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# Additional Volatiles of Black Tea Aroma

Walter Mick and Peter Schreier*

The volatiles of aqueous infusions of two black tea qualities (Indian Broken; Darjeeling Orange Pekoe) were investigated. Aroma separation was carried out by standard controlled high-vacuum distillation followed by solvent extraction (pentane–dichloromethane, 2:1). The concentrated extracts were prefractionated in four fractions by adsorption chromatography on silica gel using a pentane diethyl ether gradient. In total, 133 volatiles not described as yet as black tea aroma constituents were identified by capillary gas chromatography and coupled capillary gas chromatography–mass spectrometry, among them 11 hydrocarbons, 30 esters, 25 alcohols, 48 carbonyls, 3 lactones, 3 phenols, and 13 volatiles of miscellaneous structures. These compounds are discussed from the aspect of their formation during black tea manufacturing.

Tea is one of the most widely consumed beverages in the world, and its popularity consists certainly of its pleasant flavor combined with its stimulating effects. There are several types of tea, e.g., green tea, oolong tea, and black tea, each with several subclassifications (Eden, 1976). All types are prepared from essentially the same material, the shoot tips of the tea plant, *Camellia sinensis,* L.

Analytical techniques, especially gas chromatography and mass spectrometry, have revealed that the aroma of tea is a complex mixture of mainly trace compounds. Many reviews of black tea aroma chemistry have been provided, summarizing more than 350 identified volatiles (Sanderson, 1972, 1975; Natarajan et al., 1974; Yamanishi, 1975, 1981; Howard, 1978), but which of the black tea aroma constituents are really essential in determining the character of this beverage is still unknown. Therefore, studies of black tea aroma are further extended. In this paper, some additional volatile constituents not described as yet as black tea volatiles (Van Straten and Maarse, 1983) will be described.

## EXPERIMENTAL SECTION

**Materials.** Two black tea qualities, Indian Broken (IB) (grown at low altitude, harvested in late summer 1980) and Darjeeling Orange Pekoe (DOP) (Grown at high altitude, harvested in spring 1980), purchased from P. Schrader & Co., Bremen, West Germany, were used.

**Solvents.** All solvents used were analytical–grade materials and additionally purified by distillation.

**Aqueous Tea Extract.** An aqueous tea infusion was prepared as follows: 200 g of black tea was brewed for 3 min with 2 L of boiling water. After cooling (7 min) in an ice bath, the leaves were separated and pressed by hand with a cloth. The aqueous infusion (approximately 1.6 L) was placed in a 4-L three-neck bottle. After addition of internal standards (0.16 mg of alloocimene, 0.3 mg of

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methyl decanoate, 0.25 mg of 1-decanol, 0.25 mg of 1-dodecanol), high-vacuum distillation was started. In total, 1 kg of black tea was applied, i.e., repeating this step 5 times.

**High-Vacuum Distillation.** The sample (approximately 1.6 L) was temperature controlled at 40–50 °C in a water bath. Four cooling traps were connected in a row; traps 1 and 2 were mantel-cooled (–25 °C, $CH_3OH$–solid $CO_2$), and traps 3 and 4 were cooled with liquid nitrogen. The vacuum distillation took 2 h at 0.1–0.01 bar. The four traps were thawed, and the contents were combined (yield 1 L) and used for the following solvent extraction.

**Solvent Extraction.** The extraction with a pentane–dichloromethane (2:1) mixture was performed during 24 h (using fresh solvent after 12 h) (Drawert and Rapp, 1968). The extracts were concentrated to approximately 1 mL by means of a Vigreux column. In an experiment without any standard addition, the extract obtained after high-vacuum distillation–solvent extraction had the appropriate tea aroma properties.

**Column Chromatography on Silica Gel.** The concentrated extract from the high-vacuum distillations (1 mL) was fractionated on silica gel 60 (Merck), activity grade II, with a pentane–diethyl ether gradient (Schreier et al., 1979). Cooled (11–13 °C) glass columns, 2.0 cm i.d. × 45 cm, were used with an elution rate of 70 mL/h, and four fractions were obtained. Fraction I was eluted with 160 mL of pentane, fraction II was obtained by eluting with 160 mL diethyl ether–pentane (1:9 v/v), fraction III was eluted with 220 mL of diethyl ether–pentane (5:5 v/v), and fraction IV was obtained after elution with 200 mL of diethyl ether. All eluates were concentrated to 0.1 mL for gas chromatographic and gas chromatographic–mass spectrometrical investigation. The fractionation on silica gel destroyed the tea aroma complex; i.e., none of the fractions showed the aroma of the original extract.

