The Libyan Journal of Agriculture: volume 8, 1979

Studies on the Coloured Components of Black Tea[1]

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ABSTRACT

The present investigation was carried out to study some physico-chemical properties of the pigments of black tea after their isolation and fractionation. Tea samples from India, Cylon, as well as samples obtained by ration card from the local market in Egypt, were used for this purpose. The adulteration of tea, by adding some strange matters, (like iron powder) was also studied. It was found that black tea contains from 2.5% (Indian tea) to 3.03% (Cylon tea) colouring matter. The presence of iron powder, though increased the intensity of the colour in the visible region of spectra (7.8 times), it did not really change the character of the absorption spectrum in the UV-region. The results indicate that the tea colourants have an indicator property and contain various groups of chromophores and auxochromes, which have intense absorption in the UV spectra (272–275 nm).

The elemental analysis of the isolated polymers of the Indian tea sample was as follows: C — 40.83; H — 5.78; O — 41.91; N — 5.04; S — 3.94; inorganic residue — 2.50. An empirical formula for the repeating unit in these polymers was deduced as follows:

$$C_{26\text{-}27}\,H_{45\text{-}46}\,O_{20\text{-}21}\,N_3\,S.$$

The infrared spectrum of these polymers showed at least nineteen significant bands. Employing the gel filtration technique on a column of Sephadex G-25 the colourants isolated from Indian tea were separated into three fractions. One was of brown hue and highest molecular weight. The two others were of yellow hue and lower molecular weights. Spectroscopic examination was applied to the fractions obtained.

The results obtained give an evidence that tea colourants contain thearubigins, theaflavins, degradation products of chlorophyll and monomeric catechins.

INTRODUCTION

Tea is prepared and cured by recognized methods of manufacture from the tender leaves, unopened leaf buds and tender internodes of different varieties of the plant Thea sinensis. The use of an infusion of dried leaves as a beverage has its origin in mythology and antiquity. Aside from water, tea is the worlds most popular beverage. It is an all-

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weather drink, providing an effective and enjoyable experience whether served hot or cold.

Most of the tea imported now comes from India, Cylon and certain regions of Africa. The average consumption per Capita of tea in the Arab countries reaches about one kg annually*.

There are three general types of commercial teas: black; green and oolong determined by variations in processing methods for fermentation. The two most important constituents of a tea infusion are caffeine and the coloured oxidation products of polyphenols commonly referred to as tannin bodies. They give the tea infusion its briskness, strength, colour and body (11).

The tea pigments and their fundamental properties have long been subjected to various studies in an attempt to reveal their nature. In this connection, Bokuchava *et al.* (1) and Soboleva *et al.* (15) stated that yellow and brown tea pigments have antioxidant properties when added to margarine or confectionery fat. On the other hand, Geoffrey *et al.* (6) found that theaflavins undergo autoxidative reactions, while the nondialysable pigments increase during storage of black tea.

In Egypt, tea is sold as a mixture of various commercial grades of imported teas. These are blended and handled under the supervision of the Ministry of Supply.

The investigation described herein was planned to study some physico-chemical properties of the pigments of black tea after their isolation and fractionation. The present work also intended to investigate the effect of adding of some foreign matters, such as iron powder, Melokhya (a popular Egyptian vegetable), on the colourants of tea. These matters are occasionally added to tea in Egypt as a kind of adulteration.

MATERIALS AND METHODS

Sampling

Gross sample of five kg of black tea was obtained at random from the local market. This samples was thoroughly mixed and reduced by the standard method of quartering to give a representative sample of 50 g termed A. In the same manner, representative samples, termed B and C were prepared from black tea imported from Cylon (trade-mark MAHARAJA) and India (trade-mark LIPTON), respectively. Samples termed d and E were prepared by adding iron powder and Melokhya, respectively, to the Cylon tea.

