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Liquid Sugar from Egyptian Treacle

I. A Study of some Characteristics of the Colouring Matters Isolated from Egyptian Treacle[1]

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ABSTRACT

The present investigation was performed in an attempt to study the possibility of obtaining liquid sugar from Egyptian treacle, a matter considered to be of great practical importance in food industries. The samples of treacle were chemically analysed to determine their average composition. The colouring matters of treacle were isolated and further studied.

It was found that the colouring matters had an indicator property. The presence of coloured complexes of polyphenols with metals was predicated. The detection of six amino acids in composition of the colouring matters under study emphasized the presence of melanoidines group among their colour components. The elemental analysis of the isolated polymers was as follows: C, 41.69; H, 6.59; N, 5.04; 0, 42.36; ash, 4.32. An empirical formula for the repeating unit in these polymers, $C_{9-10}\,H_{18-19}\,O_{7-8}\,N$, was deduced from the elemental analysis.

The infrared spectrum of these polymers showed at least ten significant bands indicative of the functional groups: $=N-H$, $-C\equiv N$, $-NH_3$, $-C=C-$, $R.\,CH=CH_2$,  $O-\underset{N}{C}-O$ and $-CH_3$.

Model coloured solutions were prepared under conditions similar to those which prevail during the manufacturing process of treacle. The intensity of the developed colour was measured during the heating process of these solutions. Some physico-chemical properties of the groups of colourants obtained were also studied. The effect of Fe^{+++} and Cu^{++} ions on the acceleration of colour reactions was studied. It was found that these ions, which originated from the equipment during the manufacturing processes, changed not only the intensity of colour in the visible region of spectra, but also the character of the absorption spectra for the solutions in the U.V. region.

The results obtained gave an evidence that the colouring matters of treacle contained one or more of the groups of model colourants.

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INTRODUCTION

Egyptian treacle, locally known as black honey, is a food commodity manufactured from cane juice by evaporating and concentration the juice without the removal of any sucrose (thus differs basically from molasses). The major part of treacle is manufactured by minor producers.

The amount of sugar produced annually in Egypt does not satisfy the need of the local market. At the same time, there are many food industries in Egypt that consume considerable quantities of sucrose in liquid form. The low price of treacle — 2.5 times cheaper than sucrose — besides its nutritive value should justify its use as a source of sugars in these food industries instead of sucrose solutions. But, the large amounts of colouring matters in the treacle present a handicap to its manipulation for this purpose.

The colouring matters resulting during the manufacturing processes of the treacle from cane juice are so scomplicated that their structures for the most part are still to be determined. These polymers are formed in reaction media containing 26 amino acids and four reducing hexoses (4). These media are modified by high concentrations of sucrose and the buffering action of the salts of as many as eleven organic acids (4). Such components undergo the process of heating for a long time at a high temperature and slight acidity.

The main purpose of the experiments run in this investigation was to isolate the colouring matters from the treacle in order to study their physico-chemical nature.

It was found interesting also to compare these original colouring matters of the treacle with some constituent groups of colourants, which have been generally assumed to be formed during the manufacturing processes of the treacle.

Such a study may be of use in revealing the nature of the highly complex mixture of the colouring matters isolated from Egyptian treacle. This knowledge enables one to devise methods and processes for their reduction or inhibition.

MATERIALS AND METHODS

Sampling: Samples of treacle used in this study were obtained from a national extractor at Mallawy — in upper Egypt — during the 1976 operation season, where the cane variety Co. 310 prevails.

Analytical methods

The samples were analysed for the following: total soluble solids, sucrose, reducing substances, ash, sodium, K, Ca and iron content. The colour and the U.V. absorption spectra were measured for aqueous solutions of the treacle and also for the colouring matters obtained. Infra-red analysis and elemental analysis (C, H, O, N) were carried out for the colourants isolated from the treacle samples. All the above mentioned determinations were carried out according to the methods described in (8, 14, 15). Using: 'Abbe' Refractometer, pH meter 'Radiometer-23', 'Carl Zeiss Jena' Flamephotometer, 'Carl Zeiss Jena' Spectrocolorimeter 'Spekol', Spectrophotometer 'Unicum sp-500', and Infra red spectrophotometer UR-10.

Isolation of the colouring matters from treacle

The method applied here was in accordance with that described by Vorona *et al.* (20). After recording the weight of the obtained colouring matters they were ground to powder and stored for analysis.

