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STUDIES ON THE COLOURED COMPONENTS OF BLACK TEA

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Key words: black tea, colourins matter, physicochemical characteristics.

The pigments of black tea were isolated and further studied by the using of some elaborate techniques. It was found that they consist of a highly complex mixture of components with similar chemical structure.

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

It has long been recognized that there are general types of commercial teas: black, green and oolong determined by variations in processing methods. 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, strenght, colour and body.

Tea in Egypt market is a mixture of various commercial grades of teas imported, blended and handled under the supervision of the Ministry of Supply. Because of the relatively high price of the tea in Egypt some kinds of adulteration accasionally occur. This is done by adding iron powder, Melokhya (popular Egyptian vegetable) and other matters to the dried exhausted chopped tea leaves to compensate the loss in their pigments, so these leaves could be brewed once more.

The investigation described herein was therefore planned to study some physico-chemical properties of the pigments of black tea after their isolation and fractionation. The present work also intended to investi-

*) FAO — Agricultural Commodity-Programme 1970-1980, Vol. 1 Part II, Rome, 71.

gate the effect of adding some alien matters (iron, Melokhya) on the coloured components of tea.

MATERIALS AND METHOD

SAMPLING:

Gross sample of five kg of black tea was obtained at random from the local market by ration card. This samples was thoroughly mixed and reduced by the standard method of quartering to give a final sample of 50 g termed A. In the same manner, final samples, termed B and C were prepared from black tea imported from Ceylon (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:

A — PREPARATION OF TEA INFUSION:

Thirty grams of each tea sample were placed in one litre boiling distilling 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 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 wavelenght of 450 nm using Karl Zeiss Jena Spectrocolorimeter "Spekol".

B — ISOLATION OF THE COLOURING MATTERS FROM TEA EXTRACTS:

The tea extracts were centrifuged at 5000 r/m for 15 min. to eliminate the colloidal matters. Then the colouring matters vere absorbed 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. Then the adsorbed colorants were extracted from charcoal using 70% pyridine. The pyridine was evaporated under vacuum at 55°C. The moistened colouring matters remaining after evaporation were further dried in desiccator with of H₂SO₄. After recording the weight of the dried colouring matters, they were ground to powder and stored for the following analysis.

C — ELEMENTAL ANALYSIS:

The isolated tea colorants were subjected to elemental analysis for C,H,N and S.

D — SPECTROSCOPIC EXAMINATION:

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

E — INDICATOR PROPERTY:

Employing pH-meter "Radiometer-23", and spectrophotometer "Spekol", the relation between pH values and optical density was studied for the colouring matters isolated from tea.

F — 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 Chemical A.B. Uppsala). The Sephadex column was equilibrated with 0.015 N 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 three 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 densities for the prepared tea infusions were as follows: A-0.38; B-0.42; C-0.35; D-3.28 and E-0.92. Remarkable differences in the colour could be noticed between the five samples under study. 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 Melakhya resulted in considerable increases 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 probable due to the reaction occurring between the matters added and the polyphenols present in tea infusion. They amount to about 25% of the water soluble ingredients in tea leaves [7, 11].

A part of sample E, all the tea samples were further studied, since in that particular sample undesirable pigments were extracted from *Melokhyla* along with the tea colourants.

The method used herein for the isolation of the coloured components from tea brews did not allow any changes in their chemical structure to occur. The powder of the colouring matters obtained has a yellowish-brown colour. It is easily soluble in water at room temperature. The percentage of the isolated colouring matters was recorded as follows: A-2.64%; B-3.03%; C-2.50%; D-2.54% (of the block tea weight).

