SOME OBSERVATIONS ON CARAMELISATION

By

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Wide-ranging, many sided research work for the elucidation of the mechanism of non-enzymatic browning is centered on the study of the amino-carbonyl reaction. Much less attention is paid to changes associated with caramelisation.

In our investigations, caramel formation was chosen as model system, since the literature contains almost no, or very few, information on the fine structure of caramel.

The investigation of caramel formation has a few advantages, facilitating experimental work:

a) only one starting component must be reckoned with in the model system;

b) the processes of caramel formation can be accelerated or slowed down by the appropriate selection of the circumstances, while near identical products are formed, only in different quantities. This reduces the time of observation to a period, which can be practically followed.

Decomposition products isolated from caramel are very similar to or identical with products isolated during processes occurring in other complicated systems (e.g. methylglyoxal; 3-desoxyosons), which is possibly indicative of an identical or similar reaction mechanism. HOLTERMAND [9] too arrived on the basis of his investigations to the conclusion that during the amino-carbonyl reaction the importance of sugar decomposition is greater in alkaline medium.

It can be assumed on the basis of all this that results obtained in the investigation of caramel permit a deeper insight into the processes of non-enzymatic browning, and with due caution and under consideration of the conditions of the reactions, will be applicable also to more complicated systems.

For the preparation of caramel, glucose and fructose were selected as model compounds. It was shown earlier (ÖRSI, 1967) that the decomposition of sugar is catalysed by ions dissolved from glass, therefore, always samples of identical 1 ml volume, placed into glass ampoules of identical form were heat-treated.

The thermal treatment of the samples was undertaken by two different methods:

a) isothermally, the circumstances of which were selected so that heating up and cooling sections, could be neglected.

b) at temperatures increasing linearly with time, which has the advantage that processes taking place in the heating up and cooling sections can be taken exactly into account. Heating at a linear temperature program was performed in a derivatograph.

The derivatograph is a derivative thermobalance of automatic operation, which, in addition to the reproducible heat treatment of the sample can record the temperature (T-curve) and the change in weight of the sample (TG-curve) the direction and magnitude of the changes in enthalpy (DTA-curve), and the rate of the change in the weight of the sample (DTG-curve). The DTG-curve makes possible the exact reading off of the beginning and termination of the decomposition processes even in the case of overlapping reactions, since the curve has at this point a minimum or an inflection point.

In the course of our investigations, the glucose and fructose samples, mixed with aluminium oxide, were heated, and then in different stages of decomposition after cooling and dissolution in water the quantity of the residue, of the colouring matter, and of other components has been determined by chemical analysis. In addition, an identical sample was heat-treated in a closed system at a temperature program identical with that of the derivatograph, and the volatile decomposition products were also investigated by gas chromatography. As a result of these investigations, the derivatograms of glucose and fructose, shown in Fig. 1. and 2. are interpreted as follows:

Fig. 1 shows the derivatogram of a mixture of 500 mg glucose and 1 g aluminium oxide, recorded at a heating rate of 6°C/min. The minimum exhibited at 165°C on the DTA curve, which shows the changes in enthalpy, indicates the endothermic effect of the melting of sugar. The TG curve, representing the change in weight, shows that the weight of the sugar did not change up to its melting. With the melting of glucose, its decomposition begins, and three stages of reactions can be distinguished.

The *first stage of decomposition* begins on melting and is terminated at 240°C. This stage is characterised by an endothermic heat effect equal approximately to the heat of melting, and in this stage the course of the reactions is not affected by air. The incurred loss in weight of 25 per cent results from two principal processes. One part of glucose polymerizes to form oligo- and polysaccharides. This is accompanied by the formation of water, which evaporates at this temperature.

The rest of glucose is decomposed under formation of volatile oxo compounds and other not yet fully cleared compounds, and is converted to brown colouring matter. At 240°C no glucose or oligosaccharides can be detected,

probably, these were decomposed too, and converted to brown colouring matter.

