retail outlets or taken directly from production lines. Negative controls were as similar to the suspect samples as possible with respect to composition, packaging, source, and handling. All samples were received frozen at our laboratory, inspected, and stored at <0 °C. A 1-g aliquot of frozen food sample or a 0.5-g aliquot of frozen packaging material was cut into several small pieces and weighed into 25-mL disposable sample tubes (Tekmar Co.) that had been stored in a 200 °C oven overnight and cooled to room temperature. The aliquots of positive controls, suspect samples, and negative controls to be compared were as similar as possible with respect to composition, location in the sample package, and sample size. The sample was either analyzed immediately or the sample tube was capped with aluminum foil and stored at <0 °C until analyzed later that day. The remainder of the sample was returned to frozen storage as soon as possible.

Purge and Trap/Gas Chromatography/Mass Spectrometry. A disposable 25-mL sample tube containing either 10 μL of one of the working solutions or an aliquot of one of the samples and 10 μL of the internal standard working solution was placed on the Tekmar LSC 2000 purge and trap unit. Purge and trap analysis was accomplished using a standby temperature of 30 °C, 38 psi purge pressure, 3-min preheat time, 12-min sample purge at 100 °C, and 225 °C transfer line temperature. Recovered volatiles were then separated on a Varian Model 3400 gas chromatograph equipped with a jet separator and a 75 m × 0.53 mm i.d. (3-μm film thickness) DB624 column (J&W Scientific). Helium was used as a carrier gas at a head pressure of 24 psi at 40 °C. Both injector and transfer line temperatures were 225 °C. Separation of a broad spectrum of volatiles was accomplished by holding the column temperature at 40 °C for 10 min, heating to 225 °C at 5 °C/min, and holding at 225 °C for 2 min. The separated volatiles were analyzed on a Finnigan ITS 40 ion trap mass spectrometer in the electron impact ionization mode using a scan range of 35–350 amu, 1.5 s/scan, a 40-min acquisition time, and a 6-min filament/multiplier delay. The instrument was autotuned at 100 °C to typically give a multiplier voltage of 1300 V with a target value of 10 000. Perfluorotributylamine was used for mass calibration at m/z 69, 131, 219, 264, 414, 502, and 614.

Data Analysis. A standard curve was generated by analysis of 10-μL aliquots of each of the standard working solutions and plotting (area of the base ion)/(area of m/z 117 for internal standard) vs nanograms of analyte. A daily instrument check was accomplished by analyzing 10 μL of the naphthalene working solution and verifying the response to be within 25% of that predicted by the linear regression analysis of the standard curve. A total ion chromatogram and reconstructed ion chromatograms were generated for each analysis. Retention times and mass spectra of the analytes of interest from the analysis of the food and packaging samples were compared with those generated from the daily instrument check. Positive confirmations required a retention time match of ±0.5% and a match of relative intensity of the three most prevalent ions ±20%. To minimize carry-over of the less volatile analytes, a system blank containing only 10 μL of internal standard working solution was analyzed after each sample. If the carry-over represented more than 5 ng of naphthalene or 10 ng of methylnaphthalene, then additional system blanks were run until the carry-over levels were acceptable.

Two different statistical approaches were used to determine if the concentrations of naphthalenes in suspect samples were indicative of smoke exposure. Smoke exposure was indicated by a suspect mean total naphthalene concentration that was significantly greater than the control mean total naphthalene concentration (t-test, $\alpha = 0.05$). Smoke exposure was also indicated when any suspect sample contained a total naphthalene concentration that was greater than the mean plus three standard deviations of the total naphthalene concentration of the controls ($\alpha = 0.001$).

