Nonvolatile Components of Black Tea and Their Contribution to the Character of the Beverage

David J. Millin, David J. Crispin, and Derwent Swaine

Tasting of pure compounds and various fractions isolated from black tea liquors suggests that, with the exception of caffeine, none of the characteristic monomeric nonvolatile substances contributes significantly to the taste of the beverage. The most important of the nonvolatiles are complex products formed by oxidation and polymerization of polyphenols during processing of the leaf. The molecular weight distribution of these products, their degree of association with protein, and their interaction with caffeine are of major importance in determining the 'mouthfeel' characteristics of tea.

The tea industry employs highly skilled and experienced tasters to advise on market valuation and to formulate blends. Tasters employ a bewildering number of terms (Stahl, 1962) to describe teas. Many of these terms require a good deal of experience before their meanings can be understood and appreciated. Assuming that the volatile complex (Bondarovich et al., 1967) is responsible for all of the more subtle characteristics of flavor and aroma, there remain a good many terms which seem likely to be associated with the nonvolatile substances (Roberts, 1962). A liquor may be said to be thick or thin, full or empty, strong or weak, brisk or soft, etc. It is unfortunate that tea tasters do not use the terms "astringent" or "bitter," which can be defined more accurately. To minimize the highly subjective nature of such observations, we prefer, at least in the first instance, to amalgamate all these terms under the single heading of "mouthfeel." The task is then to establish which components make any contribution to "mouthfeel."

There are two approaches to such a task. The first is to prepare pure samples of substances of known chemical constitution which are present in tea. Attempts must then be made to describe the taste of these substances at concentrations corresponding to those in the beverage as normally prepared. Since there are, at a conservative estimate, at least 80 fairly simple, nonvolatile components of known chemical structure, this is already a formidable task. It is, however, reasonable to begin by examining those substances which are peculiar to tea or are quantitatively of major importance. Conversely, if interactions are ignored, any substances which are present at concentrations below the threshold level may be temporarily disregarded.

The second approach is to fractionate the components of the brew into various groups using mild techniques which would not be expected to affect the taste. Each fraction is then tasted alone or in combination with others to assess its significance. Any fractions judged to be important may then be subfractionated in an attempt to pinpoint the important components. This approach has the advantage that it deals equally well with constituents of known structure and those whose chemical nature remains obscure.

EXPERIMENTAL

All water used in tasting experiments was distilled and filtered through a charcoal column immediately before use.

Isolation of Flavanols. Dried green leaf (200 grams) was extracted with 80% methanol (six times with 500 ml.). The filtered extracts were combined and reduced to an aqueous solution (200 ml.) by evaporation at 40° C. under reduced pressure. Ethanol (40 ml.) was added to this solution which was washed with chloroform (five times with 250 ml.). Any residual organic solvent was removed from the aqueous phase which was then extracted with ethyl propionate (five times with 250 ml.). The organic phase from this extraction was evaporated, taken up in water, and freeze dried yielding a brown-orange powder (16.9 grams). The aqueous phase remaining after ethyl propionate extraction was extracted with ethyl acetate (five times with 250 ml.), and the organic phase evaporated, taken up in water, and freeze dried to give an orange powder (25 grams).

An ethyl propionate extract (8.0 grams) was distributed between diethyl ether and water on a steady state distribution machine (Quickfit & Quartz, Ltd., England). After a fundamental distribution of 98 upper phase transfers the contents of the tubes were monitored by investigating their ultraviolet light absorption. Evaporation and freeze drying of the two fractions so revealed gave epi-gallocatechin gallate (3.81 grams) and epi-catechin gallate (2.10 grams) both of which were free of other catechins.

The ethyl acetate extract (9.0 grams) was distributed between ethyl acetate and water. By carrying out a total of 208 upper phase and 140 lower phase transfers, fractions A (2.6 grams), B (0.5 gram), and C (0.7 gram) were obtained containing epi-gallocatechin, epi-catechin, and gallocatechin, respectively, as their sole flavanol components.

