The Libyan Journal of Agriculture: volume 8, 1979

Liquid Sugar from Egyptian Treacle

II. Chromatographic Fractionation of the Treacle Colourants with Molecular Sieves[1]

MOSTAFA A. MOHAMED[2]

ABSTRACT

The present study describes the application of gel filtration technique to the fractionation of the treacle colourants. Some properties of the fractions obtained were also studied.

Gel filtration using 'Sephadex' G – 25 provided some useful information on the molecular size range of the individual colourants. U.V.-spectroscopic examination of the individual colourants strongly suggested the presence of various groups of chromophores which have intensive absorption in the wavelength range 255–290 nm.

The results obtained give an evidence that the treacle colourants contain one or more of the following groups: melanoidines, caramels, products of degradation of reducing sugars and complexes of Cu^{++} ions with these products. The present work, however, provides a narrow but helpful basis for further investigations.

INTRODUCTION

In a previous work (6) the colourants of Egyptian treacle were isolated and some of their properties further studied. The results obtained demonstrated that treacle colourants consist of a highly complex mixture of components of very similar chemical structure.

In order to provide the relevant information on the fundamental properties and the structure of such complex mixture of colourants it is necessary to find suitable techniques for separation of the complex mixture into as many components as possible and for purification of these components. The application of some new techniques, namely, the column chromatography using cellulose ion exchangers, gel filtration through Sephadex and electrophoresis, has already shown some success in solving the problem of sugar — products colour (5, 9, 10, 11).

In the present investigation, 'Sephadex' column chromatography was employed to

[1] *This work was carried out in Egypt.*

[2] *Food Technology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt. Present Address: Food Science Department, Faculty of Agriculture, Al-Fateh University, Tripoli, S.P.L.A.J.*

fractionate the treacle colourants. Some characteristics of the fractions obtained were also studied. Gel filtration had been also applied herein to the model coloured solutions in order to identify some of the constituents or groups of constituents of treacle colourants.

The results obtained herein may be of use in ascertaining the respective contribution of these colourants to the colour problem in the manufacturing processes of Egyptian treacle. Thus, it would be rather feasible to recommend methods for their reduction or inhibition.

MATERIALS AND METHODS

The colouring matters of treacle were isolated, purified, dried and ground to powder. The method applied here was described in a previous study (6).

The model coloured solutions used in the present investigation were prepared under conditions very similar to those which prevail during the manufacturing process of treacle (6). These models are as follows: Products of the acid degradation of reducing sugars — solution No. 1, the melanoidines produced from amino — carbonyl reaction — solution No. 2; complexes of Cu^{++} ions with the products of acid degradation of reducing sugars in the presence of amino acids — solution No. 6, and caramel pigments obtained from pure sucrose.

CHROMATOGRAPHIC FRACTIONATION OF THE COLOURANTS

The colourants in both treacle and model solutions were fractionated by the gel filtration technique using 'Sephadex' G-25 (the product was purchased from Pharmacia fine chemicals A.B., Uppsala). This type of gel has an approximate exclusion limit of 5,000 (molecular weight) (7). The method applied herein was based on the technique described by many workers (5, 9, 10, 11).

A column (2×40 cm) was prepared by using 'Sephadex' G-25 as a packing material. The latter was slurried with 0.015 M NH_3 solution, poured into a chromatographic tube (2 cm, internal diameter) and the packed column showed the height of 40 cm. The column was equilibrated with 0.015 M NH_3 solution and charged with 3 ml of colourant solution. This was followed by eluting the column with 0.015 M NH_3 solution. The flow rate was about 38 ml/hr. The effluents were collected in a 3 ml portions. The optical density was measured for each portion at $\lambda = 560$ nm using Carl Zeiss Jena Spectrocolorimeter 'Spekol', and the fractionation curves were made by plotting the optical densities against corresponding fraction numbers. The U.V.-spectroscopic examination was carried out for the colour components obtained from the sephadex column, using Karl Zeiss pM-4 spectrophotometer.

RESULTS AND DISCUSSION

It is generally agreed that colour in sugar products consists of a highly complex mixture of components with very similar chemical structure. Gross (5), Smith (9), Cortis-Jones (3) and others (2, 4) reported on the use of 'Sephadex' in fractionation of sugar colourants. Gross (5), for example, found that the bulk of these colourants consists of components corresponding to molecular weight of below 5,000.