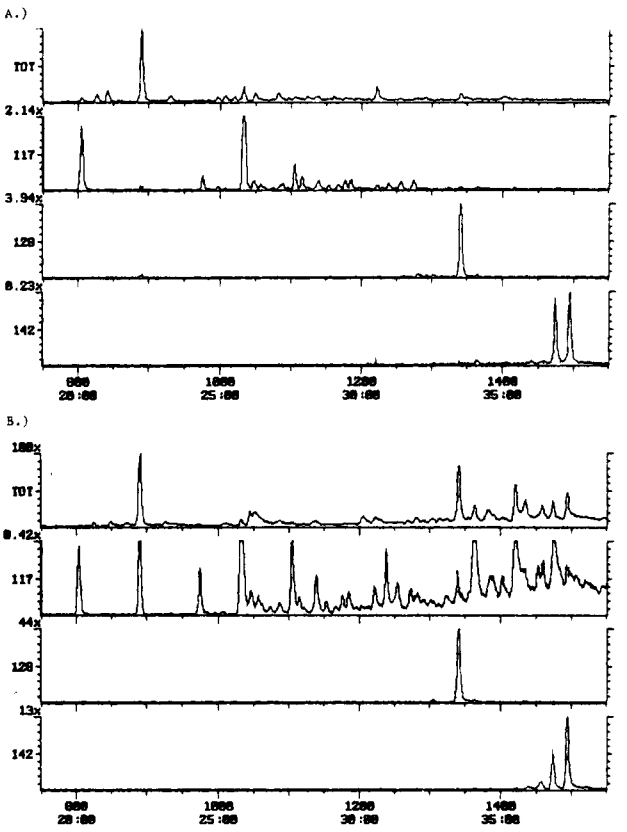

Figure 1. Total and reconstructed ion chromatograms of (A) 1 g of beef from fire-damaged container and (B) 0.5 g of fire-damaged cardboard packaging. Retention times: chlorobenzene-d_5 = 20.1 min; naphthalene = 33.1 min; 1-methylnaphthalene = 37.3 min; 2-methylnaphthalene = 36.9 min.

Table 1. Retention Times and Mass Spectral Information for Smoke Exposure Marker Compounds

compound	Rt (min)	m/z (relative intensity)
chlorobenzene-d_5	20.3	117 (100), 99 (47.0), 119 (32.0)
naphthalene	33.1	128 (100), 102 (13.0), 126 (7.6)
1-methylnaphthalene	37.3	142 (100), 141 (81.5), 115 (63.7)
2-methylnaphthalene	36.8	142 (100), 141 (81.7), 115 (59.7)

RESULTS AND DISCUSSION

The internal standard method for quantification of naphthalene and 1-methyl- and 2-methylnaphthalene residues proved to be linear (1–100 ng/analysis range) as the correlation coefficient as calculated by the linear regression analysis of the standard curves for each analyte was ≥0.995. Triplicate analyses of identical samples indicate that the standard deviation for recoveries is less than 10%. Absolute recoveries ranged from 60 to 70% for the internal standard as well as the naphthalenes. Figure 1 shows both the total ion and extracted ion chromatograms from the analysis of a 1-g sample of smoke-exposed beef and a 0.5-g sample of its cardboard package. The beef contained 162 ng of naphthalene and 44 ng of methylnaphthalene/g, while the cardboard box contained approximately 21 μg of naphthalene and 10 000 μg of methylnaphthalene/g. The minimum detectable quantity is 0.5 ng of naphthalene and 1 ng of methylnaphthalene. Though the total ion chromatograms indicate the presence of many constituents, the naphthalene and methylnaphthalene peaks are easily distinguished on the extracted ion chromatograms. Table 1 lists the retention times and relative ion intensities for the smoke exposure marker compounds as found in the standard working solution. As shown in Figure 2, the significant ions for naphthalene