Methods of analysis

Preparation of tea infusion: Thirty grams of each tea sample described above were placed in one litre boiling distilled water and the extraction was carried out for five minutes starting with boiling water. In case of samples d and E, 30 mg of iron and dry Melokhya were added respectively to the tea at the begining of extraction. The extract from each sample was then strained to remove insoluble solids and the tea leaves were squeezed for maximum yield of extract.

The colour of these extracts was measured at a wavelength of 450 nm using Karl Zeiss Jena Spectrocolorimeter 'Spekol'.

Isolation of the colouring matters from tea extracts: The procedure applied herein

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*FAO, Agricultural Commodity, Programme 1970–1980, Vol. 1, part II, Rome, 1971.

was based on the method described by Varona *et al.* (18). The tea extracts were centrifuged at 5,000 rpm for 15 min to eliminate the colloidal matters. Then the colouring matters were adsorbed on a mixture of equal parts of animal charcoal and activated vegetable carbon. After filtration, the charcoal bed was washed with hot distilled water followed by another wash of ethyl alcohol. The adsorbed colourants were extracted from charcoal using 70% pyridine. The pyridine was evaporated under vacuum at 55°C. The moistened colouring matters, remained after evaporation were further dried in desiccator on a vapour of H_2SO_4. After recording the weight of the dried colouring matters, they were ground to powder and stored for analysis.

Elemental Analysis: The isolated tea colourants were subjected to elemental analysis for C, H, N, and S.

Spectroscopic examination: The UV- and IR-absorption spectra were measured for the colouring matters under study using spectrophotometer PM-4 and IR-spectrophotometer UR-10, respectively.

Indicator property: Employing pH-meter 'Radiometer-23, and spectrocolorimeter 'Spekel', the relation between pH values and optical density was studied for the colouring matters isolated from tea.

Chromatographic fractionation of the colorants: The isolated tea colourants were fractionated by the technique known as gel filtration using 'Sephadex' G-25 (the product from Pharmacia fine chemicals A.B., Uppsala). The Sephadex column was equilibrated with 0.015 M NH_3 solution and charged with three ml of colourant solution, then eluted with 0.015 M NH_3 solution. The flow rate was about 40 ml/hr. The effluents were collected in a 3 ml portions. The optical density was measured for each portion at 450 nm and the fractionation curves were made by plotting the optical densities against corresponding tube numbers.

RESULTS AND DISCUSSION

The optical density and colouring matters content of the prepared tea infusions are shown in the following table. Remarkable differences in the colour could be noticed between the five samples investigated.

The optical density and colouring matters content of teas.

Tea sample	Optical density D	Colouring matters content % of the black tea weight
A	0.38	2.64
B	0.42	3.03
C	0.35	2.50
d	3.28*	2.54
E	0.92	—**

*Diluted 10 times to get the optical density of 0.328.

**Excluded because of the interference of "Melokhya" pigments.

Data show that tea obtained in Egypt by ration card (sample A) had an intermediate optical density between Cylon tea (B) and Indian tea (C). The adding of matters like iron or Melokhya resulted in considerable increase in the colour of tea brews. For instance, the colours of sample d and E were respectively 7.8 and 2.2 times higher than the colour of sample B. This is probably due to the reaction occuring between the matters added and the polyphenols present in tea infusion, which are generally referred to as tea tannins. They amount to about 25% of the water soluble ingredients in tea leaves (7, 11).

Excluding sample E, all the other tea samples were further studied, since in that particular sample undesirable pigments were extracted from Melokhya along with the tea colourants.

The method used herein for the isolation of the coloured components from tea brews did not permit any changes in their chemical structure to occur (18). The powder of the colouring matters obtained has a yellowish-brown colour. It is easily soluble in water at room temperature.

A loss of about 16% in the colouring matters content of tea could be noticed after the addition of powdered iron (samples B and d in the table). This is probably due to the iron-polyphenolic coloured complexes not adsorbed on charcoal.

