Revue Roumaine de Chimie, **2008**, *53*(5), 379–382

5-HYDROXYMETHYLFURFURAL - A POSSIBLE INDICATOR IN THE PREZERVATION ABILITIES OF THE THERMALLY PROCESSED FOOD

Maria CIOROI

"Dunărea de Jos" University Galaţi, Faculty of Science, Chemistry Department, Domneasca str., 47, Galaţi - 800008, Roumania
Maria.CIOROI@ugal.ro

Received June 8, 2007

The Maillard reaction (MR) involves the reaction between carbonyl groups of monosaccharoses or polysaccharoses and amino groups of amino acids or proteins. The MR plays an important role in nutritional value of foods. This work is mainly focused on presenting the results from the gas chromatographic and the mass spectrometry analysis in order to identify the 5-hydroxymethylfurfural (5-HMF) from the glucose-lysine model system. Studies were carried out on the glucose-lysine model of 1:1 mole ratio treated at 100 ^{o}C. Spectrophotometric measurements were done relative to the optical density (DO) variation in a certain heat treatment time intervals. The dynamic quantitative estimation of 5-HMF was made by the HPLC method. Correlating the results from experiments a significant increasing in the amount of 5-HMF was noticed.

INTRODUCTION

Major part of food products when are subjected to heating treatment and storaged at inadequate temperature, may generate different furfural derivatives because of Maillard reaction.

The non enzymatic browning reaction, the Maillard reaction, takes place in the food products during cooking, storing and technological processing and implies deep chemical reactions between the carbonyl groups and nitrogen compounds (α-amino acids, peptides, proteins, biogene amines and other organic compounds with free amino groups). The Maillard reaction consists in generating of a very large number of compounds of low molecule weight such as carbonyl and heterocyclic compounds as well as of high molecule weight such as polymers, premelanoidins and melanoidins.[1]

This reaction has a strong effect on the food quality and it is responsible for the modification of aroma, taste, colour and nutrition value, stability and guaranty terms.[2] Hydroxymethylfurfural (HMF) is an indicator of quality deterioration as a result of excessive heating or storage in a wide range of food containing carbohydrates and amino acids/ proteins. HMF occurs in many foods in high concentrations, sometimes exceeding 1g/kg in certain dried fruits and caramel products. European Union Standards limits the level of HMF in fruits and vegetable juices and concentrates(less than 20 mg/kg).[3] In this way it is important to study the presence of HMF in food rich in proteins and carbohydrates. On sugar-amino acid models were carried out investigations in order to study the factors influencing the Maillard reaction[4,5] as well as to determine the resulting products.[6]

In the present work it had in view the evolution of amount of 5-HMF from glucose-lysine model system. It was intended to establish the proper reaction conditions so as to provide standardization of the analysis method applied to preserve food products rich in carbohydrates and proteins.

EXPERIMENTAL

1. Model system preparation

The glucose-lysine model was prepared by using lysine monochloride (SIGMA Italy), D(+)-glucose anhidrous (RPE-ACS reagent, Carlo Erba, Milano, Italy) and bidistilled water. The glucose and lysine were dissolved separately in bidisltilled water and were further stirred in a graded vessel to provide a solution of 1M from each component. The solution was then adjusted to pH=7 with NaOH 1M (pH-meter Mettler Toledo, MP 220).

For the spectrophotometric measurements and high performance liquid chromatography (HPLC) investigations the mixture was divided into volumes of 10 mL and poured into glass vessels of 20 mL. The vials were sealed and heat treated

at 200^{O} C for 30-120 minutes. Then the samples were cooled with water jet and kept to temperatures below 0^{o}C to be brought to the ambiant temperature for the analysis.

As reference a water glucose solution of 1 M concentration was prepared, heat treated as the glucose lysine system.

2. Gas Chromatography-Mass Spectrometry(GC-MS) analysis of 5-hydroxymethylfurfural [7]

For the GC-MS analysis the samples were extracted by etylacetate from the heat treated glucose-lysine model from which the volatile compounds had been previously separated.

In order to confirm the presence of 5HMF in the etylacetate analysis GC-MS were made. The samples of etylacetate extract were dry evaporated and treated with methylic alcohol. One volume of 0.2 mL methanolic solution was poured into a grade vessel of 10mL. From this solution, 0.05 mL were mixed with 0.05 mL eicosan solution in penthane as internal standard.

The CG-SM analysis was made with a VARIAN gas cromatograph, model 3400, connected to a mass spectrometer VARIAN SATURN (IT. DMS).

