

EFFECTS OF METHOD OF MANUFACTURE ON THE OXIDATION OF POLYPHENOLS AND CHLOROPHYLLS

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Comparisons have been made of the proportions of oxidised to unoxidised and partially oxidised polyphenols in teas manufactured by different processes, *viz* black tea manufactured by conventional, Rotorvane, CTC* and Clivemeare methods, Japanese green tea and samples of 3 different soluble teas. In black tea manufacture, more drastic methods of leaf maceration reduce the degree of oxidation undergone by polyphenols during manufacture. Quantitative measurements showed that the proportion of oxidised and partially oxidised polyphenols was low in CTC and Clivemeare teas. Semi-quantitative chromatography indicated that CTC teas contained a relatively high content of partially oxidised compounds. A compound, tentatively identified as belonging to the group of true tannins appears during manufacture to a larger extent in CTC than in other teas. The conversion of the chlorophylls of tea leaf to breakdown products during manufacture is described. It was found that this breakdown is less during Rotorvane and CTC than conventional manufacture, and it is suggested that this may contribute to the relative brownness of teas made by the former processes.

Introduction

The changes undergone by polyphenols during the manufacture of tea by conventional methods have been studied in detail and discussed by Roberts (1962). He obtained evidence that the products of fermentation are derived to a large extent from epigallocatechin and its gallate, which are converted successively to bis-epigallocatechin and its gallate, theaflavin and theaflavin gallate, and a heterogeneous group of compounds referred to as thearubigins. Furthermore, Roberts and Smith (1961) observed that the theaflavins played a role in determining the quality of black tea. Following these observations, Wickremasinghe and Swain (1965) found that a differential extraction of the unoxidised and partially oxidised polyphenols from the oxidised polyphenols of tea could be effected by the use of anhydrous and aqueous organic solvents respectively, and this technique afforded a method of determining the relative extent of oxidation of polyphenols in black teas manufactured by different methods. This paper gives the results of analyses of samples of tea manufactured by conventional, rotorvane, Clivemeare and CTC methods, together with those of analyses of one sample of Japanese green tea and samples of three different soluble tea preparations—'Nestea', hot-soluble 'Ceytea' and cold-soluble 'Ceytea'. The differences in the various preparations of tea are discussed.

Samples of black tea manufactured by conventional, Rotorvane and CTC methods are also compared for their chlorophyll contents during the different stages of manufacture. It was seen that the green chlorophylls of flush undergo transformation to brown phaeophytins during manufacture, and that this transformation was less in CTC and Rotorvane than in conventionally manufactured teas.

Methods

Extraction procedure

Polyphenols: The different samples of tea were successively extracted three times with boiling absolute methanol (5 ml/g material), after which the residue was re-extracted three times with aqueous (60% v/v) methanol.

* CTC teas are manufactured by methods not comparable with Indian or African CTC teas.

Chlorophylls: 10 g material were minced in a homogenizer with acetone, (3 ml/g material) and the acetone solution filtered. The residue on the filtrate was extracted twice more and the acetone extracts pooled.

Quantitative determination

Polyphenols were estimated by the methods described by Swain and Hillis (1959), and chlorophylls by measuring absorption at 665 m μ in a Beckman DU spectrophotometer.

Chromatography

Polyphenols were separated by 2 dimensional paper chromatography, and chlorophylls by thin layer chromatography on plates coated with silica gel.

Results and discussion

The results shown in Table I represent the quantities of polyphenols and ratios of fully oxidised to partially oxidised and unoxidised polyphenols in samples of black tea manufactured by different methods, together with the analytical values for

TABLE 1—*Polyphenols in different samples of made tea*

Made tea	POLYPHENOLS		
	Unoxidised	Oxidised	Oxidised/ unoxidised
Conventional	135	135	1.0
Rotorvane	150	125	0.8
CTC	184	90	0.5
Clivemeare	190	90	0.5
Green tea	88	124	1.4
'Ceytea'—cold-soluble	302	172	0.6
'Ceytea'—hot-soluble	255	170	0.7
'Nestea'	38	223	6.5

All values expressed as mg/g dry weight in terms of D—Catechin

samples of Japanese green tea and instant teas. It is seen that the proportion of oxidised polyphenols is less in methods which use drastic maceration of the leaf (Clivemeare, CTC) than those prepared by conventional and rotorvane methods. The reason for this difference is due partly to the shorter fermentation periods for Clivemeare and CTC teas and partly to the relatively higher levels of polyphenols liberated by drastic methods of breaking the leaf, which would result in insolubilization of the enzyme systems responsible for oxidation (Wickremasinghe and Swain (1965). The results with the samples of Japanese green tea and instant teas are not

comparable because the starting material was different in each case. However, the relatively high proportion of oxidised polyphenols in the green tea sample is, at first sight, anomalous, because the method of manufacture does not involve a fermentation step during which enzymic oxidation of the polyphenols occurs. In green tea manufacture, inactivation of the enzymes is effected by steaming the leaf and it is probable that the polyphenols are bound to various cell constituents (*eg* protein) during this process, and are, therefore, not extracted by absolute alcohol, although they are extracted by aqueous alcohol. 'Nestea' was found to contain a very high proportion of oxidised polyphenols, indicative of its origin from black tea extracted at an elevated temperature, or the possible addition to the product of extraneous material which reacts with the reagents used in estimation. The samples of 'Ceytea' are seen to contain a relatively low proportion of oxidised polyphenols.

