STUDIES ON THE QUALITY AND FLAVOUR OF TEA

2 – THE CAROTENOIDS

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Carotenoids are a group of pigments which are found in all plants. It is possible that the changes which these pigments undergo during the manufacture of tea may play a part in determining quality and flavour. These effects may be due to the golden yellow colour of carotenoids, and to their ease of oxidation to volatile compounds which could contribute to the aroma complex of black tea. This paper describes the separation of the carotenoid compounds present in tea and the results of preliminary studies of their changes during manufacture. Fourteen carotenoids have been shown to be present and their concentration found to decrease during manufacture. The products of their breakdown have not been fully identified as yet, but studies on the clone DTI showed the appearance, during manufacture, of compounds which could possibly contribute to tea aroma.

Introduction

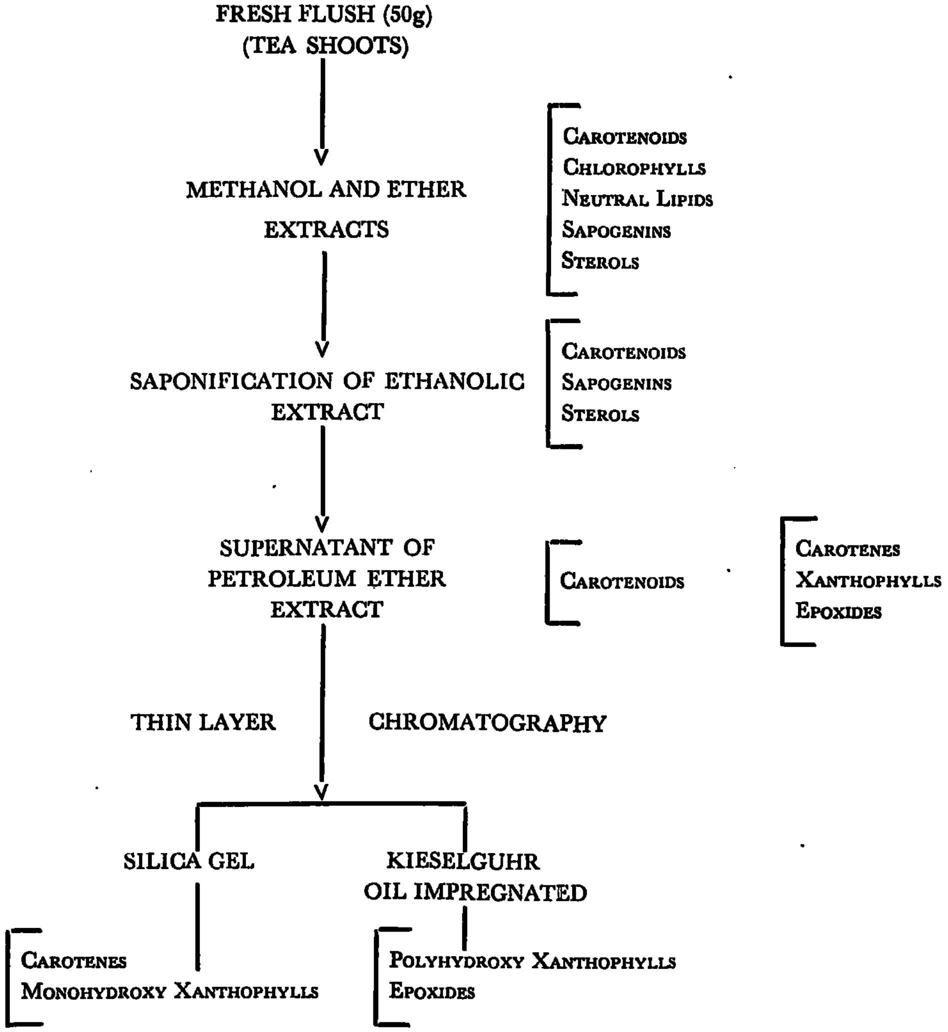
The carotenoids are a group of pigments which are of universal occurrence in plants and to which various functions have been ascribed, *eg* energy absorption, oxygen transport, reproduction, storage and antioxidation (Calvin 1955; Cholnoky *et al* 1956; Stanier *et al* 1955). The majority of these pigments are golden yellow in colour and susceptible to oxidation. Carotenoids are long chain unsaturated hydrocarbons usually having the composition $C_{40}H_{56}$. Most of these are characterized by the presence of a ring at one or both ends of the hydrocarbon chain. This ring is structurally related to the compounds α – and β –ionone, which are of importance in the perfume industry. The structure of a carotenoid may be considered to be derived from eight isoprene units. These units are also of importance in the biosynthesis of terpenes, sterols and rubber.