Amino acids were qualitatively determined in the colouring matters obtained by paper chromatography using Whatman paper No. 1 in descending system. The solvent employed was n-butanol: glacial acetic acid: water in the volume ratio (120; 30; 50). The preparation of the hydrolysate and the development of the chromatograms were applied as described by Block *et al.* (6).

Preparation of model solutions of colourants

The following coloured model solutions were prepared under conditions very similar to those which prevail during the manufacturing processes of treacle:

Solution No. 1 — Products of acid degradation of reducing sugars-using citrate buffer solution with pH-5.25.

Solution No. 2 — Melanoidines — using the amino acids glycine, aspartic acid and glutamic acid with invert sugar.

Solutions No. 3 and 5 — Complexes of Fe^{+++} ions with solutions No. 1 and No. 2, respectively, using Ferric chloride.

Solutions No. 4 and 6 — Complexes of Cu^{++} ions with solutions No. 1 and No. 2, respectively, using Cupric chloride.

The solutions No. 1–6 were heated in flasks equipped with reflux condensers on water bath for 8 hours. Samples were withdrawn for analysis at hourly intervals during the period of heating.

Caramel pigments — An aqueous solution was prepared from caramel products obtained by heating pure sucrose at 150°C for five hours.

Complexes of polyphenols with Cu^{++} and Fe^{+++} ions — Successive volumes of both $FeCl_3$ and $CuCl_2$ were added to two separate volumes of 100 ml of aqueous solutions of 0.1% tannic acid. The optical density of the coloured complexes obtained was measured after each addition.

RESULTS AND DISCUSSION

The average composition and characteristics of Egyptian treacle are represented in Table 1. It is evident that the high sugar content beside the presence of other elements of value, such as iron and calcium may explain the using of treacle as a popular food commodity in Egypt. The total soluble solids, as reported in Table 1, show that the cane juice was concentrated about 5 times during the manufacturing process of treacle.

Table 1 Average composition and characteristics of Egyptian treacle.

Characteristic measured	
Total soluble solids %	78.90
Sucrose %	47.82
Reducing sugars %	12.91
Total ash (carbonated) %	1.59
Na ... mg/100 g	39.00
K ... mg/100 g	637.00
Ca ... mg/100 g	49.00
Fe ... mg/100 g	4.10
Colouring matter %	1.42
Colour (attenuation index)	4.46
pH value	5.15

It is observed that the total sugars calculated as equivalent invert sugars are about 63.0%. Hence, reducing sugars comprise about one fifth of the total sugars. This would explain why crystallization of sucrose does not take place in treacle in spite of its high content.

The color of treacle, expressed as the attenuation index, was 4.46. Such high value of colour reveals the necessity of clarification of the treacle before using as a liquid sugar in food industries.

The method used herein for isolation of the coloured components from treacle does not allow any changes to occur in their chemical structure (20). The powder of the colouring matters obtained has a yellowish-grey colour as well as an odour characteristic of the treacle. It is easily soluble in water at room temperature. The percentage of the colouring matters isolated from treacle was 1.42%.

The elemental analysis of the isolated colorants was as follows: C, 41.69; H, 6.59; N, 5.04; 0, 42.36 (by difference); ash 4.32. The empirical formula for the repeating unit of such polymers was deduced as $C_{9-10}\,H_{18-19}\,O_{7-8}\,N$.

It has been generally assumed that colour in sugar products consists of many groups, the most important one of which is the melanoidins produced from amino-carbonyl reactions (5, 10, 16, 19). Figure 1 shows the chromatogram of the amino acids content of the colouring matters isolated from treacle. From this chromatogram the presence of the following amino acids is observed: aspartic acid, glutamic acid, alanin, valine, leucine and Histidine. The detection of these amino acids in the hydrolysate of the isolated colouring matters emphasizes the presence of melanoidins group among their coloured components.

