Seasonal Variations in the Composition of the Volatile Constituents of Black Tea. A Numerical Approach to the Correlation between Composition and Quality of Tea Aroma

Maurizio A. Gianturco,* Robert E. Biggers, and Brigitte H. Ridling

A study of the seasonal variation of the volatile constituents of teas from three estates over a period of 15 months has evolved into a scheme for the classification of samples according to the quality of their aroma. The scheme is based on parameters derived from gas chromatographic data.

Previous communications from this and other laboratories have brought forth information with regard to the structures of some polyphenols formed by enzyme-catalyzed oxidation during black tea manufacturing (Ferretti et al., 1968; Takino et al., 1965, 1966, 1967), and with regard to the composition of the "aroma complex" of tea (Bondarovich et al., 1967). One of these studies has further shown that at least quantitative differences exist between the volatile constituents of teas of different origin (Bondarovich et al., 1967). More recent advances in the understanding of the aroma complex and of reactions occurring during the process of tea manufacture have been reviewed (Sanderson, 1972), and it is of interest that several studies link together polyphenols and volatile constituents by pointing to the intermediacy of some transient oxidized species of tea polyphenols in the formation of volatile compounds from amino acids (saturated aliphatic aldehydes and phenylacetaldehyde), long-chain unsaturated acids (trans-2-hexenal), and carotenoids (β-ionone; 2,2,6-trimethylcyclohexanone, etc.).

The purpose of the present communication is twofold: namely, (1) to illustrate the seasonal variation of some key constituents of the "aroma complex" of tea, and (2) to describe what promises to be a simple method for coarse classification of teas of different aromatic characters. The proposed classification is based on parameters derived from gas chromatographic data.

Concepts and computer techniques applied in the study of classification are similar to those fully described in our previous investigation of coffee, which led to a simple analytical method for the unambiguous differentiation of Coffea Arabica and Coffea Robusta (Biggers et al., 1969). Similar concepts and computer techniques are finding application elsewhere in the characterization of normal and abnormal human metabolic profiles (Teranishi, 1972).

However, the computational work was modified in the present study so as to permit bringing to bear human judgment with regard to peaks that should be considered together on the basis of their odor qualities. This approach has the inherent advantage of giving understandable physical meaning to the differentiating parameters, as opposed to their being considered merely as "numbers" (Gianturco, 1970), which was the case in the study of coffee. For instance, without physically smelling the samples and other things being equal, one should be able to create a mental image of the odor difference existing between tea samples by considering a set of numbers which reflect: (a) the concentration of some key compounds (this serves as an indicator of overall odor intensity); and (b) the ratios of the concentration of one or a small number of key compounds with a certain type of odor notes (for instance, delicately floral notes) to the concentration of one or more compounds with a different type of odor notes (for instance, coarse vegetable notes).

Once correlations between analytical data and odor qualities have been determined through computer manipulation of the data, utilization of the findings is straightforward. Thus, it is likely that researchers working at experimental stations in tea-producing countries will find useful the information given below, especially in their attempts to devise objective methods for the evaluation of the effect of agricultural and manufacturing practices upon the aromatic characteristics of tea. It is expected that some of the observations will also be generally pertinent to the study of the biogenesis of volatile compounds in plants.

Seasonal Variations of Some Volatile Constituents of Black Tea. For the first part of this study, dealing with seasonal variations of the volatile constituents of tea, analyses were carried out on monthly samples produced over a period of 15 months (January, 1970–March, 1971) on two estates, one located in India (Estate A, high elevation) and the other in Ceylon (Estate B, medium elevation). Both estates produce tea of good aromatic character, but it is the opinion of professional experts skilled in the evaluation of tea that the flavor of samples from Estate A is generally of finer quality than that of samples from Estate B. Accordingly, throughout the period of this study, teas from the former estate were usually, but not always, priced higher than those from the latter. Also analyzed were 15 samples produced over a period of 17 months (July, 1970–November, 1971) on a second Indian estate (Estate C, low elevation), which is known to produce tea of aromatic character coarser than and distinctly different from that of teas from Estates A and B.

The most detailed available picture of the true complexity of the aromatic fraction of tea is afforded by the chromatogram of tea oil reproduced in Figure 1. However, to utilize such a high degree of resolution for survey work is impractical and confusing. Figure 2 represents, instead, bar graphs of the principal gas chromatographic peaks of steam distillates obtained under controlled conditions from teas from the above three estates; attention is called to the fact that peak intensities in the 1 to 10% and in the 10 to 40% ranges are plotted on increasingly compressed scales. The bar graphs, although based on average values from the chromatograms of all the available monthly samples from Estates A, B, and C, are generally representative of teas from each of the three estates. Only those peaks due to compounds, the concentration of which in the aroma complex is greater than approximately 0.1%, were considered in the preparation of the bar graphs. It should be borne in mind that the total content of volatile constituents of black tea is not greater than 0.02% (Bondarovich et al., 1967). Therefore, a compound representing 0.1% of the aroma complex constitutes no more than 0.00002% of black tea and is expected to be present in a tea beverage in concentrations not exceeding 3 ppb. This simplified approach to survey work seems to be well

