Analysis of Neutral Volatiles of Mayonnaise by Direct Gas Chromatography and Mass Spectrometry[1]

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ABSTRACT

Simple procedures have been developed for analyzing neutral volatiles from mayonnaise by direct gas chromatography and combined direct gas chromatography-mass spectrometry. For gas chromatographic analysis, a glass liner containing glass wool coated with alkali in the lower quarter and plain glass wool in the remaining space is placed in the heated inlet of a gas chromatograph, and mayonnaise and water are injected onto the plain packing. Neutral volatiles eluted from the mayonnaise by the combined action of water, carrier gas, and heat collect on the cool column of the gas chromatograph, but acetic acid is trapped by the alkaline glass wool and thus does not interfere with the analysis. After removal of the liner with the spent sample, the temperature of the column oven is programmed to resolve the volatiles. For mass spectrometric analysis, neutral volatiles are passed directly from a chromatograph inlet to a second inlet liner containing a porous polymer that traps most organic compounds but has low affinity for water. These neutral organic volatiles are desorbed from the porous polymer in the inlet of a chromatograph interfaced with a mass spectrometer for analysis. This procedure allows components resolved by the gas chromatograph to be identified by mass spectrometry without interference from either water or acetic acid. A total of 21 neutral volatile compounds was identified in mayonnaise.

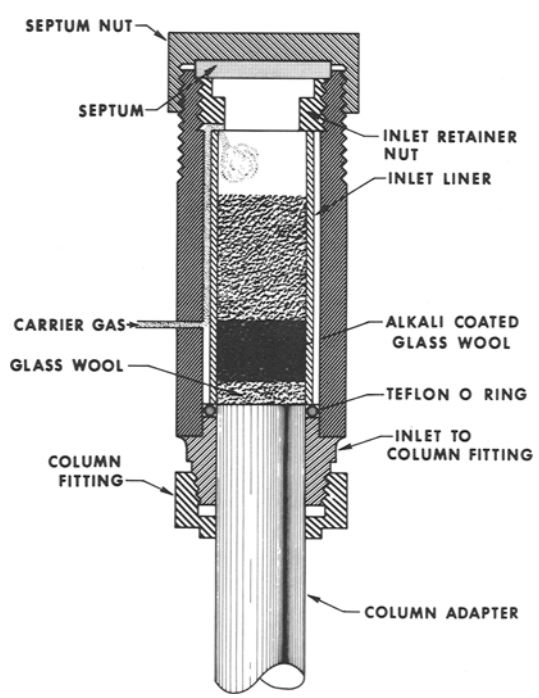

FIG. 1. Cross section of gas chromatograph inlet, showing inlet liner packed with alkali-coated and plain glass wool.

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INTRODUCTION

Although over 100 million gallons of mayonnaise are produced annually in the United States (1), the literature contains little information on its volatile components. Kawase et al. (2) identified 23 volatile components in a concentrate from oxidized mayonnaise, but a simpler procedure is needed to facilitate research on mayonnaise flavor and to monitor product quality.

Direct gas chromatographic methods were used to analyze volatiles in vegetable oils and peanut butter (3-6), and, in combination with mass spectrometry, to identify volatile components in vegetable oil (5,6). However, the presence of large quantities of acetic acid and water interfered with the use of these methods for analyzing mayonnaise. This paper describes simple techniques for removing acetic acid to facilitate analysis of other mayonnaise volatiles by direct gas chromatography or mass spectrometry, and for eliminating water prior to identifying volatiles by mass spectrometry.

EXPERIMENTAL PROCEDURES

Materials

Tenax GC (a 2,6-diphenyl-*p*-phenylene oxide porous polymer), 60-80 mesh, and Poly MPE (poly-*m*-phenoxylene) were obtained from Applied Science Laboratories, State College, PA. Column packing of 7% Poly MPE on Tenax GC was prepared by soaking 10 g of Tenax GC in 90 ml of a 2% solution of Poly MPE in ethyl acetate for 16 hr, then removing the excess solution by filtration and air drying the packing, and finally preconditioning the packing for 16 hr at 290 C. Porapak P (a styrene-divinylbenzene porous polymer), 80-100 mesh, was manufactured by Waters Associates, Framingham, MA. Teflon O-rings (Alltek Associates, Arlington Heights, IL), sandwich type silicone septums (Hamilton Co., Reno NV), and Pyrex brand glass wool (Corning Glass Works, Corning, NY) were conditioned 16 hr at 200 C before use. Syringe needles, 22 gauge, 3 in. long, with side hole and conical point, were from Hamilton Co. Inlet liners, 10 x 84 mm, were cut from borosilicate glass tubing. Alkali-coated glass wool was prepared by soaking glass wool in a 5% aqueous solution of sodium bicarbonate for 1 hr, then removing excess solution by filtration, and drying the treated glass wool at 125 C for 16 hr. The treated glass wool contained ca. 1.6 meq of alkali per g. Mayonnaise samples were either experimental or commercial products.