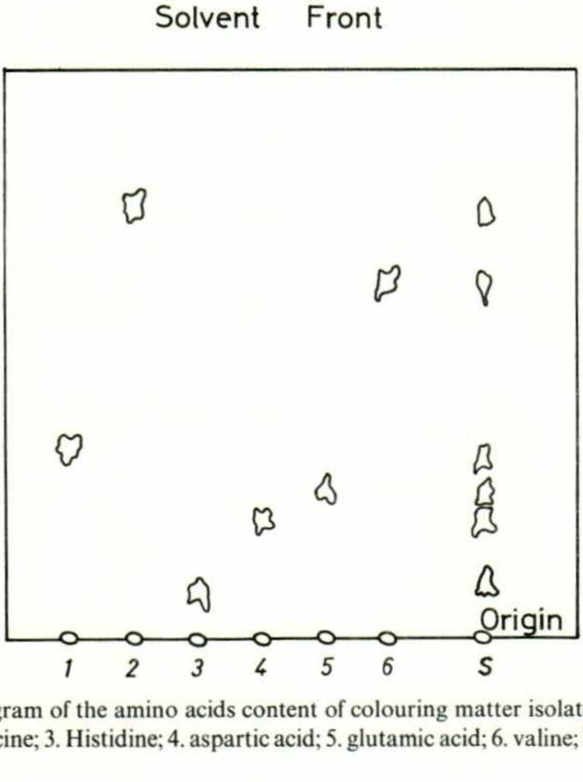

Fig. 1. Chromatogram of the amino acids content of colouring matter isolated from treacle.
1. Alanine; 2. Leucine; 3. Histidine; 4. aspartic acid; 5. glutamic acid; 6. valine; S. hydrolysate of the colouring matter.

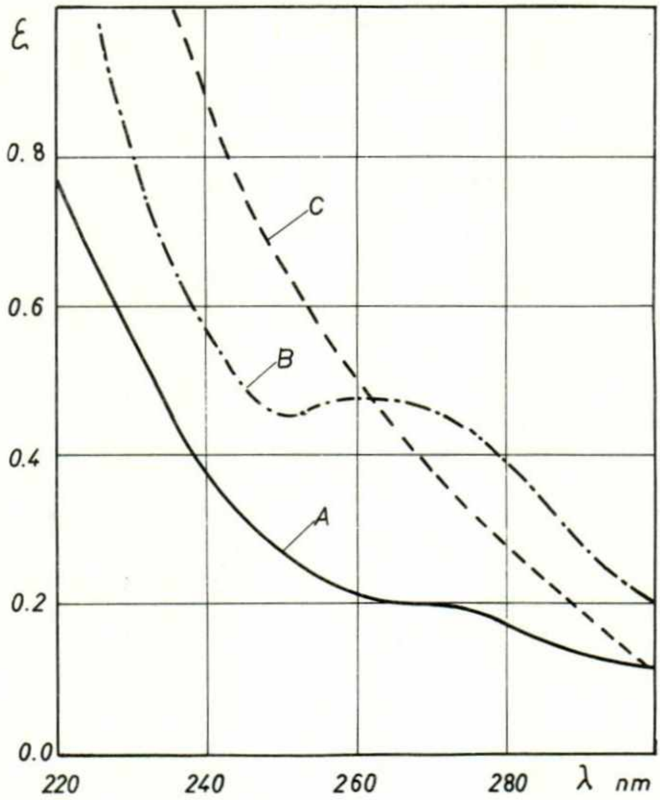

Fig. 2. U.V. absorption spectra of: A. treacle; . . . original sample; B. colouring matter isolated from treacle; C. diluted treacle after isolation of the colouring matter.

Figure 2 illustrates the U.V. absorption spectra for: the original sample of treacle (A); the colouring matter isolated from it (B) and the diluted treacle after isolation of the colouring matters (C). This figure shows a shoulder in the wavelength range of 260–275 nm for the optical absorption curve A. Also it can be noticed that an intensive absorption in the wavelength range of 255–280 nm for the curve B is present. This may be attributed to the existance of different groups of chromophores, which have intensive absorption in the preceding two wavelength ranges of spectra.

The absence of any shoulder in the case of curve C verified the complete separation of all amounts of the colouring matters present in the treacle.

The infrared spectrum of the colouring matters isolated from the treacle is shown in Figure 3. The pattern of these colorants displayed at least ten significant bands: at 3350 CM^{-1} a very strong, very broad band characteristic of $=N—H$ or NH_2 free; at 2910 CM^{-1} a medium broad band indicative of $—CH_3$ and $>CH_2$ absorption; at 2350 CM^{-1} a weak broad band in the $—C\equiv N$ region of spectrum; at 1650 CM^{-1} a medium broad band characteristic of $—C=C—$, $>C=0$, $—NH_2$; at 1430 CM^{-1} a weak broad band for ionized carboxyl absorption; at 1250 CM^{-1} a weak broad band for $C—O$ absorption (C unsaturated or aromatic); at 1070 CM^{-1} a strong broad band indicative of $O—H$ absorption; at 930 CM^{-1} a medium sharp band for $R.CH=CH_2$ absorption; at 870 CM^{-1} a medium sharp band for $R_2.C=CHR$ absorption and at 850 CM^{-1} a weak sharp band in