It is generally agreed that UV-spectroscopic examination provides some useful information on the principal functional groups of compounds (3, 10). The UV

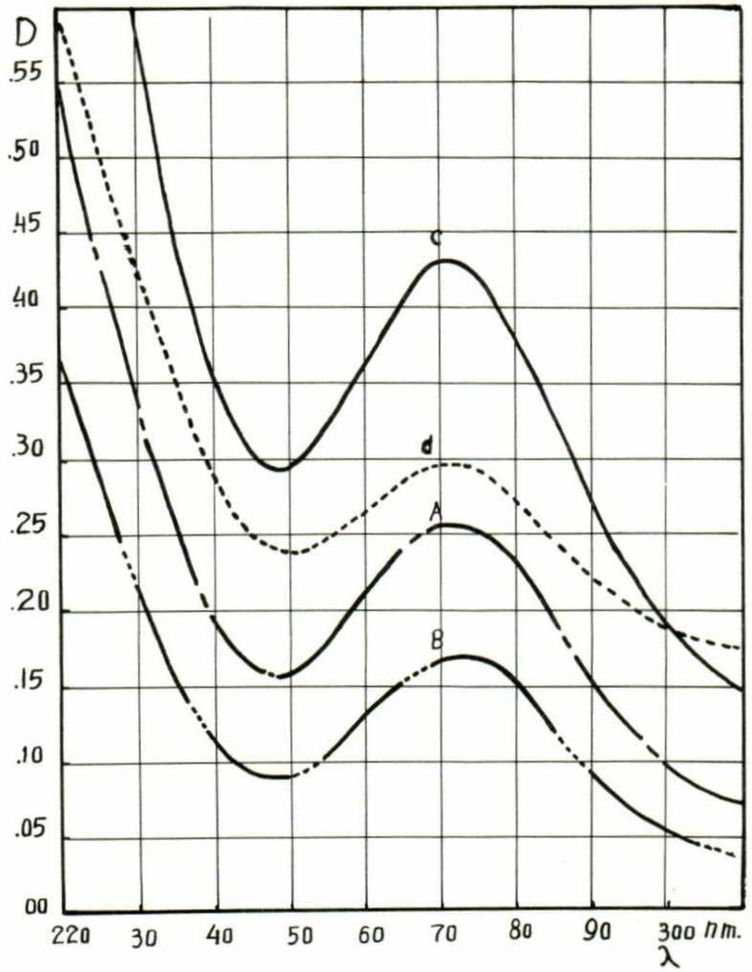

Fig. 1. U.V. absorption spectra for different sorts of tea: A. tea obtained from the local market by ration carde, B. tea imported from Ceylon, C. tea imported from India, d. sample B + iron powder.

