

CHROMATOGRAPHIC STUDIES ON THE KINETICS OF THE PRODUCTION OF CAMEL

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CAMEL is a mixture of nitrogen-free organic compounds and is one of the colouring matters that are produced during the manufacture of cane sugar, chiefly due to overheating of sugars.¹ Caramel not only affects the colour of sugar but also retards crystallisation.² It is manufactured on a large scale for purposes of colouring liquors, sweetmeats and gravies.^{3,4}

There are three main components in cane juice which are responsible for the production of caramel during the manufacture of cane sugar: these are sucrose, glucose and fructose. The process of conversion of sucrose into caramel acquires appreciable velocity only at $\text{pH} \gtrsim 9$,⁵ while in the manufacture of cane sugar, the different processes are operated, after the initial purification of the juice, nearly at neutral pH. Cane juice contains 0.5 to 2 per cent. of a mixture of glucose and fructose; these have been found to be the most important sources for the production of the colouring matter during sugar manufacture.⁶ The production of caramel from glucose has been studied by Geils,⁷ while the same from fructose has been examined by Ripp⁸ and others.

The exact chemical composition of caramel is not yet available. Schumaker and Buchanan⁹ reported that when solid sucrose was heated at about 200° C., three periods of foaming occurred, indicating the splitting of water at each stage and resulting in the formation of anhydrides with the empirical formulæ, $\text{C}_{12}\text{H}_{20}\text{O}_{10}$, $\text{C}_{24}\text{H}_{36}\text{O}_{18}$ and $\text{C}_{36}\text{H}_{50}\text{O}_{25}$. Recently, Luckesch¹⁰ chromatographically analysed caramel prepared from sucrose and reported that it contained glucose, fructose, glyceryl aldehyde, dihydroxy acetone, pyruvic acid, hydroxy methyl furfural and methyl glyoxal. Authors' chromatographic studies¹¹ of the caramels prepared by heating glucose, fructose, maltose, sucrose and liquid glucose, in alkaline solutions, indicated the presence of four reducing compounds constituting caramel, with the following R_f ($\times 100$) values: 0, 1.4, 4.4 and 6.5 (standard reference value = levulose — 13.6). The observation of a spot on the reference line on the chromatogram (R_f value = 0), which could not be separated from the base line even by running the chromatogram for more than 5 days, indicated

the presence of one or more reducing compounds of very large molecular weight.¹¹ Authors' chromatographic analysis indicated the presence of no acids in the caramels.

Although attempts as mentioned above have been made to elucidate the composition of caramel, very few investigations seem to have been undertaken to study the kinetics or the mechanism of the production of caramel. The present communication reports data on chromatographic studies on the kinetics of the production of caramel from glucose.

§ 2. EARLIER WORK

The first attempt to investigate the kinetics of the development of colour in sugar solutions seems to have been made by Doss and Ghosh.¹² These authors employed a spectro-photometer (Spekker) and studied the time development of colour in the invert sugar in alkaline solutions; they observed that "double the concentration of invert sugar in the reaction mixture nearly doubled the rate of colour development"; and inferred, therefore, that the colour development in alkaline solutions at ordinary temperatures is essentially of the first order. The present authors¹³ also studied spectrophotometrically (using Unicam S.P. 350 D.G. spectrophotometer) the development of colour in alkaline glucose solutions, at different temperatures in the range 35°–70° C.; and found that the following equation was applicable to the data on the time development of the colour in glucose at temperatures greater than 50° C.:

$$a - r = ae^{-kt}$$

where a is the total initial concentration of the substance undergoing change or the value of the optical density proportional to the concentration thereof; r , the amount decomposed upto time t ; and k , the characteristic velocity constant of a first order reaction. This observation suggested that the production of colour or caramel from glucose, when heated in alkaline solutions at temperatures above 50° C., is first order in nature. Employing the familiar Arrhenius equation, the apparent energy of activation of the process involved has been computed from the data obtained at different temperatures; an average value of this appeared to be 11.9 kcal.

An interesting observation recorded by the present authors¹³ and also by Doss and Ghosh,¹² was that the initial portion of the curves representing the variation of the optical density with time was concave in shape towards the X-axis (*see* Figs. 1 and 2); this feature is clearly seen from the data obtained at low temperatures, say 40° C. (*see* Curve 1, Fig. 2). At higher temperatures the concavity is noticeable only at very low time intervals.

§ 3. PRESENT INVESTIGATION

The data in Figs. 1 and 2, especially the observed concavity towards X-axis of the optical density — time curves, suggested the following two important deductions¹³:

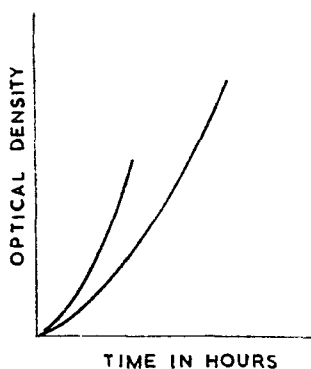


FIG. 1.