Figure 1 illustrates the elution diagram of a fractionation of the treacle colourants and some model solutions on 'Sephadex' G-25. Considering the theoretical principles of gel chromatography (1, 7), it can be mentioned that the colourants of both treacle and model solutions were separated into three fractions depending upon their molecular

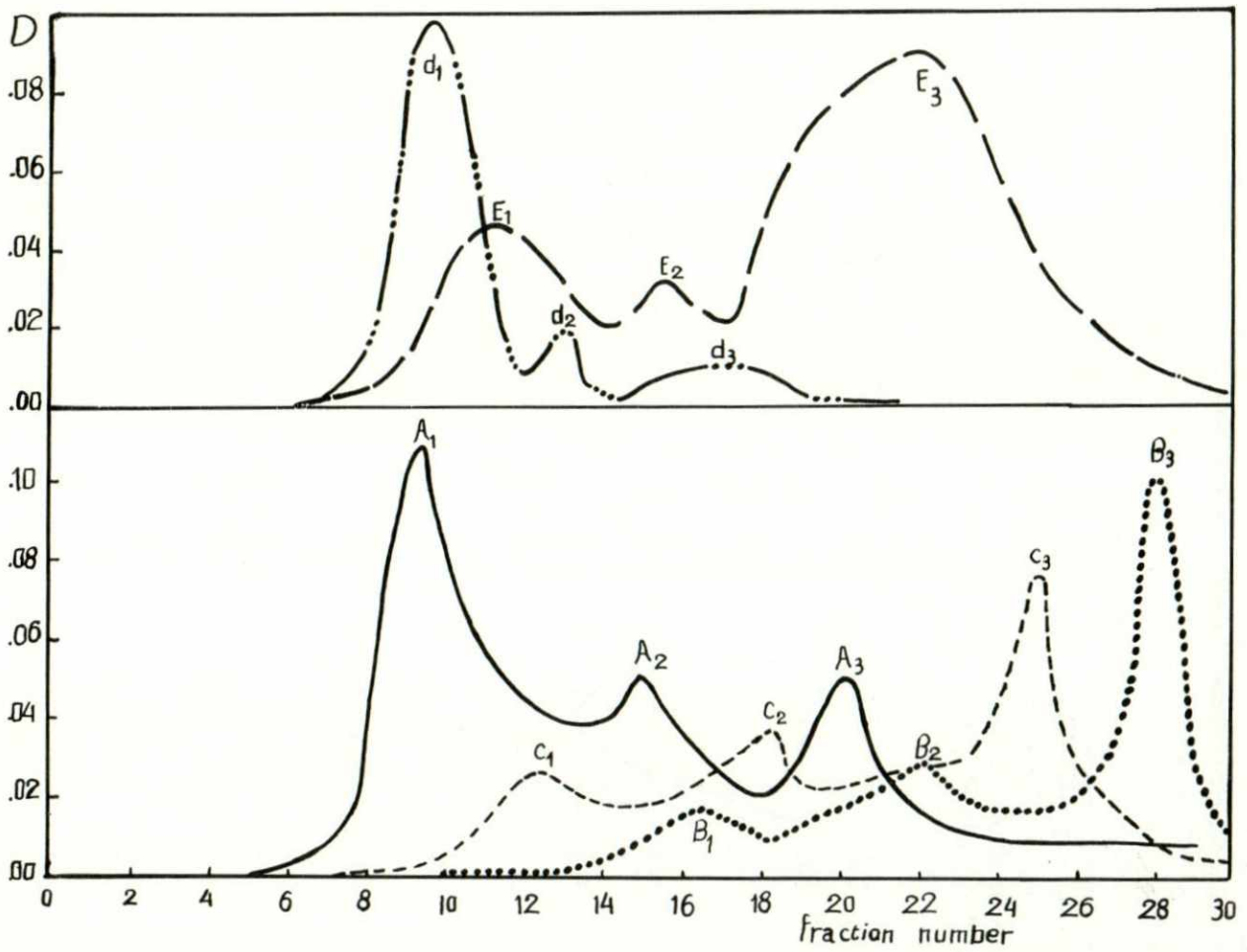

Fig. 1. Fractionation of the colouring matters of treacle and model coloured solutions on Sephadex G-25 where:

A_1, A_2, A_3, ... fractions of treacle, B_1, B_2, B_3 ... fractions of solutions No. 1, C_1, C_2, C_3 ... fractions of solution No. 2; d_1, d_2, d_3 ... fractions of caramels; E_1, E_2, E_3 ... fractions of solution No. 6.

weights. Fractions A_1, B_1, C_1, d_1 and E_1 — of brown hue- have larger size molecules than those of fractions A_3, B_3, C_3, d_3 and E_3 — of yellow hue.

