Two Methods to Determine If Styrene Monomer Is Present in Milk

by J. W. FINLEY and J. C. WHITE
Department of Food Science
Cornell University
Ithaca, New York

Since the development of polystyrene food cartons, much interest has been aroused by various regulatory agencies concerning the possibility of residual styrene monomer in food. Polystyrene containers often have a pronounced odor of monostyrene, and we became concerned as to the amount of monostyrene which could be detected in milk purchased in polystyrene containers. For this reason a technique was developed to analyze for trace amounts of styrene monomer in milk after storage in polystyrene cartons.

Here are described two techniques for this analysis, one with a sensitivity of five parts per million and the other a sensitivity of 0.01 parts per million.

Styrene monomer was obtained by distillation at 145°C from polystyrene. The styrene monomer was then triple redistilled on a fractionating column. Standard samples were purified by preparatory gas chromatography on an Aerograph Autoprep. Conditions for preparative chromatography were: column temperature 150°C, Helium flow 100 ml per min on a 3/8 in diameter, 20 ft stainless steel column packed with 20% carbowax 20M on chromosorb W. Collection bottles were cooled by ice water in a Dewar flask at the collection port. This standard was dissolved in methylene chloride and

Bulletin of Environmental Contamination & Toxicology,
Vol. 2, No. 1, 1967, published by Springer-Verlag New York Inc.

injected into an analytical gas chromatograph, an F & M model 609 electrometer equipped with a model 400 oven and a flame ionization detector. Conditions of chromatography were, for all samples and standards, an oven temperature of 100°C with a helium flow of 60 ml per min on a six ft glass column packed with 20% carbowax 20M plus 2.5% KOH on 60-80 mesh Gas chrom R. At all times the injection volume was five microliters. All solvents were redistilled on a fractionating column to assure a high degree of purity.

Method I

To prepare samples for chromatographic analysis, milk was first inoculated with concentrations of styrene monomer ranging from 100 ppm to 0.1 ppm. Two hundred milliliter aliquots of these milk samples were saturated with ammonium sulfate and centrifuged to separate the bulk water phase. The water was then removed by filtration; the sample was heated to 50°C for 15 minutes and cooled to room temperature. Upon cooling, 50 ml of 95% ethanol were added to the curd and thoroughly mixed in. The slurry was then centrifuged and a 25 ml sample of the alcoholic phase was pipetted with a pipette equipped with a U-shaped tip. To this 10 ml of 0.1 N alcoholic KOH was added and the solution was brought to boiling for 10 minutes. Next 20 ml of water and 2 ml of methylene chloride were added. The methylene chloride was allowed to separate and the solution was injected into the F & M gas chromatograph to detect the styrene recovery.

Results showed that styrene can be detected at levels as low as 5 ppm and a linear response at all levels between 100 ppm and 5 ppm. Below this level, detection is possible but the degree of linearity is open to question in that the peak height at 5 ppm is only 3% of the recorder scale at maximum sensitivity.

Method II

Standard solutions of milk containing styrene monomer were prepared with styrene concentrations ranging from 10 parts per million to 0.01 parts per million. Forty milliliter aliquots from each sample were pipetted into 50 ml centrifuge tubes and centrifuged at 19,500 rpm for 40 min. For the first 30 min the temperature was held at 30°C and for the last 10 min, it was held at 3°C to allow for solidification of the fat phase. After the centrifuge coasted to a stop, the fat layer was carefully removed and immersed in 10 ml of 0.1 N alcoholic KOH and held at boiling for 7 min to allow saponification of the fat to take place. After cooling to room temperature, 10 ml of water and 2 ml of carbon tetrachloride were added; the sample was shaken and let stand for separation of the carbon tetrachloride phase. After the CCl_4 phase had separated, 1 ml was removed with a syringe. Five microliters of this sample were then injected for chromatographic analysis.

The gas chromatograph used to detect the styrene monomer was an F & M model 609 electrometer with a model 400 oven equipped with a hydrogen flame detector. In all work, a helium flow rate of 60 ml per min and oven temperature of 100°C were employed. Three different columns were used for this work: a 20% carbowax plus 2.5% KOH on 60-80 mesh Gas Chrom R, a 10% EGS column, and a 10% Apiezon L on 80-100 mesh firebrick. The latter proved to give the best separation of the monomer from the carbon tetrachloride and other decomposition products as a result of the clean-up procedure.

Results and Discussion

The lower detection limit by the first method is five parts per million. Detector responses are observed as low as one part per million, but quantitation is impossible because they are so slight at these levels. However, from five parts per million to 100 parts per million, excellent linearity is observed as shown in Figure 1 where detector response is expressed in square centimeters of peak area.

