Determination of Styrene in Selected Foods

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Styrene levels in 12 commodities were determined. The foods tested were wheat, oats, peanuts, pecans, coffee beans, tomatoes, peaches, strawberries, cinnamon, beef, chicken, and milk. The samples were collected in a manner that avoided contact with styrene or any type of plastic. Analytical measurements were performed using a dynamic heated headspace purge-and-trap extraction technique followed by quantification by selected ion monitoring capillary gas chromatography/mass spectrometry. Method blanks, duplicate samples, and samples fortified with benzene and styrene were used to assess method performance. The limit of detection of the method varied between food types but was generally less than 2 ng/g for styrene. The highest measured concentrations of styrene were found in cinnamon (169–39 200 ng/g). Styrene concentrations in beef samples ranged from 5.25 to 7.85 ng/g and in coffee beans from 1.57 to 7.85 ng/g. Wheat, pecans, oats, strawberries, and peaches showed no styrene concentrations greater than 3 ng/g. No detectable styrene was found in tomatoes, milk, or chicken.

Keywords: *Styrene; packaged food; analysis; GC/MS*

INTRODUCTION

Styrene was first discovered in 1827 via the pyrolytic decarboxylation of organic acids of Storax balsam. Storax (styrax) is a pathological exudate produced in sapwood (balsam) and bark tissues of the trees *Liquidambar orientalis* and *Liquidambar styraciflua* (Fenaroli, 1971).

Since that time, styrene has been identified as a natural constituent in a wide variety of foods and beverages (Maarse, 1992). The formation of styrene in foods and beverages can occur in several ways. It can be formed, for example, by bacteria in mold-ripened cheese, during the storage of grain (Wilkins and Scholl, 1989; Wasowicz and Kaninski, 1988), or during the fermentation of grapes. The following having been suggested as possible precursors of styrene in food products: carotenoids (MacLeod and Cave, 1975; Adda et al., 1989; Johnson et al., 1969); long-chain hydrocarbons (Min et al., 1977); fatty acids, shikimic acid (Ducruet, 1984); methyl arachidonate (Taylor and Mottram, 1990); 2-phenylethanol (Maarse and Visscher, 1989, 1990, 1991); glucose and phenylalanine (Westphal and Cieslik, 1981); and aldehydes (Min et al., 1977; Maarse and Visscher, 1991). Since styrene is structurally similar to a wide variety of flavorant molecules that occur naturally (e.g., cinnamic acid, cinnamic aldehyde, cinnamyl acetate, cinnamyl alcohol, cinnamyl benzoate, and cinnamyl cinnamate), it is also possible that styrene can be formed during the biodegradation of those substances.

The French scientist Pierre Berthelot discovered a method for making styrene synthetically early in the 19th century, and by the early 1900s, styrene was recognized as an impurity of industrial processes using coal tar and petroleum cracking. Styrene was commercially unimportant until World War II, when the U.S. government initiated a major program to make synthetic rubber. Since that time, styrene has become an important commodity chemical used in the manufacture of numerous types of plastics, glass fiber-reinforced resins, protective coatings, and ion-exchange resins in addition to synthetic rubber. Styrene is also an FDA-approved flavoring agent used, for example, in ice cream and candy. Approximately two-thirds of styrene monomer production is used in the manufacture of polystyrene. In the United States, polystyrene is used extensively in food packaging or food service applications.

In recent years, questions have been raised about trace levels of styrene monomer in foods or beverages that have been in contact with polystyrene packaging. It is, therefore, important to determine the extent to which styrene occurs in raw agricultural commodities that have never been in contact with styrene-based polymers, so that a more thorough understanding of the sources and significance of very small quantities of styrene monomer in the diet may be determined.

The purpose of this study was to determine the amount of styrene present in several important raw agricultural commodities. Particular care was taken in sample collection to ensure that only indigenous styrene was measured. This involved the collection of samples at the production site where possible and the use of collection, handling, and shipping procedures designed to avoid contact with styrene-containing materials.