Crystalline compounds were obtained when each of the flavanol fractions was separated from contaminating flavonols, acids, and oxidation products by chromatography on a column of Sephadex LH-20 (Pharmacia, Sweden), equilibrated, and eluted with methanol. Satisfactory physical data were determined to confirm the identity of the substances (Vuataz et al., 1959).

Synthesis of Theaflavin. The method was essentially that employed for the synthesis of 1′,2′-dihydroxybenzotropolone (Takino et al., 1967). epi-Gallocatechin (0.031 gram) and epi-catechin (0.073 gram) gave a red solid which was purified by partition chromatography on cellulose with 2% acetic acid as eluent. Crystallization of the product from water gave a substance (0.008 gram) whose spectral and physical constants were consistent with those reported for theaflavin (Takino et al., 1967).

Caffeine. Commercial caffeine was purified by sublimation.

Brooke Bond Liebig Research Centre, Sonning Common, Reading, Berks, England

Isolation of a Mixture of Flavonols. A modification to the procedure used by Roberts (1956) was that the precipitate formed with lead acetate at pH 8.5 was decomposed with the hydrogen form of a cation exchange resin and the resulting material was separated from nonflavonol substances by chromatography on a column of Sephadex LH-20 equilibrated and eluted with 60% acetone.

Polysaccharides. (Gudnason *et al.*, 1966). A tea brew (approximately 3% solids) was treated with acetone (two volumes) and allowed to stand overnight. The clear supernatant was decanted and the precipitate suspended in 95% ethanol for several hours. The precipitate was then filtered and washed thoroughly with ethanol and ether. A sample (200 mg.) was applied to a column of Sephadex G.25 (80 × 2.5 cm.) and eluted with water.

CHEMICAL COMPOSITION OF TEA

It is convenient to consider first the chemical composition of the green tea shoots at plucking (Millin and Rustidge, 1967; Roberts, 1962; Stahl, 1962) (Table I). The chemistry at this stage is much simpler than that of black tea obtained after the leaves have been processed. The most striking feature is the very large amount—up to 30% of the dry weight—of polyphenolic substances. These may be grouped into four classes: flavanols or catechins, flavonols, flavandiols or leucoanthocyanins, and phenolic acids and their derivatives. Recently the presence of small amounts of flavones has also been reported (Sakamoto, 1967). Quantitatively the most important are the flavanols. The major phenolic acid is theogallin, representing 1 to 2% of the dry weight. The major amino acid, theanine, is a characteristic compound accounting for up to half of the weight of the amino acids. Caffeine is also a well-known major component.

Processing of the green leaf into black tea includes a so-called "fermentation" step. This is basically an enzymic oxidation in which the phenolic substances represent the primary substrates. As a result of processing, therefore, many of these substances disappear partially or completely, being converted into a wide variety of complex oxidation products, the chemistry of which still remains rather obscure.

An estimated composition of the soluble components of black tea is shown in Table II. Although the flavanols are drastically reduced to less than 10% of their original level, they can still represent quite important components of the soluble solids. The flavonols are reduced by only about 20% (Ulyanova, 1966), and among the phenolic acids, theogallin is also partially consumed (Bhatia and Ullah, 1965). The simple monomeric flavandiols are completely oxidized (Roberts *et al.*, 1956). The oxidation products include bisflavanols, theaflavins, and many other colored products collectively termed "thearubigins."

ISOLATION AND TASTING OF PURE COMPONENTS

Flavanols. The structures of the catechin gallates occurring in tea are shown in Figure 1. The parent flavanols without the ester linked gallic acid are also important components and smaller amounts of the isomeric D-(+)-catechin and (+)-gallocatechin are also present. The ability to synthesize *epi*-gallocatechin and to form galloyl esters of catechins in large quantities seems to be more or less confined to the tea plant. Consequently, unfermented green tea leaf has been used as a source of these materials.