When a solution of methylene chloride containing 10 ppm styrene monomer was run on a chromatogram, the peak from the styrene monomer was measured and compared to equal concentrations isolated from milk by the procedures described.

	Control	Method I	Method II
Peak area @ 10 ppm	37.5 cm^2	10.9 cm^2	34.5 cm^2

The second method obviously gives better recovery of the monomer. Linear detection by this method goes down to 0.1 ppm and will give detectable responses at the level of 0.05 ppm but since these peaks are extremely small, quantitation is impossible.

Figure II shows the response in square centimeters plotted against concentration in parts per million.

Using this data, milk which had been stored in polystyrene cartons for up to eight days was analyzed and no styrene monomer was detected. Therefore, it can be assumed that this particular carton is suitable for holding foods.

Summary

It can therefore be concluded that the second method is better for clean-up of styrene monomer but does require the expense of a high speed centrifuge which is not required by the first method which requires only common laboratory apparatus and reagents.

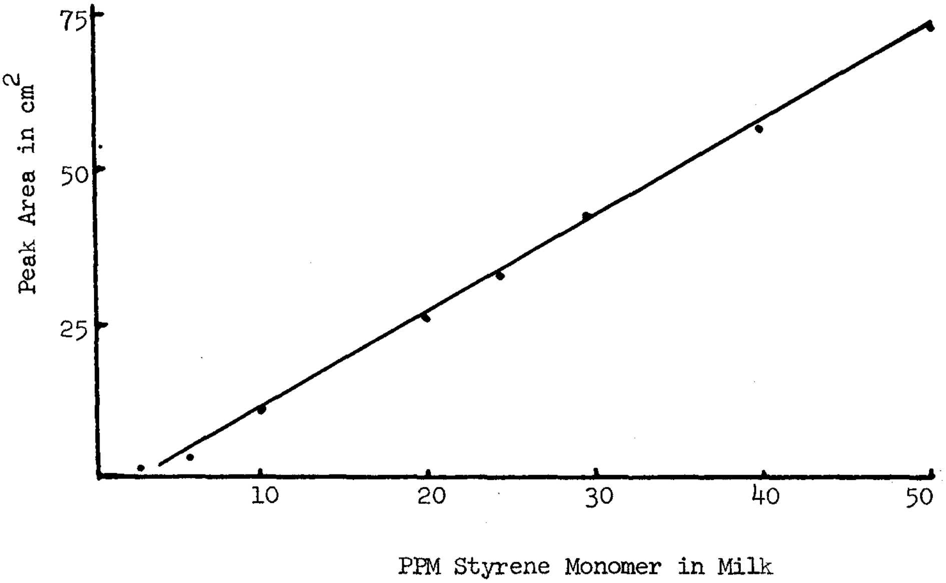

FIGURE 1. Calibration curve for determining styrene monomer in milk by ammonium sulfate, ethanol, methylene chloride extraction and gas chromatography

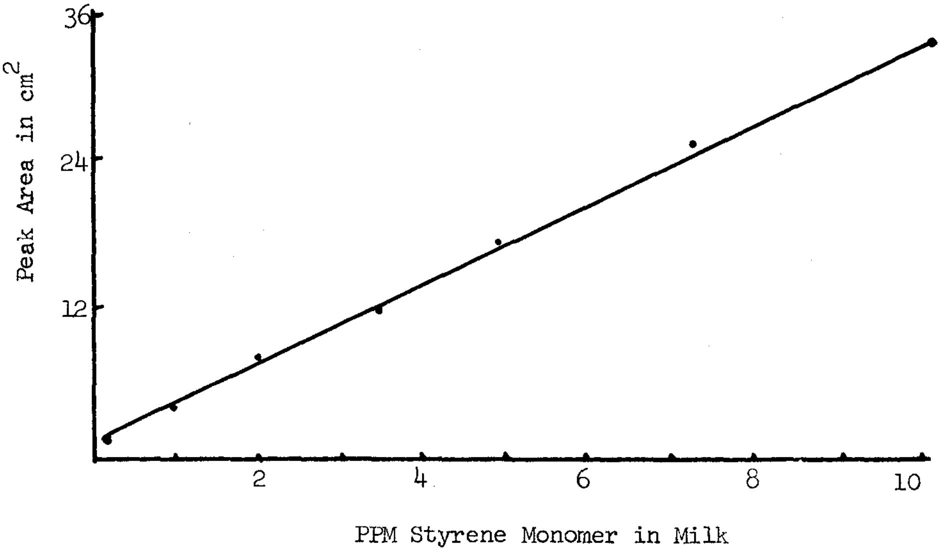

FIGURE 2. Calibration curve for determining styrene monomer in milk by high speed centrifugation, extraction with carbon tetrachloride and gas chromatography