**Gas–Liquid Chromatography.** A Carlo Erba Fracto-vap 4160 gas chromatograph equipped with a J & W fused silica wide-bore CW 20 M capillary column (30 m, 0.31 mm i.d.) was used. On-column injection with an air-cooled

**Table I. Different Methods of Sample Preparation to Analyze Black Tea Volatiles**

| method | sample | literature references |
|---|---|---|
| static headspace | dry tea | Heins et al. (1966) |
| | tea extract | Reymond et al. (1966); Yamanishi et al. (1968) |
| dynamic headspace with polymer adsorption | tea extract | Wickremasinghe et al. (1973); Vitzthum and Werkhoff (1978) |
| solvent extraction | dry tea | Kobayashi et al. (1965); Yamanishi et al. (1965); Saijo and Kuwabara (1967); Sato et al. (1970); Saijo (1977) |
| | tea extract | Selvendran et al. (1978) |
| CO_2 extraction | dry tea | Grimmett (1981) |
| extraction with supercritical gases | dry tea | Vitzthum et al. (1975); Hubert and Vitzthum (1980); Coenen et al. (1982) |
| steam distillation | dry tea | Müggler-Chavan et al. (1966); Bondarovich et al. (1967); Yamanishi et al. (1972); Gianturco et al. (1974) |
| steam distillation-extraction | tea extract | Brandenberger and Müller (1962); Kozhin and Treiger (1973); Renold et al. (1974); Cloughley et al. (1982) |

injection system was employed. The temperature program was 50–240 °C at 2 °C/min. The flow rates for the carrier gas were 0.5 mL/min $N_2$, for the makeup gas 30 mL/min $N_2$, and for the detector gases 30 mL/min $H_2$ and 300 mL/min air, respectively. The detector temperature was 220 °C. Volumes of 0.1 µL were injected.

Qualitative analyses were carried out by means of retention and mass spectral data of reference substances. Quantitative determinations were performed by standard controlled calculations using a Hewlett-Packard 3388 A laboratory data system without consideration of distillation and extraction yields (calibration factor for all compounds, $F = 1.00$).

**Gas Chromatography–Mass Spectrometry.** A Varian Aerograph 1400 gas chromatograph coupled by an open-split connection to a mass spectrometer, Finnigan MAT 44, was used. The apparatus was equipped with a Mega CW 20 M fused silica wide-bore capillary column (25 m, 0.32 mm i.d., thick film). On-column injection with a water-cooled injection system was employed. The temperature program was 5-min isothermal at 60 °C and 60–240 °C, 2 °C/min. The carrier gas flow rate was 1.0 mL of He/min. The temperature of ion source and all connection parts was 200 °C. The electron energy was 70 eV; the cathodic current was 0.8 mA. Injection volumes were 0.1 µL.

RESULTS AND DISCUSSION

Many different methods have been described for the isolation of black tea volatiles (Table I). As the isolation and enrichment procedures essentially influence the results (Teranishi, 1981), many findings in black tea aroma studies are not directly comparable. In our study, two different qualities of black tea were used, namely, Darjeeling Orange Pekoe (highland, plucked spring 1980) (DOP) and Indian Broken (plain, plucked summer 1980) (IB). From these samples aqueous infusions were prepared and high vacuum distilled. The aqueous distillates were then extracted by a pentane–dichloromethane mixture (2:1), and after careful removal of the solvent by distillation on a Vigreux column, the volatiles were preseparated in four fractions by using adsorption chromatography on silica gel with a pentane–diethyl ether gradient (Schreier et al., 1979). The analysis of volatiles was carried out by capillary gas chromatogra-
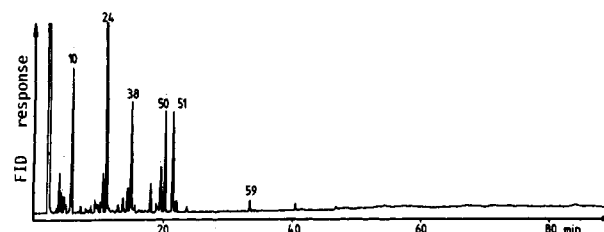