Suitable extracts of green leaf are first partitioned be-

Table I. Approximate Composition of Water Soluble Substances in Green Tea Shoots (Assam variety)

		% Dry Wt. of Leaf
Flavanols: *epi*-Gallocatechin gallate		9–13
epi-Catechin gallate		3–6
epi-Gallocatechin		3–6
epi-Catechin		1–3
Other flavanols		1–2
Flavonols and their glycosides		3–4
Flavandiols (leucoanthocyanins)		2–3
Phenolic acids and depsides (including 1 to 2% theogallin)	*ca.*	5
Total phenolic substances	*ca.*	30
Caffeine		3–4
Amino acids (including up to 2% theanine)		4
Simple carbohydrates		4
Organic acids		0.5
Volatile substances		0.01–0.02
Partially soluble substances		
Polysaccharides	*ca.*	14
Protein	*ca.*	15
Inorganic material (ash)	*ca.*	5

Table II. Estimated Composition of Black Tea[a] Components Soluble in Water

		% Dry Wt. of Leaf		% of Total Soluble Solids
Flavanols (mainly *epi*-gallocatechin gallate)		1–3		3–8
Flavonols and flavonol glycosides[b]		2–3		6–8
Phenolic acids and depsides[b]		4		11
Theaflavins		1–2		3–6
Nondialyzable phenolic substances		1.5		4
Other phenolic substances (bisflavanols, dialyzable thearubigins, other fermentation products)		2–4		6–10
Caffeine		3–4		8–11
Amino acids and peptides		5		14
Simple carbohydrates		4		11
Organic acids		0.5		1.5
Partially soluble substances				
Protein	*ca.*	15		1
Polysaccharide		14		4
Nucleic acid		0.09		0.1
Mineral salts (ash)	*ca.*	5	*ca.*	10

[a] Assam type, orthodox manufacture; assuming 35% soluble solids.
[b] Assuming 20% loss during fermentation (Bhatia and Ullah, 1965; Ulyanova, 1966).

tween ethyl propionate and water and then between ethyl acetate and water. Ethyl propionate preferentially extracts the bulk of the galloyl esters and ethyl acetate most of the remaining catechins. Originally both extracts were chromatographed on polyamide to remove flavonols and oxidized materials and subsequently the individual catechins were separated by partition chromatography on silica gel (Bradfield *et al.*, 1947; Bradfield and Penney, 1948). A preferred method, however, is countercurrent distribution employing transfer of both phases (Fleetwood, 1966). The ethyl propionate extract is distributed between ether and water and the ethyl acetate extract between ethyl acetate and water. The five flavanols, *epi*-catechin gallate (I, R = H), *epi*-gallocatechin gallate (I, R = OH), *epi*-gallocatechin, *epi*-catechin, and gallocatechin, have been obtained free from each other by this technique. The final stages of purification consist of chromatography
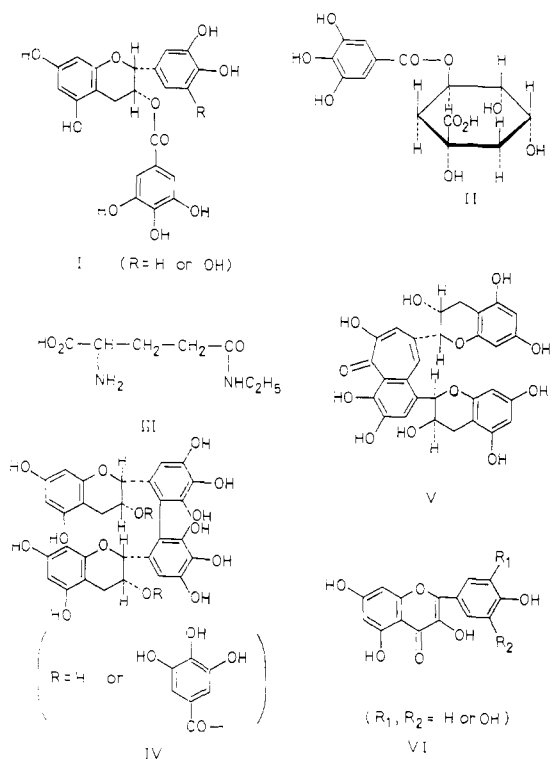
Figure 1. Structural formulas of some tea constituents

on Sephadex LH-20 after which the products crystallize readily.