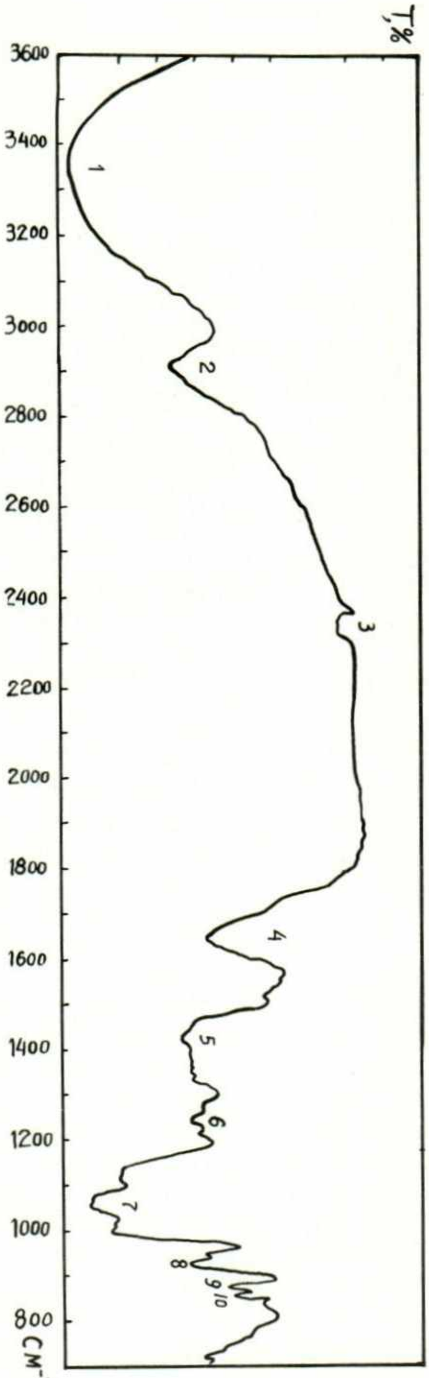

Fig. 3. Infrared spectrum of the colouring matters isolated from treacle.

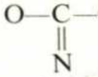 $O{-}\underset{\overset{\|}{N}}{C}{-}O$ region of spectrum. Identification of the previous functional groups has been performed according to Barnes *et al.* (3).

Figure 4 shows the increase in the optical density D of the model solutions during the process of heating. In general, it is evident that the optical density increases with the heating time; but there are wide differences in the rate of increase between the solutions under study. This may be accounted for by the accumulation of coloured