TEA OIL

COLUMN: 80 M, 0.29 MM ID
          GLASS CAPILLARY
          EMULPHOR
TEMPERATURE: COL 40°-180°C
                  INJ PORT 280°C
CARRIER GAS: N_2, 0.5 ML/MIN
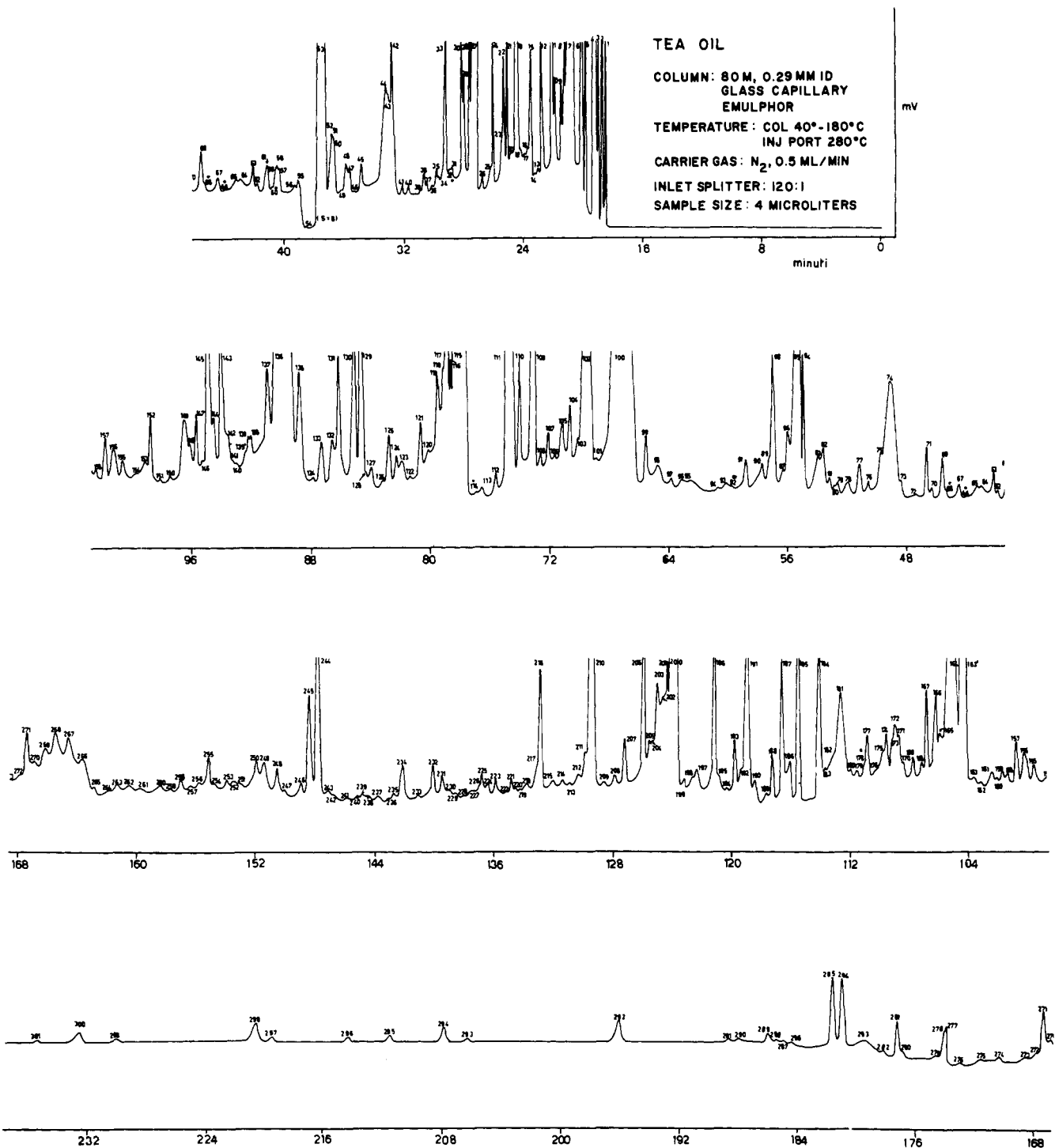INLET SPLITTER: 120:1
SAMPLE SIZE: 4 MICROLITERS

Figure 1. High-resolution chromatogram of the aroma complex of tea.

justified in the case of tea because there is no evidence that the essential odor of this plant material is due to volatile constituents of particularly low threshold value. It is possible, however, that the finer nuances observed in the multitude of tea aromas recognized by experts (Sanderson and Graham, 1973) may well depend on compounds present in low concentrations. For instance, it has been reported that certain Ceylon teas processed in the "flavory" season differ from the same teas processed at different times of the year, inasmuch as the former contain low but perhaps important concentrations of a few compounds which are organoleptically related to jasmine (Yamanishi et al., 1974).