MATERIALS AND METHODS

The determination of levels of styrene in the food samples was conducted using an isotope dilution technique and heated

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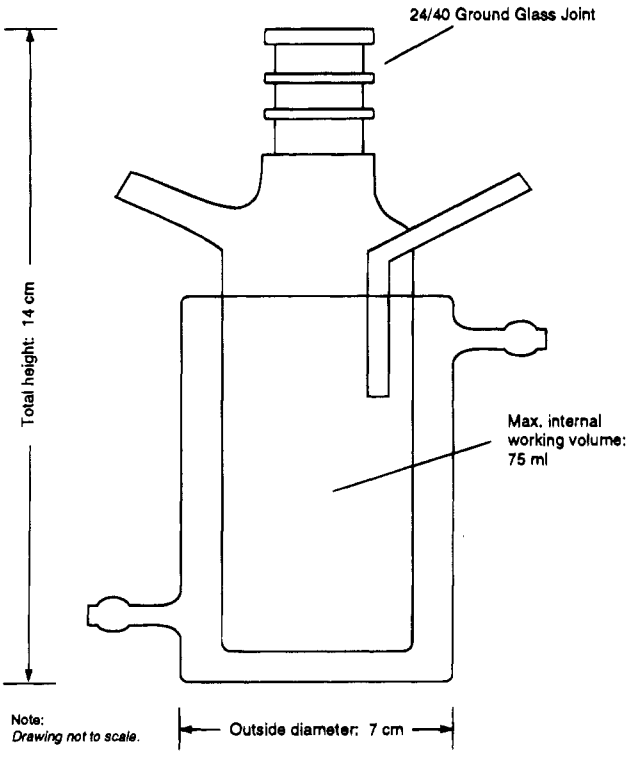

Figure 1. Modified Wheaton purge vessel.

dynamic headspace purge-and-trap/capillary gas chromatography/selected ion monitoring mass spectrometry. Quantification was performed by internal standardization with deuterated analogs of benzene and styrene.

Upon receipt, samples were placed in a freezer and stored frozen until analysis. Samples were prepared for analysis by homogenizing them in a glass blender with a measured amount of volatile-free water. A weighed aliquot of the homogenized mixture was added to specially designed purge vessels. The design of this vessel is shown in Figure 1. Deuterated styrene was added to serve as an internal quantitation standard. All sample preparation activities were conducted in a volatile-free laboratory dedicated to the preparation of samples for trace volatiles analysis. The prepared samples were transported to the GC/MS facility in an ice-filled cooler and analyzed within 2 h of preparation.

A schematic drawing of the analysis system is shown in Figure 2. Collection of the styrene in the prepared samples was accomplished by heating the purge vessel and continuously sweeping the headspace with nitrogen. Volatiles in the headspace were collected on an absorbent trap. Following the purge-and-trap cycle, the trap was thermally desorbed into the GC/MS system, where chromatographic separation and quantitation of the analytes were performed. Comparison of the areas of native analyte peaks to the internal standard peaks was used for quantification. Absolute recoveries of the internal standards were determined using a recovery standard (ethylbenzene-d_{10}) added to the purge tower immediately prior to sample analysis.

Sample Collection. Twelve agricultural commodities were collected by Stewart Agricultural Research Services (Macon, MO) from typical production sites. The commodities collected were cinnamon, coffee beans, strawberries, peanuts, pecans, wheat, oats, tomatoes, peaches, raw milk, ground beef, and chicken. The samples were collected from a total of eight states in the United States, as well as from one country in South America. At least three different samples were taken for each commodity. Two subsamples, designated A and B, were collected for each of these samples. The samples collected for strawberries, peanuts, pecans, wheat, oats, tomatoes, and peaches were each taken from different states. Imported coffee beans and cinnamon were collected in the United States from sites of market distribution. Samples collected in the United States for cinnamon, coffee, raw milk, ground beef, and chicken were each taken in one state. Two coffee samples from different growers were collected in Colombia, South America. Different varieties were sampled for imported cinnamon and coffee. While different breeds of dairy cattle were used for milk samples, only one breed of cattle was sampled for the ground beef. In addition, only one chicken breed was sampled.

The samples were collected in a manner that avoided contact with plastic or other potential sources of styrene. All samples were collected solely by hand or by machine (metal) and then by hand. Each sample was placed in a 1-L clean glass jar to prevent any potential contact with plastic or styrene. After collection, the samples were frozen (except the South American coffee samples) and shipped to the laboratory by refrigerated truck. Upon receipt, the samples were inventoried, checked for breakage, assigned sample numbers, sealed with evidence tape, and stored in a locked freezer in a restricted-access laboratory. All samples were received in good condition, with the exception of one sample of chicken, which arrived in a broken container and was discarded.

Sample Preparation. The variety of samples resulted in different handling. Sample handling was kept to a minimum to avoid compromising sample integrity. Coffee beans, peanuts, wheat, ground beef, and oats were drawn from the center of the container and were analyzed whole. The strawberries and pecans were analyzed by removing one or two berries or nuts from the containers. Approximately 10 g of each was processed by grinding and included stems, shells, etc. The tomatoes and peaches were pulled from the container and sliced through the center to obtain the appropriate weight. The peach pit was not included in the sample. The raw milk was shaken before an aliquot was removed for analysis. The chicken was analyzed by slicing through the skin, meat, and bone to obtain the sample; however, no cartilage from the end bones was used. The samples that required thawing (milk, ground beef, and chicken) before sampling were placed in an ice-filled cooler and allowed to thaw under these conditions until sampling could take place.