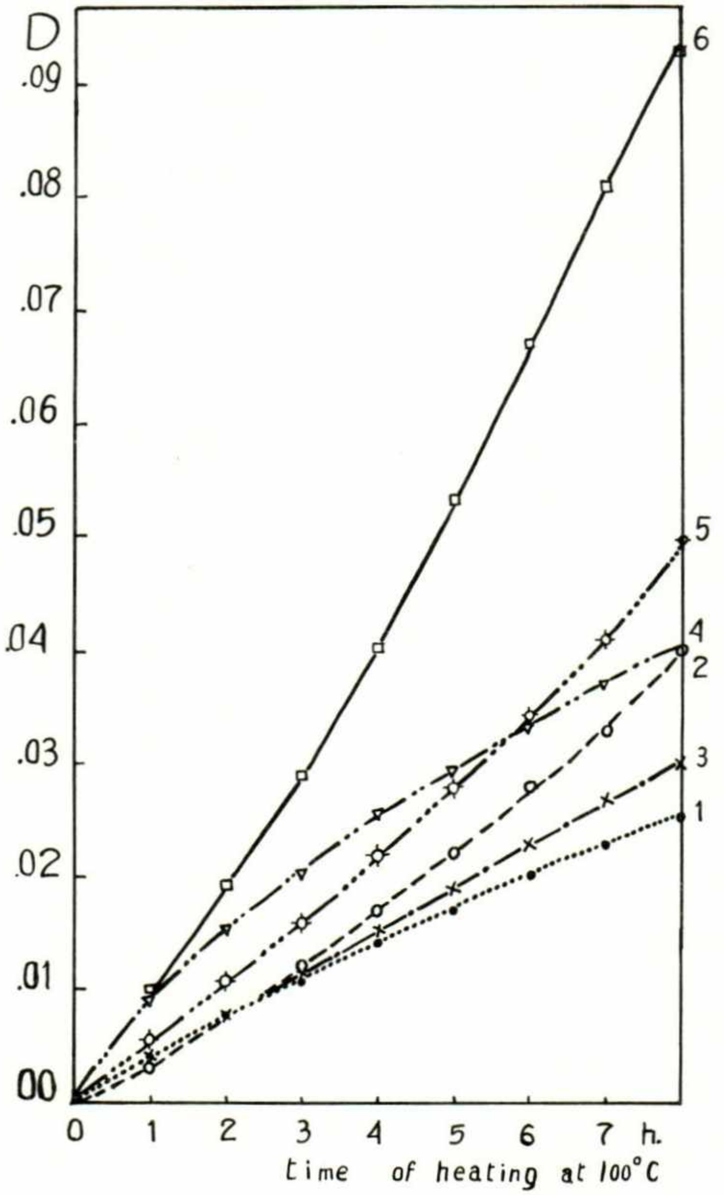

Fig. 4. Increase in the optical density of the models solutions during the heating process:
1. solution No. 1; 2. solution No. 2; 3. solution No. 3; 4. solution No. 4; 5. solution No. 5; 6. solution No. 6.

substances, which differe qualitatively and quantitatively in the investigated solutions. As shown in Figure 4, the optical density of solution No. 2, at the end of the heating process, is about 1.60 times higher than that of solution No. 1. This may confirm the significant role of melanoidines produced from amino-carbonyl reaction in formation of the coloured substances. The significance of melanoidine group in the formation of colour has been reported by many investigators (1, 3, 5, 9).

The effect of Fe^{+++} ions on the accumulation of the colouring matters can be observed when comparing the optical densities of the solutions No. 1 with No. 3 and also No. 2 with No. 5 at the end of the heating time. It is clear that solution No. 3 has an optical density 1.20 times higher than that of solution No. 1, while solution No. 5 has more intensive colour than solution No. 2(1.25 times). The Cu^{++} ions have also the same effect upon the colour of the investigated solutions. Thus, for instance, the optical density of the solution No. 4 is 1.60 times higher than that of the solution No. 1, while the solution No. 6 has an optical density 2.32 times higher than that of the solution No. 2. It is of interest to note that the effect of Cu^{++} ions upon the accumulation of the coloured substances is more considerable than that of the Fe^{+++} ions.

Regarding the effect of metal ions on the reactions of colour formation, there is no general agreement in the literature (2, 12, 17, 18). For example, M. Athenstedt (2) reported that in the absence of oxygen the iron has no effect on the intensity of colour of solutions. At the same time, M. Fleming (9). H. Tsai (18) and Kapa — Murza *et al.* (12) confirmed that in the presence of iron the reactions of colour formation proceed more

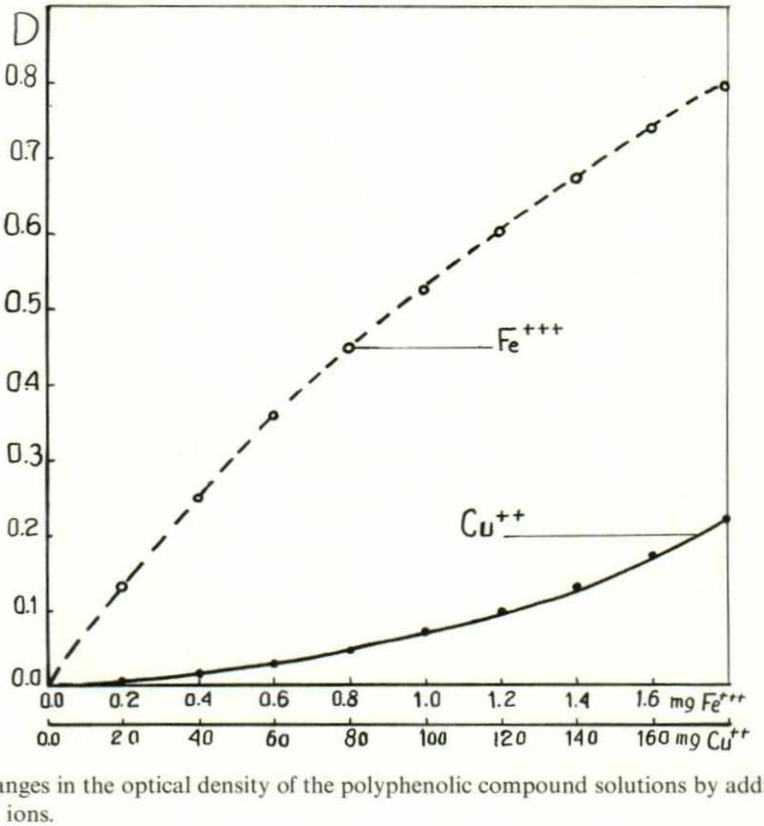

Fig. 5. Changes in the optical density of the polyphenolic compound solutions by adding Cu^{++} and Fe^{+++} ions.