In the further stage of decomposition, the brown colouring matter and polysaccharide accompanying it are decomposed. The brown colouring matter becomes insoluble, and the polysaccharides are also converted to insoluble

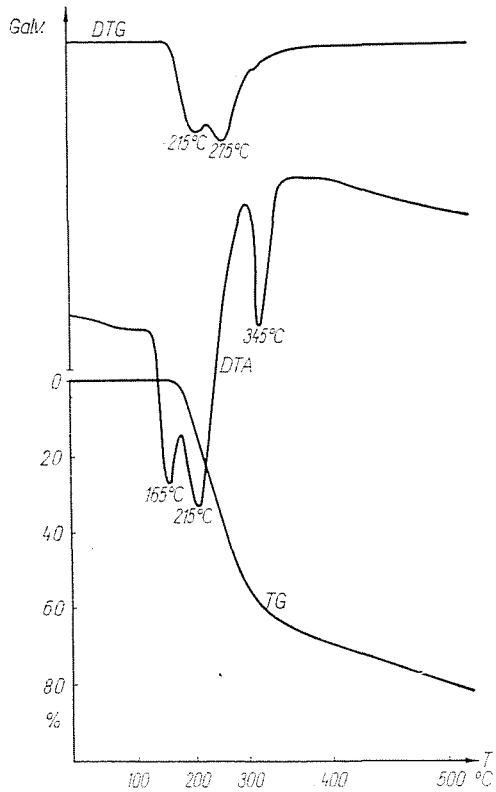

Fig. 1. Derivatogram of a mixture of 500 mg glucose and 1 g Al_2O_3; heating rate: 6°C/min

colouring matter. The decomposition products formed, consisting of very differing compounds, become inflamed in the presence of air, and finally the whole residue burns.

Fig. 2 shows the derivatogram of 500 mg of fructose, recorded under identical conditions with glucose. In the case of fructose, similar stages can be observed, too but fructose melts already at 120°C, and the stage of decomposition, which terminates in the case of glucose at 240°C, proceeds here accord-

ing to the DTG curve of fructose in two steps. The *first step* up to 180°C proceeds at a lower rate with a loss in weight of about 10 per cent, and mainly oligosaccharides of diheterolevulosane type are formed. These are decomposed in the *second step* of decomposition from 180 to 240°C, together with the residual

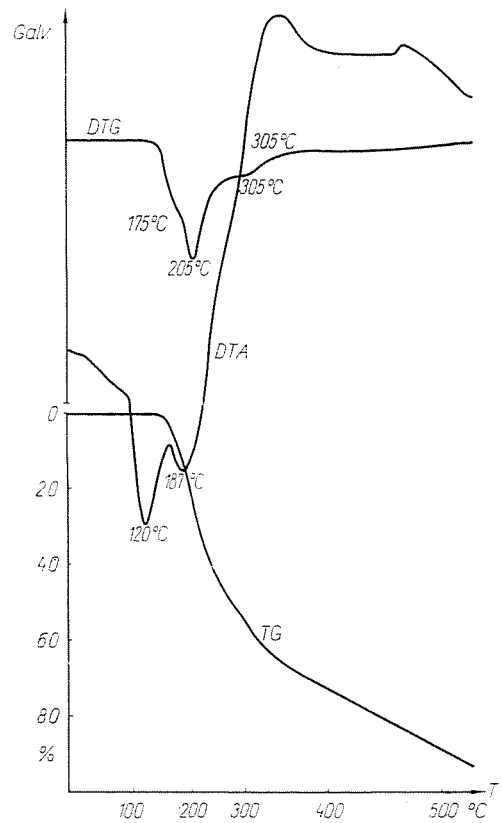

Fig. 2. Derivatogram of a mixture of 500 mg fructose and 1 g Al_2O_3; heating rate: 6°C/min

fructose, into brown colouring matter, while in the volatile part formation of several oxo and other compounds can be observed.

Since in the case of fructose no polysaccharides are formed in the next section of decomposition from 240 to 400°C, less decomposition products can be found, but the total loss in weight attains 68 per cent similarly to that of glucose.

The final section of decomposition is completely identical with that of glucose.

