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Methylglyoxal determination from different carbohydrates during heat processing

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Heat induced formation of methylglyoxal (MG) was examined in the caramelisation and Maillard reaction. Mono-, oligo- and polysaccharides (i.e. D-glucose, dextrin 15, starch) were caramelised using three different conditions. MG concentration was analysed with HPLC as a methylquinoxaline derivate. The concentration of MG formed from dextrin 15 was higher than that of starch because of the presence of more reducing end groups.

The effect of Maillard reaction on the formation of MG was examined. The different carbohydrates were heated in the presence of o-phenylenediamine (o-PD) and glycine. In the formation of MG there was a catalytic effect of the amino group, so the MG concentration was higher than in caramelisation.

When carbohydrates were heated with glycine, the MG concentration was not as high because of the base strength of the amino acid.

Both in caramelisation and Maillard reaction the concentration of MG was higher in the presence of water.

INTRODUCTION

Heat processing (roasting, frying, pasteurising, sterilising, drying, evaporation etc.) has significant functions in food technology in preparation of both end-products and intermediates. Both enzymatic (Maillard reaction) and non-enzymatic (caramelisation) reactions are responsible for the browning effect.

The decomposition of sugars leads to the formation of volatile (caramel aroma) and brown coloured compounds (caramel colours).

The colours and the aromas depend on the sugar used (i.e. whether mono, oligo- or polysaccharide) and formed mostly through deoxyosuloses, O-heterocyclic and carbocyclic intermediates as well as low molecular weight sugar fragments (Heynes *et al.*, 1966; Kroh, 1994).

A summary of the degradation process of the carbohydrates started by polymeric carbohydrates is shown in Fig. 1.

The formation of MG is more complicated in the aminocatalysed degradation of carbohydrates than in the caramelisation process (Fig. 2) (Belitz & Grosch, 1992).

In the caramelisation and in Maillard reaction, the most important intermediates are the osuloses such as 3-deoxyosulose. It is assumed that the 3-deoxyosulose could form MG as well as Maillard and caramelisation products. The structures of coloured products of caramelisation are still not fully understood.

MG has two oxo-groups and very flexible H of the methyl-group, these easily condense with the intermediates such as 3-deoxyosulose building low-molecular weight colour compounds. This idea is supported by the determination of MG in different browning systems.

MG seems to play a central role in the formation of coloured compounds and in building the low- and high-molecular-weight colour compounds.

Determination of methylglyoxal

MG is water soluble and polymerises readily, so the most commonly used method to analyse MG includes derivatisation. MG was analysed as a methylquinoxaline derivate in cigarette smoke (Moore-Testa & Saint-Jalm, 1981) and in rat tissues (Ohmori *et al.*, 1987).

MG has mutagenic (Kasai & Nishimura, 1986) and cytotoxic (Ueno *et al.*, 1991) effects as well. It has been found in different food products such as bread, boiled potatoes (Kajita & Senda, 1972), coffee (Ohmori *et al.*, 1987), wine and various beverages (Hayashi & Shibamoto, 1985), vegetable oils (Hirayama *et al.*, 1984), and in various bakery products (Henle *et al.*, 1994).

There are also reports determining MG formed from lipids as a photodegradation product of fatty acids (Niyati-Shirkhodaee & Shibamoto, 1993) and from heated carbohydrates, for example from fructose (Örsi *et al.*,

1995). There are, however, no reports on their formation from oligo- and polysaccharides.

METHODS AND MATERIALS

Monosaccharides, D-glucose (FLUKA), oligosaccharides dextrin 15 (dextrin) from MERCK (where 15 equal the number of D-glucose unit) and polysaccharides (starch from MERCK) were used both in the caramelisation and Maillard model system. The concentration of the components are indicated in Table 1.

Caramelisation model system

Different concentrations of carbohydrates were sealed in 5 ml ampoules and heated in an aluminium block thermostat for 10 to 60 min at different temperatures. This heat treatment of carbohydrates (caramel) was prepared in four different temperatures in two parallel series.