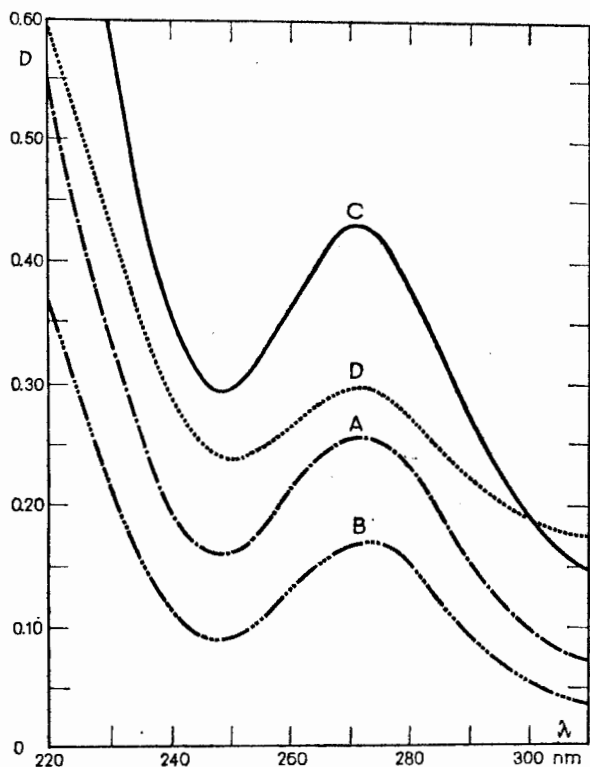


Fig. 1. U.V. absorption spectra for different sorts of tea; A — tea obtained from the local market; B — tea imported from Ceylon; C — tea imported from India; D — sample B + iron powder

It is generally agreed that UV-spectroscopic examination provides some useful information on the principal functional groups of compounds [3, 10]. Fig. 1 and Fig. 2 represent UV absorption spectra for tea infusions as well as the colouring matters isolated from them, respectively. The extinction curves of tea samples were quite similar in their character and configuration (Fig. 1). They have a minimum absorption in the wavelength range of 245-250 nm and an intense absorption in the

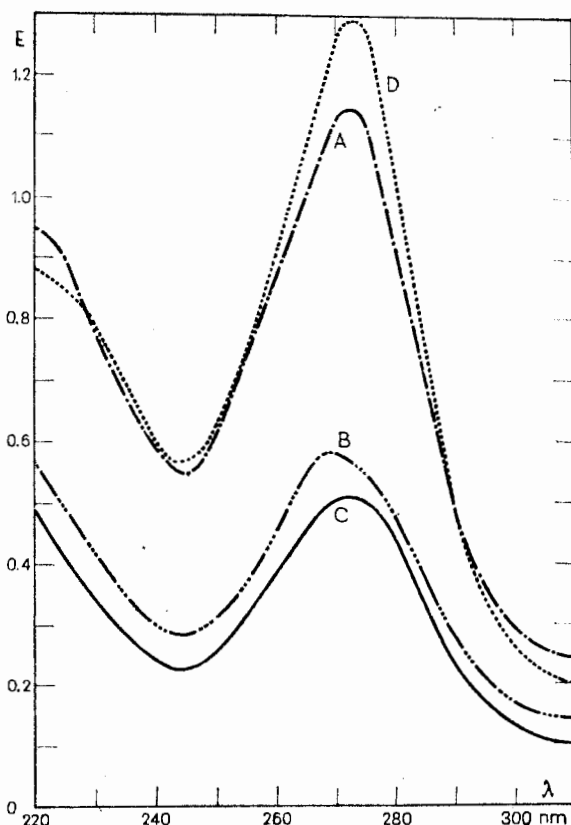


Fig. 2. U.V. absorption spectra for the color components isolated from different sorts of tea; A — tea obtained from the local market; B — tea imported from Ceylon; C — tea imported from India; D — sample B + iron powder

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 Fig. 1.

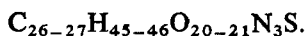
The comparison between the extinction curves in Fig. 1 and Fig. 2 indicates that the absorption spectra for the tea colourants after isolation have not undergone any changes in their character, except that their absorption peaks became relatively more sharp and more distinct.


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 range 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 flavonols, catechin and its derivatives undergo oxidation and condensation. The oxidized flavonol products obtained, such as thearu-

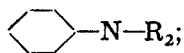
begins, are coloured compounds with an intense absorption in the UV-region of spectra [8, 9, 11, 12, 18, 19]. In this connection, Farber and Carpenter [5] have found that chlorogenic acid and its hydrolysis product caffeic acid have an absorption peak in the ultraviolet. Other investigators stated that caffeine has characteristic absorbance at 272-273 nm [14, 17].


It is generally agreed that tea colorants 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, C-41.91 (by difference), N-5.05, S-3.94, inorganic residue — 2.50. The empirical formula for the repeating unit of such polymers was deduced as:





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]. Fig. 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 band characteristic of $>C = NH$ or $HN = C$ ($O - R/2$), at 2870 cm^{-1} a medium rather sharp band indicative of $CH_3 - CCH_2 -$, 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 1550 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 vinyl $-CH = CH_2$, at 1335 cm^{-1} a medium sharp band indicative of $R - SO_2 - R$ or



at 1275 cm^{-1} characteristic of $(CH_3) - N$, at 1225 cm^{-1} a strong sharp band for -CHO, at 1050 cm^{-1} two weak sharp bands characteristic of

, at 785 cm^{-1} a weak sharp band for  at 774 cm^{-1} and 760 cm^{-1} two bands in the $-CH_2 - CH_2 - CH_2 - CH_2 -$ region of the spectrum at 620 cm^{-1}

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] and [10].

