

Artifacts in Flavor Isolates Produced by Steam Vacuum Distillation and Solvent Extraction of Distillate

Artifacts contributed to gas chromatographic flavor profiles by vacuum distillation techniques and subsequent solvent extraction of distillate were studied. Artifacts entering the flavor profile came from the antifoaming agent, distilled laboratory water, chromatograph septum bleed, vacuum grease, and extracting solvent. The antifoaming agents, septum bleed, and vacuum grease all contributed volatile silicone compounds to the gas chromatographic flavor profile. The extracting solvent (redistilled dichloromethane) contained cyclohexane, acetone, chloroform, and vinylidene chloride (tentative) as trace impurities. The laboratory distilled water contributed two minor peaks to the flavor profile that we could not identify. Suggestions are made on how to minimize the presence of these contaminants in flavor extracts obtained through steam vacuum distillation.

The generation of artifacts during the isolation of flavor extracts for instrumental analysis poses a serious problem. One can never be absolutely certain that the compounds identified were actually present in the food sample. They may be the result of chemical decomposition of the food product during isolation, enzymatic action by enzyme systems still active in the food system, reaction of individual flavor chemicals during isolation, artifacts introduced by the isolation equipment or procedure, etc. A study of the stale flavor in ultrahigh temperature processed milk under way in our laboratory necessitated the investigation of artifacts contributed to gas chromatographic profiles by steam vacuum distillation and subsequent solvent extraction.

While Teranishi et al. (1971) mention several examples of artifact formation encountered in flavor research, one seldom finds publications devoted to the study of artifacts. This information is generally buried within other major studies and it is, therefore, difficult to review pertinent literature in this area. Ferretti and Flanagan (1973) suspected that benzothiazole in a vacuum steam distillate of stale nonfat dry milk might originate from short sections of rubber tubing used to connect parts of the vacuum system. Arnold et al. (1966) reported that the two major peaks in their chromatogram probably came from the antifoam emulsion used. However, they did not specify the amount or the type of antifoam used. Bingam (1964) reported that a large chromatographic peak observed by Patel et al. (1962) from the vacuum distillate of sterilized concentrated milk was an artifact from Antifoam AF emulsion.

The objective of this study was to investigate the sources of artifacts introduced into flavor extracts through steam vacuum distillation and subsequent solvent extraction.

EXPERIMENTAL SECTION

Steam Vacuum Distillation. The apparatus used for the steam vacuum distillation consisted of a glass steam generator, vapor trap, 12-l. sample flask, large condenser, distillate collection flask, Davies condenser, and three liquid nitrogen Dewar traps. The steam generator was kept between 45 and 50°C during the distillation by manipulating a manostat for vacuum control. Ice water was used to cool the condensers.

Isolation of Volatiles. Six liters of sample was placed in the 12-l. round-bottomed flask of the distillation system, with or without added antifoam agents. The sample was either distilled water from our laboratory or fluid milk. Antifoaming agents used were Antifoam A concentrate (Sigma Co.; 25 ppm), Dow-Corning Antifoam C emulsion (40 ppm), or tristearin (Eastman Kodak Co.) which was twice dissolved in dichloromethane and precipitated by ethanol and subsequently stored overnight under high

vacuum in a freeze-dryer. The distillation was performed for 3 hr and approximately 300 ml of the distillate collected in the ice water trap. After completing distillation, the distillate was transferred to a separatory funnel. The liquid nitrogen traps were rinsed with dichloromethane (Mallinckrodt, Nanograde) which had been redistilled with an Allihn condenser (60 cm long) filled with stainless steel wool. The dichloromethane rinses of the Dewar traps were poured into the separatory funnel containing the distillate from the ice water trap. The distillate was extracted five times with dichloromethane. A total of 200 ml of dichloromethane was used. The dichloromethane extract was dried with 0.5 g of anhydrous magnesium sulfate and filtered and then the filtrate was reduced to 0.2 ml under a stream of nitrogen gas on a warm heating plate. Five microliters of this concentrate was injected into the gas chromatograph.

Gas Chromatography. A Hewlett-Packard Model 7620A Research Gas Chromatograph equipped with a hydrogen flame ionization detector was used in this study. Separation of the volatiles was accomplished using a 3 m × 0.32 cm o.d. stainless steel column packed with 10% Carbowax 20M on 80-100 Gas Chromosorb P (Applied Science Laboratories, Inc., State College, Pa.). The column was programmed from 50 to 195°C at 4°C per minute with a 2-min post-injection hold and a 22-min hold at a final limit. The carrier gas (helium) flow was 35 ml/min and the injection port temperature was 200°C. Range was set to 10² and attenuation set to 20 on a Hewlett-Packard Model 3370B integrator.

Mass Spectrometry. A LKB Model 9000 combined gas chromatograph-mass spectrometer was used to identify the compounds arising from the solvent used and to observe the mass spectral characteristics of other artifacts. All mass spectra were obtained at 70 eV with a constant accelerating voltage of 3500 V.