Sampling

About 10 g of mayonnaise was placed in a 1-oz jar and mixed thoroughly. The sample jar was tamped against a rubber stopper to remove air bubbles. A 100-μl syringe was filled with mayonnaise, the Luer tip was cleaned, and the needle was attached and filled from the syringe. The needle was removed, the syringe refilled, the tip cleaned, and the mayonnaise-filled needle was replaced. The sample was then ready to be injected into the inlet of the gas chromatograph.

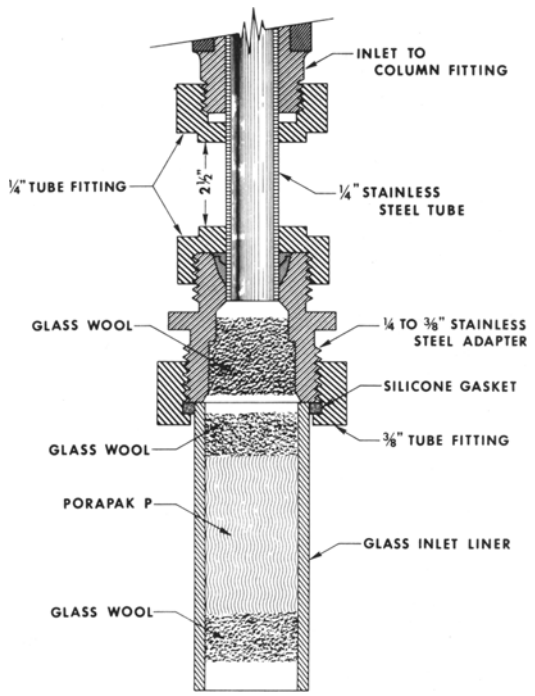

FIG. 2. Cross section of trap used to separate water and collect neutral, dry, organic volatiles for gas chromatographic-mass spectrometric analysis.

Direct Gas Chromatography

Volatiles profiles of mayonnaise were obtained on a Tracor MT 222 gas chromatograph with flame ionization detector, Westronics MT 22 recorder, and Columbia Scientific Industries Supergrator 2 integrator. An inlet liner was packed with ca. 0.2 g of alkali-coated glass wool and ca. 0.5 g of plain glass wool (Fig. 1) and inserted into the inlet of the gas chromatograph. The inlet retainer nut was replaced and tightened to press the lower lip of the liner against a Teflon O-ring at the base of the inlet and form a seal between the inlet's base and liner. When the inlet was closed with the septum and septum nut, the carrier gas was forced to flow upward and then down through the liner. First 100 µl of water was injected into the inlet liner, and then 100 µl of mayonnaise was injected by a technique designed to spread the mayonnaise over the glass wool 0.5 to 2 in. from the top of the inlet liner. The syringe needle was inserted into the inlet to a depth of 2.5 in. and slowly withdrawn 1.5 in. while 50 µl of mayonnaise was discharged. The syringe was rotated 180°, and the remainder of the sample was discharged as the needle was reinserted to 2.5 in. Neutral organic volatiles that were eluted from the mayonnaise by the combined action of carrier gas, steam, and heat from the inlet collected on the cool column of the chromatograph, but acidic volatiles were trapped by the alkaline glass wool. A wet towel was wrapped around the column to keep it at ca. 20 C while the volatiles were collected. After 20 min the liner with the spent sample was removed from the inlet, and the volatiles were resolved by programming the temperature of the column oven to 220 C.

A 10 ft x 1/8 in. OD (0.093 in. ID) stainless-steel U-tube packed with Tenax GC coated with ca. 7% Poly MPE (7,8) was used to resolve the volatiles. The column oven was heated to 90 C in about 3 min, held there for 9 min, then heated at 3 C per min to 220 and held there for 10 min. The inlet temperature was set at 125 C and the detector at 300 C. The flow of nitrogen carrier gas was set at 20 ml per min, hydrogen at 20 ml per min, and air at 34 liters per hr. The electrometer attenuation was 10 x 8.