The aroma of the tea leaf is due to the presence of volatile compounds. Some of these compounds, *eg* vanillin, p – hydroxybenzaldehyde, cinnamic aldehyde and coniferyl aldehyde, are known to occur free in the tea leaf (Skobeleva *et al* 1958). Other volatile compounds may be formed by the oxidation of the amino acids by the quinones which arise during tea manufacture (Bokuchava and Popov 1954; Wickremasinghe and Swain 1964). A wide range of volatile compounds (unsaturated aldehydes and ketones) may also be formed by the oxidation of the carotenoids, and hence carotenoid breakdown products may possibly contribute to tea aroma. In order to understand the nature of the volatile constituents of carotenoid oxidation, it is first necessary to make a study of the carotenoids themselves. This paper gives the results of a preliminary qualitative investigation of the carotenoids in the tea leaf.

Experimental and Results

The flush of clone DT1 used in this study was obtained during the months of February and March 1965. At least fourteen carotenoids have been detected in the tea leaf. As the majority of these carotenoids were present only in trace amounts, chemical data such as absorption maxima, partition ratios and iodine isomerisation tests, necessary for the absolute identification of the carotenoids, could not be obtained at the present stage. The extraction procedure is outlined in the chart given below.

EXTRACTION PROCEDURE



It is probable that some of these carotenoids, *eg* lutein (3, 3′ – dihydroxy – α carotene) and cryptoxanthin (3 – hydroxy – β –carotene), originally occurred as esters which were hydrolysed in the saponification stage of the extraction procedure.

Thin Layer Chromatography

Thin layer chromatography was used for the separation of the carotenoid pigments in preference to commonly used column chromatography. This new method gave clear cut separations of the pigments, and possessed the additional advantage that it required comparatively small quantities of starting material. The carotenes and the monohydroxy xanthophylls, or oxygenated carotenes, were separated on silica gel plates while the polyhydroxy xanthophylls and the epoxides were separated on Kieselguhr plates impregnated with oil. Mixtures of organic solvents were used for development in both cases. The rate of migration of the carotenoids in the oil impregnated Kieselguhr plate was the reverse of that in the silica gel plate.

Identification

Since the only marker available was β-carotene, the other carotenoids (Figure 1) were identified by comparing their Rf values with the major component of extracts of different plant materials, which had been previously analysed by various workers (Table 1). Carotene A was identified as β-carotene by the determination of the absorption maxima in two different solvents and by co-chromatography with an authentic marker. The Rf value of the carotenoid G is slightly lower than that of F and is probably related to it. The carotenoids H and J were reddish in colour and gave a brown colour reaction with dinitrophenyl hydrazine followed by ethanolic potassium hydroxide, indicating that these are probably keto carotenoids (Smith 1960). A similar colour reaction was given with $SbCl_3$ in chloroform. These two carotenoids were partly overlapped by another unidentified carotenoid 1 which was yellow in colour. It is perhaps noteworthy that appreciable quantities of the two reddish carotenoids were present in the low quality clones PA 22 and TRI 26.