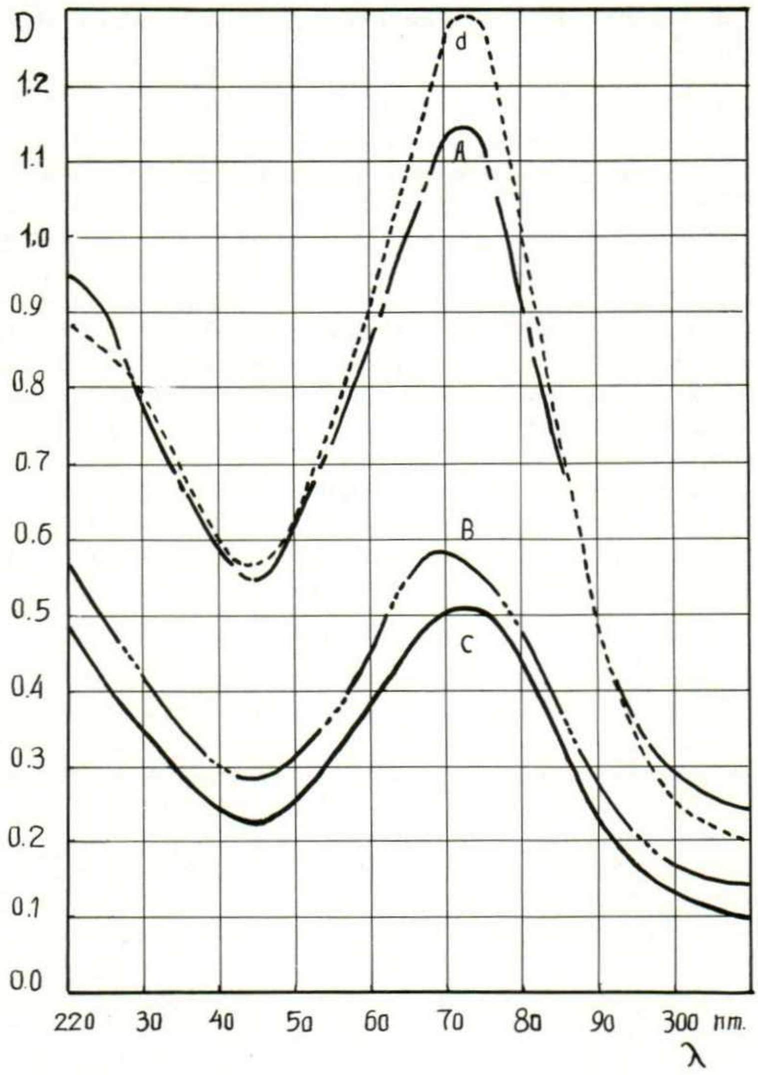

Fig. 2. U.V. absorption spectra for the colour components isolated from different sorts of tea: A. tea obtained from the local market by ration carde, B. tea imported from Ceylon, C. tea imported from India, d. sample B + iron powder.

absorption spectra for tea infusions as well as the colouring matters isolated from them are represented in Figures 1 and 2, respectively; the extinction curves of tea samples were quite similar in their character and configuration. They have a minimum absorption in the wavelength range of 245–250 nm and an intense absorption in the wavelength range of 272–275 nm. It is interesting to note that the presence of iron powder, though increased the colour intensity in the visible region of spectra (7.8 times), it did not really change the character of the absorption spectrum in the UV-region as shown in Figure 1.

The comparison between the extinction curves in Figures 1 and 2 indicates that the absorption spectra for the tea colourants after isolation have not undergone any changes in their character, except that their absorption peaks became relatively more sharp and more distinct.

The characteristic features observed in the above curves are, generally, attributed to the presence of various groups of chromophores which have intense absorption in the preceding wavelength ranges of spectra (3, 10). The recent reports on black tea have shown that as a result of its manufacturing processes, withering, rolling and fermentation, the flavanols catechin and its derivatives undergo oxidation and condensation. The oxidized flavanol products obtained, such as thearubigins, are coloured compounds with an intense absorption in the UV-region of spectra (8, 9, 11, 12, 19, 20). In this connection, Farber and Carpenter (5) have found that chlorogenic acid and its hydrolysis product caffeic acid have an absorption peak in the ultraviolet. Other investigators stated that caffeine has characteristic absorbance at 272–273 nm (14, 17).

It is generally agreed that tea colourants have more than one chromophore. Consequently, they would exhibit the characteristic absorption bands of each of the chromophores present (10, 16).

The colouring matters isolated from the Indian tea were subjected to elemental analysis. The results obtained were as follows: C — 40.83; H — 5.78; O — 41.91 (by difference); N — 5.04; S — 3.94; inorganic residue — 2.50. The empirical formula for the repeating unit of such polymers was deduced as:

$$C_{26\text{-}27}\ H_{45\text{-}46}\ O_{20\text{-}21}\ N_3S$$

It has long been recognized that infrared absorption analysis is a powerful diagnostic tool for the identification of molecular structure of any unknown compound (3, 10). Figure 3 illustrates the infrared spectrum of the colouring matters isolated from sample C. The pattern of these colourants displayed at least nineteen significant bands: At 3320 cm^{-1} a very strong broad band characteristic of $>C{=}NH$ or $HN{=}C\ (O{-}R)_2$; at 2870 cm^{-1} a medium rather sharp band indicative of $CH_3{-}C$ or $-CH_2-$; at 1690 cm^{-1} a strong sharp band in the conjugated $>C{=}CH-$ region of spectrum; at 1640 cm^{-1} a very strong sharp band characteristic of conjugated $>C{=}CH_2$; at 1150 cm^{-1} a medium sharp band for vicinal , at 1480 cm^{-1} a weak sharp band characteristic of $CH_2{-}C$ or $-CH_2-$; at 1430 cm^{-1} and 1400 cm^{-1} two weak sharp bands for venyl $-CH{=}CH$; at 1335 cm^{-1} a medium sharp band indicative of $R{-}SO_2{-}R$ or  $-N-R_2$; at 1275 cm^{-1} characteristic of $(CH_3)_2{-}N$; at 1225 cm^{-1} a strong sharp band for  $-CHO$; at 1050 cm^{-1} and 1025 cm^{-1} two weak sharp bands characteristics of ; at 785 cm^{-1} a weak sharp band for ; at 775 cm^{-1} and 760 cm^{-1} two medium sharp bands for n-propyl,  or ; at 735 cm^{-1} a weak sharp band in the $-CH_2{-}CH_2{-}CH_2{-}CH_2-$ region of the spectrum; at 620 cm^{-1} a medium sharp band indicative of $CH_2{-}O{-}CH_2$ absorption; and at 480 cm^{-1} a medium sharp band characteristic of $(SO_4)^{--}$ ionic sulfate. The identification of the previous functional groups has been performed according to (3, 10).

Figure 4 represents the dependency of optical density upon the pH values for the colourants isolated from the Indian tea — sample C. It is clear that tea colorants have an indicator property, i.e. their colour changes depending upon pH values. This property may be associated with the polyphenols, such as theaflavins and thearubigins present in the molecules of these pigments (5, 11). Moreover, the indicator property of tea colourants would explain the lightening occurs to the colour of tea drinks after adding lemon slices to them.

In the present study, the colourants isolated from the Indian tea sample were fractionated on a colomn of Sephadex G-25. Figure 5 illustrates the elution diagram