TABLE 2—Comparison of chromatograms of different samples of made tea

Made tea	TF	EG	EGG	Triacetidin	'Galloyl glucose'	Chlorophyll
Conventional	5	2	2	1	1	0
Rotorvane	5	2	2	1	1	0
CTC	2	4	4	3	3	0
Green tea	0	5	10	0	0	10
'Ceytea'—cold-soluble	1	2	2	1	1	0
'Ceytea'—hot-soluble	5	2	2	2	1	0
'Nestea'	1	1	1	0	0	0

Values are comparable, but approximate and arbitrary

TF = theaflavin + theaflavin gallate
 EG = epigallocatechin
 EGG = epigallocatechin gallate

Paper chromatographic examination of the extracts gave the results summarized in Table 2. As expected, the samples of Japanese green tea, the manufacture of which does not involve a fermentation stage, did not contain theaflavins. This observation is in accord with the detection of relatively high amounts of epigallocatechin and its gallate, which are the precursors of the theaflavins in black tea. The chlorophyll content too of Japanese green tea was much greater than that of black tea and, as shown below, this is due to the complete conversion of chlorophyll to pheophytin and other compounds during fermentation and firing of tea. It is noteworthy that hot soluble 'Ceytea' had a higher theaflavin content than the cold-soluble product. Both samples of 'Ceytea' contain a compound which has been tentatively identified as a galloylated glucose. This compound is not present in flush, withered leaf or black tea but appears during fermentation and there are indications that its concentration is higher in CTC than in conventional or Rotorvane treatments. Galloyl glucose belongs to the group of true tannins (Haworth 1961), and its appearance during tea manufacture has not been reported earlier. It is of potential importance because it belongs to the group of substances (*eg* oak bark tannin, sumach tannin), used in the leather industry. Tanning of leather depends on the combination of such tannins with the protein of animal skins and compounds of the nature of galloyl glucose would also 'tan' enzyme protein and, therefore, play an important role in the enzymic processes occurring during fermentation.

Visual inspection of the chromatograms of the fermented tea leaf samples after conventional, Rotorvane and CTC treatment showed that the amounts of unoxidised epigallocatechins were least in the CTC sample. This observation is in agreement with that of Bahtia and Ullah (1965) who carried out careful quantitative estimations of the epigallocatechins and found that their concentrations were less in CTC manufactured teas than conventional teas. However, quantitative estimations reported here in Table 1 show that the amount of unoxidised material is higher in the CTC teas. This apparent discrepancy is very probably due to the presence in CTC teas of partially oxidised compounds derived from the epigallocatechins, which are soluble in absolute alcohol. One such compound is triacetidin, the concentration of which was seen to be high in fermented leaf samples obtained after CTC treatment, and low in those after Rotorvane and conventional treatments.

Changes in chlorophylls during manufacture

Thin layer chromatographic methods were used in the studies of chlorophylls. Chlorophyll *a* and chlorophyll *b*, together with small quantities of the corresponding brown coloured phaeophytins were found to occur in the flush. The results of quantitative determinations of the chlorophylls at different stages of tea manufacture of clone DT 95 by conventional, Rotorvane and CTC methods are given in Table 3.

TABLE 3—Quantitative changes in chlorophyll during manufacture of Clone DT 95

Method of manufacture	STAGE OF MANUFACTURE				
	Flush	Withered	Partly fermented	Fully fermented	Fired
Conventional	0.314	0.274	0.265	0.178	0.015
Rotorvane	0.314	0.273	0.246	0.240	0.012
CTC	0.314	0.242	0.246	0.237	0.029

Figures represent absorption values of extracts expressed per g dry wt of material

These results, together with the thin layer chromatography observations, which are not depicted here, showed that there is a transformation (enzymic) of chlorophylls to phaeophytins during withering and fermentation, and that the degree of transformation is less in CTC and Rotorvane than in conventionally manufactured teas. Firing leads to a considerable drop in both the chlorophyll and phaeophytin contents, probably due to chemical destruction at the elevated temperature. It is possible that the higher content of chlorophyll before firing, in Rotorvane and CTC teas may contribute to the relative brownness of the teas made by these processes.

Summary

- 1 – A comparison has been made of the ratio of oxidised to unoxidised and partially oxidised polyphenols in black teas manufactured by different methods, in Japanese green tea and in soluble teas.
- 2 – Drastic methods of leaf maceration employed in CTC and Clivemear processes lead to a reduction in the oxidation of polyphenols during manufacture, as compared with conventional or Rotorvane methods.

- 3 – A compound tentatively identified as galloyl glucose has been detected in extracts of fermented leaf samples. The concentration of this compound is higher during CTC than conventional or Rotorvane manufacture.
- 4 – The concentration of triacetidin is higher in CTC than in conventional or Rotorvane teas.
- 5 – The chlorophylls are transformed to phaeophytins during withering and fermentation and broken down almost completely during firing.
- 6 – The transformation of chlorophylls to phaeophytins is less in CTC and Rotorvane than in conventional processing.

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