Approximately 20 g of the solid samples was weighed and placed in a blender. A weighed amount of volatile-free water was added to facilitate blending. The amount of water added depended on the food type and ranged from 40 to 70 mL. Each sample was blended until a finely divided and visually homogeneous mixture was obtained. The time required for homogenization ranged from 30 to 60 s, depending on the food type. Aliquots of the homogenized mixtures representing 10 g of sample were accurately weighed directly into a custom purge vessel (see Figure 1). Milk, which was the only liquid sample, was shaken manually for approximately 30 s and added directly to the purge vessel. Due to the high levels of styrene present in the cinnamon samples, 10-mg aliquots were taken for analysis of styrene in these samples. Benzene levels in cinnamon were measured using 20 g of sample.

Each sample was spiked with 50 ng of each internal standard, yielding internal standard concentrations corresponding to 5 ng/g of sample. A Teflon-coated stir bar was added, and the purge vessel was immediately sealed, placed in an ice-filled cooler, and analyzed within 2 h of preparation.

For spiked samples, approximately 40 g of sample was transferred to the blender for homogenization. The sample was homogenized, and two individual 10-g aliquots were prepared. One of the aliquots was analyzed immediately to determine the native levels of benzene and styrene in the sample. The second 10-g aliquot was then spiked with both analytes at approximately twice the level of the analyte with the highest concentration in the unspiked sample.

A laboratory method blank was prepared for each analysis day and each commodity type by blending volatile-free water for the same time as for the samples (30–60 s), transferring an appropriate amount of the water to the purge vessel and adding internal standards at the same levels as for the samples. The amount of water used to prepare the method

