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New Volatile Constituents of Black Tea Aroma

The volatile components of black tea were isolated by extraction with supercritical CO₂ under pressure followed by atmospheric steam distillation and enrichment of steam volatiles on Porapak Q. The total black tea aroma fraction was separated into basic and neutral components. A total of 56 constituents, mainly pyridines, pyrazines, quinolines, thiazoles, aromatic amines, and carbonyls, have been identified for the first time in black tea

aroma by using a combination of glass capillary gas chromatography and mass spectrometry. Identifications of the new compounds were accomplished by comparison of their mass spectra with authentic reference spectra measured on the same instrument and with mass spectral data given in the literature. GLC retention times were used to confirm identifications.

Many authors have carried out studies on volatile components of black tea, with the result that no single key aroma compound could be identified which shows a distinctly black tea like character. In recent years Japanese investigators have been studying the flavor of black tea (Ina and Sakato, 1968; Ina and Eto, 1972; Yamanishi et al., 1972, 1973a). The available information on the chemistry and biochemistry of black tea aroma formation was reviewed by Sanderson and Graham in 1973. Two recent publications by Cazenave et al. (1974) and Cazenave and Horman (1974) deal with studies on the composition of the aroma of black tea. Renold et al. (1974) identified 68 volatile constituents for the first time in a black tea aroma concentrate using the coupling technique of a mass spectrometer with a glass capillary column.

The present paper reports on the identification of hitherto unknown volatile black tea aroma ingredients, mainly nitrogen-containing constituents, which were separated by using a new mild isolation technique which is generally applicable in food odor research (Vitzthum et al., 1975).

MATERIALS AND METHODS

A commercial blend of black tea was extracted with supercritical CO₂ under pressure. The CO₂ system used in the authors' laboratory is shown schematically in Figure 1 and the CO₂ extraction procedure is briefly described. Dense gas phases, e.g. CO₂ above its critical conditions, are able to solve a great variety of substances. This solubility depends upon pressure and temperature of the compressed CO₂. In general, with increasing pressure solubility rises, too. On the other hand, with decreasing pressure a separation of the soluted substances from the gas phase occurs.

Vessel A contains air-dried black tea and a flow of compressed supercritical CO₂, by passing the tea, is charged with the aroma and flavor and a supercritical "solution" is formed. The pressures are of the magnitude of 100-300 bars. The gas flow is then fed into vessel B by expansion, and the separation mentioned above takes place at pressures in the range of 50-70 bars. The pure, now extract-free

CO₂ is exhausted from B by the pump and compressed again in vessel A, etc. After careful removal of the remaining CO₂ a concentrate with a very intensive tea aroma is obtained in B.

Experimental details concerning operating parameters were previously described by HAG AG (1974b) and Vitzthum et al. (1975). The aroma concentrate was subjected to atmospheric steam distillation and fractionated into basic and neutral components essentially by the same procedure as described previously for coffee by Vitzthum and Werkhoff (1974a,b). Gas-liquid chromatographic analyses were performed on a Carlo Erba GI Model 450 gas chromatograph with an all-glass system using the direct injection technique on high-resolution glass capillary columns. The analytical chromatogram shown in Figure 2 was obtained with a 100 m × 0.30 mm i.d. glass capillary column coated with polypropylene glycol. The GC-MS equipment was similar to that described previously by Vitzthum and Werkhoff (1974a) except that a 200 m × 0.31 mm i.d. glass capillary column was coupled to the mass spectrometer.

N-Ethylacetamide and *N*-ethylpropionamide were prepared by reaction of aqueous ethylamine with acetyl chloride and propionyl chloride, respectively, according to the general procedure of D'Alelio and Reid (1937).

2,4-Dimethylthiazole was synthesized by the method of Schwarz (1945) by the reaction of thioacetamide and chloroacetone. 2,5-Dimethylthiazole and 2,4,5-trimethylthiazole were obtained as described by Kurkijy and Brown (1952). 2,5-Dimethyl-4-ethylthiazole was prepared by a modification of a method described by Takahashi and Hayami (1961). All other compounds identified in our laboratory were from reliable commercial sources.