intensively. Sapronov (16) found that in the presence of iron or copper plates the accumulation rate of coloured substances increases. Bugaenko and Mohamed (7) after extensive experiments concluded that iron ions take part in the reactions of colour formation not only as catalysts, but also as constituent elements in the structure of the molecules of the coloured substances.

Zerban and Freeland, cited in (11), emphasized the importance of polyphenols present in sugar cane, since they react, particularly with iron dissolved from mill equipment, and oxygen, to form dark coloured compounds. These polyphenols include: tanin, phenolic hydroxyls and saccharretin.

It is interesting to point out that the extraction of cane juice for treacle manufacture in Egypt is usually performed using iron mills. Moreover the extracted juice is evaporated and concentrated in copper pans. That is why the presence of iron and copper ions is to be expected in the final product.

In the present study, the reaction between polyphenolic compound present in sugar cane (tannic acid) and metals has been examined. Data obtained is illustrated in Figure 5. The resulting intensive colour, in this case, may be attributed to the formation of coloured complexes of polyphenols with Cu^{++} and Fe^{+++}. It is clear, from Figure 5, that the tendency of the ions of iron to form these coloured complexes was higher than that of the copper ions.

It is now well established that the coloured complexes of organic compounds with metals are characterized by changing their colour depending on the pH values of their solutions (13). The concentration of hydrogen ion is considered to be one of the most important factors, which define the equilibrium of the coloured complexes (13).

Figure 6 shows changes in the optical density depending upon pH values for the

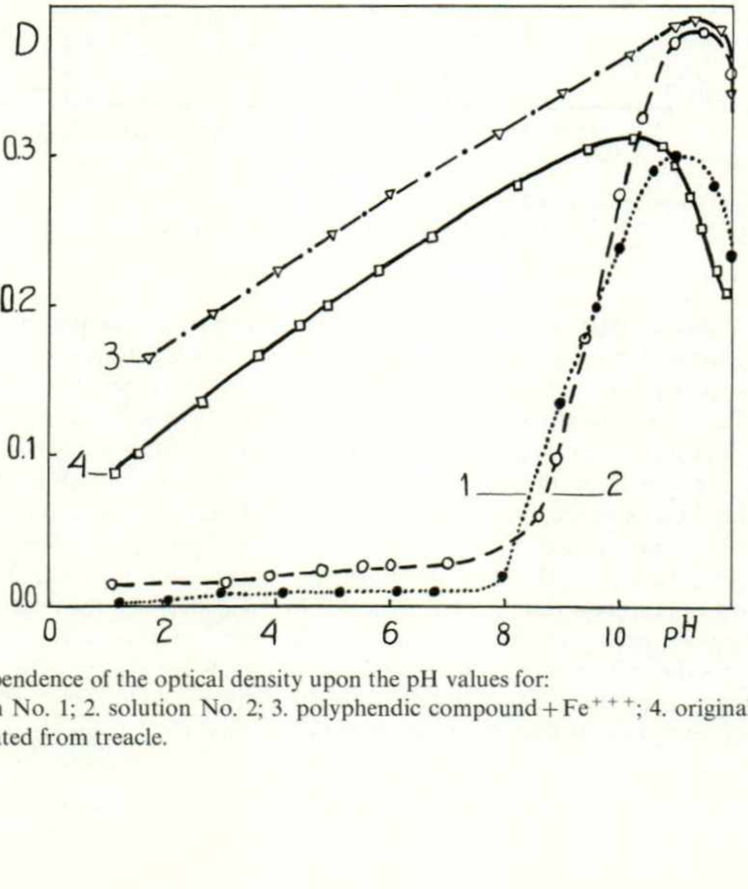

Fig. 6. Dependence of the optical density upon the pH values for:
1. solution No. 1; 2. solution No. 2; 3. polyphendic compound + Fe^{+++}; 4. original colouring matters isolated from treacle.