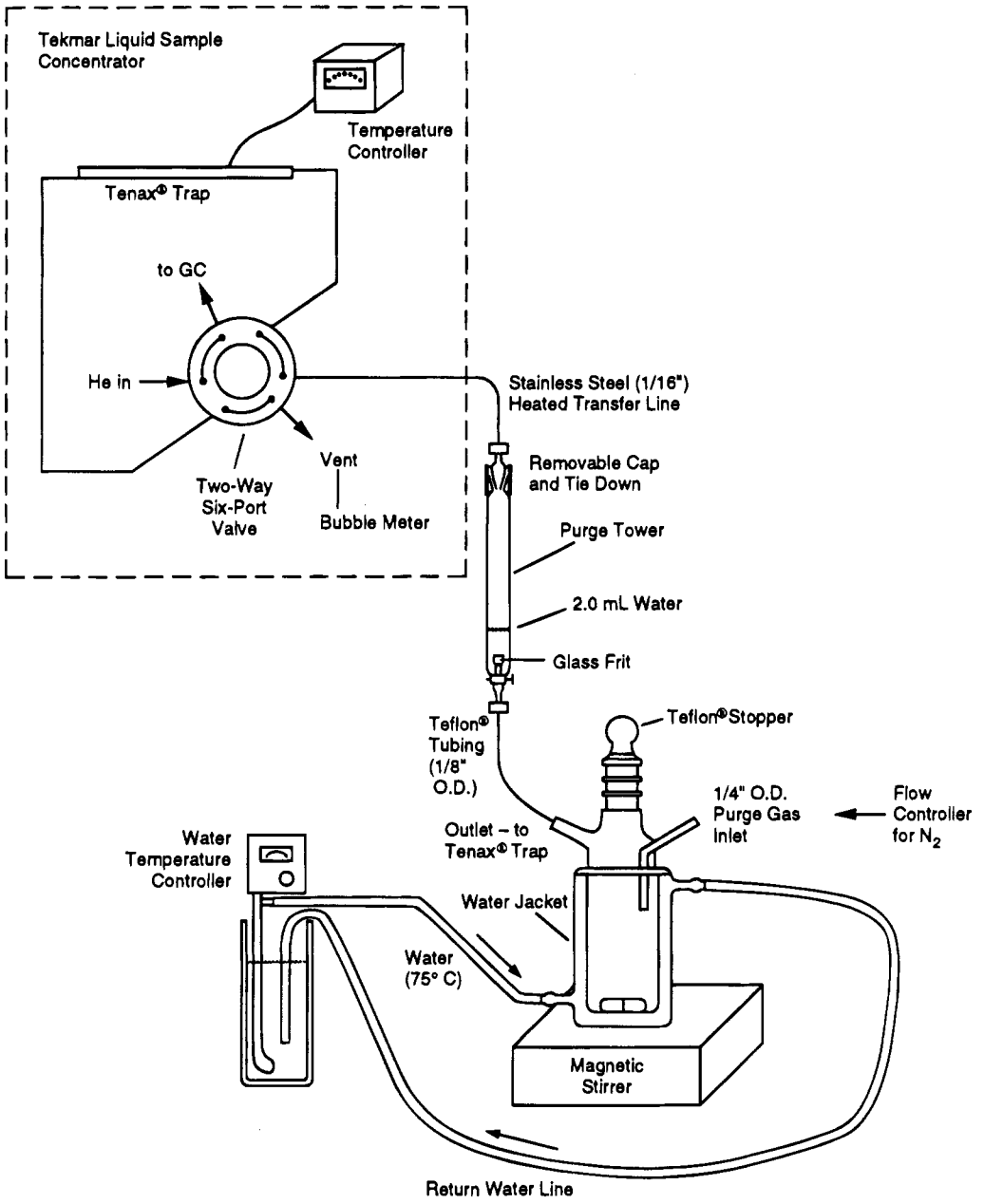

Figure 2. Heated dynamic headspace purge and trap system.

blank for each commodity type was the same as that used to prepare the commodity for analysis.

Sample Analyses. A dynamic heated headspace purge-and-trap procedure was used to purge volatile organic compounds from the samples and transfer them into a gas chromatograph/mass spectrometer (GC/MS) for analysis. The GC/MS system (Hewlett-Packard Model 5890 GC/Finnigan OWA 1050 MS) was operated in the selected ion monitoring mode and was calibrated over a range of 1–500 ng for styrene, representing levels in the food products of 0.1–50 ng/g, based on analysis of 10-g aliquots of samples. Instrument calibration was performed by adding standards to the purge tower, along with ethylbenzene-d_{10} as a recovery standard. Relative response factors were calculated for styrene using the deuterated analog (styrene-d_8) as the internal standard. The response factor for the internal standard was used to calculate absolute internal standard recoveries during sample analysis. Internal standard recoveries varied according to food types. Peanuts, pecans, and coffee beans typically exhibited low internal standard recoveries which ranged from 9% to 53%. The internal standard recoveries for grains and meats ranged from 21% to 65%. All other commodities (fruits, vegetables, and milk) had internal standard recoveries ranging from 72% to 112%. The method detection limit is based on the sample size and internal standard recovery as well as the instrumental response for styrene. Samples that yielded low internal standard recoveries and no responses for styrene resulted in higher detection limits than the value based on the calibration data. The data for samples that exhibited low recoveries of the internal standard and quantitative measures of styrene, however, are considered accurate measures of concentration as a result of the isotope dilution quantitative method.

Upon completion of the setup and calibration procedures, 50 ng of the system recovery standard was added to the purge tower. The purge vessel containing the previously prepared samples was attached to the purge-and-trap device. The stirred sample was heated to approximately 75 °C and purged for 20 min. Upon completion of the purge cycle, the sorbent trap was rapidly heated to approximately 180 °C. The desorbed analytes were directed onto the GC column for separation and mass spectral detection. Identification of the target analytes was accomplished by comparing peak areas of the ions monitored (abundance ratios) and retention times of the ions monitored and retention times of peaks in the samples with those of the corresponding internal standards. The primary and secondary ions monitored for styrene were