RESULTS AND DISCUSSION

The compounds identified from the black tea aroma concentrate are listed in Table I. The chief advantage of the low-temperature CO₂ procedure (40°) consists in its rapidity and in the fact that it eliminates the risk of contaminating the aroma complex with solvents and their impurities

Table I. Compounds Identified from Black Tea^a

Compound	Fraction	Authentic ref compd	Evidence		MS reference
			MS	GLC	
Pyridines					
Pyridine	A	+	P ^a	+	Cornu and Massot (1966); Stenhagen et al. (1969); MSDC ^a
2-Methylpyridine	A	+	P	+	Cornu and Massot (1966); Stenhagen et al. (1969); MSDC
3-Methylpyridine	A	+	P	+	Cornu and Massot (1966); Stenhagen et al. (1969); MSDC
4-Methylpyridine	A	+	P	+	Cornu and Massot (1966); Stenhagen et al. (1969); MSDC
2-Ethylpyridine	A	+	P	+	Ledl and Severin (1973); Stenhagen et al. (1969); MSDC
3-Ethylpyridine	A	+	P	+	Cornu and Massot (1966); Stenhagen et al. (1969); MSDC
2,6-Dimethylpyridine	A	+	P	+	Cornu and Massot (1966); Stenhagen et al. (1969); MSDC
2,5-Dimethylpyridine	A	+	P	+	Cornu and Massot (1966); Stenhagen et al. (1969); MSDC
2-Methyl-6-ethylpyridine	A		P		Cornu and Massot (1966); MSDC
2-Methyl-5-ethylpyridine	A	+	P	+	Cornu and Massot (1966); MSDC
3-Methoxy-pyridine	A		P		Lacey et al. (1971)
4-Vinylpyridine	A	+	P	+	Cornu and Massot (1966); Stenhagen et al. (1969); MSDC
2-Acetylpyridine	A	+	P	+	Ferretti and Flanagan (1971); Stenhagen et al. (1969); MSDC
3- <i>n</i> -Butylpyridine	A		P		Lightner et al. (1970); Stenhagen et al. (1969)
2-Phenylpyridine	A		P		Neurath and Duenger (1969)
3-Phenylpyridine	A		P		Neurath and Duenger (1969)
Pyrazines					
Methylpyrazine	A	+	P	+	Cornu and Massot (1966)
2,6-Dimethylpyrazine	A	+	P	+	Ferretti et al. (1970)
2,5-Dimethylpyrazine	A	+	P	+	Ferretti et al. (1970)
2,3-Dimethylpyrazine	A	+	P	+	Ferretti et al. (1970)
Ethylpyrazine	A	+	P	+	Kinlin et al. (1972)
2-Ethyl-6-methylpyrazine	A	+	P	+	Kinlin et al. (1972)
2-Ethyl-5-methylpyrazine	A	+	P	+	Kinlin et al. (1972)
Trimethylpyrazine	A	+	P	+	Kinlin et al. (1972)
Tetramethylpyrazine	A		P		Kinlin et al. (1972)
2-Ethyl-3,5-dimethylpyrazine	A	+	P	+	Kinlin et al. (1972)

Table I (Continued)

