

during baking has been demonstrated (6, 13, 19). Rothe (19) reacted 14 individual amino acids with xylose, and in each case an aliphatic aldehyde and furfural were formed. He also demonstrated that these reactions take place in the crust of rye bread during baking. The most reactive amino acids were isoleucine, leucine, valine, methionine, alanine, and phenylalanine which formed 2-methylbutyraldehyde, isovaleraldehyde, isobutyraldehyde, methional, acetaldehyde, and phenylacetaldehyde, respectively. Simultaneously, furfural was formed from pentoses, and HMF from hexoses. It has also been reported that the addition of amino acids or nonfat dry milk solids increases crust browning (3, 24). Furthermore, the Amadori rearrangement has been shown to take place in bakery products (5, 17).

However, as suggested by Pomeranz *et al.* (18), the possibility of caramelization reactions of various sugars as an additional source of brown pigments and flavor compounds cannot be overlooked. That such reactions may take place at temperatures even below those generally encountered during baking has been reported (7). Also, the extensive formation of methylfurfural from rhamnose does not appear to be accompanied by a corresponding decrease in amino acids,

thus suggesting the formation of methylfurfural directly from the sugar.

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COFFEE AROMA

Mass Spectrometric Determination of the Volatile Components from Ground Coffee

INTEREST in the chemical composition of coffee aroma has been increasing greatly in recent years arising mainly from the desire to improve the acceptability of soluble coffee products and also to afford a means of quality evaluation and control.

Early work on coffee aroma was carried out more than 50 years ago, by employing coffee roaster condensates as samples. The complexity of these condensates and the lack of sensitive analytical methods made complete analysis extremely difficult, if not impossible. In the past decade, several papers have appeared reporting further attempts to gain more complete information about the volatile components which comprise

a coffee aroma (2, 3, 8, 9, 12-15, 17, 18). In reviewing the rather voluminous literature which has appeared on this subject, a wide divergence is found with respect to the nature of the samples taken, the methods of analysis used, and the results obtained. In one report (6), as many as 70 compounds are listed as identified. Consideration of the methods employed, however, raises considerable doubt as to the reliability of some of the identifications.

The answer to this complex and perplexing problem appears to lie in the utilization of more modern instrumental methods of analysis. The results reported here are based entirely on mass spectrometric analysis. In other current research (14, 15, 18), gas chromatography and mass spectrometry are also being used.

Chemical changes responsible for development of coffee flavor and aroma are generally conceded to be effected

exclusively in the roasting process. The majority of previously reported studies on coffee aroma have, therefore, been made in connection with these roasting products, and the sampling procedures have employed heat, steam, water extraction, or solvent extraction, individually or in combination. The introduction of solvents and the use of steam or water might also be expected to cause chemical alteration of the volatile components. In addition, steam, water, or solvent extraction methods of obtaining a sample all suffer the disadvantage that nonvolatile but extractable materials may be collected, and the problem of subsequent separation of these impurities as well as the solvents and their associated impurities needlessly complicates the procedure. The method of sampling which employs a carrier gas to sweep out the coffee sample is apparently a satisfactory one but requires considerable extra time, and very

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Mass spectrometric analysis has led to the identification of several components of coffee aroma. Volatile compounds from ground roasted coffee are collected by distillation under high vacuum at room temperature into a receiver at liquid nitrogen temperature. Direct fractionation of a center cut on the mass spectrometer and subsequent analysis has shown the presence of more than 20 compounds. Among the compounds identified are furans, aldehydes, esters, alcohols, nitriles, and sulfur compounds. The methods of sampling and fractionation used, together with direct mass spectrometric analysis, offer minimum sample handling and avoid the possibility of loss and/or contamination of the sample. By using vacuum distillation without heat, a more representative sample has been obtained.

volatile components may be lost by failure to be condensed in the cold trap.

In this study, volatile components are considered to be those compounds which may be distilled under vacuum at room temperature from the dry, ground, roasted coffee. Samples collected in this way are believed to be more representative of a characteristic sample of aroma.

Methods

The methods employed are basically those described previously for the analysis of volatile components from irradiated meat (7).

The apparatus employed to collect the sample of total condensate from coffee is shown in Figure 1. One pound of freshly ground, medium grade coffee was placed in a flask attached to a vacuum manifold, cooled to liquid nitrogen temperature, and the air pumped from the system until a final pressure of about 1 micron was attained. The stopcock to the vacuum pump was then closed and the coffee allowed to come to room temperature. A dewar of liquid nitrogen was placed around the receiver flask, and distillation was allowed to proceed for approximately 120 hours. Since this investigation was concerned mainly with qualitative results, no studies were made of the exact collection times, and the sample was allowed to distill for a relatively long period of time to achieve a nearly exhaustive collection of the volatile materials. The mass spectral data from which ultimately 30 volatile components were identified were obtained from two such 1-pound samples of coffee.