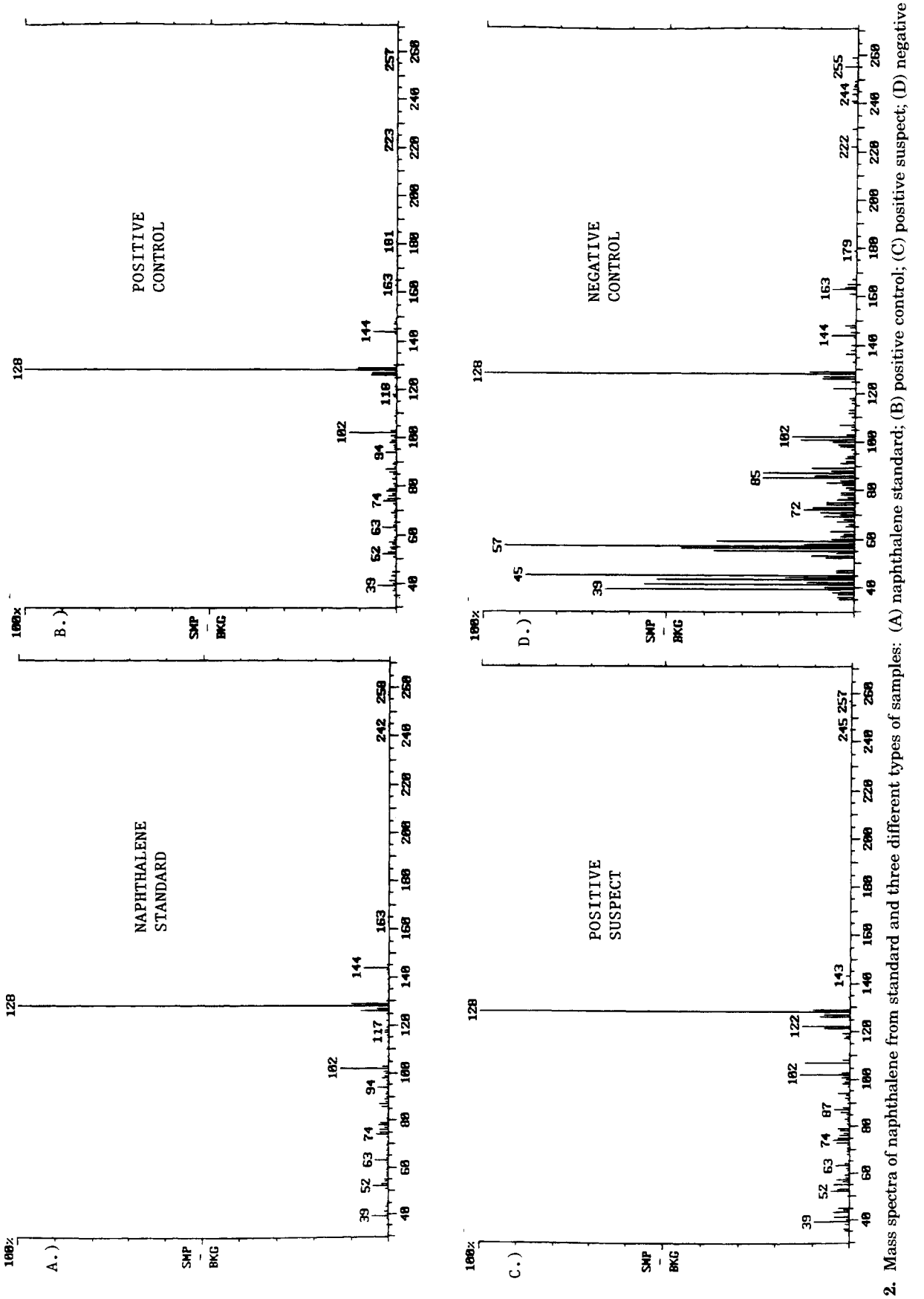

Figure 2. Mass spectra of naphthalene from standard and three different types of samples: (A) naphthalene standard; (B) positive control; (C) positive suspect; (D) negative control.

Table 2. Naphthalene Residues in Smoke-Exposed Food and Packaging from Food Storage Facility and Retail Control Samples

sample[a]	ppb of naphthalene	ppb of Me-naphthalene	comments
boneless turkey breast (S)	5	1	package odor
boneless turkey breast (C)	4	0	package odor
young turkey breast (S)	24	10	no odor
young turkey breast (C)	17	6	no odor
smoked chicken (S)	20	36	package odor
smoked chicken (C)	5	13	package odor
boneless beef (S)	9	3	no odor
boneless beef (C)	5	2	no odor
cooked beef (S)	4	2	package odor
cooked beef (C)	3	2	package odor
corned beef (S)	4	3	package odor
corned beef (C)	3	2	package odor
ice (S)	2	3	smoky odor
ice (C)	1	0	smoky odor
TV dinner box (P)	4200	1300	strong smoky odor
TV dinner box (C)	140	130	strong smoky odor
cardboard box (P)	95	250	slight smoky odor
cardboard box (C)	35	63	slight smoky odor
hot dog (P)	67	28	faint smoky odor
hot dog (C)	25	5	faint smoky odor
fried chicken (P)	300	66	moderate smoky odor
fried chicken (C)	26	27	moderate smoky odor

[a] (S) suspect; (C) control; (P) positive.

(m/z 128, 102, and 126) are evident in the mass spectra from a standard, positive control, suspect sample, and negative control. The spectra for methylnaphthalenes are of similar intensity. It is quite evident from the chromatograms and mass spectra that this PT/GC/MS technique can detect low levels of the smoke exposure marker compounds, naphthalene and methylnaphthalenes, in complex matrices, even in the presence of significant amounts of fat.

Table 2 lists the marker compound concentrations for a variety of suspect and known positive samples from the food storage cave and their retail control samples. In every case the naphthalene and methylnaphthalene residue levels are lower in the control samples. Again, the difference between the known positives and their controls is greater than the difference between the suspect samples and their controls. As indicated by the low levels of naphthalenes in most of the control samples we analyzed and by the appreciably higher levels in control packaging (Tables 2–5), it appears that the migration of these compounds from packaging to food is responsible for the background levels of naphthalenes. Thus, to determine whether a suspect sample has been contaminated by smoke, it is essential to compare its analytic results with those from the analysis of an appropriate control sample. The samples presented in Table 2 were also analyzed by another laboratory and the results agreed within ±10% for naphthalene and 45% for methylnaphthalene. We believe these differences are quite acceptable as these were nonhomogeneous smoke-contaminated real samples rather than artificially prepared fortified blanks.