The tastes of these components in water at the levels occurring in black tea were described as slightly astringent and metallic. In addition epi-gallocatechin was said to be sweetish and to impart a burning sensation. Owing to the very persistent aftertastes it was not possible to carry out any meaningful tests to establish threshold levels. Taste tests were also carried out on tea liquors to which the individual catechins had been added in quantities at least as great as those already present. The taste panel was unable to detect the presence of the added catechins. It would therefore seem that the simple monomeric flavanols are of little significance in the taste of tea. It may also be confidently stated that the raw green flavor associated with under-fermented tea cannot be ascribed to the unoxidized polyphenols.

Theogallin and Related Acids. The structure of theogallin is shown in Figure 1 (II). This is 3-galloylquinic acid, first identified in tea (Roberts and Myers, 1958). Theogallin is the major representative of the phenolic acids and is present to the extent of 2 to 5% in the soluble solids of tea, and has been obtained by the published procedures (Roberts and Myers, 1958; Vuataz et al., 1959). Again, it seems to be rather insignificant to the taste of tea. The pure substance was described as faintly astringent with a slight nutty taste and it could not be detected when added to tea liquors as described above.

Sufficient amounts of other phenolic acids, chlorogenic, p-coumarylquinic, and caffeic, were made available for tasting as by-products from the isolation of the flavanols. Even when tasted at levels far exceeding those encountered in a brew of tea, their taste was regarded as insignificant.

Theanine. The major amino acid of tea has the structure shown in Figure 1 (III)—i.e., γ-N-ethylglutamine. Again this represents 2 to 5% of the soluble tea solids. Theanine may be readily obtained by synthesis (Furuyama

et al., 1964). Tasting showed that it was present in tea brews at somewhere near the threshold level; only 25% of the panel could detect it. Theanine has been said to be important in the taste of green tea (Sakato, 1957). If this is the case it must presumably be interacting with other components or serving as a flavor potentiator.

Theaflavins and Other Fermentation Products. All of the products mentioned so far are constituents of the green leaf which persist to varying degrees in black tea. The distinct contrast between the taste of black tea and green tea, which latter tends to be somewhat thin and insipid, already suggests that the fermentation products are likely to be of particular importance.

Unfortunately, relatively few of the nonvolatile fermentation products have been properly characterized. Three bisflavanols of the type shown in Figure 1 (IV) have been described (Roberts and Myers, 1959a; Vuataz and Brandenberger, 1961). Their structures have only recently been confirmed (Ferretti et al., 1968). They are presumed to be formed by condensation of the appropriate quinones followed by reduction. These substances are only found in rather small amounts and as yet we have not obtained sufficient quantities for taste evaluation.

The only colored fermentation products which have been at all adequately characterized are the theaflavins. The structure of theaflavin itself (Figure 1, V) was first suggested by Takino's group (Takino et al., 1964) in Japan and subsequently proved by Takino et al. (1965, 1966), and independently by Brown et al. (1966). Gallates of theaflavin are certainly also present in black tea (Roberts and Myers, 1959b) but rigid proof of their structure has not, as yet, been published. Theaflavin has been prepared by coupled oxidation of epi-catechin and epi-gallocatechin derived from green leaf. The oxidation was carried out using hydrogen peroxide and horseradish peroxidase (Takino et al., 1967).

Pure crystalline theaflavin has a very fierce astringency and several tasters remarked that the solution had a distinct tea-like character which was very difficult to describe in precise terms. In the synthetic product this could scarcely be brought about by contamination with volatiles, which is an otherwise ever present danger.

Caffeine. Solutions of caffeine at concentrations equivalent to those in tea of normal drinking strength are unpleasantly bitter and not at all tea-like. This can, no doubt, be explained by the interaction of caffeine with other components, notably the phenolic substances.