Fractionation of the total condensate was accomplished by means of usual procedures employing low temperature-high vacuum distillation into a water fraction, a carbon dioxide fraction, and a center cut (7). A schematic diagram of the fractions obtained is shown in Figure 2.

The carbon dioxide fraction comprised about 95% of the total condensate. Only low molecular weight gases of relative unimportance are expected to be found in this fraction since it contains

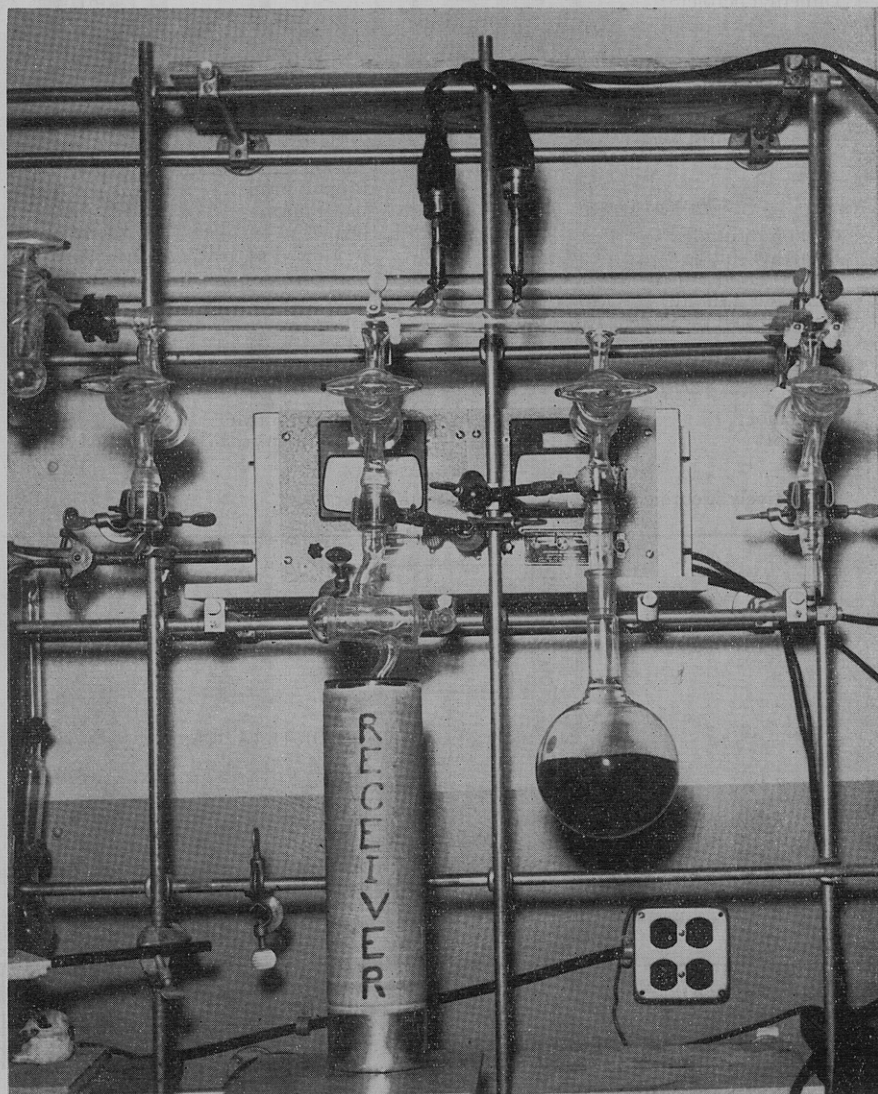


Figure 1. Collection apparatus for coffee volatiles

only compounds that are volatile at a temperature below -145° C. For this reason, no systematic analysis of the carbon dioxide cut was made. The water fraction was approximately 10 ml. in volume and had a pale yellow color which turned darker on standing. Since techniques for the analysis of water fractions have not yet been fully developed, no attempts were made to identify the compounds present at this time. Accordingly, the analyses reported here

are based entirely on the analysis of the center cut which contains the components of intermediate volatility.