A very good separation of the xanthophylls and their epoxides was obtained on kieselguhr plates impregnated with oil. Five such compounds were detected in appreciable concentrations. Of these compounds, K, L and M were identified as 5, 6-epoxides, since they gave the characteristic pale greenish blue colouration on treatment with concentrated HCl. These epoxides were further characterized by the observation of a shift of their absorption maxima to a lower wavelength, on the addition of a drop of ethanolic 0.05 HCl. This hypsochromic shift is due to their isomerisation to 5, 8-epoxides (Goodwin 1955). The carotenoid L gave a deep blue colour with concentrated HCl which colour is usually given by 5, 8-epoxy compounds, but other studies indicated that it was violaxanthin (5, 6, 5′, 6′ diepoxyzeaxanthin). The blue colour was due to its immediate conversion to auroxanthin (5, 8, 5′, 8′ diepoxyzeaxanthin) in the presence of concentrated HCl. Its identity as violaxanthin was confirmed by the indigo blue colour with concentrated H_2SO_4, the green colour with glacial acetic acid (Dawson *et al* 1959), and the identity of its Rf value with the major xanthophyll of yellow pansies (*Viola tricolor*).

Changes during manufacture

This preliminary survey of the carotenoids was followed by a study of the qualitative changes during manufacture. The flush was withered, minced and fermented in the laboratory, after which it was fired in the factory. Preliminary quantitative estimations indicated that there was an appreciable increase in the total carotenoids during withering, and that the concentration of these carotenoids fell during the subsequent stages of manufacture. During fermentation, the disappearance of the keto-carotenoid H was accompanied by the appearance of trace amounts of another reddish carotenoid P which had a higher Rf value on the silica gel plate. The carotenoids present in trace amounts disappeared during manufacture.

The identity of the volatile compounds formed as a result of the degradation of the carotenoids are not yet known. Preliminary work carried out on clone DT1 showed the presence of an aldehyde in the flush and the appearance of a second aldehyde during fermentation. Another compound found only after 45—90 minutes of fermentation, gave a positive result for a diterpene test. It has yet to be shown whether these observations have any relation to the oxidation of the carotenoids and the contribution that the oxidation products may make to the aroma of tea.

TABLE 1 — *Identification of Carotenoids*

	Carotenoid	Plants containing compounds of similar Rf values	Source	Probable identity	Reference
Carotenes	B	*Delonix regia* (flamboyant)	Sepals	Phytoene (Lycopene derivative)	Jungalawa & Cama, 1962
	C	—do—	—do—	Phytofluene (Lycopene derivative)	—do—
	D	*Solanum lycopersicum* (tomato)	Fruit	Lycopene	Willstatter & Escher, 1910
	E	*Cuscuta* sp	Stems	γ - carotene	Mackinney, 1935
Xanthophylls	F	*Carica papaya* (papaw)	Fruit	Cryptoxanthin (3-hydroxy β - carotene)	Subbarayan & Cama, 1964
	L	*Viola tricolor* (yellow pansy)	Petals	Violaxanthin (5, 6, 5′, 6′, diepoxyzeaxanthin)	Kuhn & Winterstein, 1931
	N	—	Egg yolk	Lutein (3, 3′ -dihydroxy - α - carotene)	Mayer, 1943
	O	*Zea mays* (maize)	Corn	Zeaxanthin (3, 3′ -dihydroxy - β -carotene)	Karrer & Jucker, 1950