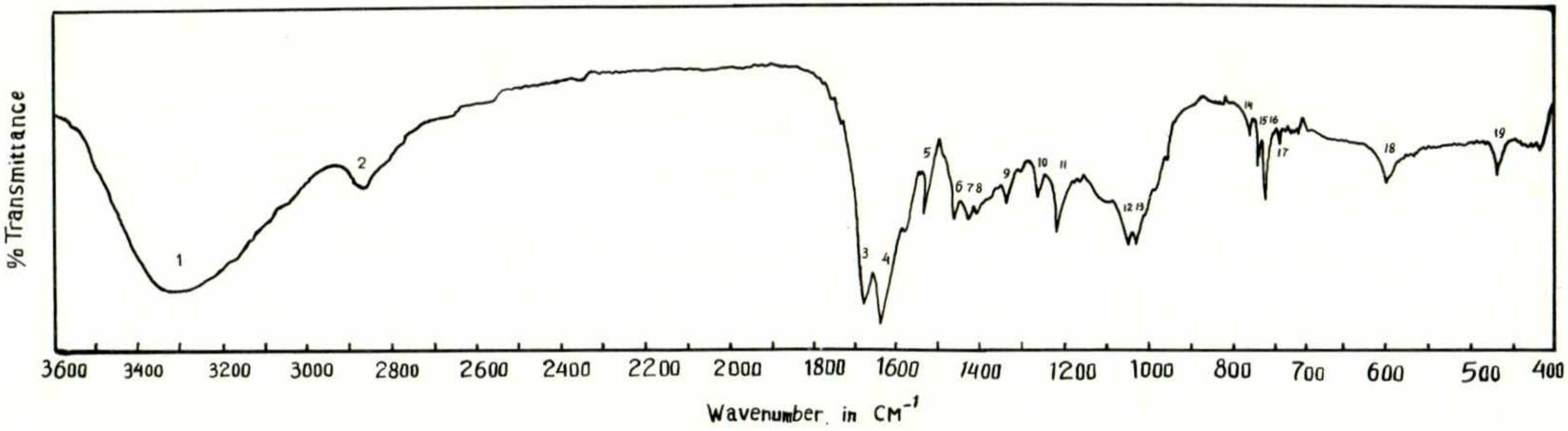

Fig. 3. Infrared spectrum of the coloring matters isolated from tea.

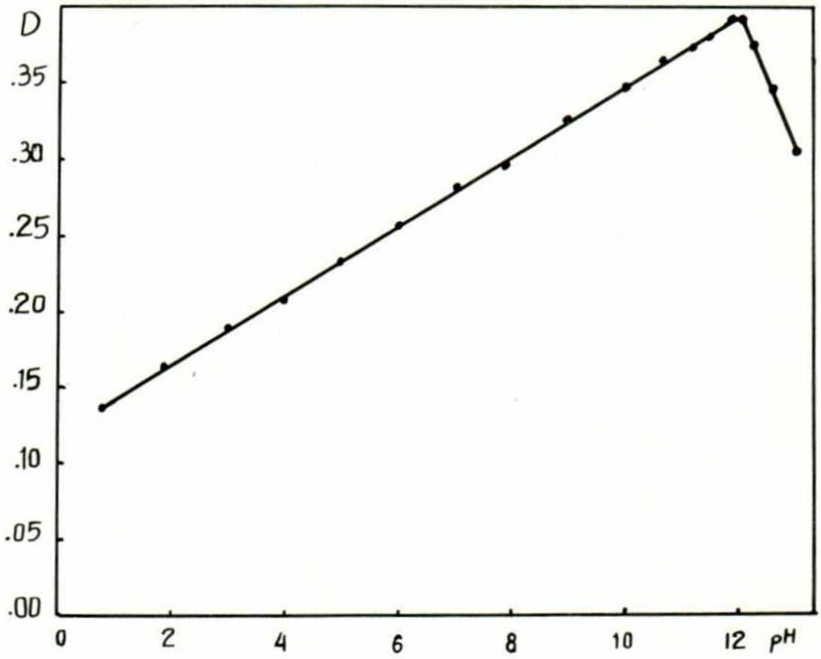

Fig. 4. The dependency of optical density upon the pH values.