Compound	Fraction	Authentic ref compd	Evidence		MS reference
			MS	GLC	
2-Ethyl-3,6-dimethylpyrazine	A	+	P	+	Kinlin et al. (1972)
Thiazoles					
2,4-Dimethylthiazole	A	+	P	+	Vitzthum and Werkhoff (1974a)
2,5-Dimethylthiazole	A	+	P	+	Vitzthum and Werkhoff (1974a); Tabacchi (1974)
5-Methylthiazole	A		P		Vitzthum and Werkhoff (1974a); Tabacchi (1974)
2,4,5-Tri-methylthiazole	A	+	P	+	Vitzthum and Werkhoff (1974a); Tabacchi (1974); Pittet and Hruza (1974)
2,5-Dimethyl-4-ethylthiazole	A	+	P	+	Vitzthum and Werkhoff (1974a); Tabacchi (1974)
Benzothiazole	A	+	P	+	Cornu and Massot (1966); MSDC
2-Methylbenzothiazole	A	+	P	+	Cornu and Massot (1966); MSDC
Quinolines					
2-Methylquinoline	A	+	P	+	MSDC
6-(or 7)-Methylquinoline	A		T		MSDC
2,6-Dimethylquinoline	A		P		Stenhagen et al. (1969); MSDC
2,4-Dimethylquinoline	A		T		Stenhagen et al. (1969)
4,8-Dimethylquinoline	A		T		Stenhagen et al. (1969)
3- <i>n</i> -Propylquinoline	A		T		
4- <i>n</i> -Butylquinoline	A		T		
Aromatic amines					
Aniline	A	+	P	+	Cornu and Massot (1966); MSDC
<i>N</i> -Methylaniline	A	+	P	+	Cornu and Massot (1966); MSDC
<i>N</i> -Ethylaniline	A	+	P	+	Cornu and Massot (1966); MSDC
<i>o</i> -Toluidine	A	+	P	+	Cornu and Massot (1966); MSDC
<i>N,N</i> -Dimethylbenzylamine	A	+	P	+	Stenhagen et al. (1969)
Amides					
<i>N</i> -Ethylacetamide	A	+	P	+	MSDC
<i>N</i> -Ethylpropionamide	A	+	P	+	
Miscellaneous					
Benzoxazole	A		P		Ogura et al. (1970)
1,4-Diacetylbenzene	B		P		Cornu and Massot (1966); MSDC
1,3-Diacetylbenzene	B		T		
2,4-Dimethylacetophenone	B		P		Cornu and Massot (1966); MSDC
<i>p</i> -Ethylacetophenone	B		P		Cornu and Massot (1966); MSDC
2,4-Dimethylpropiophenone	B		T		
<i>p</i> -Ethylpropiophenone	B		T		
3,4-Dimethoxyacetophenone	B		P		Cornu and Massot (1966); MSDC

^a A, basic fraction; B, neutral fraction; MSDC Mass Spectrometry Data Centre, AWRE, Aldermaston Reading, RG 7 4 PR, U.K.; P, positive; T, tentative.

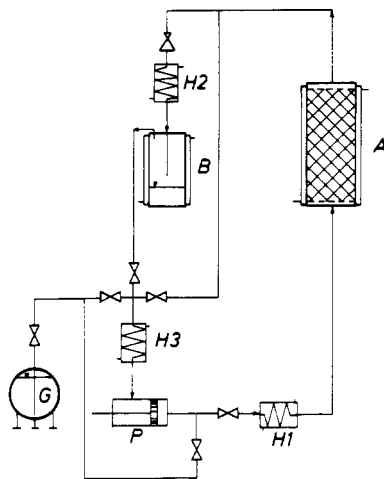


Figure 1. (A) Pressure tube (= extraction vessel), equipped with a jacket for heating fluids, filled with black tea leaves. (B) Separating vessel, equipped with a jacket for cooling or heating fluids, partially filled with liquid CO₂, containing the separated tea aroma. (P) Pump for liquified gas. (G) Liquid gas tank. (H1-H3) Heat exchanger.

and prevents artifact formation as much as possible. Furthermore, extracts obtained by this method possess the full and complete aroma. We have studied previously the applicability of the carbon dioxide technique to the extraction of various foods, for example hop (HAG AG, 1974a) and black tea (HAG AG, 1974b). The identified black tea aroma compounds include 16 pyridines, 11 pyrazines, 7 quinolines, 7 thiazoles, and some miscellaneous compounds. By adding the new compounds resulting from the present investigation to those summarized by Sanderson (1974), it can be concluded that at least 325 volatile constituents occur in black tea aroma.

Identifications of the components are based on comparison of the observed spectral data with spectra obtained on authentic compounds in our laboratory or with literature data. Additional confirmation was provided wherever possible by determining GC retention times. In this investigation the main emphasis has been placed on the identification of the nitrogen-containing compounds.