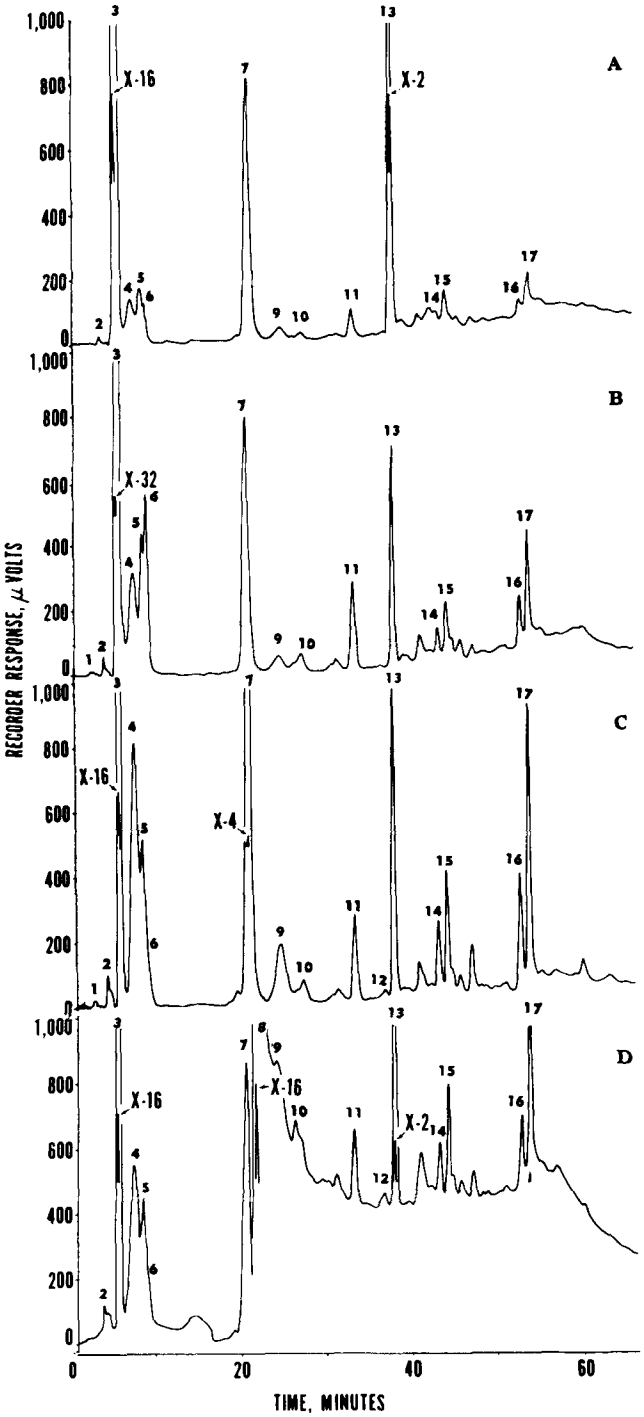

FIG. 3. Neutral volatiles profiles for three lots of mayonnaise: A, 3-week-old; B, 7-month-old; and C, 3-year-old. D, same as C but retaining acidic volatiles. 1 = methanol; 2 = acetaldehyde; 3 = ethanol; 4 = pentane; 5 = propanal; 6 = acetone; 7 = ethyl acetate; 8 = acetic acid; 9 = 3-methylbutanal; 10 = pentanal; 11 = hexanal; 12 = furfural; 13 = allyl isothiocyanate; 14 = *trans*-2,*cis*-4-heptadienal and benzaldehyde; 15 = *trans*-2,*trans*-4-heptadienal; 16 = *trans*-2,*cis*-4-decadienal; 17 = *trans*-2,*trans*-4-decadienal.