The experiments verified in the case of both sugars that the use of temperatures above 200°C is to be avoided in the investigation of the decomposition process of sugars because in this temperature range already the pyrolysis of colouring matter takes place, the decomposition products of which complicate and confuse the composition of the reaction mixture.

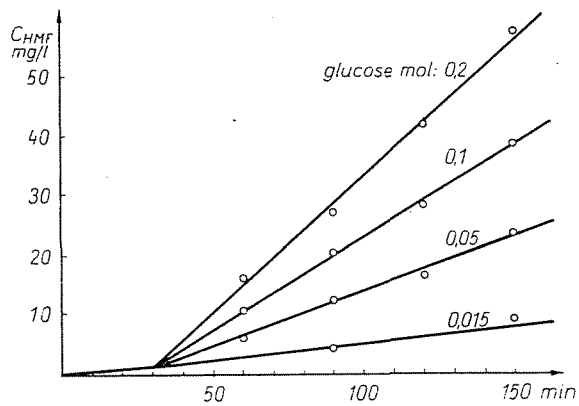

Fig. 3. Change of 5-hydroxymethyl furfurol concentrations in heat-treated glucose-solutions

Out of the processes of the reaction referred to three steps have been investigated more thoroughly:

1. the formation of 5-hydroxymethylfurfurol, since even today several authors consider the furane compounds as the precoursors of brown colouring matter.

2. The formation of a low molecular (< 5000) fraction of colouring matter, separated by gel filtration, and

3. the formation of a high molecular (> 5000) fraction of colouring matter, separated also by gel filtration, because, as verified by our earlier investigations, these are the steps of the formation process of insoluble colouring matter.

The quantity of 5-hydroxymethylfurfurol has been determined by Winkler's method modified earlier.

During the heat-treatment of glucose, the increasing concentration of 5-hydroxymethylfurfurol as a function of time is represented by a straight line, which does not start from the origo. This means that for the formation of an induction period is needed 5-hydroxymethylfurfurol *(Fig. 3)*.

From fructose already within the induction period 5-hydroxymethylfurfurol was formed in detectable quantities, so that here the induction period

appears to be characterized by a smaller slope, when the concentration of 5-hydroxymethylfurfurol is plotted as a function of the time of thermal treatment. *(Fig. 4).*

The fact that after the induction period the concentration of 5-hydroxymethylfurfurol changes approximately linearly with time, makes the assump-

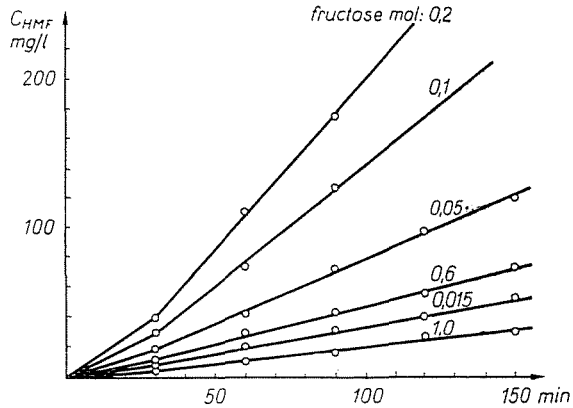

Fig. 4. Change of 5-hydroxymethylfurfurol concentratio ns is heat-treated fructose, solutions

tion justified that the rate of the reaction is determined by the rate of formation of the intermediate products, and the concentration of the intermediates does not change essentially during the investigations. Thus, leaving out of consideration the induction period, the rate of the reaction can be calculated from the slope of the linear section.

The logarithm of the rate of 5-hydroxymethylfurfurol is plotted in *Figs 5* and *6* as a function of the logarithm of the starting sugar concentration. It can be seen from the figures that up to a sugar concentration of about 5.3 mole/litre (corresponding to a mole fraction of 0.2) the rate of reaction increases linearly with the sugar concentration, and begins to decrease at a concentration higher than this. The reason for this is that in concentrated solutions reversion products are formed from the sugars, though the lower mobility of the molecules may also play a role. The slope gives the order of the reaction, and this was found to be 1.