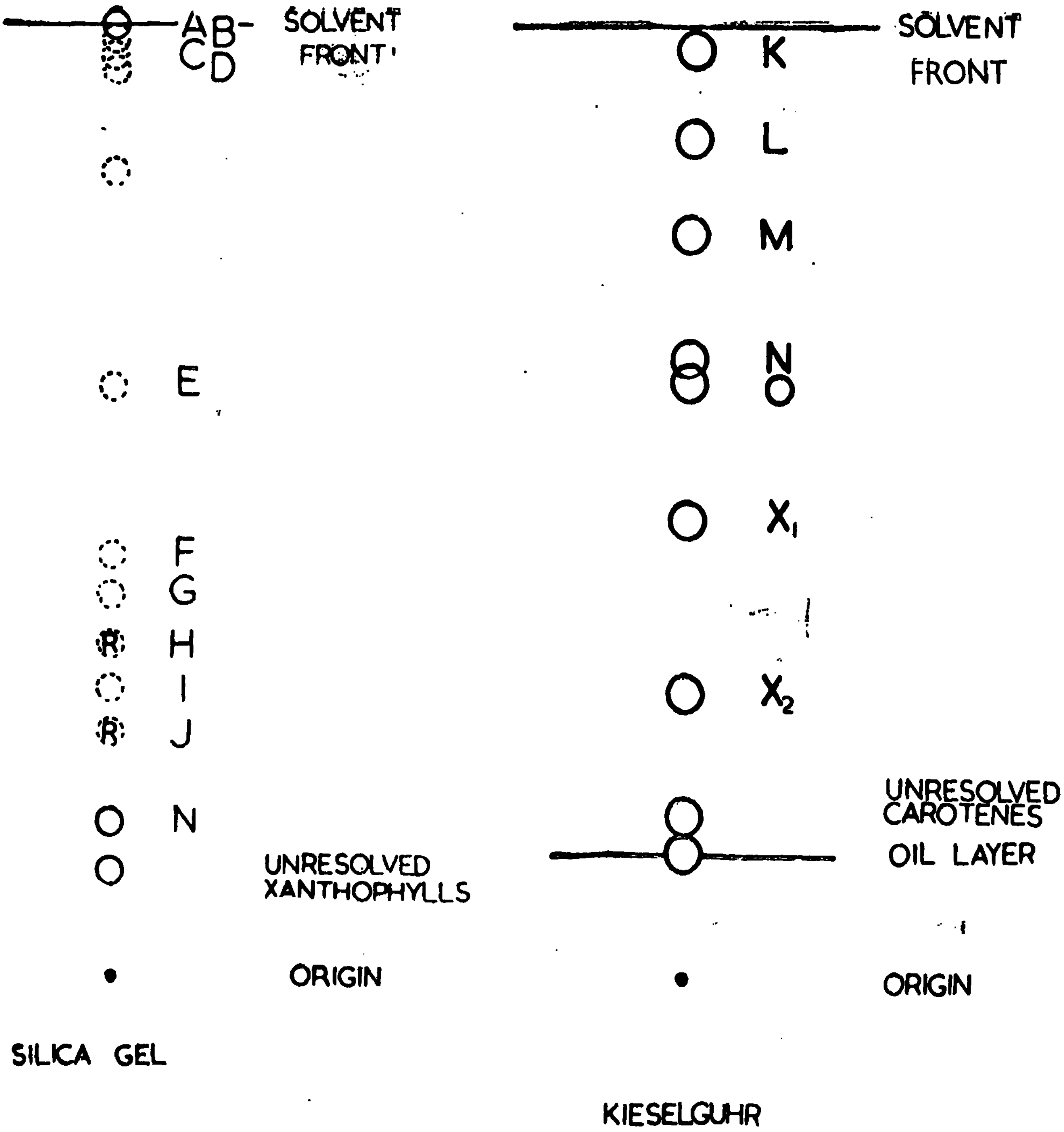


FIGURE 1

A — *Separation of carotenes and monohydroxy xanthophylls* B — *Separation of polyhydroxy xanthophylls and their epoxides*

KEY:—A β-carotene (6.05); B Phytoene (6.03); C Phytofluene (5.97); D probably Lycopene (5.87); E probably γ carotene (3.87); F Cryptoxanthin (2.78); G unidentified (2.52); K 5, 6 epoxy carotenoid (1.49); L Violaxanthin (1.35); M 5, 6 epoxy carotenoid (1.20); N Lutein (1); O Zeaxanthin (0.96); X_1 unidentiged (0.74); X_2 unidentified (0.46)—A dotted circle indicates a trace amount—The figures in brackets are the Rf values measured with the value of lutein (1) taken as the standard