The seasonal variations of certain key volatile substances, represented by chromatographic peaks, are illustrated in Figure 3. Each of the peaks was normalized to the value of the same peak in the chromatogram of the highest priced tea in the series under study (February, 1971, sample from Estate A), which was of very fine and delicate aromatic character. The peaks were chosen among those deemed, in this laboratory, to be of particular organoleptic importance. For instance, peaks 23, 25, and 28 are endowed with a delicately sweet and floral character, and the generally pleasant odor of peak 19, as well as those of peaks 7, 9, 15, and 20 (not shown in Figure 3), is reminiscent of fresh leaves. By contrast, peaks 12 and 32 are associated with rather coarse and heavy odors of vegetable matter and dead roses or hyacinths, respec-
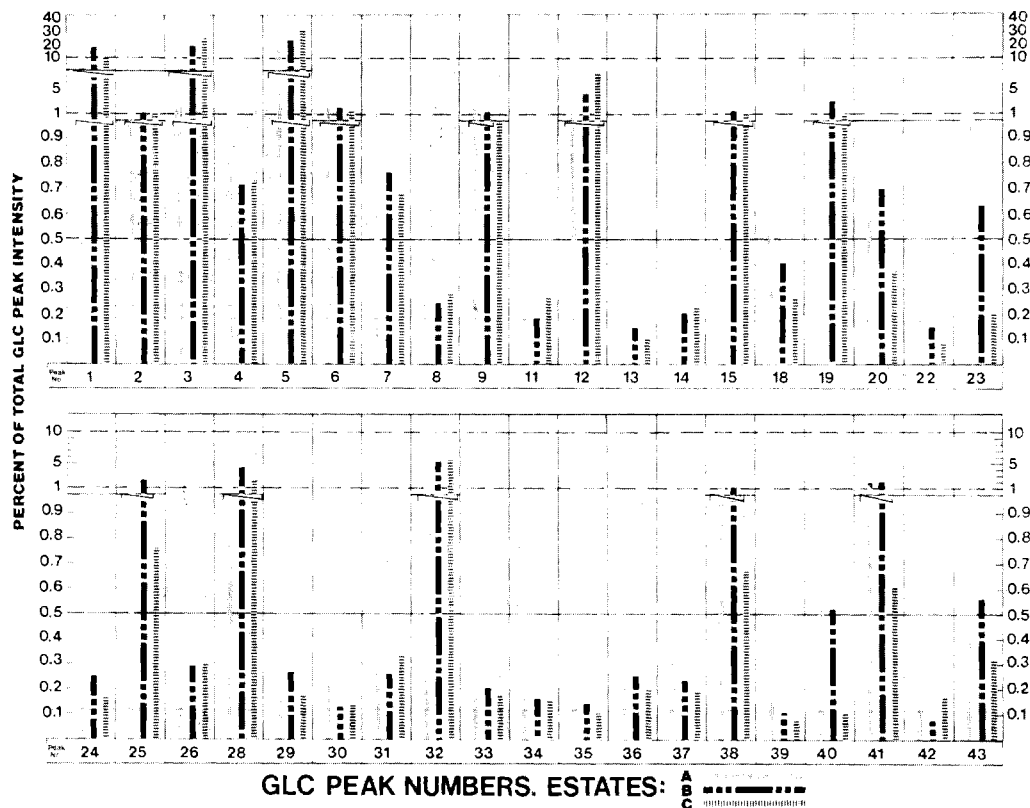
Figure 2. Bar graphic representation of simplified average chromatograms of the steam distillates of teas from three different estates.

tively. Therefore, the seasonal variations and the differences between estates which are illustrated by Figure 3 have a definite and easily understandable organoleptic significance.

It is noteworthy from a phytochemical viewpoint that tea bushes on the estates located at high and medium elevations are heavier producers of cis-3-hexen-1-ol, of the two five-membered linalool oxides, and linalool (peaks 19, 23, 25, and 28) than the bushes of the low elevation estate. This phenomenon appears to have its origins in true biosynthetic differences. On the other hand, the concentrations of some other volatile constituents of black tea are likely to be heavily dependent on manufacturing practices. For instance, two published studies have shown that the content of trans-2-hexenal (peak 12 of Figures 2 and 3) increases dramatically with increasing degree of "fermentation" (Saijo and Kuwabara, 1967; Yamanishi et al., 1966); this has been confirmed in this laboratory. Other work (unpublished) has shown that the concentration of phenylacetaldehyde (Peak 32 of Figures 2 and 3) also increases with fermentation time. Presumably, as fermentation proceeds, conditions become favorable for the Strecker degradation of phenylalanine, which is a known precursor of phenylacetaldehyde in tea (Saijo and Takeo, 1970). Evidence is also growing (unpublished work from this laboratory) that substantial amounts of some compounds, phenylacetaldehyde being among these, are formed during the "firing" step (see also Sanderson, 1972).