The temperature dependence of the reaction rate constant of 5-hydroxymethylfurfurol formation has been investigated under the assumption of the validity of the Arrhenius equation. The logarithm of the measured rate constants has been plotted against the reciprocal of absolute temperature. Points plotted for a wide temperature interval lie in both diagrams along a straight line.

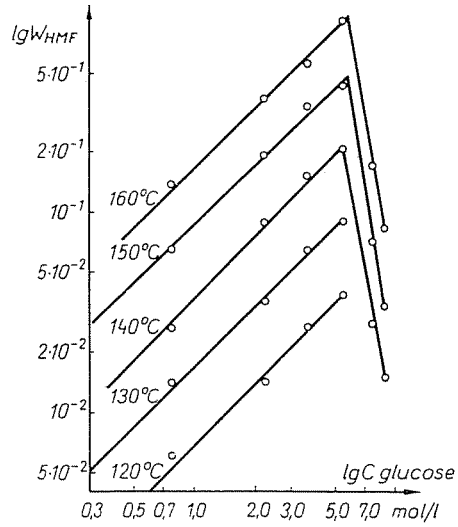

Fig. 5. Logarithmic correlation of 5-hydroxymethylfurfurol formation with the starting glucose concentration on various temperatures

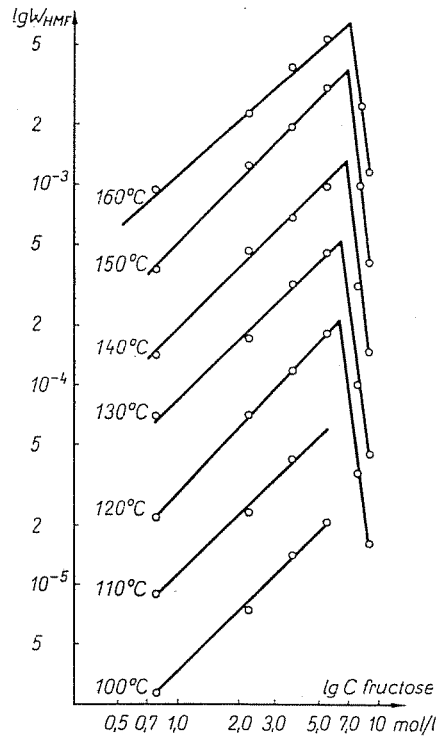

Fig. 6. Logarithmic correlation of 5-hydroxymethylfurfurol formation with the starting fructose concentration on various temperatures

In summary *(Fig. 7)* it can be said that the activation energy of 5 HMF formation in glucose solution of 0.015 to 0.2 mole fraction is 28 kcal, which agrees with the value of the activation energy determined for the enolization of glucose. Thus, it can be assumed that the rate of the reaction is determined in this range by the rate of endiol formation.

In more concentrated glucose solutions, the activation energy of 5-hydroxymethylfurfurol formation is larger, and agrees rather with the results

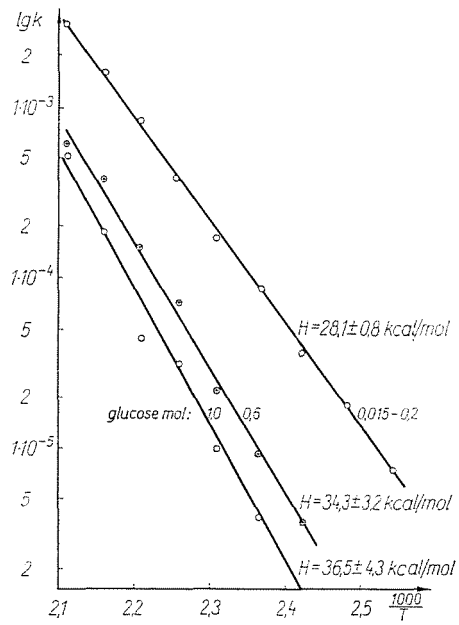

Fig. 7. Correlation of the measured rate constants of 5-hydroxymethylfurfurol formation with the reciprocals of abs. temperatures in glucose solutions

of investigations in acid medium. Presumably, this can be ascribed to the fact that the rate of reaction is determined under such conditions by the decomposition of the reversion products, which requires a higher activation energy.