The analysis of the center cut was accomplished by direct fractionation on the mass spectrometer using the procedure which has been described previously (7). The fractionation was accomplished in steps of 5° C. from -145° C. to room temperature, and a mass spectrum was obtained for each fraction. For some of the fractions

Table I. Summary of Mass Spectral Analysis of Coffee Aroma

Summary of Composition			
Aldehydes		50.7%	
Ketones		21.5	
Esters		10.3	
Heterocyclic compounds		7.0	
Sulfur compounds		4.3	
Alcohols		2.4	
Nitriles		1.7	
		<u>97.9%</u>	
Aldehydes		Ketones	
Acetaldehyde	26.4%	Acetone	0.5%
Isovaleraldehyde	13.7	Methyl ethyl ketone	12.7
Propanal	8.9	Methyl vinyl ketone	0.4
Propenal	0.8	Biacetyl	7.8
α -Methyl, β -thyl acrolein	0.9	Acetylacetone	0.1
Dimethyl acrolein ^a	...		<u>21.5%</u>
	<u>50.7%</u>		
Esters		Heterocyclic Compounds	
Methyl formate	5.3%	Pyrrole	0.5%
Methyl acetate	5.0	Furan	2.3
	<u>10.3%</u>	2-Methyl furan	4.0
		2,5-Dimethyl furan	0.2
Sulfur Compounds		Propyl furan ^a	...
Carbon disulfide	0.2%	Butyl furan ^a	...
Dimethyl sulfide	0.7	Dimethyl pyrrole ^a	...
Methyl ethyl sulfide	0.2	<i>N</i> -Methyl pyrrole ^a	...
Dimethyl disulfide	3.2		<u>7.0%</u>
Methyl ethyl disulfide ^a	...		
	<u>4.3%</u>		
Alcohols		Nitriles	
Methanol	2.0%	Propene nitrile	0.6%
Ethanol	0.4	3-Butene nitrile	1.1
	<u>2.4%</u>		<u>1.7%</u>

^a Tentatively identified due to lack of calibration spectra.

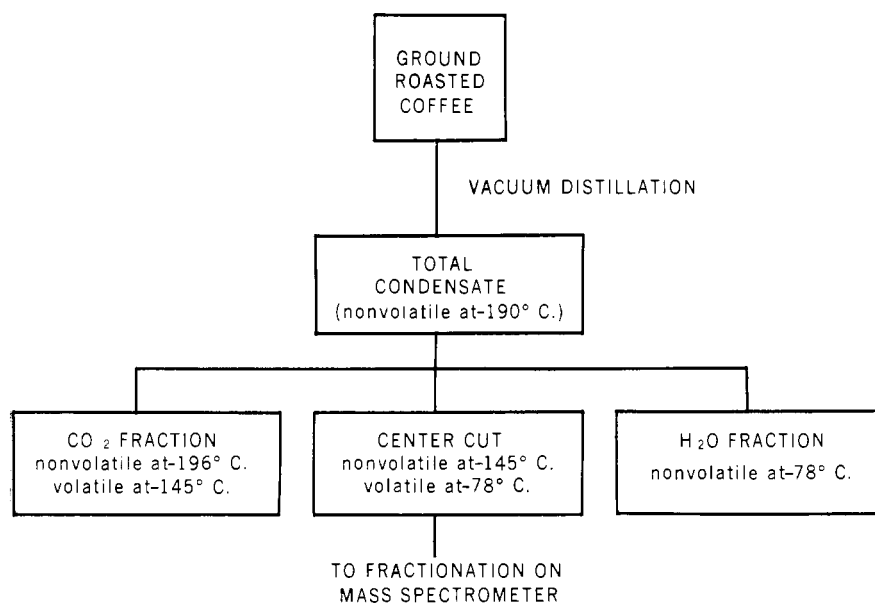


Figure 2. Diagram of the fractionation of a sample of coffee aroma

which contained as many as eight or 10 compounds, the interpretation of the spectrum was simplified by employing the low ionization voltage technique (7).

Results and Discussion

Results of mass spectral analysis of the coffee center cuts are summarized in

Table I. The occurrence of 12 compounds previously reported (Table II) is confirmed, and 18 additional compounds have been identified, six of these tentatively. (The criterion for positive identification is that the mass spectrum of the sample component matches exactly the mass spectrum of a pure reference compound. Identification is considered tentative if no reference spectrum

is available, but the structure can be ascertained by interpretation of the mass spectrum obtained.)