**Figure 1.** Fused silica capillary gas chromatographic separation of black tea infusion volatiles (silica gel fractions II-IV) after on-column injection using a J & W Scientific CW 20 M wide-bore column. Further conditions are outlined under Experimental Section. The peak numbers correspond to the numbers in Table II.
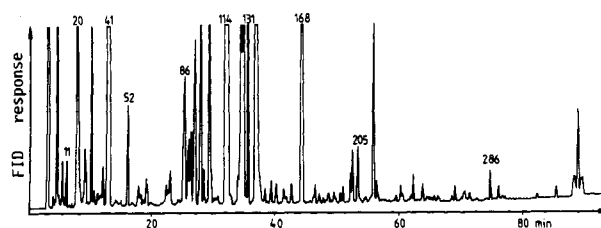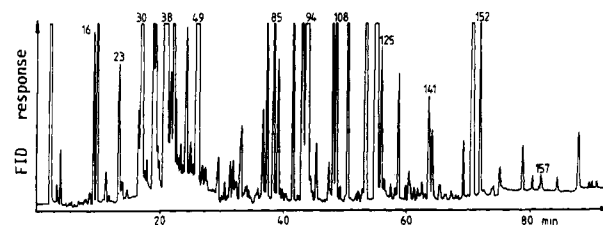


**Figure 2.** Compare Figure 1.



**Figure 3.** Compare Figure 1.

phy and coupled capillary gas chromatography–mass spectrometry.

Figures 1–3 show the gas chromatographic separations of volatiles in silica gel fractions II–IV obtained from the black tea infusion of IB quality. In Table II the volatiles identified in the IB quality by gas chromatography–mass spectrometry are summarized that were not known as yet as volatile constituents of black tea infusion. Where no reference samples were available, substances were designated as tentatively identified (cf. Table II). As to the IB quality, the names of compounds given in Table II correspond to the numbered peaks in Figures 1–3. In Table II the volatiles newly identified by the above-mentioned methods in the infusion of DOP tea quality are also included but without a corresponding figure.

As it can be seen from Table II, in both tea qualities 11 hydrocarbons, 30 esters, 25 alcohols, 48 carbonyls, 3 lactones, 3 phenols, and 13 volatiles of miscellaneous structures were identified, which have not been described as yet as black tea aroma components. From the data outlined in Table II, it cannot be concluded that the volatiles detected occur specifically in the mentioned tea quality. It has to be considered that all the volatiles newly detected were present in quantities <10 µg/kg of infusion, and in spite of the preseparation by adsorption chromatography, the tea aroma composition in the individual fractions remained very complex, i.e., complicating the detection of individual components. Relations between black tea quality and quantitative distribution of volatiles have already been discussed elsewhere (Schreier and Mick, 1984).

Black tea is fermented tea, and the tea manufacturing process essentially consists of four steps, i.e., withering, rolling, fermentation, and drying (Yamanishi, 1981).

**Table II. Additional Volatiles Identified by HRGC and HRGC-MS in Silica Gel Fractions I–IV Obtained after High-Vacuum Distillation–Solvent Extraction of Black Tea Infusions (IB, Indian Broken; DOP, Darjeeling Orange Pekoe)**