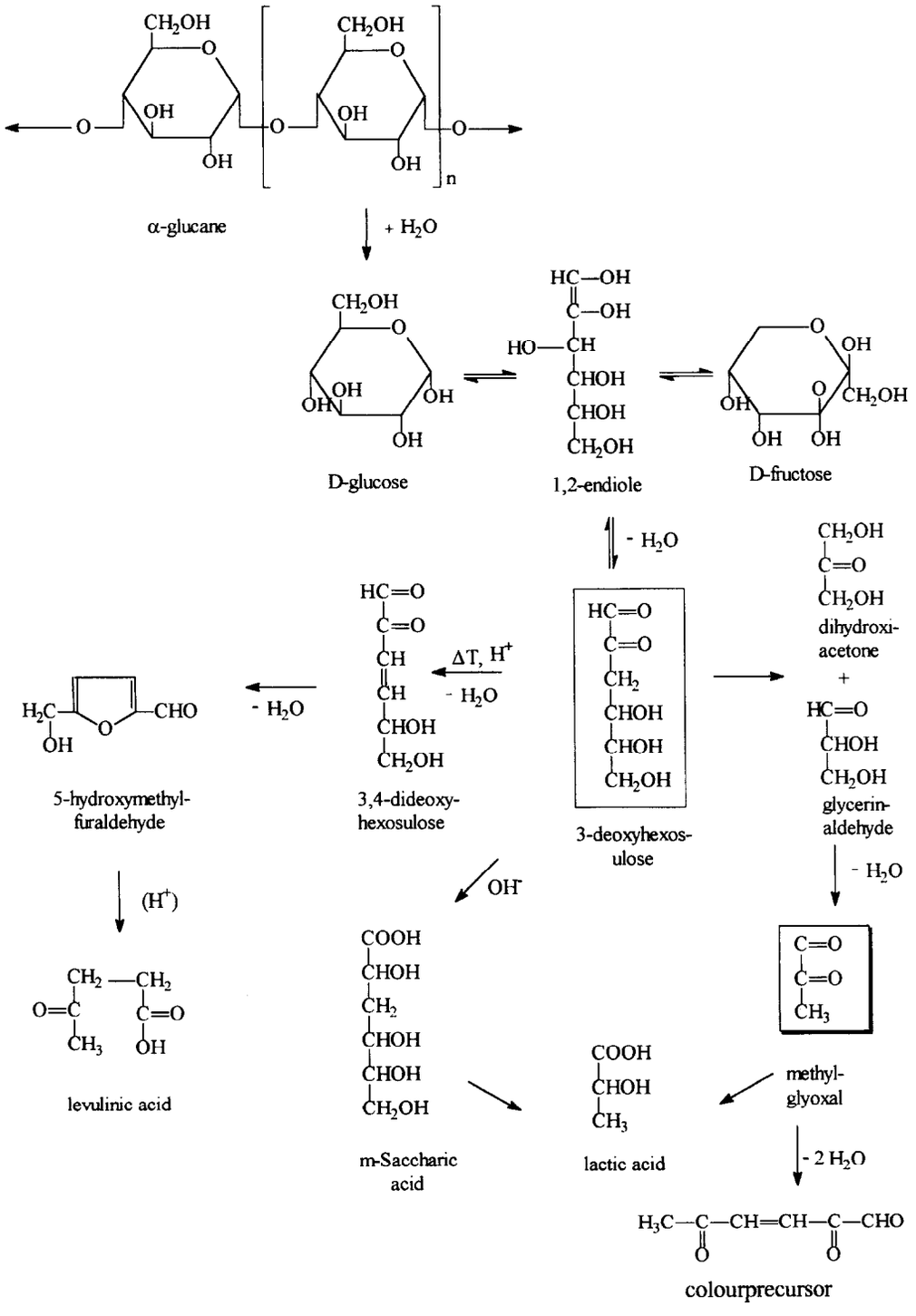

Fig. 1. Degradation of carbohydrates by caramelisation.

D-glucose was heated at 160, 170, 177 and 182°C, dextrin and starch were heated at 170°C.

After heating, the samples were cooled to room temperature and stored at −20°C. Before the HPLC analysis the tubes were diluted to 5 ml with destilled water and filtered if any unsoluble colouring matter was present.