Table 1. Sample Analysis Results

food type	origin	styrene (ng/g)	% rec styrene-d_8	food type	origin	styrene (ng/g)	% rec styrene-d_8
wheat	Missouri	0.442	63	pecans	Missouri	ND (2.56)	9
		0.576	56			ND (1.66)	14
	Oregon	1.71	22		Georgia	ND (1.25)	19
		3.52	57			ND (1.24)	19
	New York	0.484	30		Texas	ND (1.58)	15
		0.529	65			ND (1.79)	13
peanuts	Texas	2.14	22	strawberries	Oregon	2.54	98
		2.17 mpc[a]	16			2.78	84
	Georgia	1.20	29		Washington	0.770	94
		3.36	40			3.09	90
	Oklahoma	1.02	20		California	0.397	98
		1.18	21			0.370	106
coffee beans	imported	6.44	30	cinnamon	China[e]	2330	101
		6.18	41			2720	102
	Colombia, South America[b]	1.82	46		Sri Lanka[f]	169[h]	96
		1.57	30			175[h]	105
		4.79	49		Indonesia[g]	39200	94
	Colombia, South America	1.88	53			36500	94
		1.90	36	milk	Missouri	ND (0.44)	72
	Colombia, South America	1.99	46			ND (0.41)	81
		1.99	41		Missouri	ND (0.40)	84
	Colombia, South America	7.14	32			ND (0.38)	89
		3.55	52		Missouri	ND (0.44)	73
						ND (0.43)	74
tomatoes	Missouri	ND (0.21)[c]	96				
		ND (0.22)	90	beef	Missouri	6.35	39
	Oregon	ND (0.21)	96			5.25	52
		ND (0.23)	66		Missouri	6.07	40
	New York	ND (0.22)	93			5.91	37
		ND (0.19)	103		Missouri	7.85	38
						5.42	43
peaches	Georgia	ND (0.20)	91				
		ND (0.21)	82	chicken	Missouri	ND (0.95)	24
	New York	ND (0.19)	96			ND (0.68)	34
		ND (0.18)	102		Missouri	ND (0.94)	24
	Oregon	0.233	112			ND (1.07)	21
		0.302	101				
oats	New York[d]	0.568	49				
		1.24	29				
	New York[d]	ND (0.79)	26				
		ND (0.77)	31				
	Oregon	ND (0.65)	32				
		1.64	48				
	Missouri	0.827	34				
		0.870	35				

[a] mpc, maximum possible concentration. Response noted for quantitation ion at correct retention time, however, ion ratio with other characteristic ion did not meet qualitative criteria. [b] Three jars of this commodity received and analyzed. [c] ND, not detected. Value in parentheses is the estimated detection limit based on actual sample size and lowest calibration point. [d] Two jars of each received. [e] *Cinnamomum cassia* Tung Hing from China. [f] *Cinnamomum cassia* Zeylanicum from Sri Lanka. [g] *Cinnamomum cassia* Korintji, grade A, from Indonesia. [h] Ion abundance ratio out of criteria for SIM but confirmed with full-scan MS.

m/z 104 and 78, respectively. For deuterated styrene, the primary and secondary ions were m/z 112 and 84, respectively.

Quantitative determination of peaks that passed the identification criteria was conducted using the relative response factors obtained during instrument calibration, the integrated area counts of the quantitation ions for the target analytes, and the internal standards obtained during sample analysis. A final sample concentration was obtained by dividing the mass of analyte found by the mass of sample taken for analysis.

Limits of detection for each individual food type were calculated at 3 times the concentration of styrene found in the laboratory method blanks. Generally, the limits of detection were less than 2 ng/g. Method precision was determined by analyzing duplicate samples for each food type. Method precision, calculated as the relative percent difference between the two samples, ranged from 3.84% for wheat to 28.9% for beef. These duplicate analyses were performed at levels approaching the limit of detection, where the analytical method can be expected to show the greatest variability.

Method accuracy was demonstrated through the analysis of samples fortified with styrene for each food type. Accuracy, expressed as percent recovery, ranged from 59% to 126%.

RESULTS

The results of the analysis of select food types are given in Table 1. Detectable levels of styrene were found in all food types with the exception of tomatoes, pecans, milk, and chicken. The highest levels of styrene were found in samples of cinnamon, for which concentrations ranging from 169 to 39 200 ng/g were measured. Beef samples showed styrene concentrations ranging from 5.25 to 7.85 ng/g, and coffee beans had styrene concentrations ranging from 1.57 to 7.14 ng/g. Wheat, peanuts, oats, and strawberries had lower styrene concentrations, with no samples showing levels above 3 ng/g. Styrene was detected in only one of three samples of peaches at a concentration of approximately 0.2–0.3 ng/g.

Due to the high levels of styrene measured in cinnamon, full-scan mass spectra were obtained to confirm the presence of styrene in the samples. A spectrum of the peak identified as styrene in a cinnamon sample was consistent with a literature spectrum of styrene. Coelution with the internal standard and the mass spectrum obtained confirmed the identity of this peak as styrene.

DISCUSSION

Styrene was detected in 8 of 12 of the selected food types. The presence of styrene in the raw agricultural commodites could not be due to migration from packaging since specific measures were taken to ensure that the food samples were not in contact with styrene or any type of styrene-based polymer or copolymer.

High concentrations of styrene (up to 39 200 ng/g) were found in the cinnamon samples. This result is not surprising in view of the close structural similarity between styrene and cinnamic aldehyde, the principal constituent of cinnamon flavoring.

The results of this study indicate that styrene may be a natural constituent of many foods. The occurrence of styrene in processed food cannot, therefore, be automatically assumed to be related to contact with styrene-based polymers. This fact must be taken into consideration when the origin and significance of low levels of styrene monomer in the diet are determined.

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