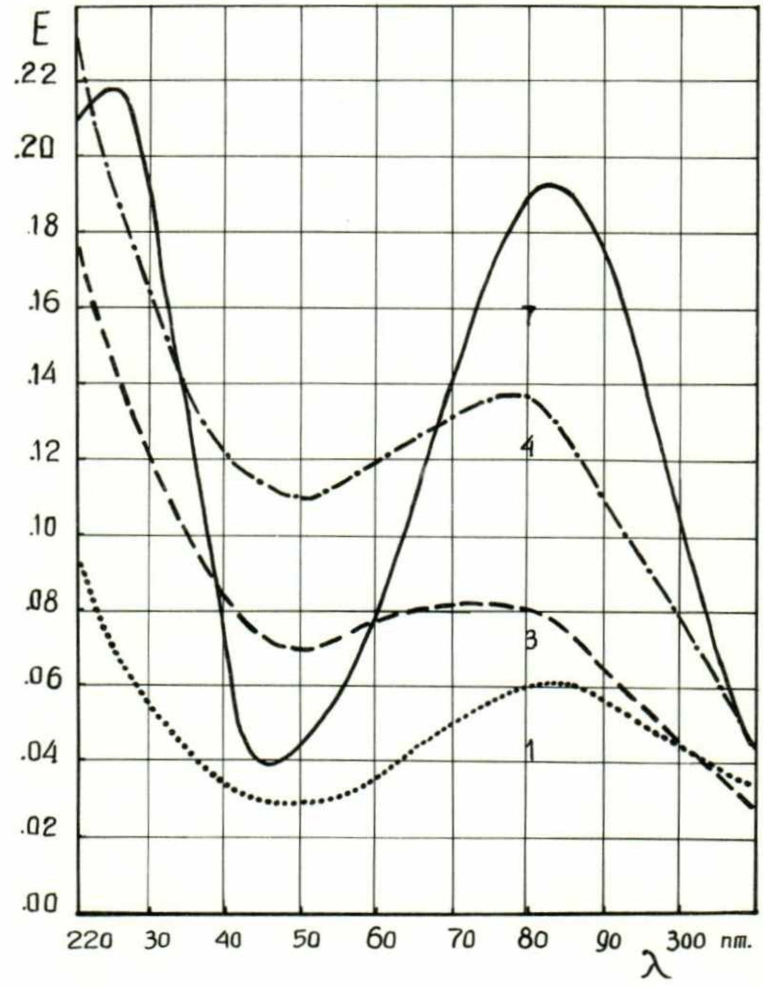

Fig. 7. U.V. absorption spectra for solutions:
1. solution No. 1; 3. solution No. 3; 4. solution No. 4; 7. caramels.

colouring matters under study. It is of interest to compare the configuration and the extreme points of curve 3 in this figure with that of curve 4 illustrated for the colouring matters isolated from treacle. The similarity present between the two curves strongly suggests that the coloured complexes of polyphenols with metals are considered to be one of the constituent groups of the colouring matters isolated from the treacle.

Figures 7 and 8 show the U.V. absorption spectra for the model solutions which were prepared under conditions similar to those which prevail during treacle manufacture. The U.V. absorption spectrum of furfural-derivative product from acid degradation of reducing sugars — is also illustrated in Figure 8 for comparison.

This spectrophotometric study shows slight differences between the configurations of the absorption spectra for the groups of colourants under study. This may be attributed to the unfavourable conditions, under which the reactions of colour formation were carried out i.e. temperature and pH value.

It is interesting to note that the presence of Fe^{+++} and Cu^{++} ions changed not only