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, since these pigments stabilized the fatty acids composition and were effective in retarding peroxide formation. On the other hand, Geoffrey et al [6] studied the chemical changes occurring in tea pigments during storage. They found that theaflavins undergo autoxidative reactions, while the nondialysable pigments increase during storage of black tea. Moreover, Faber et al [5] and Pintauro [11] reported on the pH sensitivity of tea pigments.

Fig. 4 represents the dependency of optical density upon the pH values for the colorants isolated from the Indian tea — sample C. It will

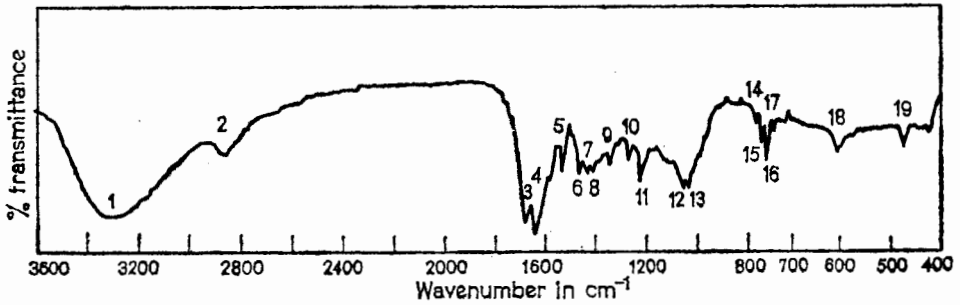


Fig. 3. Infrared spectrum of the coloring matters isolated from Indian tea

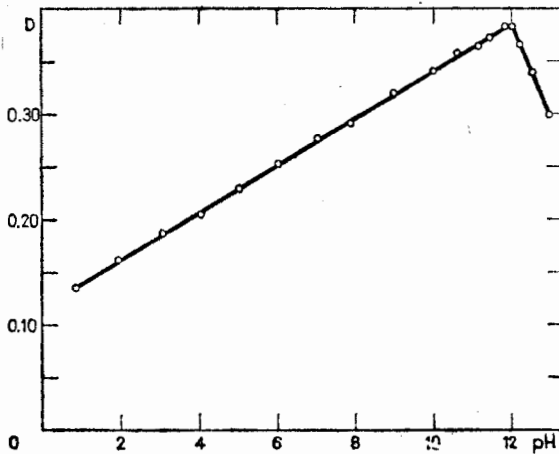


Fig. 4. The dependency of optical density of the colorants from Indian tea upon the pH values

be noted 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. Moreover, the indicator property of tea colorants would explain the lightening occurs to the colour of tea drinks after adding lemon slices to them.

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 1500 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]. In the work of Sanderson [12], over 140 compounds, including some coloured oxidized flavanols, have been identified in black tea aroma.

In the present study, the colorants isolated from the Indian tea sample were fractionated on a column of Sephadex G-25. Fig. 5 illustrates the elution diagram obtained. Considering the theoretical principles of gel chromatography [10], it can be mentioned that the tea colorants 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.

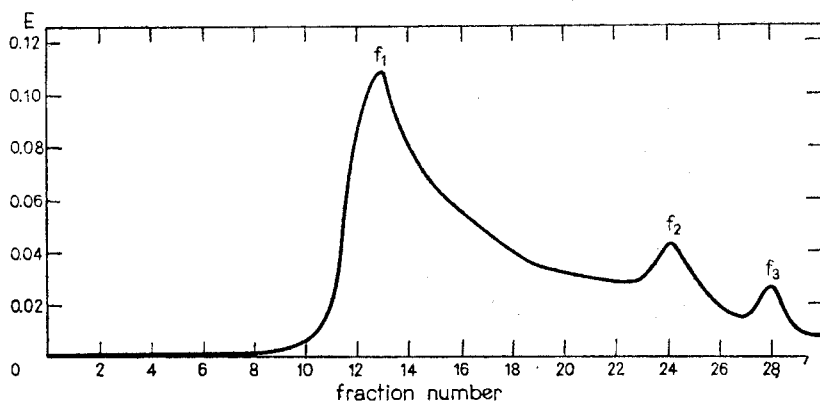


Fig. 5. Fractionation of the color components of tea on Sephadex G-25

Fig. 6 represents UV-absorption spectra for the fractions separated. It can be observed that the extinction curves for these fractions were considerably different in their character and configuration. For instance, the fraction f_1 showed a plateau in the wavelength of 255-270 nm, while