Regarding the sites of colourant fractions in the elution diagram, a kind of similarity can be noticed between treacle and some model solutions. This may give an evidence that treacle colourants contain one or more of certain coloured groups very similar in their molecular weight to those of model colourants. However, it was previously found (6), that each of the groups: melanoidines, caramels and degradation products of reducing sugars possesses its characteristic contribution to the colour of treacle. These findings would allow one to recommend some measures for reducing the formation of such colouring groups during the manufacturing processes of treacle. Boiling temperature, heating time, pH of the medium, and the presence of traces of amino acids and metals are factors of practical significance in this subject.

It has been shown that the yellow fractions, A_2, A_3, B_2, B_3, C_2, C_3, d_2, d_3, E_2 and E_3, most probably consist of polyphenolic — flavonoid — compounds (5). As reported in

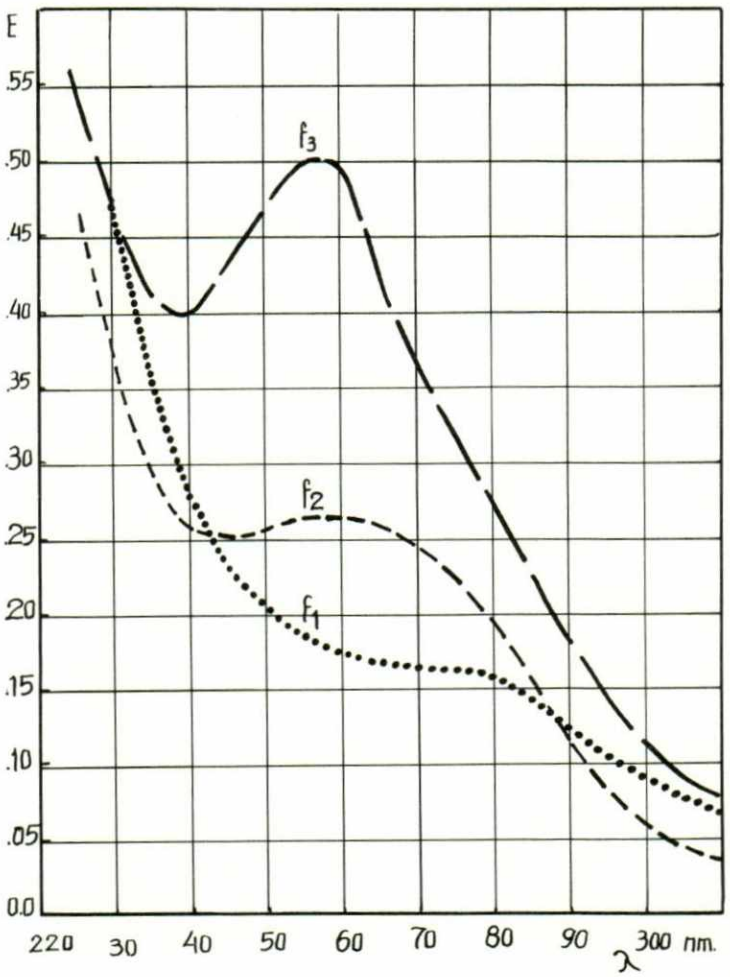

Fig. 2. U.V. absorption spectra for the fractions of the colouring matters of treacle separated by gel filtration.