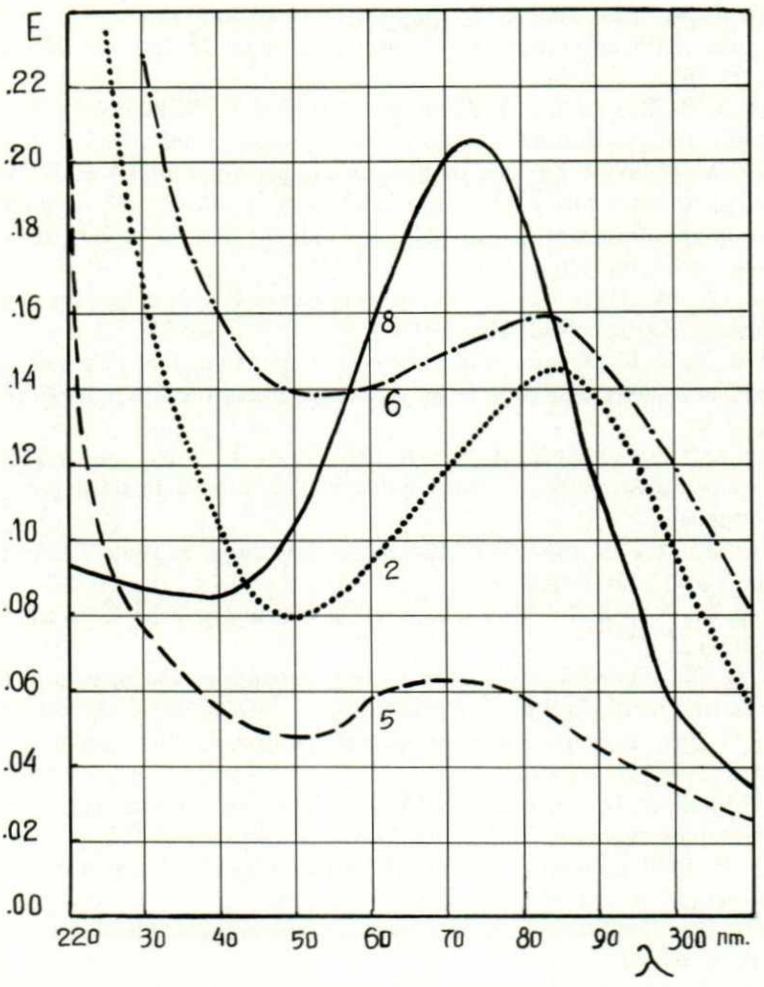

Fig. 8. U.V. absorption spectra for solutions:
2. solution No. 2; 5. solution No. 5; 6. solution No. 6; 8. pure furfural.

the intensity of the colour in the visible region of spectra (Fig. 4), but also the character of the absorption spectra in the U.V. region as shown in Figures 7 and 8 (curves 1, 3, 4 and 2, 5, 6). These results are in agreement with the findings reported by Bugaenko *et al.* (7).

It is important here to point out that the U.V. absorption spectra of these model colourants are very similar — in their character and configurations — to the spectrum of the colouring matters of the treacle (illustrated in Fig. 2). This may give an evidence that the colouring matters of the treacle contain one or more of these groups of model colourants. At any rate, this question needs further study.

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محاليل سكرية نقية من العسل الأسود المنتج في مصر

الدراسة بعض الصفات المميزة للمواد الملونة المفصولة من العسل الأسود المنتج في مصر

د. مصطفى عبد المنعم محمد

المستخلص

أجرى هذا البحث كمحاولة لدراسة إمكانية الحصول على محاليل سكرية نقية من العسل الأسود المنتج في مصر — الأمر الذى سيكون له أهمية تطبيقية في الصناعات الغذائية .

أجرى تحليل عينات من العسل الأسود تحليلا كيميائيا لمعرفة تركيبه . كما تم فصل المواد الملونة من العسل الأسود في صورة مسحوق نقي وذلك تمهيدا لدراسة خواصها الطبيعية والكيميائية فيما بعد . ويتميز مسحوق المواد الملونة المفصولة بلون رمادى مائل للصفرة ورائحة العسل الأسود وهو يذوب جيدًا في الماء على درجة حرارة الغرفة . وقد أجرى تحليل كروما توجرافي للأحماض الأمينية التي تدخل في تركيب هذه المواد الملونة ، كما أجرى تحليل عنصرى لها واقترح رمز تجريبي للوحدة المتكررة لها وهو ك$_{٩-١٠}$ يد$_{١٨-١٩}$ أ$_{٧-٨}$ ن .

ثم تحضير محاليل نموذجية ملونة تحت ظروف قريبة الشبه من الظروف التي تم فيها صناعة العسل الأسود في مصر كما درست بعض الخواص الفيزيوكيمائية لمجاميع المواد الملونة الناتجة .

أثبتت النتائج أهمية الدور الذى تقوم به مجموعة الميلانويدين في التفاعلات الخاصة بتلوين المحاليل . وقد درس هذا البحث أيضا تأثير أيونات كل من الحديد والنحاس على سرعة إتمام التفاعلات اللونية . وقد أيدت النتائج المتحصل عليها وجود معقدات ملونة من الفينولات العديدة المتحدة مع المعادن في تركيب المواد المفصولة من العسل الأسود .

وأخيرا فإن نتائج الدراسة السبكتروفوتومترية للمحاليل الملونة الناتجة تعطى الدليل على احتواء المواد الملونة المفصولة من العسل الأسود على واحدة أو أكثر من هذه المجموعات الملونة المحضّرة صناعيا .