Differentiation of Teas from Estates A, B, and C by Means of Analytical Parameters. Computational methods were employed to determine which peaks or combination of peaks would unambiguously differentiate teas from each of the three estates A, B, and C. This appeared a worthwhile goal in view of the aforementioned differences in the quality of the aroma of the three sets. As in the case of the previously mentioned work on coffee, ratios

were computed of every peak to every other peak in every chromatogram of the series:

$$\left(\frac{P_1}{P_2}, \frac{P_1}{P_3} \cdots \frac{P_1}{P_n}; \frac{P_2}{P_1}, \frac{P_2}{P_3} \cdots \frac{P_2}{P_n}; \cdots \frac{P_n}{P_1}, \frac{P_n}{P_2} \cdots \frac{P_n}{P_{n-1}}\right)$$

Also computed were ratios involving the sum of two peaks to a third peak

$$\left(\frac{Px + Py}{Pz} \text{ or } \frac{Pz}{Px + Py}\right)$$

with the following constraints: (a) Px is added to Py only if either peak is no less than 20% of the other, and if the odors of Px and Py are of the same general type; and (b) computer-generated ratios are taken into consideration only when the odor qualities of peaks in the numerator and denominator are distinctly different. Selected ratios involving more than three peaks were computed manually so as to avoid unduly complicating the computer programs.

Judging from the frequency with which they appear in ratios capable to differentiate teas from the three estates, peaks 7, 9, 12, 15, 19, 20, 23, 25, and 28 are among the most important differentiators. Among these, peaks 28 and 12 appear in such ratios with the greatest frequency in the numerator and denominator, respectively. Note is taken that trans-2-hexenal, the main constituent of peak 12, can be generated from linolenic acid in the course of enzyme-mediated reactions taking place during grinding and steaming of fresh plant material in the presence of oxygen (Major and Thomas, 1972; Major et al., 1963; Nye and Spoehr, 1943). However, a drying operation called firing, which is part of the manufacturing process of black tea, leads to inactivation of the enzymatic system; therefore, correlations based on the measured concentration of trans-2-hexenal are considered valid in the case at hand.
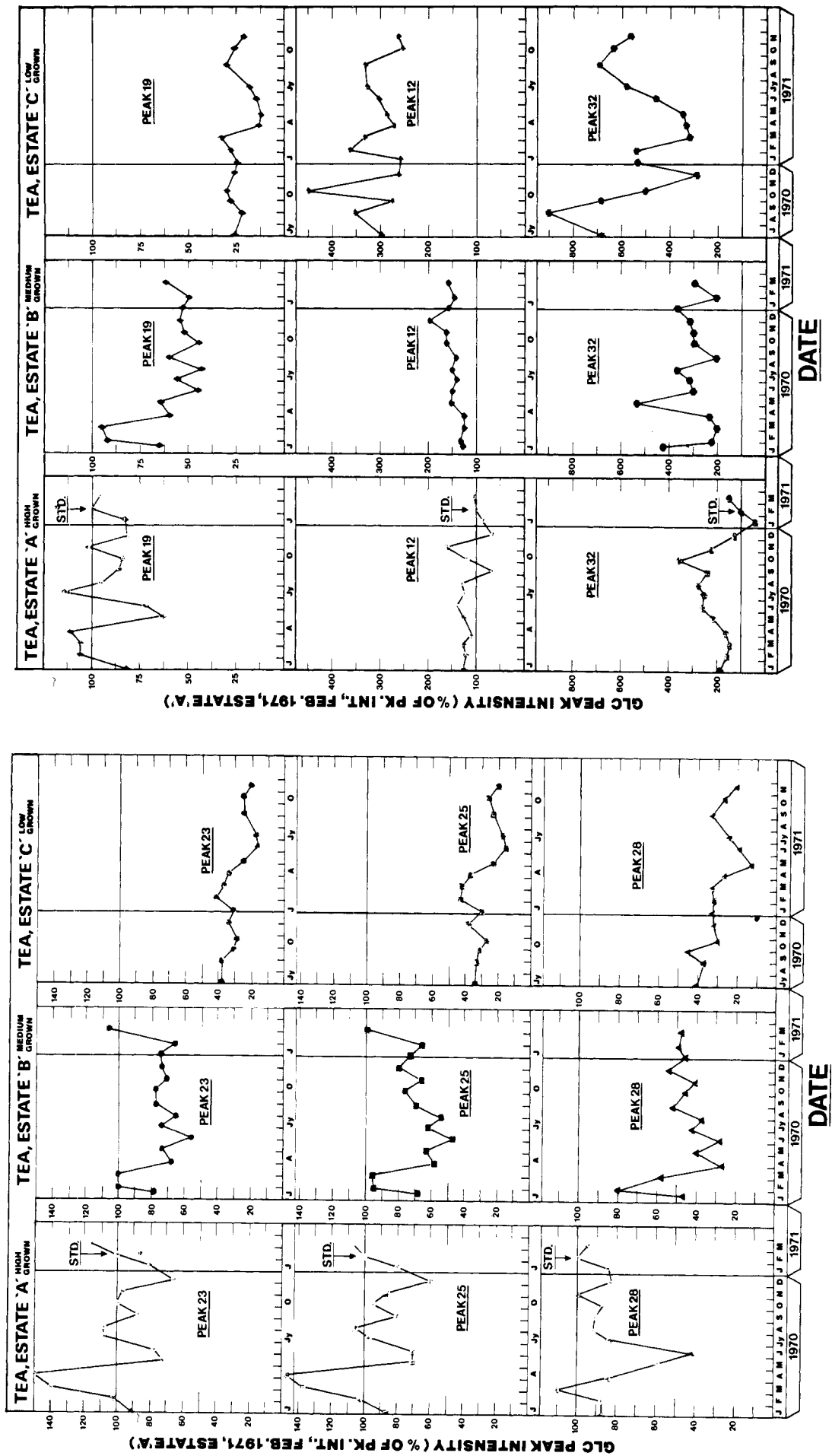
Figure 3. Seasonal variations in the concentration of some volatile constituents of teas from three different estates.