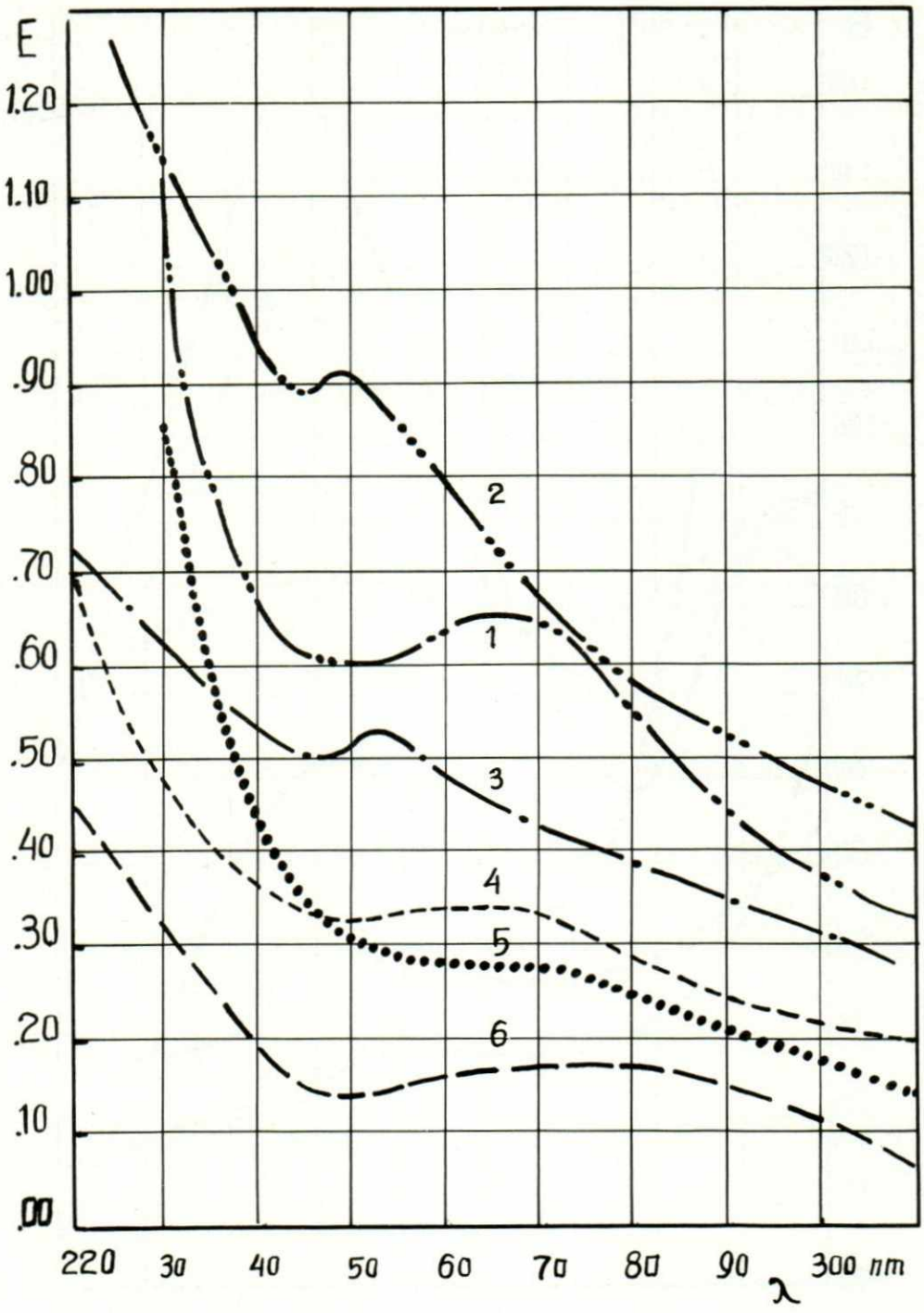

Fig. 3. U.V. absorption spectra for the fractions of the model coloured solutions:
1, 2, 3 ... fractions of solution No. 1, 4, 5, 6 ... fractions of solution No. 2.