| peak no.[a] | compound | identified in silica gel fraction[b] | mass spectral data[c] |
|---|---|---|---|
| | **Hydrocarbons** | | |
| 9 | α-pinene | II | 93, 91, 92, 41 |
| * | camphene | II | 93, 41, 79,121 |
| * | Δ-3-carene | II | 93, 41, 91, 77 |
| 20 | α-phellandrene | II | 93, 91, 77, *136* |
| 25 | β-phellandrene | II | 93, 77, 79, *136* |
| 21 | α-terpinene | II | 93, 121, 91, *136* |
| 30 | γ-terpinene | II | 93, 91, 77, 121 |
| 35 | p-cymene | II | 119, 91, 134, 117 |
| 61 | β-elemene | III | 121, 93, 41, 79 |
| 46 | 1,2,4,5-tetramethylbenzene | II | 119, *134*, 91, 123 |
| 48 | ethinylbenzene | II | *102*, 76, 103, 51 |
| * | 1,1'-(1,2-ethynediyl)-bis(benzene) (tent.) | II | *178*, 179, 176, 89 |
| 55 | (?)-dimethylstyrene (tent.) | II | *132*, 117, 131, 115 |
| 58 | (?)-tetrahydronaphthalene (tent.) | II | 104, *132*, 91, 51 |
| 63 | (?)-trimethyl-(?)-dihydronaphthalene (tent.) | II | 157, *172*, 142, 141 |
| * | 1,7-dimethylnaphthalene (tent.) | II | *156*, 141, 155, 157 |
| * | 1,6-dimethyl-4-methylethylnaphthalene (tent.) | II | 183, *198*, 184, 169 |
| | **Esters** | | |
| * | 1-hydroxy-2-propanone acetate | IV | 43, 87, 42, 44 |
| 17 | butyl acetate | III | 43, 56, 41, 73 |
| * | phenyl acetate | III | 43, 94, 58, 42 |
| * | neryl propanoate | III | 57, 69, 41, 93 |
| 174 | neryl methylpropanoate | III | 59, 43, 41, 93 |
| 7 | methyl butanoate | III | 43, 71, 74, 41 |
| 58 | 2-hexyl butanoate | III | 71, 43, 56, 84 |
| 193 | neryl butanoate | III | 59, 58, 93, 80 |
| 55 | ethyl 3-hydroxybutanoate | III | 43, 45, 42, 60 |
| 109 | (Z)-3-hexenyl pentanoate | III | 67, 82, 57, 41 |
| 23 | methyl hexanoate | III | 74, 87, 59, 99 |
| * | (Z)-2-pentenyl hexanonate | IV | 99, 43, 41, 71 |
| * | (E)-2-pentenyl hexanoate | IV | 99, 43, 41, 71 |
| 47 | methyl (Z)-3-hexenoate | III | 41, 68, 74, 69 |
| * | methyl (E)-2-hexenoate | III | 55, 41, 68, 87 |
| * | (Z)-3-hexenyl nonanoate | III | 82, 67, 55, 41 |
| 242 | (Z)-3-hexenyl decanoate | III | 82, 67, 55, 43 |
| * | methyl tetradecanoate | III | 74, 87,43, 41 |
| 264 | methyl pentadecanoate | III | 74, 87, 43, 41 |
| 273 | ethyl pentadecanoate | III | 88, 101, 43, 41 |
| * | methyl heptadecanoate | III | 74, 87, 43, 41 |
| 292 | methyl octadecanoate | III | 74, 87, 43, 55 |
| 294 | ethyl octadecanoate | III | 88, 101, 43, 55 |
| * | ethyl benzoate | III | 105, 77, 51, 122 |
| * | benzyl benzoate | III | 105, 91, 77, 51 |
| * | 3-methylbutyl 2-hydroxybenzoate | III | 120, 43, 138, 121 |
| 267 | methyl 2-acetoxybenzoate | III | 120, 92, 43, 121 |
| * | methyl 4-methoxybenzoate | III | 135, 77, *166*, 92 |
| 243 | methyl 2-methoxybenzoate | III | 135, 77, 133, 92 |
| 167 | methyl phenylacetate | III | 91, *150*, 65, 59 |
| | **Lactones** | | |
| * | 2-methyl-4-butanolide | IV | 41, 56, 42, *100* |
| 113 | (?)-methyl-4-heptanolide | IV | 85, 41, 55, 42 |
| 219 | 5-octanolide | IV | 42, 99, 71, 55 |
| * | 5-nonanolide | IV | 42, 99, 41, 43 |
| | **Phenols** | | |
| 246 | 2-ethylphenol | III | 107, *122*, 77, 79 |
| * | 3-ethylphenol | III | 107, *122*, 77, 79 |
| 130 | 4-aminophenol | IV | *109*, 80, 53, 107 |
| | **Alcohols** | | |