Table I. Some Analytical Parameters of Teas from Different Estates[a]

Estate	Parameter A, peaks 23, 25, 28	Parameter B, (peaks 23, 25, 28/ peak 12) × 100	Parameter C, (peaks 7, 9, 15, 19, 20/ peak 12) × 100	Parameter D, (peak 9/peak 12) + (peak 19/peak 12) × 100
C	58 (79/33)	43 (65/25)	51 (69/36)	25 (34/14)
B	101 (126/67)	152 (196/101)	165 (234/127)	96 (131/70)
A	174 (232/99)	359 (573/160)	329 (501/250)	205 (320/157)

[a] The underscored figures represent average values; adjoining dropped and raised numbers represent minima and maxima, respectively.

Additional peaks, ignored in the present study, may have to be considered in any scheme of finer classification since they also show some power of differentiation. Computer treatment of the analytical data indicates that prominent among these are peaks 11, 13, 22, 32, and 38.

A combination of the numerical and of a simply intuitive approach has led to the following observations.

1. The sum of the heights of the peaks corresponding to linalool and its two five-membered oxides (peaks 28, 23, 25) averages 58 arbitrary units, with a minimum of 33 and a maximum of 79, in the chromatograms of teas from Estate C. The corresponding figures are 107, with a minimum of 67 and a maximum of 166, for Estate B, and 173, with a minimum of 99 and a maximum of 232, for Estate A. The relationship between concentrations and the arbitrary units employed for the measurement of the above peaks is given in the Experimental Section.

2. The above sum averages 43% of the height of peak 12, which is mostly due to *trans*-2-hexenal, with a minimum of 25 and a maximum of 65%, in the chromatograms of teas from Estate C. The corresponding figures are 164%, with a minimum of 101 and a maximum of 280%, for Estate B, and 357%, with a minimum of 160 and a maximum of 573%, for Estate A.

3. The sum of the heights of peaks 7, 9, 15, 19, and 20 averages 51% of the height of peak 12, with a minimum of 36 and a maximum of 69%, in the chromatograms of teas from Estate C. The corresponding figures are 175%, with a minimum of 127 and a maximum of 280%, for Estate B, and 330%, with a minimum of 220 and a maximum of 501%, for Estate A. Peaks 7, 9, 15, 19, and 20 are due to mixtures of compounds consisting predominantly of hexanal, 1-penten-3-ol, *cis*-2-penten-1-ol, *cis*-3-hexen-1-ol, and *trans*-2-hexen-1-ol, respectively.

4. The sum of the ratios of the height of peak 9 to that of peak 12 and of the height of peak 19 to that of peak 12, multiplied by 100, averages 25, with a minimum of 14 and a maximum of 34, in the chromatograms of teas from Estate C. The corresponding figures are 104, with a minimum of 70 and a maximum of 170, for Estate B, and 205, with a minimum of 157 and a maximum of 320, for Estate A.

The parameters of two samples from Estate B were quite similar to those of teas from Estate A. These two samples were among the highest priced of the teas from Estate B. Conversely, one sample from Estate A, which was among the lowest priced of the teas from this estate, showed parameters similar to those of teas from Estate B. With these exceptions, the situation can be summarized as in Table I.

