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GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC ANALYSES OF "FLAVORY" AND "NON-FLAVORY" CEYLON BLACK TEA AROMA CONCENTRATES PREPARED BY TWO DIFFERENT METHODS

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SUMMARY

Aroma concentrates of Ceylon black tea were prepared by two methods, (1) steam distillation and (2) adsorption on charcoal of the head-space vapor over a tea brew followed by elution with diethyl ether. The former method was superior to the latter, perhaps partly due to formation of artefacts on the charcoal. Comparison by gas chromatography of aroma concentrates from "flavory" and "non-flavory" tea showed marked qualitative and quantitative variations in the components detected. Twenty-one of these components were identified by mass spectrometry.

INTRODUCTION

A black tea with flavor is, economically, of much greater value than one which is devoid of flavor, and many investigations have been made of the components in the black tea aroma complex which are responsible for this flavor. Aroma concentrates of tea obtained by steam distillation have been separated by gas chromatography (GC) and the components identified by mass spectrometry (MS) and other methods by YAMANISHI *et al.*¹, BONDAROVICH *et al.*², and MUGGLER-CHAVAN *et al.*³. YAMANISHI *et al.*¹ used steam distillation under slightly reduced pressure, and obtained a concentrate, which was fractionated by silica gel column chromatography, prior to GC separation and identification by MS and infrared analyses.

GC-MS analyses of the head-space vapor over dry tea has been carried out by HEINS *et al.*⁴, while REYMOND *et al.*⁵ investigated the composition of head-space volatiles over brewed tea. Adsorption of head-space volatiles on charcoal prior to analysis has not been previously used for tea, although the use of charcoal was reported for the collection of the aroma complexes of apples by SCHULTZ *et al.*⁶, onions by CARSON AND WONG⁷, apricots by TANG AND JENNINGS⁸, peas by RALIS *et al.*⁹ and pears by HEINZ *et al.*¹⁰. WEURMAN¹¹ reviewed methods for the isolation and concentration of food volatiles and suggested that artefact formation on charcoal was probably not a serious problem.

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YAMANISHI *et al.*¹² compared the composition of volatiles in steam distillates of black tea from different geographical locations, and found variations in the relative amounts of linalool plus linalool oxides, and in the ratios of the sum of the peak areas of components having retention times lower than that of linalool to the sum of the peak areas of components appearing after linalool. Analyses by YAMANISHI *et al.*¹³ of the head-space vapor over "flavory" and "non-flavory" Ceylon black tea showed a high proportion of linalool for the flavory tea sample, whereas the non-flavory sample showed a high proportion of components having retention times lower than that of linalool.

In the present study, the head-space volatiles over brewed tea were trapped on charcoal and eluted therefrom prior to GC-MS analyses, and the results obtained are compared with those for an aroma concentrate prepared by steam distillation under reduced pressure. Using these two procedures for the preparation of aroma concentrates, the volatile components of a Ceylon black tea sample, judged to be flavory by a panel of professional tea tasters, were compared with those from a sample which was considered to have no flavor.

EXPERIMENTAL

Materials

Duplicate samples of two fresh, unblended, high-grown black tea samples, manufactured in two different factories in Ceylon, were the material used in this investigation. One of the teas was flavory and the other non-flavory, as judged by a panel of professional tea tasters. The aroma concentrates obtained by steam distillation under slightly reduced pressure were prepared at Ochanomizu University, Tokyo, Japan. Those obtained by adsorption on charcoal were prepared at the M.I.T., Cambridge, Mass., U.S.A. All aroma concentrates were investigated by GC-MS in the M.I.T. laboratories.

Preparation of aroma concentrates

Steam distillation under slightly reduced pressure. Black tea (200 g) was placed in a three-necked 3-litre flask fitted with an inlet for steam, a funnel for the introduction of boiling water and an outlet for steam-volatile compounds. Distillation was carried out for 1 h, during which period the volatiles were condensed in two water-cooled condensers, and collected in two traps cooled with a dry ice-acetone mixture. Conditions of reduced pressure were obtained by connecting the outlet of the trap to an aspirator. The procedure was repeated five times. The combined steam distillates were extracted with peroxide-free diethyl ether, dried over anhydrous Na_2SO_4 and concentrated by distillation at 40° in a fractionating column. The aroma concentrate obtained from the flavory tea sample was designated I-F, and that from the non-flavory tea I-N.