RESULTS AND DISCUSSION

The chromatogram obtained from the steam vacuum distillation of distilled water using 25 ppm of Antifoam A concentrate is shown in Figure 1A. Dow-Corning Antifoam C emulsion showed the same pattern of chromatogram as Antifoam A concentrate. Figure 1B shows the volatiles present in concentrated (1000×) redistilled dichloromethane. A 1000-fold concentration of the dichloromethane is typical for our flavor isolates prior to gas chromatography. This chromatogram suggests that peaks 1, 2, 3, 4, and 5 of Figure 1A originated from the solvent. Peaks 1, 2, 4, and 5 were identified as cyclohexane, acetone, dichloromethane, and chloroform, respectively, by mass spectrometry and cochromatography of known compounds with the unknown peaks. Peak 3 was tentatively identified as vinylidene chloride by mass spectrometry. Redistillation

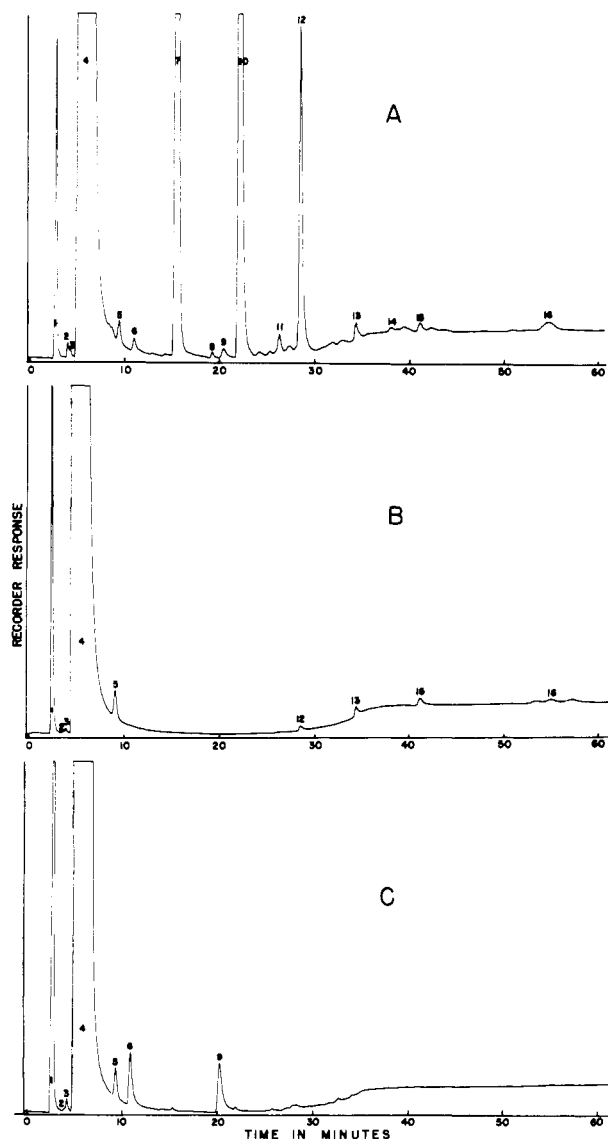


Figure 1. Gas chromatograms obtained from (A) steam vacuum distillation of Antifoam A concentrate in distilled water, (B) concentrated (1000 \times) re-distilled dichloromethane, and (C) direct solvent extraction of distilled water.

of Nanograde dichloromethane using the Allihn condenser packed with steel wool considerably reduced the amount of extraneous compounds present in the solvent but, as shown in Figure 1B, did not remove them completely.

While redistilled dichloromethane did not contain detectable levels of acetone, vinylidene chloride, or chloroform, they appeared in the 1000-fold concentrate of the solvent. The concentrated redistilled dichloromethane contained the following impurities at the stated concentration: cyclohexane (0.83%), acetone (0.02%), vinylidene chloride (0.01%), and chloroform (0.02%).

In addition to increased concentrations of the compounds identified, the undistilled dichloromethane also contained a trace of toluene. This appeared to be completely removed by the redistillation process. In addition, a blank run indicated that peaks 12, 13, 15, and 16 were coming from septum bleeding. While the use of Teflon-backed septa minimizes bleed, septa contamination can still present a problem. In Figure 1A, peaks 6 and 9 probably originated from contaminants in distilled water. This is suggested by Figure 1C which was obtained from distilled water by directly extracting 3 l. of the water with the same volume of dichloromethane used for extracting volatiles from the distillates. Peaks 7, 8, 10, 11, 12, 13, and

14 (Figure 1A) primarily resulted from the antifoams used. The huge metastable ion peaks and isotope peaks typical of silicone compounds indicate that these are all silicone polymers used in the antifoaming agent.

These peaks represent about 17% of the total amount of Antifoam A concentrate added to the distilled water [peaks 7 (8.27%), 8 (0.02%), 10 (6.82%), 11 (0.21%), 12 (1.59%), 13 (0.17%), and 14 (0.02%)]. This estimation was based on the peak area (percent), the weight of the isolate, and the antifoam agent added. Peaks 15 and 16 probably also come from the antifoams but we did not attempt to identify their source. It is noteworthy that peaks 12, 13, 15, and 16 overlap with those coming from the septa bleed (Figure 1A and B). Peaks 7, 10, 13, and 14 (Figure 1A) could also be obtained from dichloromethane exposed to Dow-Corning high vacuum grease for 1 min and subsequently concentrated. This suggests that direct contact between dichloromethane and high vacuum grease in the joints of the distillation apparatus should be avoided.

In an effort to minimize artifacts observed above, we searched for alternate antifoaming agents. Brunner (1950) reported several antifoaming agents available for skim milk and whey in the vacuum-condensing operation. We have found tristearin to be an adequate nonvolatile antifoam agent. While others might also be considered nonvolatile, they were required at higher concentrations (e.g., 0.1%). Our experience has indicated that floating as low as 50 ppm of tristearin on the top of the fluid milk sample was sufficient to suppress foaming in milk during the steam vacuum distillation. We did not observe any extraneous peaks due to tristearin on a gas chromatogram obtained from the steam vacuum distillation of distilled water using 100 ppm of purified tristearin. Extracting distilled water with a suitable solvent (e.g., dichloromethane) and subsequently boiling the water for about 30 min were sufficient to eliminate organic contaminants and residual solvent in the distilled water.

We also feel that Teflon sleeves can be substituted for high vacuum grease at the joints of distillation apparatus which contact solvents during solvent extraction if the high vacuum grease is not completely inert to the solvent being used.

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