Preparations of phenolic substances rich in theaflavins from black tea were tasted with and without the addition of caffeine. Caffeine was found to decrease astringency and some tasters thought it increased "body" or "thickness." At the same time the samples with added caffeine were said to be only slightly bitter. Addition of caffeine to pure theaflavin modifies both the astringency of the theaflavin and the bitterness of the caffeine. The resulting sensation is again very difficult to describe. Perhaps, as suggested by Roberts (1962), it may be equated with "briskness," a term employed by professional tea tasters and described as "liveliness on the palate" (Stahl, 1962).

Using this rather limited approach there seems to be no doubt that theaflavin and caffeine are by far the most significant of the compounds tasted. Although not all of the characteristic nonvolatiles of known structure have, in fact, been tasted, these observations go some way towards justifying the great emphasis placed upon the theaflavins
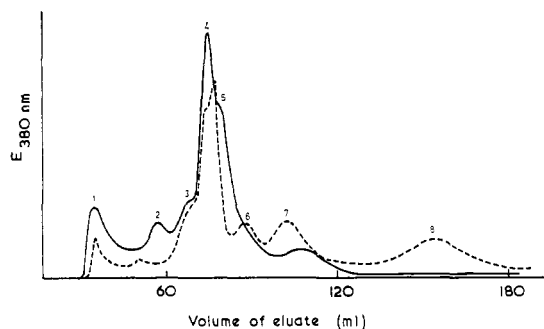
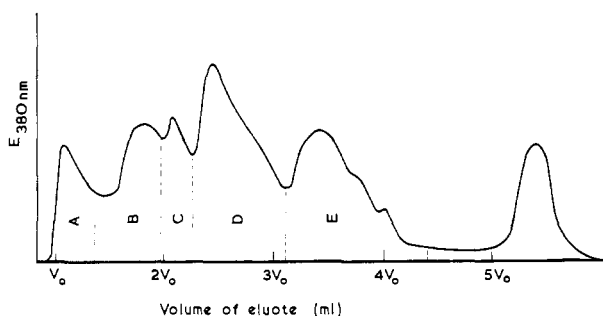Figure 2. Chromatography of tea extracts on Sephadex LH-20 (- - - tea A; ——— tea B)

Figure 3. Chromatography of a partially dialyzed tea brew on Sephadex LH-20

by Roberts (1962), Roberts and Smith (1963), and Smith and White (1965).

FRACTIONATION OF TEA BREW SOLIDS

Flavonols. A complex mixture of flavonols and their 3-glycosides having the general structure shown in Figure 1 (VI) occurs in green leaf (Roberts, 1956). Since these compounds are apparently only changed slightly during fermentation (Ulyanova, 1966), they are quantitatively quite important components of soluble tea solids (Table II). A mixture of flavonols and their derivatives has been obtained free from other components by slight modifications of the published procedures (Roberts, 1956). When tasted at the appropriate concentration these were described as slightly astringent, metallic, woody, and sulphurous. It seems probable that, like the other monomeric flavonoids mentioned earlier, these substances play only a relatively minor role in the taste of tea.

Polysaccharides. If a tea brew is treated with acetone an almost colorless precipitate is obtained which includes the bulk of the polysaccharides present in the brew (Gudnason et al., 1966). The precipitate contains about 80% carbohydrate and 3% nitrogen and has a pleasant malty taste. "Maltiness" is recognized by the professional tea tasters and regarded as a rather desirable character.

This fraction could be readily separated into two components by gel permeation chromatography on Sephadex G-25. The fraction excluded by the gel consisted of 80% polysaccharide and 20% protein and was completely tasteless. The malty taste was entirely associated with the lower molecular weight fraction, which was predominantly a mixture of sugars.

Nondialyzable Fraction. By employing the technique of thin film countercurrent dialysis (Craig and Stewart, 1965), we have shown that some 10 to 12% of the soluble solids of tea are nondialyzable (Millin et al., 1969). It

includes the polysaccharide material mentioned above (34% by weight), smaller amounts of protein (6%) and nucleic acid (ca. 1%), and a substantial proportion of phenolic substances (32%). This material is of considerable interest since it can be responsible for up to 40% of the color ($E_{450\,nm}$) of a typical black tea infusion. It is, however, virtually tasteless but has a soft "mouthfeel." Addition of the nondialyzable fraction to a normal brew imparts a definite "softness" or "flatness" to the taste.