Most of the components of the basic fraction of black tea volatiles are pyrazines, pyridines, and quinolines. Furthermore, there were a few compounds with an odd numbered molecular weight and also indicating the presence of sulfur

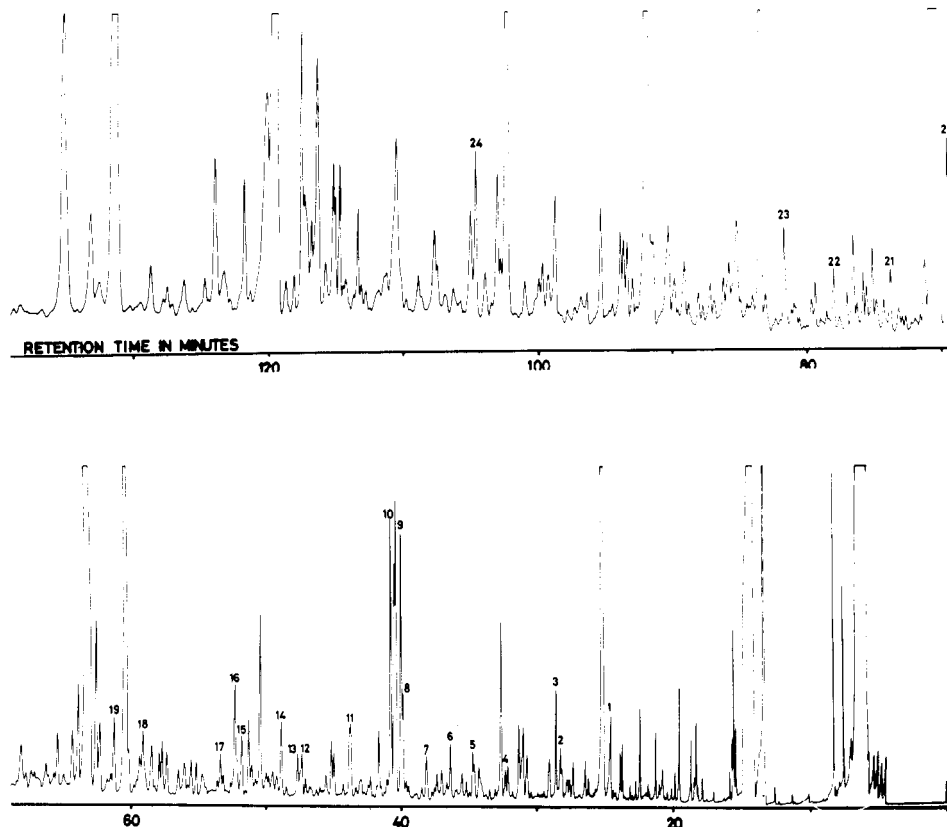


Figure 2. Gas chromatographic separation of basic steam volatiles of black tea. Direct injection of 0.5 μ l of a dilute ether solution: 100 m \times 0.30 mm i.d. glass capillary column coated with polypropylene glycol; carrier gas flow, 2.5 ml/min of hydrogen. The temperature was held for 5 min at 20°, 8 min at 50°, then programmed at 1°/min up to 160° and isothermal thereafter; peak No. 1, pyridine; 2, 2-methylpyridine; 3, methylpyrazine; 4, 2,4-dimethylthiazole; 5, 4-methylpyridine; 6, 2-ethylpyridine; 7, 2,5-dimethylthiazole; 8, 2,5-dimethylpyridine; 9, 2,6-dimethylpyrazine; 10, 2,5-dimethylpyrazine; 11, 3-ethylpyridine; 12, 2,4,5-trimethylthiazole; 13, 2-ethyl-6-methylpyrazine; 14, 2-ethyl-5-methylpyrazine; 15, 4-vinylpyridine; 16, trimethylpyrazine; 17, 2,5-dimethyl-4-ethylthiazole; 18, 2-ethyl-3,6-dimethylpyrazine; 19, 2-acetylpyridine; 20, aniline; 21, *N*-methylaniline; 22, *N*-ethylaniline; 23, *o*-toluidine; 24, 2-methylquinoline.