For MG determination 0.5 ml filtrated solution, 0.5 ml water and 30 μl 30mg/ml freshly prepared o-Phenylendiamine (o-PD) was used and the amount of o-PD was in surplus.

Maillard model system

The heat processing was the same as in caramelisation model system except different carbohydrates were heated in the presence of o-PD and glycine.

Before heating 30 mg o-PD and 30 mg glycine were added to each carbohydrate solution, which were heated to 170°C in the presence of water. The water content was the same. If the sample contained o-PD before heating only dilution by destilled water was necessary.

The parameters of HPLC

Instruments:

(a) Pharmacia LKB HPLC Column Oven 2155
(b) Temperature: 40°C
(c) Column: Spherisorb ODS 25μm, 250 * 4,6 mm
(d) Eluent: acetonitril:water = 70:30
(e) Pharmacia LKB HPLC Pump 2248
(f) Flow: 0.7 ml/min
(g) Pressure: 4.6 Mpa

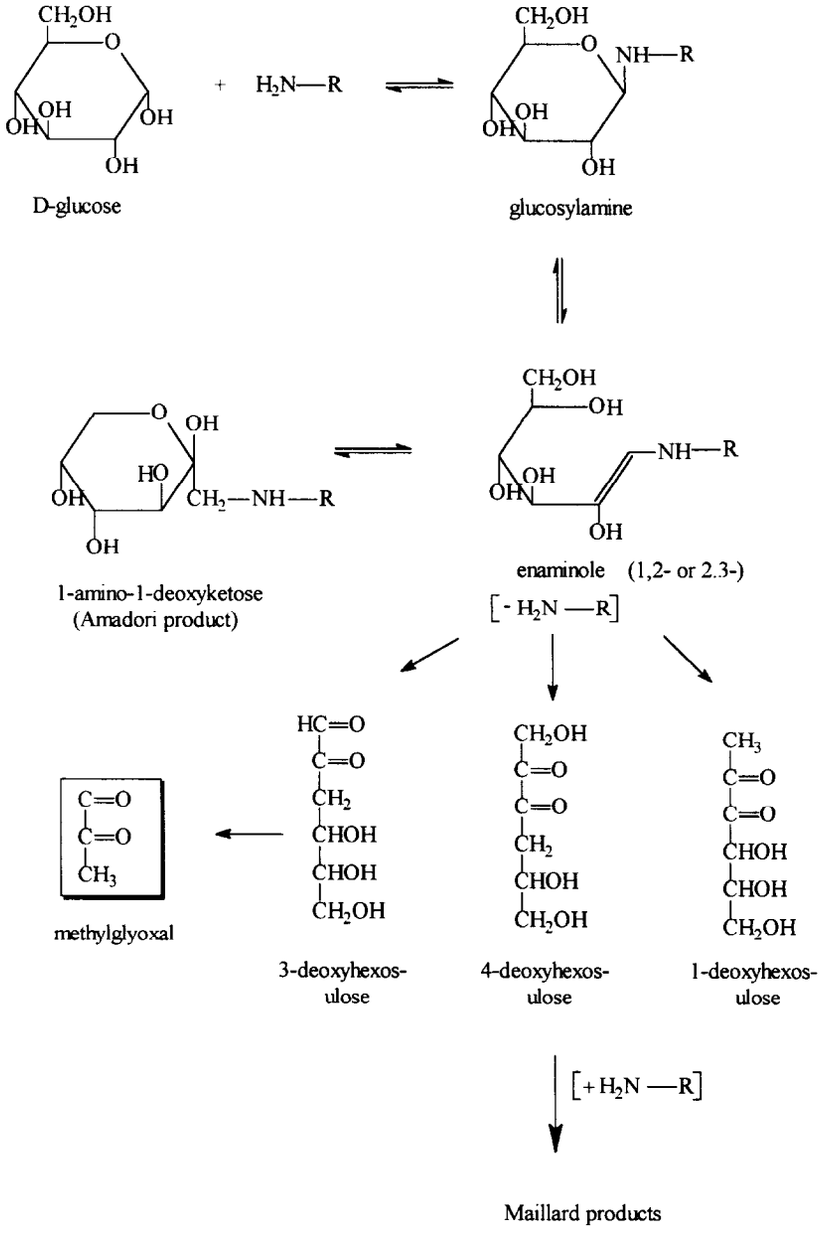

Fig. 2. General Maillard reaction.