Combined Gas Chromatography-Mass Spectrometry

Sample preparation: The gas chromatograph inlet was prepared as shown in Figure 1. The trap, an inlet liner containing 0.4 g of Porapak P between two plugs of glass wool, was connected to the inlet by means of an adaptor as shown in Figure 2. Flow rate for the carrier gas, nitrogen, was 35 ml per min. The column oven was heated to 200 C for 1 hr to remove residual volatiles from the Porapak P,

TABLE I

Analysis of Neutral Volatiles in Mayonnaise by Direct Gas Chromatography[a]

Component	Peak area, millivolt sec		Standard deviation (%)
	Range	Mean	
Ethanol	196-219	207	4.5
Pentane	6.5-7.5	7.1	4.8
Ethyl acetate	25-29	27	5.9
Hexanal	2.4-2.8	2.6	5.7
Allyl isothiocyanate	30-36	33	6.8
t-2,*t*-4-Decadienal	1.7-2.3	2.0	11

[a]Six replicates of a 5-month-old mayonnaise sample were analyzed to verify reproducibility of the method.

and then cooled to room temperature. After the inlet temperature indicator had remained at 125 C for 15 min, a piece of wet cloth was wrapped around the trap and the lower fitting of the adaptor. The 100 µl of water and 300 µl of mayonnaise were injected by the technique described for direct gas chromatographic analysis. After 15 min the inlet liner with the spent sample was removed, and the inlet was closed. The wet cloth was removed from the lower part of the adapter at this time but not from the trap. After another 2 min, the trap containing the neutral volatiles adsorbed on Porapak P was disconnected from the adaptor, stoppered with Teflon-covered corks, placed in a screw cap vial, and stored in a refrigerator (up to 48 hr) until it was inverted and placed in the inlet of a combined gas chromatograph-mass spectrometer for analysis.

Analysis: Analyses were made on a Tracor 222 gas chromatograph interfaced with a Hewlett Packard Model 5930 A (quadrapole) mass spectrometer with a silicone membrane separator. An inlet liner containing volatiles from 300 µl of mayonnaise was sealed in the gas chromatographic inlet at 160 C for 20 min. Volatiles collected on the gas chromatographic column, a 1/8 in. OD, 0.085 in. ID, stainless-steel U-tube packed with Tenax GC coated with Poly MPE, that was kept at 35 C. After the liner with the Porapak P was removed from the inlet, the column oven was heated to 90 C in 3 min, held there for 5 min, then heated to 190 C at 3 C per min and held there for 20 min. The flow rate for the helium carrier gas was 35 ml per min. For the mass spectrometer, ionization potential was 70 eV and scan range was 29-235 AMU. Data were processed by an INCOS 2000 mass spectrometer system.

Flavor Evaluation

Samples were submitted in pairs to an informal panel of five tasters known to have sensitivity for detecting rancidity in oils. They were asked to rate the quality of the mayonnaise, indicate the degree of rancidity, if any, and indicate which of the pair they preferred. At least four of the five tasters agreed on all of the evaluations reported here.

RESULTS AND DISCUSSION

Several problems had to be solved before good profiles of neutral volatiles of mayonnaise were obtained. The most obvious, removal of acetic acid, was readily accomplished by placing a small plug of alkali-coated glass wool in the inlet liner of the gas chromatograph and injecting water and mayonnaise onto plain glass wool above this plug (Fig. 1). In Figure 3, curve D is a profile for mayonnaise obtained without removal of acetic acid, and curve C is a profile for neutral material from the same mayonnaise sample. Comparison of these two profiles shows that acetic acid is removed effectively by this procedure.

The amount of added water and the inlet temperature were both important in determining the amount of volatiles eluted from mayonnaise in the chromatograph inlet. The amount of volatiles increased as more water was added up to 75 µl, and remained constant from 75 to 150 µl, but volatiles eluted increased as inlet temperature increased over the entire range tried, 80-150 C. An inlet temperature of 125 C was selected because it was well below the temperature of ca. 135 C where the mayonnaise darkened noticeably. To reproduce results satisfactorily, the inlet temperature had to be the same for all analyses. With the Tracor MT 222 gas chromatograph, about 15 min was needed for the temperature in the interior of the inlet to equilibrate after the pyrometer indicated 125 C. Since the inlet temperature was not uniform from top to bottom, it was necessary to inject all samples into the same portion of the inlet and to spread them as evenly as possible over the glass wool in the area selected. Spreading the sample over the glass wool, instead of injecting it at one spot, also improved reproducibility and increased the amount of volatiles eluted. Table I shows reproducibility of major peak areas for six replicate analyses of 5-month-old mayonnaise.