Values of activation energy obtained in the investigation of fructose *(Fig. 8)* differ only slightly from those of glucose, however, the value of the *action constant* is by about two orders of magnitude larger, than in the case of glucose. Values obtained are in good agreement with the notion that decomposition proceeds in the case of both sugars in the same way. This is indicated by the identical activation energy, however, the higher value of the action constant shows that the number of active molecules in the case of fructose is by about two orders of magnitude larger.

The end-product of the heat treatment of mono- and oligosaccharides is a brown, water-soluble product of undefined composition *called caramel.*

For the separation of caramel, the molecular sieves "Sephadex" have been used with success. These sieves do not adsorb the colouring matter, but nevertheless, do separate it according to the molecular size into two fractions: into low < 5000 and high > 5000 molecular fractions.

The quantity of colouring matter fractions, eluated from the Sephadex column with distilled water, has been characterised by their light absorption

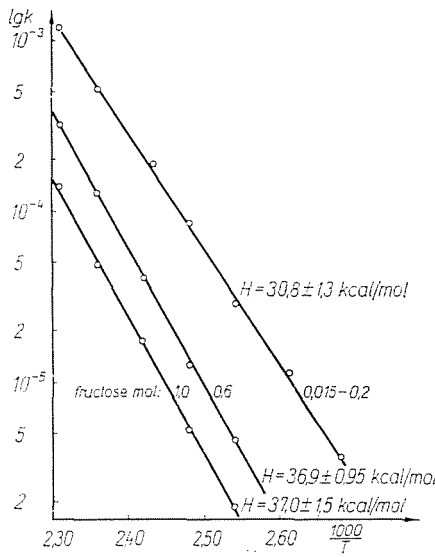

Fig. 8. Correlation of the measures rate constants of 5-hydroxymethylfurfural-formation with the reciprocals of abs. temperatures in fructose solutions

measured at 400 nm. The values of specific extinction have been determined for each of the colouring matter fractions after separation.

The characteristic picture of the course of colouring matter formation in time will be shown using glucose and fructose solutions of 0.1 molar fraction in the following Figs 9 and 10.

Fig. 9 shows the change in quantity of the *low* molar fraction during the heat treatment of glucose. The colouring matter is formed without an induction period in a quantity proportional to time.

It also shows the change in quantity of the *high* molecular fraction. In course of the heat treatment of glucose, formation of that fraction begins only after a distinct induction period, which is shorter at high temperatures. After the induction period, the quantity of the high molecular fraction increases linearly with time.

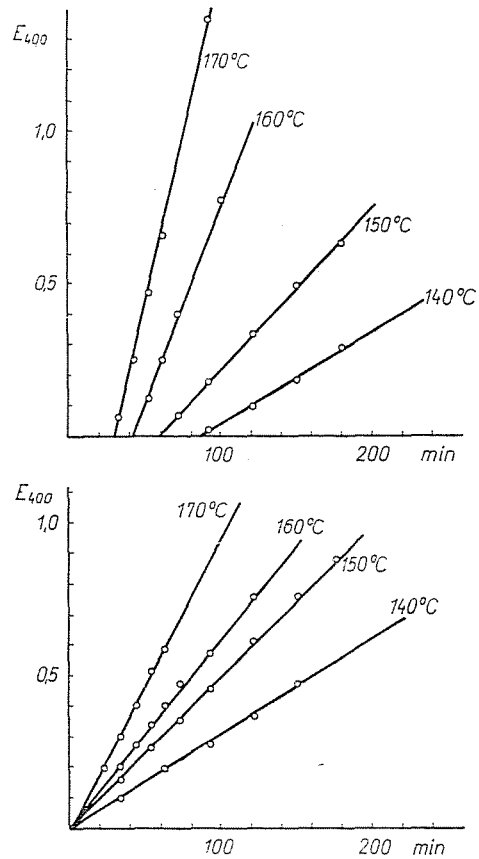

Fig. 9. Change of low molecular (under) and high molecular (above) fractions during the heat treatment of glucose-solutions

Fig. 10 shows the change in quantity of the low molecular fructose fraction with time. Here decomposition can be observed already at lower temperatures, and an induction period is exhibited in the formation of the low molecular colouring matter. The change in quantity of the high molecular fraction is similar to that of glucose, however, it can be seen that when the fructose has been used up on appearance of the insoluble caramel colouring matter fraction the curve becomes less steep.