| * | 2-methyl-3-pentanol | III | 59, 73, 41, 55 |
| 25 | 2-pentanol | III | 45, 41, 43, 73 |
| 11 | 2-methyl-2-pentanol | IV | 59, 45, 43, 87 |
| 33 | 3-methyl-2-pentanol | III | 45, 41, 56, 69 |
| 18 | 4-penten-1-ol | IV | 68, 67, 41, 53 |
| * | 2-hexanol | IV | 45, 43, 41, 69 |
| 19 | 2-methyl-2-hexanol | IV | 59, 43, 101, 41 |
| 46 | 1-hexen-3-ol | III | 57, 43, 72, 41 |
| * | 2-heptanol | III | 45, 43, 55, 41 |
| 38 | 4-heptanol | III | 55, 73, 43, 41 |
| * | 1,5-octadien-3-ol | III | 57, 70, 55, 41 |
| * | 5-nonanol | IV | 69, 41, 87, 43 |
| 133 | 5-undecanol | III | 55, 83, 101, 41 |
| 258 | 1-tetradecanol | III | 43, 55, 41, 57 |
| 279 | 1-pentadecanol | III | 43, 55, 41, 57 |
| 287 | 1-hexadecanol | III | 43, 55, 41, 57 |
| 299 | 1-octadecanol | III | 43, 55, 41, 57 |
| 65 | 2,4-dimethyl benzyl alcohol | IV | 118, 91, 121, *136* |
| 112 | teresantalol | III | 121, 93, 94, 92 |
| 122 | fenchyl alcohol | III | 81, 80, 69, 82 |
| * | myrtenol | III | 79, 91, 41, 43 |
| 187 | p-cymen-8-ol | III | 135, 91, 65, *150* |
| 197 | p-mentha-1,4-dien-7-ol | III | 79, 43, 91, 92 |
| 91 | dihydrocarveol | IV | 41, 43, 55, 93 |
| * | carveol | IV | 119, 91, 134, 92 |
| | **Carbonyls, Aldehydes** | | |
| 234 | thiophene-2-acetaldehyde | III | 43, 55, 84, 41 |
| 21 | (E)-2-methyl-2-butenal | III | 55, *84*, 41, 53 |
| * | 2-methylpentanal | III | 43, 58, 41, 71 |
| * | 3-methylpentanal | III | 56, 41, 43, 44 |
| * | 2-methyl-2-pentenal | III | 41, 98, 69, 55 |
| * | 4-methyl-2-pentenal | III | 41, 56, 69, 55 |
| * | 2,4-dimethyl-(?)-2,4-heptadienal (tent.) | III | 109, 41, 67, *138* |
| 204 | (E)-2-tridecenal | III | 44, 43, 41, 55 |
| 121 | 4-methylbenzaldehyde | III | 91, 119, *120*, 65 |
| 157 | 2,4-dimethylbenzaldehyde | III | 133, *134*, 105, 77 |
| 162 | (?)-dimethylbenzaldehyde (tent.) | III | 133, *134*, 105, 91 |
| 196 | 2,4,6-trimethylbenzaldehyde | III | 147, *148*, 119, 91 |
| 205 | 2-ethoxybenzaldehyde | III | 121, 65, *150*, 13 |
| 211 | 3-ethoxybenzaldehyde | III | 121, *150*, 65, 104 |
| 224 | 4-ethoxybenzaldehyde | III | 121, *150*, 65, 104 |
| 280 | (?)-dimethoxybenzaldehyde (tent.) | III | *166*, 165, 95, 120 |
| 270 | 2-pentylcinnamaldehyde | III | 117, 91, 129, 145 |
| 145 | thiophene-2-aldehyde | III | 111, *112*, 45, 83 |
| 172 | 3-methylthiophene-2-aldehyde | III | 125, *126*, 45, 53 |
| | **Miscellaneous** | | |
| * | 2-butylfuran | II | 91, *124*, 82, 53 |
| 185 | 3-phenylfuran | III | *144*, 115, 81, 63 |
| 41 | 2,5-furandione | IV | 54, 53, *98*, 41 |
| * | 2,6,6-trimethyl-2-vinyltetrahydropyran | III | 68, 67, 110, 43 |
| 60 | 2,5-dimethylpyrrole | III | 94, 95, 42, 80 |
| 129 | 1-methyl-2-formylpyrrole | III | *109*, 108, 53, 80 |
| 183 | 1-methyl-2-ethylpyrrole | III | 94, *109*, 42, 41 |
| * | propylpyrazine | IV | 94, 107, *122*, 121 |
| * | vinylpyrazine | IV | *106*, 52, 79, 53 |
| 146 | (?)-dimethylquinoline (tent.) | IV | *157*, 156, 142, 115 |
| 12 | 2-methylthiophene | II | 97, *98*, 53, 99 |
| 14 | 3-methylthiophene | II | 97, *98*, 45, 59 |
| 190 | 2-propionylthiophene | III | 111, *140*, 83, 112 |
| 7 | tetrahydrothiophene | IV | 60, *88*, 45, 46 |