A Scheme for the Classification of Teas. *The Physical Meaning of the Analytical Parameters.* The different and changing concentrations of and balance between the quantitatively and organoleptically important substances associated with the chromatographic peaks of Table I are likely to be related to some of the flavor differences observed in teas produced in different geographic areas or on a given estate at different times of the year. This suggested that the parameters of Table I could constitute the basis for a simple scheme of coarse classification of teas with a broad spectrum of aromatic characteristics.

Consequently, analytical data were obtained for an additional 144 teas, grown in the following countries: Argentina, Brazil, Ceylon, Congo, Ecuador, India, Indonesia, Kenya, Portuguese East Africa, and Uganda. The results, which include those obtained with teas from Estate A, B, and C, are given in Table II in the form of a proposed scheme of coarse classification.

It must be pointed out explicitly that the classification

Table II. Proposed Coarse Group Classification of Teas Based on Parameters Derived from Gas Chromatograms[a]

	Parameter A, Σ peaks 23, 25, 28, light floral aroma	Parameter B, (Σ peaks 23, 25, 28/ peak 12) × 100, (light floral aroma/coarse vegetable aroma)	Parameter C, (Σ peaks 7, 9, 15, 19, 20/peak 12) × 100 (fresh leafy aroma/ coarse vegetable aroma)	Parameter D, (peak 9/peak 12) + (peak 19/peak 12) × 100 (fresh leafy aroma/ coarse vegetable aroma)	Number of teas investigated falling into group (%)
Group 1	55 (95/19)	60 (100/21)			
Subgroup 1A			75 (126/36)	36 (72/14)	58 (30.8)
Subgroup 1B			178 (249/127)	83 (133/50)	29 (15.4)
Group 2	75 (136/31)	138 (196/101)			
Subgroup 2A			100 (126/50)	50 (78/29)	20 (10.6)
Subgroup 2B			207 (400/127)	110 (185/65)	50 (26.6)
Group 3	134 (232/45)	384 (1050/214)	381 (788/176)	223 (442/106)	31 (16.5)

[a] The figures underscored by a full line represent average values; adjoining dropped and raised numbers represent minima and maxima, respectively. The figures underscored by dotted lines constitute the basis for the classification system (see text).

stems from letting the minimum and maximum values of parameter B of group 2, as well as the minimum value of parameter C of the same group, be identical with the corresponding experimental values for teas from Estate B (*cf.* Tables I and II). The aromas of the teas from Estate B are considered to be good but not excellent.

With these anchor points defined, the experimental values of all the other teas studied led to the subdivision of Group 2 into two subgroups and to the establishment of Groups 1 and 3. Consideration of the experimental data further suggested the subdivision of Group 1 into two subgroups; this was done by arbitrarily letting the minimum value for Parameter C of one of the two subgroups (subgroup 1B) be identical with the corresponding value for subgroup 2B.

It will be noted that the values for the key parameter B form essentially a continuum from group to group. Therefore, common sense and some practical experience are necessary when using the proposed classification scheme.

The physical meaning associated with the different parameters, which can be gleaned from consideration of the general odor descriptions given above for the pertinent peaks or groups of peaks, is succinctly described in Table II. In this context, it must be borne in mind that while parameters B, C, and D relate only to the *balance* of some important odor notes, parameter A (to which the others are directly or indirectly linked) gives an indication of the *intensity* of odor. Experiments conducted in this laboratory indicate that, with some practice, an interested person can learn not only to differentiate tea samples having adequately different analytical parameters but also to describe the observed differences in terms consistent with the odor of the compounds the concentrations of which determine the numerical value of the parameters. However, since there are considerable differences in individual odor perception and discrimination, how different the parameters must be for a given person to differentiate in a consistent manner between samples can be determined only by experiment.

On the basis of experience to date, the authors expect that people trained in flavor evaluation will find that: (a) a coarse note of common vegetable matter is an important component of the aroma of teas in subgroup 1A and, to a lesser extent, of that of teas in subgroup 2A; (b) in general, the green leafy odor component is more pleasant and better balanced in teas of the subgroups B than in those of the corresponding subgroups A; (c) the teas with the most delicate lightly floral aroma are to be found among those placed in group 3 by the parameters; and (d) in each of the groups, the relative impact of the light floral odor component increases with increasing value of parameter A, *i.e.*, with increasing content of linalool and its two five-membered oxides.

A particular case is that of teas from the Darjeeling district of India, which are known for their highly valued and typical aroma. A preliminary study has shown that these teas, placed in group 3 by parameters A–D, have particularly low concentrations of *trans*-2-hexenal and unusually high concentrations of some compounds with characteristic floral qualities, *i.e.*, geraniol and phenyl ethyl alcohol. It has been noted, however, that still higher concentrations of geraniol and phenyl ethyl alcohol are encountered in some teas of heavy and rather undistinguished aroma (Yamanishi *et al.*, 1968). These latter teas are not placed in the top groups by the parameters described in this communication, and this constitutes further evidence of the importance of taking into account not only absolute but also relative concentrations, *i.e.*, of utilizing peak ratios, in studies of odor classification.