(h) Pharmacia LKB VWM 2141 UV detector
(i) Wawe length: 200 nm
(j) Data evaluation:Pharmacia LKB Integrator 2221
(k) Injector: 20μL

Calibration

From the original methylquinoxaline (FLUKA) a 50 μg/ml solution was prepared. Several dilutions of the 50 μg/ml methylquinoxaline stock were injected into HPLC. The calibration curve was prepared for the concentrations and the peak area.

The parameters of the linear curve were as follows:

$$C = a * X + b$$

$$R^2 = 0.99948, a = 1277.79, b = 1.91$$

where:

R^2 = determination coefficient
C = concentration
X = peak area
a = slope
b = Y interception.

The retention time, t_R = 5.84 min.

RESULTS AND DISCUSSION

Caramelisation model system

In the first part of our experiment we caramelised monosaccharides. MG was determined as a methylquinoxaline

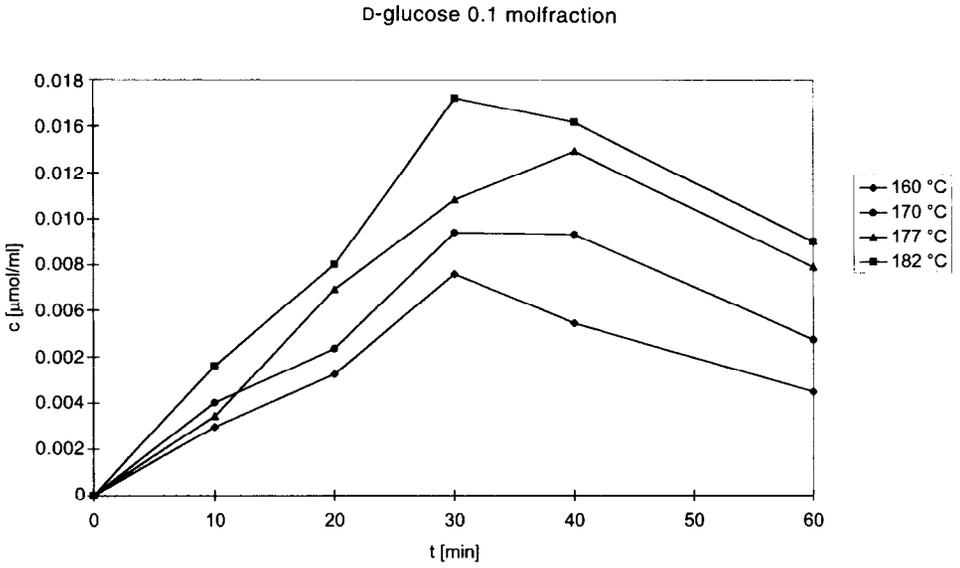

Fig. 3. Formation of methylglyoxal in 0.1 molfraction D-glucose caramel-solution vs. the period of heat treatment and different temperatures.