Additional Volatiles of Black Tea Aroma

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**Table II** (Continued)

| peak no.[a] | compound | identified in silica gel fraction[b] | mass spectral data[c] | peak no.[a] | compound | identified in silica gel fraction[b] | mass spectral data[c] |
|---|---|---|---|---|---|---|---|
| | Carbonyls, Ketones | | | * | 5-octene-2-one (tent.) | III | 43, 68, *126*, 41 |
| * | phenyl-1-propan-1-one | III | 105, 77, 51, *134* | * | 2,3-octadione | IV | 43, 71, 99, 41 |
| 27 | 3-hydroxy-2-butanone | IV | 45, 43, *88*, 42 | 107 | 3,3-dimethyl-2,7-octadione (tent.) | IV | 43, 69, 109, 86 |
| 9 | 4-methyl-2-pentanone | III | 59, 43, 41, 58 | | | | |
| 32 | 3-hydroxy-2-pentanone | IV | 59, 43, 41, 58 | 57 | 3-nonanone | III | 43, 57, 72, 85 |
| 71 | 4-hydroxy-4-methyl-pentan-2-one | III | 43, 59, 58, 42 | 71 | 2-undecanone | IV | 43, 58, 71, 41 |
| | | | | 150 | 2-dodecanone | III | 43, 58, 71, 41 |
| 11 | 1-penten-3-one | III | 55, 84, 56, 57 | * | (?)-methyltetradecan-3-one (tent.) | III | 57, 72, 43, 73 |
| * | 3-methylcyclopent-2-en-1-one | IV | 96, 53, 67, 81 | * | 4-methylacetophenone | III | 119, 91, 92, *134* |
| * | 5-methyl-2-hexanone | III | 43, 58, 41, 71 | 170 | 2-hydroxyacetophenone | III | 105, 77, 51, 122 |
| 61 | 2,3-dimethylcyclo-hexanone | III | 55, 83, 41, 82 | 130 | 2-(methoxymethyl)-acetophenone (tent.) | IV | 131, 149, *164*, 103 |
| 63 | (?)-dimethylcyclo-hexanone (tent.) | III | 56, 41, 98, 69 | 293 | benzophenone | III | 77, 105, 51, 50 |
| 43 | 3-hydroxycyclohexanone | IV | 43, 44, 68, 42 | * | 2-furfuryl methyl ketone | III | 43, 81, 82, 53 |
| 132 | 4-hydroxy-2,6,6-trimethylcyclo-hexanone | IV | 83, 57, 41, 69 | * | 5-methyl-2-acetylfuran | II | 109, *124*, 53, 43 |
| | | | | 48 | 2-methyltetrahydro-furan-3-one | III | 43, 72, *100*, 42 |
| 124 | 3,5,5-trimethylcyclohex-2-en-1-one | III | 82, *138*, 41, 55 | * | 1-indanone | III | *132*, 104, 78, 77 |
| 43 | 6-methyl-2-heptanone | III | 43, 58, 41, 71 | 110 | 1-acetylpyrrole | III | 67, *109*, 43, 41 |
| * | 6-methyl-3,4-heptanedione (tent.) | IV | 57, 85, 41, 43 | 77 | 1-methyl-2-acetylpyrrole | IV | 108, *123*, 53, 80 |
| | | | | 74 | 2-acetylpyrazine | IV | 43, 52, 53, 94 |
| * | (E)-3-hepten-2-one | III | 55, 43, 41, 97 | * | fenchone | III | 81, 68, 51, *152* |
| * | 5-(methylethyl)-(E)-3-hepten-2-one | III | 43, 97, 112, 55 | 99 | camphor | III | 95, 81, 41, 108 |
| | | | | 152 | pulegone | III | 81, 67, 109, 41 |