Although water does not interfere with volatiles profiles, it does interfere with identification of components by combined gas chromatography-mass spectrometry. We found that dry organic volatiles could be collected on Porapak P and then desorbed in the inlet of the combined gas chromatograph-mass spectrometer. Although the low affinity of Porapak P for water was used to effect the final drying of the organic volatiles, about 0.1 g of water condensed on glass wool in the adaptor before reaching the Porapak P trap. Efforts to prevent condensation of water in the adaptor by eliminating the glass wool packing, shortening the adaptor tube from 2 ½ to ½ in., and cooling only the trap, not the lower fitting of the adaptor, resulted in condensation of droplets of water (up to 0.1 g) in the trap. However, the condensed water, analyzed by gas chromatography, contained only traces of other volatiles.

Volatiles were identified by comparing their gas chromatographic retention times and mass spectra with data for known compounds obtained on our instruments or with published data. Components identified in commercial mayonnaise are shown in Figure 3. Ethyl acetate, from vinegar (9), and allyl isothiocyanate, from mustard used to flavor mayonnaise (10), were major neutral constituents of

TABLE II

Peak Areas for Major Volatile Components of Mayonnaise (Quality Range from Good to Very Bad)

Quality	Degree of rancidity	Peak area, millivolt sec					
		Ethanol	Pentane	Ethyl acetate	Hexanal	Allyl isothiocyanate	*t*-2,*t*-4-Decadienal
Good	None detected	90	0.7	8.6	0.6	32	0.5
Fair	Incipient	194	5.4	31	2.5	27	1.5
Bad	Slight	243	28	25	2.4	47	13
Very bad	High	122	113	24	22	12	43

all mayonnaise samples analyzed. Other constituents of special interest – pentane, hexanal, the heptadienals, and the decadienals – are cleavage products of oxidized soybean oil (11). Although hexanal is no more abundant in the 3-year-old sample than in the 7-month-old sample, the rest of these oxidation-cleavage products increased with sample age (Fig. 3, curves A, B, C). Pentene was not resolved from propanal and acetone by gas chromatography, but was identified in the 3-year-old sample by combined gas chromatography-mass spectrometry.

Samples of two other brands of mayonnaise were also analyzed. Their volatiles profiles were similar to those in Figure 3, and no different compounds were identified. Other compounds were found in an experimental cottonseed oil mayonnaise that had been heated at 50 C for 9 days. These compounds were eluted at 29, 41, and 42 min, and were identified at 1-pentanol, *trans*-2-heptenal, and 2-pentylfuran, respectively. These three compounds are produced through oxidation of the linoleic acid moiety of oils (11).

A total of 21 neutral volatile components were identified in mayonnaise. These were methanol, acetaldyhyde, ethanol, pentane, propanal, pentene, acetone, ethyl acetate, 3-methylbutanal, pentanal, 1-pentanol, hexanal, furfural, allyl isothiocyanate, *trans*-2-heptenal, 2-pentylfuran, *trans*-2,*cis*-4-heptadienal, benzenaldehyde, *trans*-2,*trans*-4-heptadienal, *trans*-2,*cis*-4-decadienal, and *trans*-2,*trans*-4-decadienal. Kawase, et al. (2) identified ethyl acetate, pentanal, hexanal, *trans*-2-heptenal, 2-pentylfuran, benzaldehyde, *trans*-2,*trans*-4-heptadienal, and *trans*-2,*trans*-4-decadienal in oil recovered from mayonnaise, but the 13 other compounds have not been identified previously in mayonnaise.

Peak areas for major neutral volatile components from four mayonnaise samples with flavor evaluations ranging from good to very bad are shown in Table II. The peak areas of both pentane and *trans*-2,*trans*-4-decadienal increased as rancid flavor increased and as general flavor quality decreased, but hexanal content did not show a regular increase with increasing rancidity. This agrees with the observation of Dupuy et al. that flavor scores of light exposed soybean oils correlated better with *trans*-2,*trans*-4-decadienal and pentane contents than with hexanal contents of these oils (6). No relation was found between the amounts of ethanol, ethyl acetate, and allyl isothiocyanate and flavor quality, but these components, as well as acetic acid, may have to be considered when more subtle flavor evaluations are needed.

These techniques should be useful for detecting and identifying volatiles in other food products that contain large amounts of water or volatile acids, as well as for studying the relation between volatile components and mayonnaise flavor. More analyses of flavor-scored samples are needed to determine whether components of volatiles profiles can be correlated with flavor scores.

ACKNOWLEDGMENTS

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