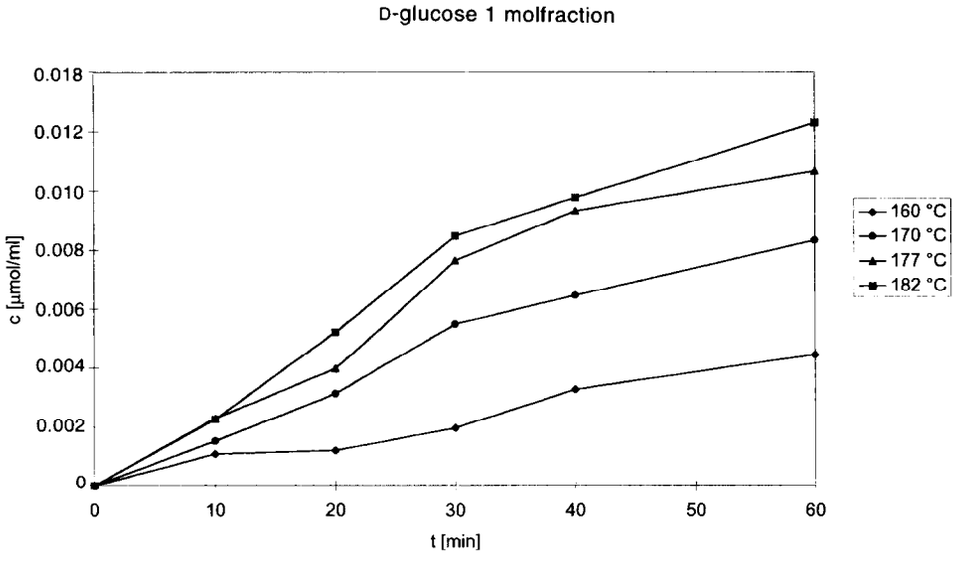

Fig. 4. Formation of methylglyoxal in 1 molfraction D-glucose caramel-solution vs. the period of heat treatment and different temperatures.

derivate, so each diagram contains MG concentration from 10 to 60 min versus the different period of heat treatment. In the presence of water (Fig. 3) MG concentration increases upto about 30 minutes of heating time, later the decreasing tendency of MG concentration was observed.

Under dry conditions (Fig. 4) the amount of MG increases slower and the maximum concentration of MG is reached later. This effect can be due to the formation of the brown colour material under caramelisation. The formation of MG from D-fructose solutions followed the same pathway as from D-glucose but at lower temperature. The different rate of MG formation could probably be explained by the particularly high reactivity of D-fructose in the enolisation reaction (Örsi *et al.*, 1995).

After monosaccharide caramelisation the MG concentration was determined in dextrin and starch caramel solutions at 170°C using the same conditions as above. Figure 5 shows the MG concentration for different period of heat treatment. Increasing the thermal heating the formation of MG grows. In aqueous solutions MG concentration is higher than in dry conditions as seen with D-glucose solution as well (Figs 3 and 4). An explanation for this effect is that dextrin has more reducing end groups especially D-glucose molecules. In dry conditions the molecular mobility is also too low (Chirife, 1979).

Figure 6 shows the formation of MG in aqueous solutions at 170°C in different model systems. Comparing the tendency of MG formation, when dextrin

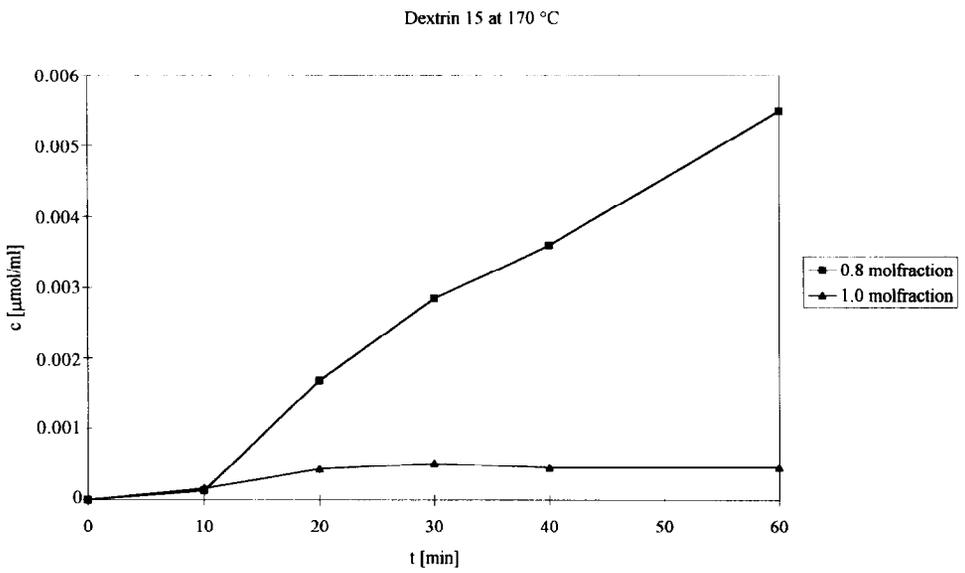

Fig. 5. Formation of methylglyoxal in water- and water free condition, in dextrin 15 caramel-solution vs. the period of heat treatment and temperatures.