[a] The peak numbers correspond to the numbers shown in Figures 1–3. Volatiles not found in the IB but in DOP quality are marked by an asterisk (*). The used internal standards are indicated as 50 and 51 [(Z)- and (E)-alloocimene, fraction II], 131 (methyl decanoate, fraction III), and 94 and 122 (1-decanol, 1-dodecanol, fraction IV). [b] Identifications were carried out by GLC retention and mass spectral data of authentic reference compounds (tent. = reference sample not available; tentative identification by mass spectra only). [c] The four highest values are represented (M$^+$ in italics).

Consequently, the aroma of black tea is composed of original green tea volatiles, which are not or only a little influenced, and of "secondary", i.e., technologically influenced volatiles formed or changed during these biological and technological steps. In the following the volatiles identified in this study will be discussed from the aspect of their formation during the manufacturing process.

The conditions chosen for silica gel fractionation of black tea infusion volatiles allowed separation of the group of alkanes and alkenes (fraction I) from that of terpene and aromatic hydrocarbons (fraction II). As the common alka(e)nes are not relevant as efficient aroma compounds, the gas chromatographic separation and the identified substances are not listed here. In fraction II, a series of monoterpene hydrocarbons was detected, which can be considered to be natural constituents of tea leaves. They show structures that are well-known from different food flavors (Van Straten and Maarse, 1983).

The alcohols identified in black tea infusions (Table II) can also be regarded as natural aroma compounds. They consist of different alkanols and alkenols as well as various terpene alcohols. Structurally, they are common volatiles of many foods.

A part of the esters detected in this study comprises natural aroma substances of tea leaves, but another one obviously arises from lipid degradation reactions during manufacturing. In particular, the identified 3-hexenyl esters have to be regarded as esterification products of $C_6$ alcohols formed as a results of mechanical injury (Saijo and Takeo, 1975) by enzymic splitting of unsaturated fatty acid hydroperoxides and subsequent reduction of $C_6$ aldehydes (Hatanaka et al., 1982).

Analogously to the esters, the carbonyls listed in Table

II constitute natural and "secondary" volatiles as well. Saturated and unsaturated ketones and aldehydes were found, among them well-known food flavor substances, but also less common volatiles such as, e.g., 3-methylcyclopent-2-en-1-one, the dimethylcyclohexanones, 3-hydroxycyclohexanone, 6-methyl-3,4-heptanedione or 5-methyl-ethyl-(E)-3-hepten-2-one, and 3,3-dimethyl-2,7-octanedione; the two latter have been found as yet only in tobacco (Aasen et al., 1973; Behr et al., 1977). In this group with several thiophene, furan, pyrrole, and pyrazine derivatives, typical secondary aroma compounds were identified. Substances of these chemical classes have also been listed in Table II under miscellaneous structures. Thiophene derivatives arise mainly from the thermal degradation of sulfur-containing amino acids alone (Merritt and Robertson, 1967) or in the presence of reducing sugars (Mulders, 1973; Mussinan and Katz, 1973; Kato et al., (1973). Sugar degradation products such as 3(2H)-furanones or furfural can react with hydrogen sulfide to also afford thiophenes (Van den Ouweland and Peer, 1975; Shibamoto and Russell, 1976). In tea, thiophenes have not been described as yet.

The existence of furans in foods have been known for some years, and their basic formation pathway through carbohydrate thermal decomposition has been postulated. Maga (1979) provided a detailed review on furans in food; the furan derivatives presented in Table II supplement these data.