FIG. 1. Variation of the optical density with time during caramelisation of invert sugar (cf. Ref. 12).

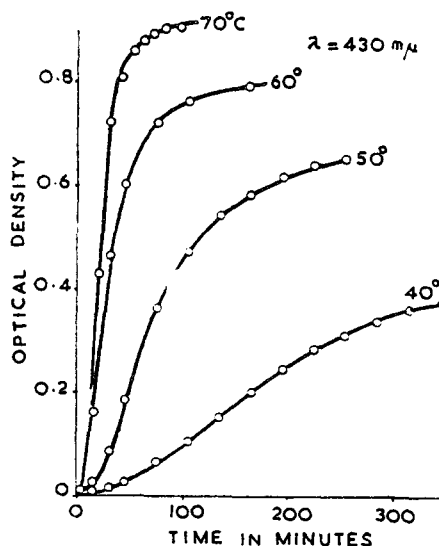


FIG. 2.

FIG. 2. Variation of the optical density with time during caramelisation of glucose at different temperatures (cf. Ref. 13).

(i) the substance or one of the substances produced initially aids or catalyses the decomposition of glucose into different substances composing the caramel; or/and

(ii) the substance or substances produced initially undergo further reaction presumably obeying a first order law, to give the final products; that is, the conversion of glucose into caramel is apparently through the formation of intermediate compound(s).

These deductions have now been examined, in some detail, employing the filter paper chromatographic technique. The procedure involved the chromatographic investigation of the reaction mixture at different intervals of time during the progress of the reaction.

§ 4. EXPERIMENTAL

Analar samples of glucose and sodium hydroxide were used. Known quantities of solutions of these substances, of desired strength, were mixed

together such that the final solution was of a pH value in the range 10–13. About 100 c.c. of the mixed solution was taken into a glass vessel which was immersed in a paraffin oil thermostat. The temperature of this last could be raised to and maintained constant ($\pm 0.1^\circ$) at any desired value.

During the development of colour in the alkaline glucose solutions at a fixed temperature, a small quantity of the reaction mixture was withdrawn at regular intervals of time and was used for chromatographic analysis. It is to be pointed out that the portion of the reaction mixture withdrawn for analysis was quickly cooled to the room temperature and thus the progress of the reaction in the sample got practically quenched.

The method employed for chromatographic analysis was essentially the same as used by Patridge.¹⁴ Strips, of suitable width, of Whatman filter paper No. 1, were used. The reaction mixture under examination was spotted on a (reference) line drawn parallel to one edge of the filter paper strip. The solvent used was the top layer of a mixture of butanol, ethanol and water (50:10:40 by volume). The bottom layer was used for saturating the chamber in which the filter paper strips were suspended. After the chromatograms were run for 70–100 hrs., they were dried and washed with a solution of silver nitrate in acetone. They were later dried again and sprayed with alcoholic KOH solution, when brown or dark brown spots referring to the reducing compounds present in the reaction mixture, became apparent. The chromatograms were later treated with ammonia, washed with continuous flow of water for 2 hrs. and finally dried.

§ 5. RESULTS

Figures 3 and 4 refer to two typical chromatograms. These refer to reaction mixtures drawn at regular intervals from the reaction vessel in which

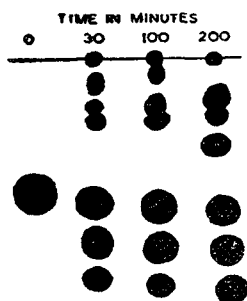


FIG. 3.

FIG. 3. Chromatographic studies of the composition of the reaction mixture at different time intervals during caramelisation of glucose at 40°C .

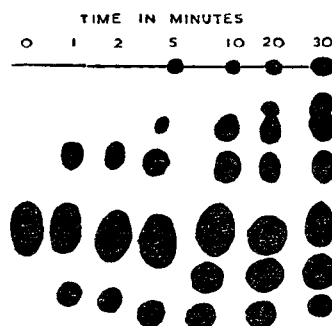


FIG. 4.

FIG. 4. Chromatographic studies of the composition of the reaction mixture at different time intervals during caramelisation of glucose at 70°C .

glucose has been heated with alkali solution at 40° and 70° C. respectively. The running time for the chromatograms of Fig. 4 was 72 hrs., while the same for the others given in Fig. 3 was 90 hrs. The R_f values for the spots observed in Figs. 3 and 4 are returned in Table I; these were computed by comparison of the spots developed for known sugars in a previous run on a separate strip of filter paper.