in the molecule. According to mass spectral fragmentation patterns and GLC retention times, these components were suspected to be alkylthiazoles. Identifications of sulfur-containing compounds in the present work were considerably supported by the authors' studies on the mass spectra of alkylthiazoles, previously detected as volatile constituents in roasted coffee (Vitzthum and Werkhoff, 1974a). The alkylthiazoles occurred at about the same order of concentration as that of many of the minor alkylpyrazines and

alkylpyridines. No pyrazine compounds have yet been found in black tea aroma, but it is noteworthy to mention that Yamanishi et al. (1973b) have investigated the composition of the aroma of Japanese roasted green tea (called Hoji-cha) and have identified 21 pyrazine compounds. The amounts of pyrazines in coffee and cocoa were considerably larger than in black tea. Many of the identified compounds can be considered to contribute to black tea flavor, but none was isolated which could be considered to constitute

the typical black tea character.

Cazenave et al. (1974) identified *N*-ethylsuccinimide in black tea and they speculate that this compound is derived from theanine by a Strecker degradation during tea fermentation. We identified *N*-ethylacetamide and *N*-ethylpropionamide and these compounds may be considered to originate from the same amino acid present in fresh tea in an unusually large amount. *N*-Ethylacetamide and *N*-ethylpropionamide were synthesized and found to be identical with respect to retention time and mass spectral fragmentation with the natural material. This finding further substantiates the importance of amino acids as precursors of tea volatiles (Sanderson and Graham, 1973). The mass spectrum of *N*-ethylpropionamide not previously reported in the literature is (*m/e* values of most significant ions; intensities in parentheses): 101 (100), 100 (5), 86 (15), 72 (65), 57 (50), 56 (6), 55 (5), 54 (6), 44 (75), 30 (38), 29 (46).

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Nona-2,4,6-trienal, an Unusual Component of Blended Dry Beans

Nona-2,4,6-trienal has been characterized in the vacuum steam volatile oil of some samples of blended dry red beans and of blended dry white beans using capillary gas chromatography-mass

spectrometry. This compound probably arises from an unusual oxidative breakdown of the linolenic acid in the bean.

Alka-2,4-dienals, particularly deca-2,4-dienal, have been found in a wide variety of foods. Reports of finding alkatrienals, however, have been much rarer. Deca-2,4,7-trienal was reported recently in fish oil (Meijboom and Stroink, 1972) and deca-2,4,6-trienal was reported recently in tea (Cazenave et al., 1974). Although nona-2,4,6-trienal has been indicated as possibly occurring in butter (Badings, 1970), to our knowledge, it has not been characterized previously in any food product with any degree of certainty.

EXPERIMENTAL SECTION

Materials. Dry red and white beans (*Phaseolus vulgaris*) were obtained from local retail markets and from an Oregon bean processor.

Synthesis of Nona-2,4,6-trienal. Hepta-2,4-dienal (0.6 ml; trans,trans; Aldrich Chemical Co.) was cooled to -5° in an ice-salt bath and acetaldehyde (0.4 ml; free from acetic acid) was added. Two drops of a 50% solution of potassium hydroxide in water were then added and the mixture was

stirred for 15 min at -5° and then for 1 hr at room temperature. The mixture was then taken up in ether (100 ml), washed first with cold dilute hydrochloric acid (50 ml, 3 *N*), and then with saturated sodium bicarbonate solution, dried over sodium sulfate, and the ether removed by distillation. The nona-2,4,6-trienal was purified by gas-liquid chromatography (GLC) using a 1-m long, 0.64 cm o.d. aluminum column packed with 80-100 mesh Chromosorb P coated with 10% Tween 20. The column was kept at 150° and had a helium inlet pressure of 8 psi. The nona-2,4,6-trienal formed a well-separated peak at 20 min. Calculation from GLC showed a yield of 10% based on the original hepta-2,4-dienal. Capillary GLC (500 ft \times 0.03 in. i.d., Tween-20) showed the material separated by packed column GLC to consist of three major isomers.

Isolation of the Volatile Oil. Dry beans (1.8 kg) were blended in portions in a laboratory blender with about three times their volume of water. They were then placed in a 12-l. flask. Additional water was added to make the