Several pyrroles have already been identified among tea aroma volatiles. Additionally, we detected some N-substituted derivatives and 2,4-dimethylpyrrole. In general, pyrroles found in foods and tobacco are regarded to be formed via Maillard or Strecker reactions between sugars and amino acids during processing (Dickerson et al., 1976;

Tressl, 1980). In model systems, N-substituted derivatives can be obtained by reaction of $\alpha,\beta$-dicarbonyl compounds, cyclic enolones, $\alpha,\beta$-unsaturated aldehydes, or 2-formylfurans with hydroxyproline (Tressl, 1980; Tressl et al., 1978).

Pyrazines occur in nature in small quantities and are considered to be most likely formed from $\alpha$-hydroxycarbonyl or $\alpha,\beta$-dicarbonyl compounds, themselves degradation products of carbohydrates and amino acids. Recently, several reviews on the occurrence and formation of pyrazines in food have been provided (Brophy and Cavill, 1980; Flament, 1981; Barlin, 1982; Maga, 1982). In tea, Vitzthum et al. (1975) identified 56 N-containing volatile substances including 11 pyrazines. The compounds newly detected in this study have already been found among the volatiles of cooked beef, cocoa, and heated eggs (Maga, 1982).

In the group of lactones, two $\gamma$- and two $\delta$-lactones not described as yet as tea aroma substances were identified in the tea infusions. The importance of this class of compounds as food flavor constituents has been fully demonstrated in the reviews provided by Maga (1976) and Ohloff (1978).

Finally, it has to be pointed out that the newly detected substances listed in Table II only comprise the part of volatiles obtained by solvent extraction of tea infusions, which were high-vacuum distilled at natural pH. The structures of volatile acids found under these conditions have already been described elsewhere (Mick et al., 1984), and the identification of volatiles detectable after alkaline treatment of the residues of high-vacuum distillations and also, high-vacuum distillation–solvent extraction is still under study. With this investigation we could further enlarge our knowledge about the chemical composition of black tea volatiles and deliver additional information for studies on correlations of sensory and analytical data.

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# Heat-Induced Changes in Sulfhydryl Groups and Disulfide Bonds in Fish Protein and Their Effect on Protein and Amino Acid Digestibility in Rainbow Trout (*Salmo gairdneri*)

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Ground fillets of frozen Alaska pollock (*Theragra chalcogramma*), a low-fat fish, and Pacific mackerel (*Pneumataphorus japanicus*), a high-fat fish, heated for 20 min at temperature intervals ranging from 40 to 115 °C showed a linear decrease in the content of -SH (sulfhydryl) groups and a concomitant increase in the content of S-S (disulfide) bonds from 50 to 115 °C. At 95 °C, the reaction was rapid and had reached an equilibrium after 20 min. These experiments indicate that temperatures higher than 50 °C are required for oxidative transformation of -SH groups to S-S bonds. Heating at 115 °C caused a loss in cystine plus cysteine. Heating to 95 °C and drum-drying caused the formation of S-S bonds from -SH groups and reduced protein and amino acid digestibility when fed to rainbow trout (*Salmo gairdneri*) as compared with the raw fish protein. Freeze-drying did not affect digestibility and no formation of S-S bonds was found. It is postulated that heat-induced S-S cross-linking from -SH oxidation causes a reduction in protein and amino acid digestibility in drum-dried samples. The effect of heating on digestibility was greater in the low-fat pollock than in the high-fat mackerel.

Sulfhydryl groups and disulfide bonds are important in maintaining structure and functions of native proteins (Saxena and Wetlaufer, 1970) and play important roles in functional properties of proteinaceous foods (Connell, 1964;

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Wolf, 1970). Disulfide cross-linking in proteins may occur from oxidation of sulfhydryl groups and sulfhydryl–disulfide interchange (Cecil and McPhee, 1959; Friedman, 1973). This has been demonstrated by heating fish (Itoh et al., 1979a,b; Itoh et al., 1980), meat (Hamm and Hofmann, 1965), soybean (Wolf, 1970), and lactalbumin (Schnack and Klostermeyer, 1980) and in frozen storage of fish (Buttkus, 1970; Tsuchiya et al., 1979). Heating that affected the status of cysteine/cystine residues was found to reduce protein utilization by animals (Rios Iriarte and Barnes, 1966; Dvorschak, 1970; Waibel et al., 1977), and