On the basis of all these observations it can be assumed that the formation of caramel colouring matter proceeds in three main steps:

1. *formation of low molecular fraction,*

2. *formation of high molecular fraction from the low molecular one and finally*

3. *formation of insoluble colouring matter.*

The *first step* (1) begins after a scarcely noticeable induction period at the beginning of the heat treatment, and lasts until the sugar is used up.

The *second step* (2) begins after a certain accumulation of the low molecular fraction at a definite concentration, and presumably by radical reactions. This assumption made by us has been verified already by the investigations

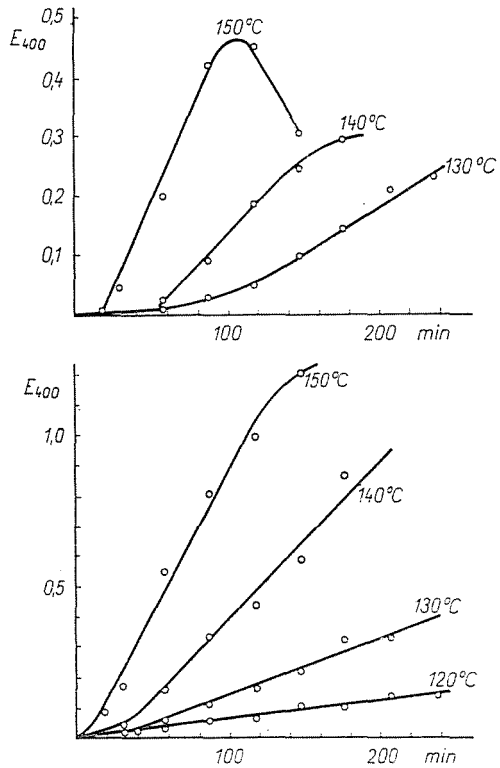

Fig. 10. Change of low molecular (under) and high molecular (above) fractions during the heat treatment of fructose-solutions

of several other authors. This reaction proceeds then at an almost constant rate until the sugar is consumed, and following this, the third step (3) begins, in which the caramel colouring matter becomes insoluble, The investigation of the radical reaction is in course.

Thus, caramel colouring matter is formed in a multistep reaction. For the clearing of the kinetic conditions, the method discussed already in connection with 5 HMF formation has been used, and the reaction rate has been calculated from the slope of the concentration-time curve, following the induction period. When the logarithms of the slope have been plotted as a

function of the logarithms of glucose and fructose concentrations, diagrams similar to that of 5 HMF formation have been obtained.

Results pertinent to the high molecular caramel colouring matter fraction have been plotted for both sugars in a common diagram, because the values measured at different temperature complement well each other. A deviation was found only at higher concentrations *(Fig. 11)*. Presumably, the decrease at higher concentrations is due to the fact that reversion products are

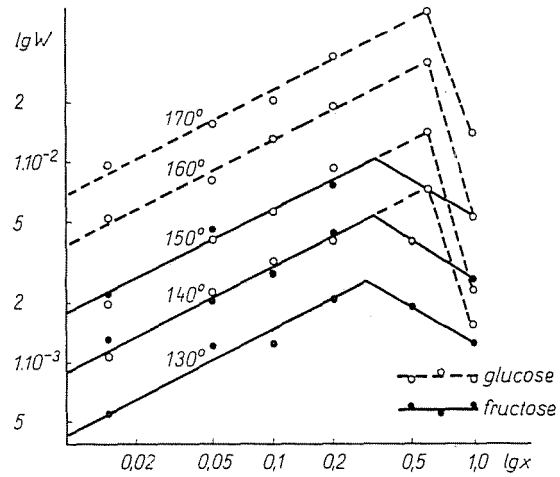

Fig. 11. Logarithmic correlation of the formation rate of high molecular fractions with the starting monosaccharide-concentrations on various temperatures

formed in equilibrium reaction from the sugars in more concentrated solutions, which decrease the actual concentration of the sugars, and thus, the reaction rate.