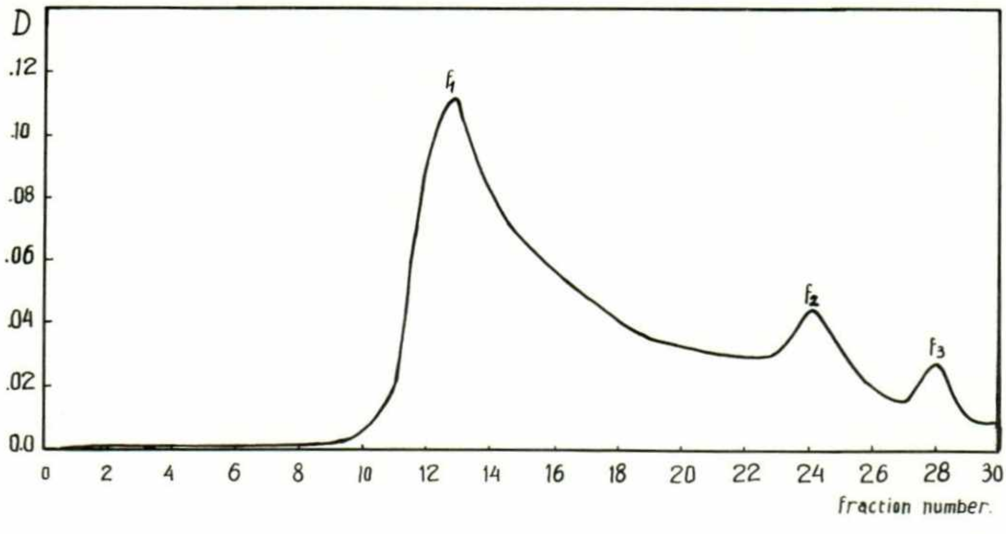

Fig. 5. Fractionation of the colour components of tea on Sephadex G-25.

obtained. Considering the theoretical principles of gel chromatography (10), it can be mentioned that the tea colourants under study were separated into three fractions f_1, f_2 and f_3, arranged in order of decreasing molecular weight. The first fraction f_1 was of brown hue, while the two other fractions were of yellow hue.

Figure 6 represents UV-absorption spectra for the fractions separated. It can be observed that the extinction curves for these fractions were considerably different in

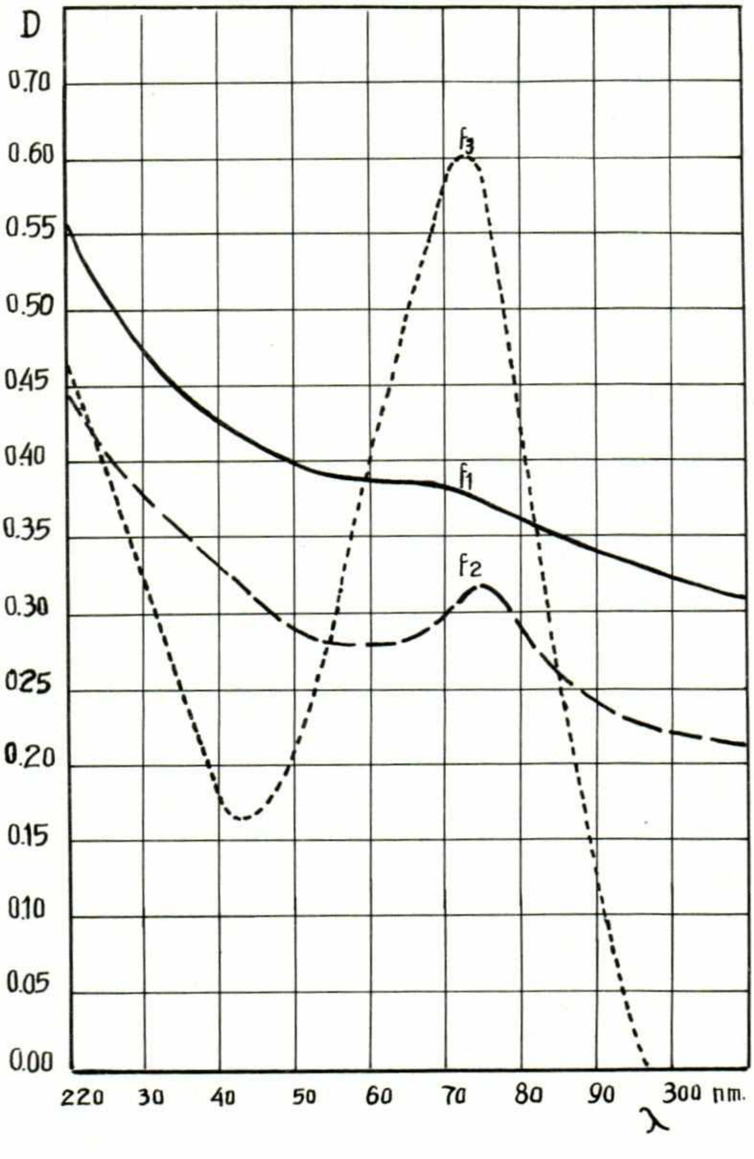

Fig. 6. U.V. absorption spectra for the fractions of the colouring matters of tea separated by gel filtration.

their character and configuration. For instance, the fraction f_1 showed a plateau in the wavelength of 255–270 nm, while the two other fractions f_2 and f_3 exhibited intense absorption in the wavelength range 272–273 nm. In this connection, the absorption peak of the second fraction f_2 is relatively more sharp and more distinct.