Table 3 lists the naphthalene and methylnaphthalene concentrations for a commercial chicken fajita microwave dinner type product that was recovered from the smoke-damaged food storage facility. The outer coated cardboard package was visibly smoke damaged. The food was contained in an inner plastic type bag. The data show that smoke penetration was hindered by the packaging as the marker compound concentrations were lowest in the food, intermediate in the middle wrapper, and highest in the outer coated cardboard box. Comparison of the naphthalene and methylnaphthalene marker levels in the suspect (25.5 and 7.8 ppb) vs control (3.4 and 2.8 ppb) food suggests that smoke penetrated the packaging and contaminated the food, even though the food appeared to be uncontaminated.

Table 3. Naphthalene Residues in Smoke-Damaged Packaged Food vs Control

sample[a]	ppb of naphthalene	ppb of Me-naphthalene
external cardboard box (S)	3700	1800
external cardboard box (C)	170	170
inner plastic wrapper (S)	200	27
inner plastic wrapper (C)	19	6
chicken (S)	26	8
chicken (C)	3	3

[a] (S) suspect; (C) control.

Table 4 lists naphthalene and methylnaphthalene concentrations for samples of beef and its packaging materials that were being transported in a commercial trailer and potentially exposed to smoke as a result of a transportation mishap. Samples that smelled of smoke or visually appeared to contain a smoke residue were used as positive controls. Suspect samples looked and smelled normal. The controls came from a different shipment that was not exposed to smoke. The levels of the marker compounds are higher in these control samples than in the control samples in Table 3 because these samples were shipped in plastic bags rather than glass. The data presented in Table 4 show that the levels of marker compounds for all samples were highest in the known positives, intermediate in the suspects, and lowest in the control samples. These data show that the residue levels for both naphthalene and methylnaphthalenes were highest in the external cardboard

Table 4. Naphthalene and Methylnaphthalene Residues in Beef and Packaging from Transportation Mishap

sample[a]	ppb of naphthalene	ppb of Me-naphthalene
external cardboard box (P)	12000	10000
external cardboard box (S)	850	400
external cardboard box (C)	440	190
inner plastic liner (P)	340	450
inner plastic liner (S)	270	260
inner plastic liner (C)	120	65
beef (P)[b]	140	120
beef (S)[c]	56	41
beef (C)[b]	26	26

[a] (P) positive; (S) suspect; (C) control. [b] Mean of five samples. [c] Mean of 20 samples.

Table 5. Naphthalene and Methylnaphthalene Residues in Breaded Chicken Patties and Packaging from Fire in Frozen Storage Area

sample	ppb of naphthalene	ppb of Me-naphthalene	comments
external cardboard	160	82	
inner wrapper	130	70	less damaged[a]
chicken patty	6	5	
external cardboard	1200	810	
inner wrapper	410	54	somewhat damaged[b]
chicken patty	20	10	
external cardboard	3200	990	
inner wrapper	1400	430	most damaged[c]
chicken patty	170	83	

[a] External cardboard contained a slight smoke odor. [b] External cardboard contained strong smoke odor. [c] External cardboard contained visual smoke residue, inner cardboard had strong smoke odor, and chicken patty contained slight smoke odor.

box, intermediate in the middle plastic liner, and lowest in the beef. While the packaging afforded some protection against smoke damage, the mean residue levels in the suspect and positive beef are significantly greater (one-tailed t-test, $\alpha = 0.025$) than the controls and indicate that these samples were smoke-contaminated even though they passed the organoleptic screening.

Table 5 lists the naphthalene and methylnaphthalene concentrations in breaded chicken patties and its packaging materials that had been exposed to smoke as the result of a different fire in a food cold storage unit. The trends are consistent with the analytical results from the other smoke-exposed food and packaging materials. Naphthalene concentrations tend to be higher than methylnaphthalene concentrations. The smoke marker residue levels increased as the level of visible smoke damage increased, and residue levels are highest in the outer cardboard packaging and lowest in the food.

While the chemical composition of smoke is affected by many variables, including nature of the combustible material, moisture, temperature, and air supply (Toth and Potthast, 1984; Tilgner and Daun, 1969), naphthalene and methylnaphthalenes proved to be excellent indicators of smoke contamination in a variety of foods and packaging materials from three different fires. It was shown that smoke can penetrate both cardboard and plastic food-packaging materials and contaminate foods. This PT/GC/MS method detected smoke contamination at low levels for which no organoleptic evidence was noted. Thus, this method is more sensitive than organoleptic screening and may offer a greater degree of consumer safety if used for screening foods that may have been exposed to undesirable chemicals as a result of exposure to smoke and/or fire.

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Registry No. Supplied by the Author: Chlorobenzene-d_5, 3114-55-4; naphthalene, 91-20-3; 1-methylnaphthalene, 90-12-0; 2-methylnaphthalene, 91-57-6.

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