The slope of the points along the linear section, belonging to the same temperature, is for both the low and the high molecular colouring matter 0.5, and indicates a reaction of 0.5 order.

From the temperature dependence of the rate constant, the activation energy of the reaction has been calculated. The activation energy of the formation of low molecular colouring matter from glucose is about 14 kcal/mole, while for fructose a value of 31 kcal/mole has been obtained. This indicates that in the case of glucose the rate determining process is not the same as in the case of fructose, and is not the endiol formation. Owing to the higher concentration of the open-chain form, a larger quantity of colourless intermediate product is formed from fructose.

A comparison of the rate of formation of colouring matter and of that of HMF yielded the remarkable result that 5-HMF is formed according to a re-

action of different order, and the rate of formation is by three orders of magnitude lower than the rate of formation of the high and low molecular colouring matter formation, so that HMF cannot be an intermediate product of the formation of coloring matter. The reaction order of 0.5 indicates that the formation of colouring matter is preceded by the decomposition of the sugar molecule into two components, of equal efficiency from the aspect of colouring matter formation. This finding also supports kinetically the role of 3 carbon atom decomposition products, as e.g. that of methylglyoxal in the formation of brown colouring matter. This is in agreement with the fact that trioses, from which only methylglyoxal but no HMF can be directly formed, can turn brown at a high rate.

In alkaline medium, the formation of brown colouring matter competes with lactic acid formation, and as is well known, lactic acid is formed from methylglyoxal.

On the basis of these results our present notions on caramelisation must be thoroughly revised.

Summary

The reaction mechanism of caramelisation was investigated on model systems containing monosaccharides. By means of derivatographic measurements it was stated that the browning reaction takes place only at temperatures under 240°C, because brown colouring substances are rapidly decomposed over this temperature. Concerning the reaction mechanism the reaction rate of the formation of 5-(hydroxymethyl)-2-furfuraldehyde is smaller than that of the colouring substances, and the latter is identical with the degradation rate of glucose. Accordingly the 5-(hydroxymethyl)-2-furfuraldehyde seems to be only a by-product of the caramelisation.

Literature

1. SONG, P. *et al.*: J. Food Sci. **31,** 906, 914, 1966., **32,** 98, 107, 1967.
2. HEYNS, K.—NOACH, H.: Chem. Ber. **97,** 415, 1964.
3. BURTON, H. S. *et al.*: Chem. and Industry **21,** 219, 1682, 1962. **22,** 693, 1963.
4. VELISEK, J. *et al.*: Z. Lebensm. Unt. Forsch. **149,** 69, 1972.
5. Symposium on non-enzymic browing of food. Liblice. 1972. X. 16—18.
6. TELEGDY KOVÁTS L. *et. al.*: Nahrung **12,** 219, 1968.
7. HIROMICHI, K.: Bull. Agric. Chem. Soc. (Japan) **24,** 1, 1960.
8. ÖRSI, F.: Thesis. Budapest, 1969.
9. HOLTERMAND, A.: Stärke **18,** 319, 1966.
10. ÖRSI, F.: Magyar Kémiai Folyóirat **73,** 1, 1967.
11. ÖRSI, F.: Acta Alimentarica. (in press)
12. ÖRSI, F.: Nahrung **15,** 43, 1971.
13. TELEGDY KOVÁTS, L. *et. al.*: Nahrung **10,** 439, 1966.
14. LAGERCRANTZ, C.: Acta Chem. Scand. **18,** 1321, 1964.
15. MITSUDY, H. *et. al.*: Agr. Biol. Chem. **29,** 751, 1965.

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