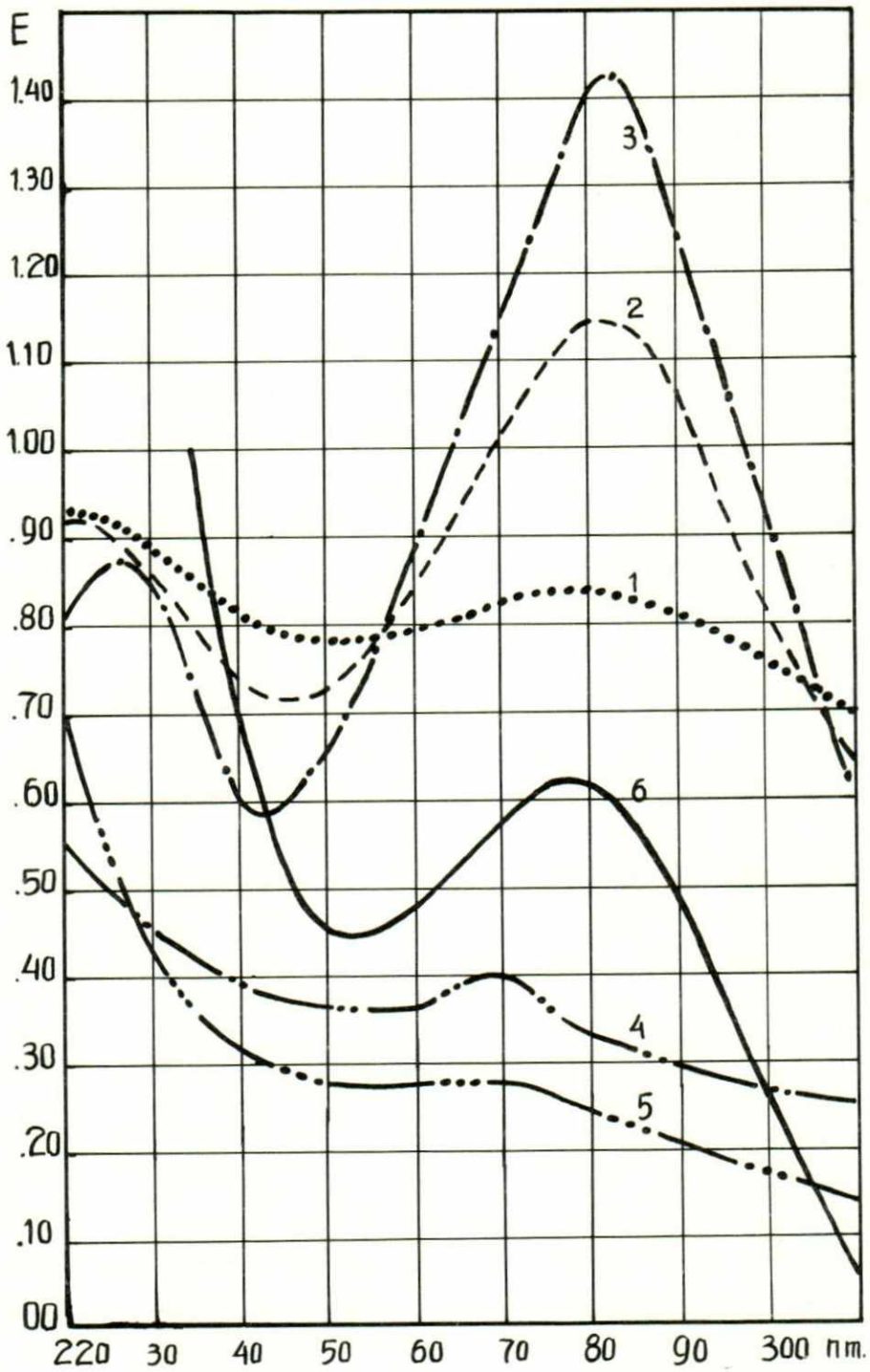

Fig. 4. U.V. absorption spectra for the fractions of the model coloured solutions: 1, 2, 3 ... fractions of caramels, 4, 5, 6 ... fractions of solution No. 6.

some recent studies, these fractions are of significantly small size, corresponding to a molecular weight of 500–1000 (1, 5).

The composition and nature of the individual colour fractions can be checked by examining the electrophoretic pattern at various pH values, indicating the ionic properties, pH sensitivity, and reacting with certain spray reagents. U.V.–spectroscopic examination may provide some useful information on the principal functional groups of these colourants. (4, 5, 8, 10, 11).

Figures 2, 3 and 4 illustrate, U.V. absorption spectra for the separated fractions of both treacle and model solutions. It can be observed that the extinction curves for treacle fractions (Fig. 2) were considerably different in their character and configurations. For instance, the first fraction f_1 — of brown hue — showed a plateau in a wavelength range of 265–280 nm, while an intense absorption in a wavelength range of 250–267 nm could be easily distinguished in the optical absorption curve for the second fraction f_2 — of yellow hue. The third fraction f_3 — of yellow hue also — had a sharp absorption peak of 258 nm. The characteristic features observed in the above curves could generally be attributed to the presence of various groups of chromophores which have intensive absorption in the preceding wavelength ranges of spectra (2, 7, 8).

The wavelength range 260–270 nm, for example, may be associated with compounds of the type R—CH=C(OH)—CHO, while the absorption at wavelength of 280 nm may indicate the presence of hydroxymethylfurfural caused by the destruction of reducing sugars (8, 10, 11). It was also suggested, that the brown fractions frequently contain nitrogen (melanoidines type) (10).