Chromatography on Sephadex LH-20. This has proved to be a most useful and informative technique when applied to tea brews. Samples of whole infusions may be applied direct to a column of Sephadex LH-20 in aqueous acetone and a useful separation of the colored components achieved. Indeed, this technique seems sufficiently useful to warrant its development for routine analysis of the coloring matter of tea brews (Crispin et al., 1968).

Chromatograms of two different teas are shown in Figure 2. Evidence of at least eight colored components can be seen. Some information has been gained on the nature of some of these components and their relationship to the work of previous authors (Millin et al., 1969). Thus peaks 1 to 3 are nondialyzable, peaks 6 and 7 are soluble in ethyl acetate and appear to be equivalent to the "S_I thearubigins" of Roberts (1962). Peak 8 represents the theaflavins. The nature of the components in the poorly resolved area around peaks 4 and 5 is obscure and complex. At least one component is only pale yellow and contributes little to the color above 400 nm. and therefore may be a mixture of flavonols. Degradation products of chlorophyll are also known to be eluted in this region.

Of the two teas depicted in Figure 2, one (tea A) is of reasonable quality and has a well-defined theaflavins peak. The other is a low quality product which has no detectable theaflavins and noticeably larger amounts of substances of high molecular weight. More than 20 years ago Bradfield (1946) correlated high quality in tea with a low proportion of nondialyzable material.

This technique, as it stands, is not particularly useful as a preparative method for taste evaluation. The colored fractions are naturally contaminated with colorless components and are particularly complex in the latter stages of elution. It has, for instance, been established that monomeric catechins are eluted just before the theaflavins, thus indicating that molecular size is by no means the only criterion involved in this separation. This picture has been somewhat simplified and attention focused on the phenolic fermentation products by carrying out partial dialysis before applying the sample to the column. If dialysis is carried out in the conventional manner for three days instead of about eight days required for exhaustive dialysis of tea brews, the product is virtually free of monomeric substances. The concentrations of all ultimately dialyzable species is, naturally, reduced in accordance with their respective rates of dialysis.

A chromatogram of such a partially dialyzed preparation is depicted in Figure 3. The fractions were arbitrarily combined as indicated and rechromatographed. Each fraction so purified had, in fact, more or less the same elution volume as in the original separation. These fractions were freeze dried and dissolved in water for tasting. If the fractions were arranged in order of elution from the column—i.e., in suspected order of decreasing molecular weight—there was a very clear gradation in mouthfeel from almost completely tasteless through to a marked

astringency. There was complete agreement on this by all tasters.

Confirmation has been sought that these fractions are, in fact, eluted in order of decreasing molecular weight. The ratios of color yields from reactions of the first three components with the vanillin and Folin-Denis reagents are 0.57, 0.73, and 0.81, respectively, and are therefore as expected (Goldstein and Swain, 1963). Similar findings have recently been reported on chromatography of condensed tannins from Crataegus on Sephadex LH-20 (Lewak, 1968).

A similar relationship between "mouthfeel" and molecular size of tannins has been suggested particularly in connection with the changes which occur during the ripening of various fruits (Goldstein and Swain, 1963; Hillis and Swain, 1959). Changes in astringency were correlated with the amounts of flavonoid compounds extracted with organic solvents or aqueous organic solvents. Color reactions were used to provide some data on relative molecular sizes. This field has been thoroughly reviewed by Joslyn and Goldstein (1964).

There seems little doubt that these substances are of considerable significance to the "mouthfeel" of tea and it is therefore relevant to summarize our present knowledge of their chemistry (Millin et al., 1968).

So far, most observations have been made on the three fractions of highest molecular weight—i.e., the nondialyzable material. All of these fractions give a positive anthocyanin test, and both cyanidin and delphinidin have been identified among the reaction products. Acid hydrolyzates show the presence of gallic acid, quinic acid, and a full range of normal protein amino acids. Further observations have indicated that the gallic acid is present both in ester links and depside links. Thus, it is probable that these substances are complex polymers involving not only catechins but also leucoanthocyanins, theogallin, and protein.