TABLE I
R_f values of the spots observed in the chromatographic studies on the kinetics of caramel formation

Spot No.	Time in Minutes						
	0	30	100	200			
Temperature of Reaction Vessel = 40° C.							
1		0	0	0			
2		2.1	2.1	2.8			
3		4.5	4.5	4.3			
4				6.5			
5	9.5	9.5	9.5	9.5			
6		12.8	12.9	13.0			
7		14.9	15.0	15.0			
Temperature of Reaction Vessel = 70° C.							
	0	1	2	5	10	20	30
1				0	0	0	0
2						2.8	2.8
3				4.3	4.4	4.4	4.4
4		7.8	7.8	7.8	7.8	7.8	7.8
5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
6		12.8	12.8		12.2	12.2	12.2
7				14.2	14.2	14.2	14.0

The chromatograms taken with the reaction mixture obtained in the beginning of the reaction, *i.e.*, at zero interval of time, indicated the presence of only one spot corresponding to glucose [with an $R_f (\times 100)$ value of 9.5]. The chromatograms referring to the reaction mixture drawn at increasing intervals of time, showed spots other than that of glucose. It was interesting to note that the reaction mixture obtained at 2 mts. intervals of time contained, as indicated by chromatographic analysis (Fig. 4), three compounds, excluding glucose, the $R_f (\times 100)$ values of these are approximately as follows: 3.0, 4.1 and 12. The production of a compound with an R_f value of 12 referring to fructose (R_f value 13 was particularly instructive in light of the finding due to Lobry de Bruyn and van Ekenstein¹⁵ who showed that during heating of sugars in alkaline solutions for caramelisation, equilibrium mixture of glucose and fructose (as also mannose) are produced; in accord with this is the observation of the authors¹¹ that whatever may be the sugar employed initially for caramelisation, the same reducing compounds have been detected in chromatographic analysis of the caramels. Further, the data in Figs. 3 and 4 show that the spaces where the original mixture was placed on the reference line for chromatographic analysis, acquired colour only after 25 mts. in the reaction mixture maintained at 40° C. (Fig. 3) and 2 mts. interval of time in the reaction mixture maintained at 70° C. (Fig. 4). This spot (R_f value = 0) on the reference line, which could not be separated from this last even by running the chromatogram for 5 or 6 days indicated, as mentioned above (§ 1), the presence of reducing substance(s) of high molecular weight. This observation suggests that the substances composing caramel are produced after the initial generation of certain substances with low molecular weight.

§ 6. DISCUSSION

The following is the resume of the important findings on the kinetics of the production of caramel:

- (i) During heating of glucose and other sugars in alkaline solutions, compounds of low molecular weight are produced initially,
- (ii) Lobry de Bruyn and van Ekenstein transformation takes place;
- (iii) the production of caramel involves autocatalysis; and
- (iv) the reaction involves a step which controls the time development of colour and which obeys a first order law.

These observations lead the authors to suggest the following mechanisms:

(A) Heating of glucose or other sugars in alkaline solutions, leads to the initial production of an equilibrium mixture of glucose, fructose and

mannose; these apparently condense together to form an intermediate compound of very high molecular weight, which decomposes unimolecularly to yield the compounds [with R_f ($\times 100$) values 0, 1.3, 4.4 and 6.6, in reference to levulose — 13.6] constituting caramel, one of which acts as a catalyst to the establishment of Lobry de Bruyn and van Ekenstein transformation.

(B) Alternatively, the rate determining step in the process is likely to be the establishment of Lobry de Bruyn and van Ekenstein transformation and the autocatalytic nature is related to the decomposition of the condensation product contemplated in (A).

The observation of the autocatalytic nature of the reaction upto 30 mts. at 40° C. and upto 2 mts. at 70° C. suggests that the mechanism (A) is to be preferred. Conclusive evidence is, however, lacking.

§ 7. SUMMARY

The kinetics of production of caramel which is a mixture of nitrogen-free organic compounds and which offers colour to sugar crystals and retards the rate of crystallisation, have been investigated by employing filter paper chromatographic technique. Chromatograms of the reaction mixture, viz., glucose heated in alkaline solutions, at different intervals of time during the progress of the reaction showed the initial production of compounds of low molecular weight, which condensed to form an intermediate compound of very high molecular weight; this last appeared to decompose unimolecularly to give the products composing caramel.

§ 8. ACKNOWLEDGEMENT

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REFERENCES

1. Zerban .. *Techn. Repts., Sugar Res. Foundation*, No. 2, 1947, 1.
2. Dass and Ghosh .. *Proc. Sugar Tech. Assocn.*, 1951, 19, 183.
3. .. *Manufacture of Confectionery*, Industry Publishers, Fifth Edition.
4. Jordan .. *Confectionery Standards*, Applied Sugar Laboratories, N.Y., 1933.