The results obtained by many investigators (2, 4, 9, 16, 19), give an evidence that the first fraction f_1 is likely to be thearubigins, while the third fraction f_3 could possible be theaflavins. The second fraction f_2 may contain a mixture of flavonols. The degradation products of chlorophyll are also known to be eluted in this region (9). In some other works (2) and (18) it has been established that catechins are eluted just before the theaflavins. It is also agreed that all of tea coloured fractions give a positive anthocyanin test (2, 9).

It is important here to point out that the research findings obtained by many investigators indicate that tea pigments consist of a highly complex mixture of components with similar chemical structure (2, 4, 13, 16, 19). Cattle *et al.* (2), for example, using sephadex chromatography obtained three coloured fractions of molecular weight of 1,500 from black tea infusion. According to Tadakazu (16), the tea pigments were separated into nine fractions when chromatographed on Sephadex LH-20. On the other hand, a number of tea flavanols with acidic; phenolic and alcoholic groups were separated by Wilkins (19). Sanderson (12), reported that over hundred fourty compounds, including some coloured oxidized falvanols, have been identified in black tea aroma.

However, the composition and nature of the individual coloured fractions should be further checked by other additional and more elaborate techniques.

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دراسات على المواد الملونة للشاى الأسود

د. مصطفى عبد المنعم محمد

المستخلص

أجرى هذا البحث لدراسة الصفات الفيزيوكيائية لصبغات الشاى الأسود بعد فصلها وتجزئتها . كما استهدف البحث أيضا دراسة تأثير إضافة بعض المواد مثل برادة الحديد على صبغات الشاى .

استخدم لهذا الغرض عينات من الشاى المستورد من كل من الهند وسيلان بالإضافة إلى عينات تم الحصول عليها من السوق المحلى بواسطة بطاقة التموين .

أفادت النتائج المتحصل عليها بأنّ الشاى يحتوى على ٢,٥٪—٣,٠٣٪ مواد ملونة وأن إضافة برادة الحديد إلى الشاى تزيد من شدة لونه بمقدار ٧,٨ مرة ولكنها لا تؤثر على منحنيات اختصاصه في منطقة الضوء فوق البنفسجي .

وقد دلت النتائج أيضا على أن الصبغات المفصولة من الشاى تمتاز بتغير لونها تبعًا لدرجة تركيز أيون الايدرو جيني كما أن لها قمة امتصاص في منطقة الضوء فوق البنفسجية (٢٧٢—٢٧٣ نم) — وقد أجرى تحليل عنصرى للصبغات المفصولة واقترح الرمز التالى للوحدة البنائية لها :—

$$\text{ك}_{٢٦\_٢٧}\,\text{يد}_{٤٥\_٤٦}\,\text{أ}_{٢٠\_٢١}\,\text{ن}_{٣}\,\text{كب}$$

كما أثبتت دراسة التحليل الطيفي في منطقة الأشعة فوق البنفسجية ومنطقة الأشعة تحت الحمراء لهذه الصبغات احتواءها على حوالى ١٩ مجموعة رئيسية نشطة .

ولقد أمكن بواسطة التحليل الكروماتوجرافي على أعمدة من الجيل تجزئة الصبغات المفصولة من الشاى إلى ثلاثة مجاميع تبعا لوزنها الجزيئي وتم دراسة كل مجموعه على حده — وتفيد النتائج المتحصل عليها احتواء صبغات الشاى على مركبات مثل : الثياروبيجينز — ثيافلافينز — منتجات هدم الكلوروفيل — وبعض صور الكاتيكينز .