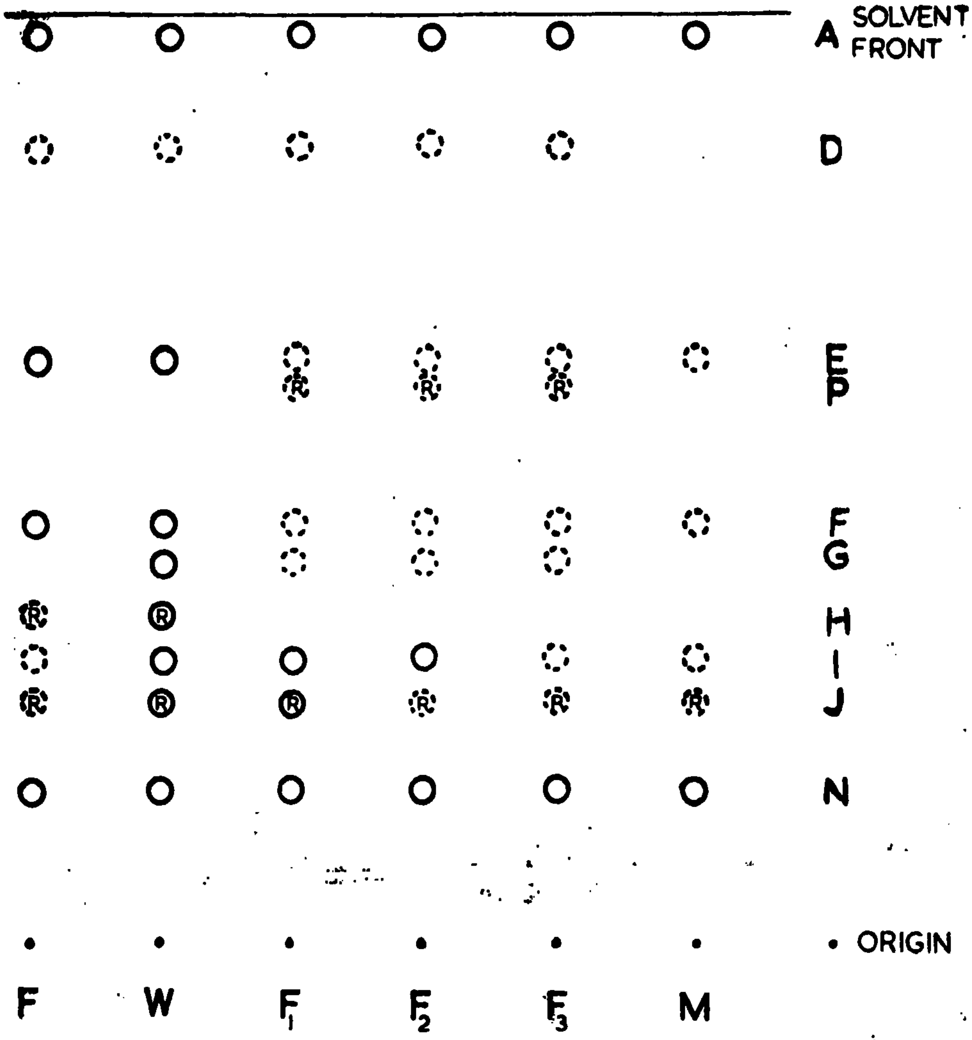

CHANGES DURING MANUFACTURE

FIGURE 2 — *Qualitative changes of the carotenoids during manufacture*

F —fresh leaf; W — withered (16 hrs); F_1—fermented (45 minutes); F_2 —fermented (1½ hrs); F_3 —fermented (2½ hrs); M — made tea; P— unidentified reddish compound; R — reddish colour —Key for other carotenoids, see page 119

Summary

1 Thin layer chromatography techniques were developed for the separation of the carotenoids of tea.

2 Nine carotenes and monohydroxy xanthophylls as well as two polyhydroxy xanthophylls, and three epoxides have been separated in this study.

3 Preliminary studies on the changes during manufacture have been made.

4 An unidentified aldehyde and a compound tentatively identified as a diterpene, have been observed to appear during manufacture.

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