Tests of the Classification Scheme. The results of a test aimed at determining whether a relationship exists between the above parameters and the expert ranking of some tea samples are given in Table III. The five samples

Table III. Expert Flavor Evaluation and Analytical Parameters of Five Samples of Tea[a]

| Sample | Expert evaluation, rank order | Parameter | | | | Group classification according to parameters A–D |
		A	B	C	D	
A	1	98	266	239	143	3
B	2	108	260	222	130	3
C	3	92	198	216	130	High 2B
D	4	75	127	137	76	Very low 2B, or 2A
E	5	76	118	120	67	2A

[a] For definition of the parameters see Table I.

(A–E) obtained from different estates in Ceylon were processed at the same time of the year and were judged to be of good flavor. The organoleptic ranking by local tea brokers was as indicated in Table III, but evaluation in this and another laboratory showed, in addition, that the first two samples (A and B) are quite similar to one another and of somewhat finer aroma than the third (C), and that a marked gap exists between the aromatic quality of the first three samples (A–C) and that of the last two (D and E). This is in good agreement with the classification based on the parameters derived from the gas chromatographic data.

The experiment described next is also informative, inasmuch as it shows that tea estate managers are quite aware of variations in the quality and in the commercial value of their products when these variations are substantial, and when the spread in the aromatic quality of samples is not very marked, the individual parameters may not rank the samples in complete agreement with the ranking by professional tasters but, nevertheless, the four parameters considered collectively seem to do so quite adequately. For this experiment, five samples produced in successive months on a high elevation estate in India were assigned a valuation on the estate, ranked according to quality by professional tea experts in England and analyzed in the authors' laboratory. The results, shown in Table IV, illustrate also the rapidity with which the volatile composition and the quality of the leaf vary following a change in weather conditions (compare ranks and estate valuations for samples A and E, produced in April and May, respectively).

Work in progress will establish whether present knowledge is adequate to discover useful correlations between analytical data and the ephemeral quality typical of those valuable teas that professional experts define as flavory. The highly valued flavory teas are produced on estates located at high elevations and only under very particular climatic conditions. The results of this study will be published in due course.

Finally, it must be noted that a report of computer-aided attempts at correlating composition and organoleptic qualities of Georgian black teas has been recently published by another group (Kozhin *et al.*, 1972). The conclusions reached by the Russian workers with regard to compounds that show positive or negative correlations with quality differ in part from those reached in this laboratory. At present, the authors are unable to determine whether or not this discrepancy is due solely to a different appreciation of certain aromatic qualities of tea.

EXPERIMENTAL SECTION

Preparation of Steam Distillate Samples for Analysis. Steam distillation of black tea samples was performed in a Garman apparatus equipped with an ice–water cooled condenser. In this apparatus (SGA Scientific Inc., Bloom-

Table IV. Expert Flavor Evaluation, Relative Estate Price Valuation, and Analytical Parameters of Five Tea Samples from One Estate[a]

Sample; date of production in [ ]	Expert evaluation, rank order	Comments of evaluators	Estate price valuation as % of valuation of sample A	Rank by parameter in [ ]			
				Parameter A	Parameter B	Parameter C	Parameter D
A [4/70]	1	Very bright; nice quality	100	205 [2]	419 [1]	368 [1]	240 [1]
B [3/70]	2	Very bright; nice quality	91	232 [1]	411 [2]	317 [3]	202 [3]
C [2/70]	3	Very bright; nice quality	100	180 [3]	333 [3]	331 [2]	207 [2]
D [1/70]	4		96	171 [4]	307 [4]	284 [4]	171 [4]
E [5/70]	5	Much inferior to other samples	73	110 [5]	196 [5]	220 [5]	129 [5]

[a] For definition of the parameters see Table I.

field, N. J., Catalog No. JD-3210), the steam is internally generated by means of a rheostat-controlled sealed-in resistance wire. The receiver was an ice-cooled 10-ml graduated centrifuge tube which was sealed with a ground glass stopper upon completion of the sample collection. Steam was passed through the black tea (23.5 g) at a rate yielding 3 ml of condensate/min until 10 ml of distillate was obtained. The samples were generally analyzed directly after collection; however, no detectable changes in composition were observed under the chromatographic conditions employed, when test samples were stored for over a month at $-20°$ in the dark. Gas chromatographic analysis indicated that this procedure, while not leading to complete removal of the volatiles from the charge of tea, is reproducible within ±5%. The procedure is therefore considered to be an acceptable compromise between completeness of volatile removal and danger of formation of artifacts upon prolonged steam distillation.