Hydrolyzates of fractions D and E (Figure 3) contain far less amino acids than those derived from the nondialyzable fractions. Indeed, fraction E contains only insignificant traces. This raises the interesting possibility that it may be the degree of association with protein or peptide material rather than the molecular weight of the phenolic moiety which brings about the striking changes in taste. If "mouthfeel" can, in fact, be ascribed to the interaction of substances with proteins in the mouth (Joslyn and Goldstein, 1964) polymeric phenolic substances already associated with protein might be expected to have a limited impact.

Minor Components. A great many nonvolatile substances occur in tea in relatively small amounts and as a result probably play no direct role in tea taste. For example, almost all the amino acids occur in quantities (Bhatia and Deb, 1965; Roberts and Sanderson, 1966) such that their concentrations in the final brew are well below the established threshold levels (Ajinomoto Co., 1966). Amino acids are, however, of considerable importance as volatile precursors. They may be converted into the corresponding aldehydes by Strecker degradation, involving quinones as the oxidizing agents. This reaction may take place during processing (Popov, 1956) and also at the time when boiling water is added to the black leaf (Roberts and Rustidge, 1962).

A further function of such minor components may be as flavor potentiators. Nucleotides, in particular, are im-

portant in this respect in other foods and beverages. Only very recently, a large increase in the concentration of nucleotides during the withering stage of black tea manufacture has been reported (Bhattycharyya and Ghosh, 1968). Since this stage is known to have important effects on quality it is possible, despite the fact that many other changes take place during this stage (Millin and Rustidge, 1967), that nucleotides also act as flavor potentiators in tea.

One further possible interaction is worthy of mention. When preparing samples of phenolic substances from tea, it is quite difficult to remove all traces of volatiles. This could indicate a possible interaction between phenolic and volatile substances. On several occasions, when tasting teas known to differ only in their volatile content, differences in astringency have been noted. Of course, certain of the volatile components may themselves be astringent, but they could conceivably be enhancing the mouthfeel of the phenolic substances.

CONCLUSIONS

With the exception of caffeine none of the simple monomeric nonvolatile substances characteristic of tea seems to be of major significance in determining the character of the beverage. Probably, however, the "malty" taste recognized in certain teas is brought about by simple sugars.

There seems little doubt that most of the "mouthfeel" of tea can be attributed to the phenolic oxidation products characteristic of black tea. Their effects are probably modified by interaction with caffeine. Of particular importance seems to be the molecular weight distribution of these oxidation products and the extent of their association with proteins. The theaflavins and other oxidation products of intermediate molecular weight having little or no associated protein are responsible for astringency and perhaps other characters such as "briskness," strength, thickness, and possibly also harsh unpleasant characters if present in too large amounts. On the other hand, the substances of high molecular weight are responsible for soft, flat, thin liquors. Clearly, for a tea of good quality a correct balance of these substances is necessary.