Adsorption on charcoal. Black tea (225 g) was brewed in 2500 ml of boiling water in a three-necked 3-litre flask fitted with a 1×10 cm charcoal adsorption column, a funnel for the introduction of boiling water and an inlet for nitrogen to sweep the head-space vapors through the activated charcoal (4.5 g, 18-40 mesh, Witco Chemical Co.). Prior to its use, the charcoal was washed well with peroxide-free diethyl ether

and left in a vacuum oven at 120° for 20 h. After boiling water had been added to the dry tea, the flask and its contents were maintained at 85° for 1 h. Immediately thereafter, the adsorption column was eluted with 250 ml of peroxide-free diethyl ether and the eluate was dried over anhydrous Na₂SO₄. The procedure was repeated five times. The combined ether eluates were concentrated by distillation at 40° in a fractionating column. The aroma concentrate obtained from the flavory tea sample was designated II-F, and that from the non-flavory tea II-N.

The efficiency of the adsorption column was high because, in preliminary experiments, no components were detected by GC analysis of ether extracts from two traps that had been attached to the column outlet and cooled in solid CO₂ and liquid N₂, respectively. Quantitation of the volatile compounds is expressed in terms of the peak area of each component as a percentage of the total peak area of all components separated in the gas chromatogram.

Analyses of aroma concentrates

Separation of the components of the aroma concentrates was effected by gas chromatography, using a 500 ft. × 0.02 in. stainless-steel capillary column, coated with Carbowax 20M and programmed from 50 to 150° at 1°/min with a helium flow-rate of 5.5 ml/min. The injector temperature was 270° and the flame ionization detector temperature was 260°. Some of the compounds in concentrates I-F and II-F were identified by combined GC-MS by using a double-focusing mass spectrometer (Hitachi Perkin-Elmer RMU-7) and procedures described by ISSENBERG *et al.*¹⁴. The peaks from extracts I-N and II-N were identified with reference to I-F and II-F on the basis of retention times alone.

RESULTS AND DISCUSSION

The aroma concentrates obtained by steam distillation under reduced pressure (I-F and I-N) had a pleasant and characteristic aroma of fresh tea, while those obtained by charcoal adsorption (II-F and II-N) had an unpleasant odor similar to that of stewed tea.

Differences between concentrates I and II

The majority of the components were present in much higher concentration in the concentrates obtained by steam distillation under reduced pressure than in those obtained by the use of charcoal. The gas chromatogram showed separation into more than 100 peaks, but only 21 of these were present in amounts sufficient for MS analysis. The identities of these compounds and their relative amounts are given in Table I. Separations of "blank" diethyl ether extracts of unused adsorption columns showed only two peaks, which eluted almost immediately after ether.

In addition to marked quantitative differences observed in the total and relative proportions of individual components in concentrates of flavory and non-flavory tea, prepared by the same method, qualitative differences resulted from the preparative procedures used. Aroma concentrates obtained by charcoal adsorption contained 17 components that were not obtained by steam distillation under reduced pressure. The converse of this situation held for 14 components. Many of these components occurred in only small or trace amounts, but they may make an important contribution to the marked difference in the aroma of the two concentrates.

TABLE I

COMPARISON OF RELATIVE AMOUNTS OF SOME COMPONENTS IDENTIFIED IN CONCENTRATES OF CEYLON BLACK TEA

I-F and I-N concentrates obtained by steam distillation under reduced pressure of flavory and non-flavory tea, respectively; II-F and II-N concentrates obtained by charcoal adsorption of head-space volatiles over brewed flavory and non-flavory tea, respectively. Values represent areas of each peak expressed as a percentage of total peak area. T = trace amount.