Gas Chromatographic Analysis of Steam Distillates. The analysis was performed by on-column injection of 40 μl of steam distillate on a 3.6 m × 0.4 cm (inside diameter) glass column packed with 15% Triton X-305 on 80–100 mesh Chromosorb W. The instrument was a Hewlett-Packard 5750 equipped with flame ionization detector; the temperature was programmed from 60 to 180° at the rate of 2°/min; injection port and detector were at 200°; flow rate was 55 ml of He/min. Peak intensities were measured as heights from consistently drawn base lines at constant retention times. Under the conditions employed, the retention times (in minutes) for the peaks used in the calculation of the numerical values of the parameters and for appropriate ethyl ester markers were as follows: peak 7, 16.3; peak 9, 20.0; peak 12, 24.8; peak 15, 30.7; peak 19, 35.4; peak 20, 36.6; peak 23, 41.2; peak 25, 43.0; peak 28, 46.6; markers: ethyl butyrate, 14.3; ethyl pentanoate, 20.7; ethyl hexanoate, 27.9; ethyl heptanoate, 35.1; ethyl octanoate, 42.3; ethyl nonanoate, 49.2.

Peak Height/Concentration Relationship for the Two Five-Membered Linalool Oxides and for Linalool (Peaks 23, 25, and 28). Calibration indicated linear detector response for the three above compounds in the range of interest; in addition, the response for the two oxides was found to be almost identical with that of linalool. The relationship between the summation of the heights of peaks 23, 25, and 28 in the chromatograms obtained from 40 μl of steam distillate and the concentration of the principal constituents of the peaks (expressed as micrograms of linalool in 10 ml of steam distillate) is shown below.

Σ (peaks 23, 25, 28) (cm) in chromatogram of 40 μl of steam distillate	Amount (μg) of linalool oxides and linalool in 10 ml of steam distillates (calculated as linalool)
50	109
100	218
150	327
200	436

LITERATURE CITED

Biggers, R. E., Hilton, J. J., Gianturco, M. A., *J. Chromatogr. Sci.* **7**, 453 (1969).
Bondarovich, H. A., Giammarino, A. S., Renner, J. A., Shephard, F. W., Shingler, A. J., and Gianturco, M. A., *J. Agr. Food Chem.* **15**, 36 (1967), and references cited therein.
Ferretti, A., Flanagan, V., Bondarovich, H. A., Gianturco, M. A., *J. Agr. Food Chem.* **16**, 756 (1968).
Gianturco, M. A., seminar presented at Underwood-Prescott Memorial Symposium, Massachusetts Institute of Technology, Boston, Mass., Sept 24, 1970.
Kozhin, S. A., Treizer, N. D., Moskaleva, N. M., Prikhod'ko, G. T., *Vestn. Leningrad. Univ., Fiz. Khim.* 147 (1972); *Chem. Abstr.* **77**, 86754 (1972).
Major, R. T., Marchini, P., Boulton, A. J., *J. Biol. Chem.* **238**, 1813 (1963).
Major, R. T., Thomas, M., *Phytochemistry* **11**, 611 (1972).
Nye, W., Spoehr, H. A., *Arch. Biochem.* **2**, 23 (1943).
Saijo, R., Kuwabara, Y., *Agr. Biol. Chem.* **31**, 389 (1967).
Saijo, R., Takeo, T., *Agr. Biol. Chem.* **34**, 222 (1970).
Sanderson, G. W., in "Structural and Functional Aspects of Phytochemistry," Academic Press, New York and London, 1972.
Sanderson, G. W., Graham, H. N., *J. Agr. Food Chem.* **21**, 576 (1973).
Takino, Y., Ferretti, A., Flanagan, V., Gianturco, M. A., Vogel, M., *Tetrahedron Lett.* 4019 (1965); Erratum, *Tetrahedron Lett.* 4024 (1966).
Takino, Y., Ferretti, A., Flanagan, V., Gianturco, M. A., Vogel, M., *Can. J. Chem.* **45**, 1949 (1967).
Teranishi, R., private communication, 1972; see also (a) *Scand. J. Clin. Lab. Invest. Suppl.* **29**, 126 (1972); (b) Teranishi, R., Robinson, A. B., Pauling, L. C., Abstracts of 164th National Meeting of the American Chemical Society, New York, N. Y., (Aug, 1972) Anal. 13; (c) Zlatkis, A., Lichtenstein, H. A., Tishbee, A., Bertsch, W., Abstracts of 164th National Meeting of the American Chemical Society, New York, N. Y., Aug, 1972, Anal. 14.
Yamanishi, T., Kawatsu, M., Yokoyama, T., Nakatani, Y., *Agr. Biol. Chem.* in press (1974).
Yamanishi, T., Kobayashi, A., Nakamura, M., Uchida, A., Mori, S., Ohsawa, K., Sasakura, S., *Agr. Biol. Chem.* **32**, 379 (1968).
Yamanishi, T., Kobayashi, A., Sato, H., Nakamura, H., Ohsawa, K., Uchida, A., Mori, S., Saijo, R., *Agr. Biol. Chem.* **30**, 784 (1966).