Differences observed in the features of the extinction curves of model solutions, Figures 3 and 4, would confirm the complexity of the chemical structure of these colourants.

It should be also pointed out that the similarity in character and configurations between model solutions spectra and those of treacle fractions could undoubtedly verify the above-mentioned findings, that treacle colourants contain one or more of the following groups: melanoidiness, caramels, products of degradation of reducing sugars and complexes of Cu^{++} ions with these products. The possibility of describing of fractions with regard to their functional groups provides a narrow but helpful basis for further investigations.

LITERATURE CITED

1. Andres, H. 1970. Investigation on Sephadex gel. Jahresber. Zuckerforschungs — Inst. (Vienna): 120–124.
2. Bugaenco, I. F., I. P. Bulgakova, and I. I. Pavlov. 1968. Fractionation of colouring matters with gel filtration. Sakharnaya Promishlennost; 7:41–44 (In Russian).
3. Cortis–Jones, B. 1962. Methods for fractionation of impurities in cane juice and mill syrups. Int. Sugar. J. 64:(762), 165–167.
4. Farber, L., F. G. Carpenter, and E. J. McDonald. 1971. Separation of colourants from cane sugar. Int. Sugar. J. 73:(870), 170–173.
5. Gross, D. 1967. The fractionation and characterization of sugar colour by modern separation methods — part I, II. Int. Sugar J., 69:(828), 323–328, 360–365.
6. Mohamed, M. A. Liquid Sugar from Egyptian treacle. I. Study of some characteristics of the colouring matters isolated from Egyptian treacle, in press.
7. Pecsok, R. L. and L. D. Shields. 1968. Modern methods of chemical analysis. John Wiley and Sons, Inc. New York, 89–110.

8. Sapronov, A. R. 1963. Spectrophotometric studies on the colouring matter in products of sugar manufacture. Ph.D. thesis. Dept. Sugar Technology Varoneg Inst. U.S.S.R. (in Russian).
9. Smith, N. H. 1966. Fractionation of sugar colourants with molecular sieves. Proc. 1966. Tech. Session Cane Sugar Refining Research: 84–102.
10. Tsuchida, H. and M. Komoto. 1969. Melanoidin produced from the reaction of glucose and ammonia part I. Chromatographic fractionation of the melanoidin on DEAE — Cellulose. Refineries Technologists. 21:89–93.
11. Tsuchida, H. and M. Komoto. 1970. The fractionation and some characterizations of the nondialysable colourants in cane sugar refinery's final molasses by DEAE Cellulose column chromatography. Proc. Res. Soc. Japan. Sugar Refin. Technol. 22:66–76.

محاليل سكرية نقية من العسل الأسود المنتج في مصر

٢ — تجزئة المواد الملونة المفصولة من العسل الأسود بواسطة التحليل الكروماتوجرافي على أعمدة من الجيل

د. مصطفى عبد المنعم محمد

المستخلص

تختص هذه الدراسة بتطبيق إحدى طرق التحليل الجزئي الحديثة وهى — التحليل الكروماتوجرافي على أعمدة الجيل — في تجزئة المواد الملونة المفصولة من العسل الأسود ثم دراسة بعض الخواص المميزة للجزئيات الملونة الناتجة وذلك في محاولة للوصول إلى أنسب الوسائل لتقليل تكوين أو تثبيط المواد الملونة المسئولة عن تلوين العسل الأسود .

ولقد أثبتت النتائج المتحصل عليها أن المواد المفصولة من العسل الأسود تتكون من ثلاثة مجاميع مختلفة — المجموعة الأولى تتميز بلونها البني وهى ذات وزن جزئي مرتفع أما المجموعتان الأخريان فتتميزان باللون الأصفر وهما ذات وزن جزئي منخفض .

وتشير نتائج الدراسات السيكتروفوتومترية للمجاميع الملونة الناتجة من التجزئة على احتواء المواد الملونة للعسل الأسود على واحدة أو أكثر من المجاميع الملونة الآتية : —

صبغات الكاراميل — المواد الملونة الناتجة من هدم السكريات المختزلة — مركبات الملانويدين .

وأخيرا فإن النتائج المتحصل عليها من هذه الدراسة تتيح الفرص لاجراء مزيد من الأبحاث المتعمقة على المجاميع الناتجة من تجزئة المواد الملونة للعسل الأسود — وهذا ما سوف تتناوله الدراسات القادمة .