Peak No.	I-F	I-N	II-F	II-N	Identity
9	3.4	42.7	25.6	66.0	Mixture in I-F
13	10.1	5.7	2.8	17.0	Mixture containing acetate
26	4.8	3.1	6.0	1.2	Isopentanal (mixture)
28	1.6	1.0	1.5	0	<i>cis</i> -2-Pentenal
34	2.1	0.5	0.7	0.02	<i>trans</i> -2-Hexenal
35	2.7	1.0	0.01	0.03	<i>cis</i> -3-Hexenal
41	0.33	3.7	2.7	2.1	Linalool oxide (<i>cis</i> -, 5-membered)
51	3.5	1.5	6.5	0.5	Linalool oxide (<i>trans</i> -, 5-membered)
57	0.18	0.1	0	0.02	Benzaldehyde
63	8.2	4.1	1.0	0.22	Linalool
65	8.0	3.8	6.3	0.23	α -Terpineol
65A	0	0	5.7	3.0	Ethylene glycol diacetate
69	0.4	0.2	0.07	T	Nerol
70	6.7	9.2	0.2	T	Linalool oxides (<i>cis</i> - and <i>trans</i> -, 6-membered)
87	0.7	0.4	T	0.02	Methyl salicylate
91	0.3	0.3	0	0	Geraniol
92	2.4	0.1	0.5	0.02	Benzyl alcohol, α -ionone
94	2.4	1.5	0	0	Phenylethanol, terpenes
99	5.4	1.0	0	0	β -Ionone (mixture)
100	0.84	0.47	0.44	0.13	<i>cis</i> -Jasmone (mixture)
102	0.1	T	T	0	Sesquiterpene alcohol

Identification by mass spectrometric analysis and comparison of retention data indicated that peak 9 was pure acetic acid in concentrate II-F, but contained a mixture of compounds in concentrate I-F. This peak was the major component of concentrate II-N and is probably an artefact of the charcoal adsorption procedure. Its origin is not known, but this relatively large amount of acetic acid may account for some of the peaks in concentrates II-F and II-N, *e.g.*, the mass spectrum of one of these was identical with that of ethylene glycol diacetate as determined by SASAK *et al.*¹⁶; its retention time was also identical with that of an authentic marker. It is presumed that this compound could arise by interaction of Carbowax 20M with the high concentrations of acetic acid. Peak 13, which is especially large in concentrate II-N, had a base peak at *m/e* 43, possibly indicating the presence of another acetate.

The relative amounts of linalool and its oxides were widely divergent in the various concentrates, which suggested that interconversions of these compounds might have occurred on charcoal. Accordingly, the respective volatile fractions from authentic linalool, and linalool oxides I and II, were trapped on charcoal and eluted under the experimental conditions used for the tea samples. GC analyses of the resulting isolates showed the formation of seven artefacts from linalool. The mixtures of linalool oxides showed formation of two artefacts, one of which had the same retention time as linalool. However, the relative extent of conversion of the authentic compounds to artefacts was small (less than 1%), and it is possible that a higher degree of artefact

formation occurs with tea owing to other factors, *e.g.*, acidic conditions. Artefact formation from linalool oxides III and IV was not examined.

It can also be seen from Table I that the relative amounts of the components is markedly different in concentrates I and II, and that this discrepancy becomes most marked for compounds with higher boiling points.

Differences between aroma concentrates from flavory and non-flavory tea

Many differences were observed in the composition of aroma concentrates from flavory and non-flavory teas, and although only concentrates I-F and I-N possessed a characteristic aroma of tea, concentrates II-F and II-N are also of interest. Evidently, a high proportion of acetic acid is detrimental to tea flavor. This may indicate that acetic acid *per se* is detrimental, or that acetic acid (or its precursor) is converted into compounds that contribute to the flavor of tea, and its accumulation is an indication that the biosynthetic reactions that are necessary for the production of flavor have not occurred. In this connection, SAIJO AND URITANI¹⁶ have shown that [2-¹⁴C]acetate is converted into nerolidol in excised tea shoots. However, other quantitative differences probably play a part in tea flavor, as many compounds, which were not identified in this study, were also found to have different relative proportions in the concentrates. Among the compounds identified, linalool and compounds with retention times higher than linalool were present in relatively higher proportions in the concentrates from flavory tea than from non-flavory tea, and the importance to tea flavor of these compounds has been already suggested by YAMANISHI *et al.*¹²; on the other hand, SAIJO¹⁷ has emphasised the contribution of some of the low-boiling tea volatiles to black tea flavor. It seems, therefore, that no single compound is responsible for black tea flavor but that this desirable characteristic is due rather to the correct balance being attained between a number of different compounds. This study has been concerned with compounds that are volatile in steam, and has not taken into consideration non-volatile compounds, which have been described by MILLIN *et al.*¹⁸.

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