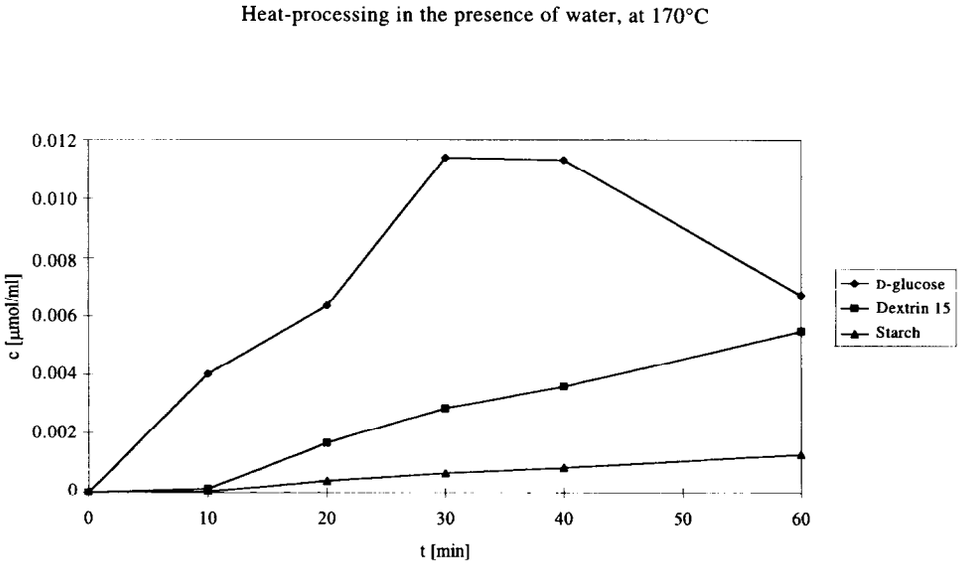

Fig. 6. Formation of methylglyoxal in aqueous solution in D-glucose, dextrin 15 and starch caramel-solution vs. the period of heat treatment at 170°C.

was caramelised, much more MG has been formed than in starch solutions, because of the higher numbers of the reducing end groups MG produced only at the active end of the dextrin chain. In dextrin caramel solution the formation of MG is not too rapid since the number of reducing end groups is less than in the D-glucose solution.

Probably the MG formation from dextrin and starch solution should follow a similar pathway as D-glucose caramelisation. When all D-glucose molecules are degraded, the MG concentration reaches a maximum and begin to transform yielding low- and high-molecular-weight colour compounds.

Maillard model system

In the second experiment we examined the behaviour of MG formation not only in the caramelisation but in the Maillard reaction as well. The rate of the Maillard reaction and the amount of low- and high-molecular-weight colour compounds formed are significantly increased by adding amino compounds or proteins.

Maillard reaction with o-phenylenediamine

In the Maillard model system the effect of o-PD was studied. Previously the formation of MG was examined