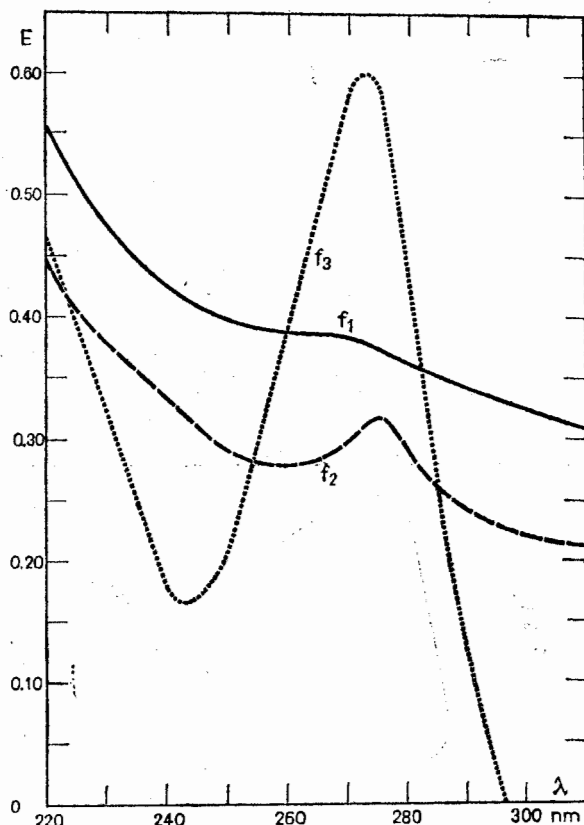


Fig. 6. U.V. absorption spectra for the fractions of the coloring matters of tea separated by gel filtration

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 possibly 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 work [2, 18] it has been established that catechine are eluted just before the theaflavins. It is also agreed that all of tea coloured fractions give a positive anthocyanin test [2, 9].

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

CONCLUSIONS

The results obtained lead to the following conclusions:

1. The adding of adulterants, such as iron, to the chopped tea leaves, though increased the colour intensity in the visible of spectra (7-8 times), it did not really change the character of the absorption spectrum in the UV-region.

2. The tea colorants possess an indicator property, which is associated with polyphenols, such as theaflavins and thearubigins, present in the molecules of these pigments.

3. The using of some sophisticated and elaborate techniques, such as gel filtration, UV — and IR — spectroscopic examinations, indicated that the tea pigments consist of a highly complex mixture of components with similar chemical structure.

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Manuscript received. July, 1979.

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BADANIA NAD SUBSTANCJAMI BARWNYMI HERBATY

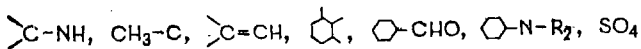
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Streszczenie

Przedmiotem badań były właściwości fizyko-chemiczne barwników czarnej herbaty Indyjskiej i Cejlońskiej oraz handlowej mieszanki herbat sprzedawanych na kartki w Egipcie. Badano także wpływ dodatków zafałszowujących na barwniki herbaty. Stwierdzono, że herbata zawiera od 2,5% (Indyjska) do 3,03% (Cejlońska) związków barwnych. Dodatek pyłu żelaza intensyfikował barwę 7-8-krotnie lecz nie zmieniał spektrum absorpcji w ultrafiolecie. Związki barwne herbaty zmieniały intensywność barwy w zależności od pH oraz zawierały chromofory i auksofory z wyraźną absorpcją w strefie 272-275 nm. Skład podstawowy wydzielonych barwników w procentach był następujący: C-40,83, H-5,78, O-41,91, S-3,94, związki nieorganiczne — 2,50. Proponowany wzór sumaryczny monomeru tych związków przedstawia się następująco:

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

Widmo w podczerwieni wskazuje na obecność co najmniej dziewiętnastu pasm charakterystycznych dla następujących grup:



Rozdział związków barwnych na Sephadex G-25 doprowadził do wydzielenia trzech frakcji, brązowej o najwyższym ciężarze cząstkowym oraz dwóch żółtych o mniejszym ciężarze. Wyniki wskazują, że substancje barwne zawierają tearubine, teaflawiny produkty rozkładu chlorofilu i pojedyncze katechiny.