The figures given at the right of the compounds listed in Table I are quantitative estimates of the relative amounts of the compounds in a center cut. The values are given as mole percentage based on an average value taken from the two coffee center cuts analyzed. More accurate quantitative data cannot be given because all the necessary reference spectra, for which calibrations have been obtained, are not at present available. However, the relative abundance of the various compounds identified can be estimated.

Furan and 2-methyl furan are believed to be among the more significant compounds in the complex which makes up typical coffee aroma.

Among the sulfur compounds only dimethyl disulfide is present in appreciable quantity. The other compounds occur to a lesser extent. The absence of mercaptans is particularly significant. By mass spectrometric analysis, it is relatively easy to distinguish between a sulfide and a mercaptan, and furthermore, mercaptans can be readily identified as a class in the presence of many other functional group type compounds. It is, therefore, reasonably certain that no mercaptans of intermediate molecular weight are present in the samples of coffee aroma isolated from dry ground coffee. The possibility exists that some higher molecular weight mercaptans may be present in the water fraction, but this has not yet been obtained. Although mercaptans have been previously reported by other workers (17, 18), in all cases the samples of volatiles have been obtained from brews or percolates. It may be reasonably assumed that the mercaptans found were formed from the disulfides during preparation of the beverage.

All the compounds identified account for 98% of the material in the center cut. The remaining 2% of the sample is distributed among those compounds which have been tentatively identified and some other compounds as yet undetermined because of the small amount present. The six tentatively identified compounds account for the bulk of this 2% remainder. From an indication of perhaps two or three other compounds which may be present, the amount unidentified can be estimated to be probably less than a tenth of a per cent of the total.

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Table II. Table of Commonly Identified Constituents of Coffee Aroma

Acetaldehyde	2,4-Dinitrophenylhydrazine 2,4-Dinitrophenylhydrazine Gas chromatography and infrared spectrum Gas chromatography and mass spectrum	Clements and Deatherage (2) Johnson and Frey (5) Rhoades (14, 15) Zlatkis and Sivetz (18)
Biacetyl	2,4-Dinitrophenylhydrazine Diacetyl dioxime with Ni salt and H ₂ NOH Bis-semicarbazone Ni dimethylglyoxime precipitate Gas chromatography and infrared spectrum Gas chromatography and mass spectrum	Clements and Deatherage (2) Prescott <i>et al.</i> (10, 11) Johnson and Frey (5) Schmalfuss and Barthmeyer (16) Rhoades (14, 15) Zlatkis and Sivetz (18)
Diethyl ketone	2,4-Dinitrophenylhydrazine 2,4-Dinitrophenylhydrazine	Clements and Deatherage (2) Prescott <i>et al.</i> (10, 11)
Methyl ethyl ketone	2,4-Dinitrophenylhydrazine Gas chromatography and infrared spectrum Gas chromatography and mass spectrum	Clements and Deatherage (2) Rhoades (14, 15) Zlatkis and Sivetz (18)
Acetone	2,4-Dinitrophenylhydrazine Salicaldehyde reaction Gas chromatography and infrared spectrum Gas chromatography and mass spectrum	Clements and Deatherage (2) Hughes and Smith (4) Rhoades (14, 15) Zlatkis and Sivetz (18)
Propionaldehyde	2,4-Dinitrophenylhydrazine Gas chromatography and infrared spectrum Gas chromatography and mass spectrum	Clements and Deatherage (2) Rhoades (14, 15) Zlatkis and Sivetz (18)
Ethanol	Naphthylcarbamate derivative Gas chromatography and infrared spectrum	Prescott <i>et al.</i> (10, 11) Rhoades (15)
Methanol	Gas chromatography and infrared spectrum Gas chromatography and mass spectrum	Rhoades (14, 15) Zlatkis and Sivetz (18)
Dimethyl sulfide	No proof offered Gas chromatography and infrared spectrum Gas chromatography and mass spectrum	Reichstein and Staudinger (12) Rhoades (14, 15) Zlatkis and Sivetz (18)
Furan	Green pine splinter reaction, brown ppt. with concd. HCl Gas chromatography and infrared spectrum Gas chromatography and mass spectrum	Johnson and Frey (5) Rhoades (15) Zlatkis and Sivetz (18)
2-Methyl furan	Gas chromatography and infrared spectrum Gas chromatography and mass spectrum	Rhoades (15) Zlatkis and Sivetz (18)
Methyl formate	Gas chromatography and infrared spectrum Gas chromatography and mass spectrum	Rhoades (15) Zlatkis and Sivetz (18)
Methyl acetate	Gas chromatography and infrared spectrum Gas chromatography and mass spectrum	Rhoades (15) Zlatkis and Sivetz (18)

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