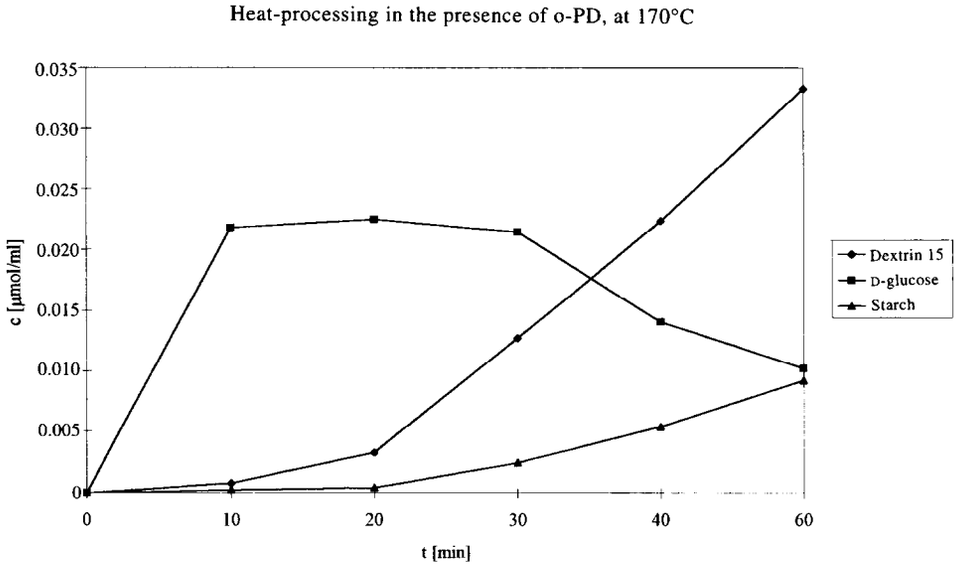

Fig. 7. D-glucose, dextrin 15 and starch caramelisation in the presence of o-phenylenediamine. Formation of methylglyoxal in aqueous solution vs. the period of heat treatment at 170°C.

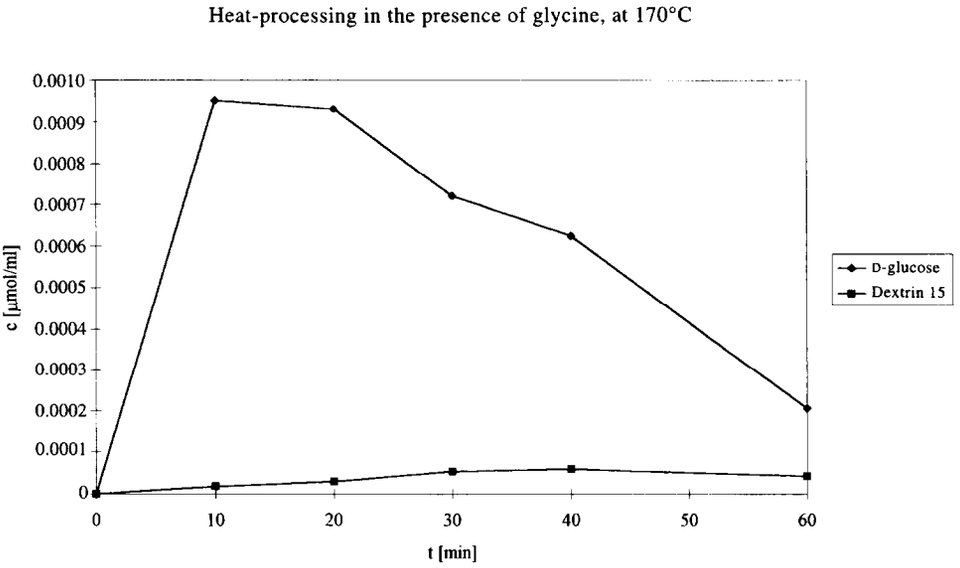

Fig. 8. D-glucose, dextrin 15 and starch caramelisation in the presence of glycine.Formation of methylglyoxal in aqueous solution vs. the period of heat treatment at 170°C.

from heated D-fructose in the presence of o-PD. It was found that the amount of MG formed and the reaction rate were higher (Örsi *et al.*, 1995).

We heated different carbohydrates in the presence of o-PD. Figure 7 shows the MG concentration formed from different sugars. After comparison with Fig. 6 it is observed that the presence of o-PD accelerates the MG formation from all of the three carbohydrates.

Using the same heating time MG formed from D-glucose, dextrin and starch were (about 2, 60 and 2 times) higher in the presence of o-PD. The starch is a more complex material, so the activation effect of o-PD takes place later.

In the beginning of heating (10 min) lots of MG was formed from D-glucose and decreased later because of the formation of volatile compounds and colour materials. A similar behaviour can be observed in the case of dextrin and starch, but in the latter the maximum MG formation occurs. Probably in dextrin solution the maximum concentration of MG is reached after about 60–80 min. This effect is retarded in starch.

Maillard reaction with glycine

Figure 8 shows the effect of glycine under heat processing in the Maillard reaction.

In the case of dextrin and D-glucose the activation effect of glycine could be observed. The concentration of MG formed is lower than in the presence of o-PD.

In the case of starch a very small activation effect was found.

When D-glucose and glycine were heated together, MG concentration was not as high as in the reaction with o-PD (Fig. 7). In the first period of the heating time, the highest amount of MG was formed and began to decrease as in the previous experiments. The difference between the formation of MG with o-PD and glycine can clearly be seen. Glycine has a lower base strenght of amino acid and the reaction with osuloses and MG occurs later. Probably glycine reacts with MG and an intermediate product could be formed.