LITERATURE CITED

Ajinomoto Co., Inc., "Amino Acid Catalogue," Part 4, p. 11, Tokyo, Japan, 1966.
Bhatia, I. S., Deb, S. B., J. Sci. Food Agr. 16, 759 (1965).
Bhatia, I. S., Ullah, M. R., J. Sci. Food Agr. 16, 408 (1965).
Bhattycharyya, A. K., Ghosh, J. J. Biochem. J. 108, 121 (1968).
Bondarovich, H. A., Giammarino, A. S., Renner, J. E., Shephard, F. W., Shingler, A. J., Gianturco, M. A., J. AGR. FOOD CHEM. 15, 36 (1967).
Bradfield, A. E., Chem. Ind. 1946, 242.
Bradfield, A. E., Penney, M., J. Chem. Soc., 1948, p. 2249.
Bradfield, A. E., Penney, M., Wright, W. B., J. Chem. Soc., 1947, p. 32.
Brown, A. G., Falshaw, C. P., Haslam, E., Holmes, A., Ollis, W. D., Tetrahedron Letters 1966, p. 1193.
Craig, J. L., Stewart, K., Biochemistry 4, 2712 (1965).
Crispin, D. J., Payne, R. H., Swaine, D., J. Chromatog. 37, 118 (1968).
Ferretti, A., Flanagan, V. P., Bondarovich, H. A., Gianturco, M. A., J. AGR. FOOD CHEM. 16, 756 (1968).
Fleetwood, J. G., Brit. Med. Bull. 22, 127 (1966).
Furuyama, T., Yamashita, T., Senoh, S., Bull. Chem. Soc. Japan 37, 1078 (1964).
Goldstein, J. L., Swain, T., Phytochemistry 2, 371 (1963).
Gudnason, G. V., Millin, D. J., Swaine, D., unpublished observations, 1966.
Hillis, W. E., Swain, T., J. Sci. Food Agr. 10, 135 (1959).
Joslyn, M. A., Goldstein, J. L., Advan. Food Res. 13, 179 (1964).
Lewak, S., Phytochemistry 7, 665 (1968).
Millin, D. J., Rustidge, D. W., Process Biochem. 2 (6), 9 (1967).
Millin, D. J., Sinclair, D., Swaine, D., unpublished observations, 1968.

Millin, D. J., Swaine, D., Dix, P. L., *J. Sci. Food Agr.*, in press, **20**, (1969).

Popov, V. R., *Biokhimiya* **21**, 380 (1956).

Roberts, E. A. H., *J. Sci. Food Agr.* **7**, 637 (1956).

Roberts, E. A. H., in "The Chemistry of Flavonoid Compounds," T. A. Geissmann, Ed., p. 468, Pergamon Press, Oxford, 1962.

Roberts, E. A. H., Cartwright, R. A., Wood, D. J., *J. Sci. Food Agr.* **7**, 253 (1956).

Roberts, E. A. H., Myers, M., *J. Sci. Food Agr.* **9**, 701 (1958).

Roberts, E. A. H., Myers, M., *J. Sci. Food Agr.* **10**, 167 (1959a).

Roberts, E. A. H., Myers, M., *J. Sci. Food Agr.* **10**, 172 (1959b).

Roberts, E. A. H., Rustidge, D. W., *Biokhim. Chain. Proizv. Akad. Nauk SSSR Inst. Biokhim.* **9**, 182 (1962); *CA* **59**, 6909e (1963).

Roberts, E. A. H., Smith, R. F., *J. Sci. Food Agr.* **14**, 689 (1963).

Roberts, G. R., Sanderson, G. W., *J. Sci. Food Agr.* **17**, 182 (1966).

Sakamoto, Y., *Agr. Biol. Chem.* (*Tokyo*) **31**, 1029 (1967).

Sakato, Y., *Proc. Symp. Phytochem. Kuala Lumpur* **1957**, p. 168.

Smith, R. F., White, G. W., *J. Sci Food Agr.* **16**, 205 (1965).

Stahl, W. H., *Advan. Food Res.* **11**, 201 (1962).

Takino, Y., Ferretti, A., Flanagan, V., Gianturco, M. A., Vogel, M., *Can. J. Chem.* **45**, 1949 (1967).

Takino, Y., Ferretti, A., Flanagan, V., Gianturco, M. A., Vogel, M., *Tetrahedron Letters* **1965**, p. 4019.

Takino, Y., Ferretti, A., Flanagan, V., Gianturco, M. A., Vogel, M., *Tetrahedron Letters* **1966**, p. 4024.

Takino, Y., Imagawa, H., Horikawa, H., Tanaka, A., *Agr. Biol. Chem.* (*Tokyo*) **28**, 64 (1964).

Ulyanova, M. S., *Teknol. Pishch. Prod. Rast. Proiskhozhd.* **1966**, 195; *CA* **67**, 81290 (1967).

Vuataz, L., Brandenberger, H., *J. Chromatog.* **5**, 17 (1961).

Vuataz, L., Brandenberger, H., Egli, R. H., *J. Chromatog.* **2**, 173 (1959).