The role of o-PD is complex. Amines can also accelerate the transformation of sugars in the pH range 4–7 without amino-ketoses being formed (Walton *et al.*, 1988). It is assumed that o-PD has also a nucleophil effect. Probably, during the heat processing the o-PD quickly bonds the formed hexosulose and MG, so their derivatives can be formed.

The applied HPLC method is suitable for determining MG in caramel and Maillard model systems. Adding o-PD and glycine to the carbohydrates MG concentration increases in the beginning of heat processing then decreases. The attained maximum concentration is highest in the case of o-PD. The concentration is lowest in the case of glycine. An explanation for this effect is that osulose reacts with MG building colour materials in the Maillard reaction. The surplus of the o-PD prevents MG further reaction building quinoxaline derivate.

REFERENCES

Belitz, H. D. & Grosch, W. (1992). Lehrbuch der Lebensmittelchemie, 4. Überarbeitete Auflage, Springer-Verlag Berlin Heidelberg, New York.

Chirife, J. (1979). Water content and temperature effect on non-enzymic browning in dried apple. *J. Food Sci.*, **44**, 601–605.

Hayashi, T. & Shibamoto, T. (1985). Analysis of mutagenetic methylglyoxal in foods and beverages. *J. Agric. Food Chem.*, **33**, 1090–1093.

Henle, T., Walter, A. W., Haeßner, R. & Klostermeyer, H. (1994). Detection and identification of a protein-bound imidazolone resulting from the reaction of arginine residues and methylglyoxal. *Z. Lebensm. Unters. Forsch.*, **199**, 55–58.

Heynes, K., Stute, R. & Paulsen, H. (1966). Bräunungsreaktionen und Fragmentierung von Kohlenhydraten. *Carbohydr. Res.*, **2**, 132–138.

Hirayama, T., Yamada, N., Nohara, M. & Fukui, S. (1994). The existence of the 1,2- dicarbonyl compounds glyoxal, methyl glyoxal and diacetyl in autoxidised edible oils. *J. Sci. Food Agric.*, **35**, 1357–1362.

Kajita, T. & Senda, M. (1972). Simultaneous determination of ascorbic acid, triose reductose and their related compounds in foods by polarographic method. *Nippon Nogei Kagaku Kaishi.*, **46**, 137–145.

Kasai, H. & Nishimura, S. (1986). Hydroxylation of guanine in nucleosides and DNA at the C-8 position by heated D-glucose and oxygen radical forming agents. *Environ. Health Perspect.*, **67**, 111–116.

Kroh, L. W. (1994). Caramelisation in food and beverages. *Food Chem.*, **51**, 373–379.

Moore-Testa, P. & Saint-Jalm, Y. (1981). Determination of α-dicarbonyl compounds in cigarette smoke. *J. Chromatogr.*, **217**, 197–208.

Niyati-Shirkhodaee, F. & Shibamoto, T. (1993). Gas chromatographic analysis of glyoxal and methylglyoxal formed from lipids and related compounds upon ultraviolet irradiation. *J. Agric. Food. Chem.*, **41**, 227–230.

Ohmori, S., Kawase, M., Mori, M. & Hirote, T. (1987). Simple and sensitive determination of methylglyoxal in biological samples by gas chromatography with electron capture detection. *J. Chromatogr.*, **415**, 221–229.

Örsi, F., Homoki, P. & Vu, Nam Phong (1995). Bestimmung von Methylglyoxal in Karamel Proben. *Die Nahrung.*, **39**, 90–97.

Ueno, H., Nakamuro, K., Sayato, Y. & Okada, S. (1991). DNA lesion in rat hepatocytes induced by *in vitro* and *in vivo* exposure to glyoxal. *Mutat. Res.*, **260**, 115–119.

Walton, D. J., McPherson, J. D. & Shilton, B. H. (1988). Evidence for nonensymatic fructosylation of human ocular lens proteins *in vivo*. XIVth International Carbohydrate Symposium, Stockholm, Sweden.