The gas used was Helium at 1.5 mL/min flow rate. The analytical results were automatically processed on a PC Filter Karnak connected to a printer EPSON LX-400. The 5-HMF was identified through the data bank as well as by comparing the mass spectrum of the sample analyzed with that of the pure sample.

3. Spectrophotometric measurements

Absorbance measurements in UV-VIS were made for all heat treated samples from the glucose-lysine model and glucose. The apparatus used was a spectrophotometer VARIAN model DMS. 80 UV-VISIBLE connected to VARIAN model 9176 Recorder. With all the measurements, dillutions adapted to the measuring range and max sensitivity limits were made.

4. The high performance liquid chromatographic analysis [8]

The investigations focused on the dynamic of 5-HMF formation in the glucose-lysine and glucose -water systems and for this purpose it was used a liquid Japan Spectroscopic CO. LTD fitted with pump type JASCO 880- PUI, detector JASCO 875-UV, integrator Data jet and a degasing system type Degasser ERC-3325.

The identification of 5-HMF was made by comparing the retention times of the sample with that of the pure standard substance (5-hydroxymethylfurfural, Fluka Chemie AG, Germany). Measurements were replicated three times for each heat treated sample.

Statistical Analysis

The Student's *t* test was applied to the experimental results. All statistical analyses were carried out using Excel 2003(SP2) software package for Windows XP.

RESULTS AND DISCUSSION

In the separations and spectra recorded by CG SM method applied to the methanolic extracts from the glucose-lysine model system it was found a peak which had a retention time identical to the pure 5-HMF.

From the mass spectrum it is noticed that the peak corresponding to the molecular ion has m/e=126. The intense molecular peak indicated the presence of the furanic heterocycle.

By HPLC method it is shown the presence of 5-HMF among the products of the Maillard reaction in the model glucose-lysine, while in the reference sample (water- glucose) its presence is insignificant (Table 1).

From all the data obtained by mass spectrometry, chromatographic analysis and UV-VIS spectrophotometry there can be drawn the conclusion that 5-HMF is a never-failing intermediate in the primary stages of the Maillard reaction between hexoses and α-amino acids.[9] This compound, while it accumulates in the system, actively takes part in the formation of the coloured condensed structures of humic type. From HPLC analysis as regards the dynamics of the 5-HMF formation it can be noticed an increase in the amount of 5-HMF in the heated model samples (for longer times) as compared with the reference sample treated in the same way. By the method of adding the internal standard it was found that the peak of 17.26 retention time corresponds to 5-HMF.

Table 1

The amounts of HMF from glucose-lysine and glucose-water model systems heated at 200^{o}C

Time heating, minutes	HMF (gl-lys) (mM) $\bar{x} \pm s$	HMF (gl-water) (mM) $\bar{x} \pm s$
30	2,300 ± 0,030	$(0.005 \pm 0.001) \cdot 10^{-3}$
45	6.958 ± 0,051	$(0.013 \pm 0.001) \cdot 10^{-3}$
60	16.029 ± 0,02	$(0.088 \pm 0.021) \cdot 10^{-3}$
75	25.156 ± 0,034	$(0.191 \pm 0.050) \cdot 10^{-3}$
90	26.721 ± 0,006	$(0.367 \pm 0.013) \cdot 10^{-3}$
120	29.835 ± 0,052	$(0.465 \pm 0.023) \cdot 10^{-3}$

The experimental results are pointed out by mean ± standard deviation; at 2 degrees of freedom the mean values of the two set of experimental data are statisticaly the same at 95% confidence level.

By correlating the data on the amounts of HMF from the two systems the results are presented in Table 1. It can be seen that in the case of glucose water solution, the amount of HMF is much less than in the glucose-lysine system. In the glucose system the amount of HMF increases slowly up to 0.088 · 10^{-3} mmol/L after 60 minutes of heat treatment.

It' s very likely that after 120 minutes of heat treatment higher concentrations of 5-HMF occur, but these have no significance as regards the preservation conditions because the product is already heat damaged.

The absorbance measurements of the heat-treated model samples(glucose-lysine and glucose alone) to 200°C indicate an increase in the DO values with the heat treatment time (Fig. 1).

We established that between the maximum absorbance values at 280 nm and the heat treatment times there is no strictly linear relation in the primary phase of Maillard reaction; however after 90 minutes the concentration of the coloured substances increases exponentially (intense batochrome effects occurs further to the cycling).[10]

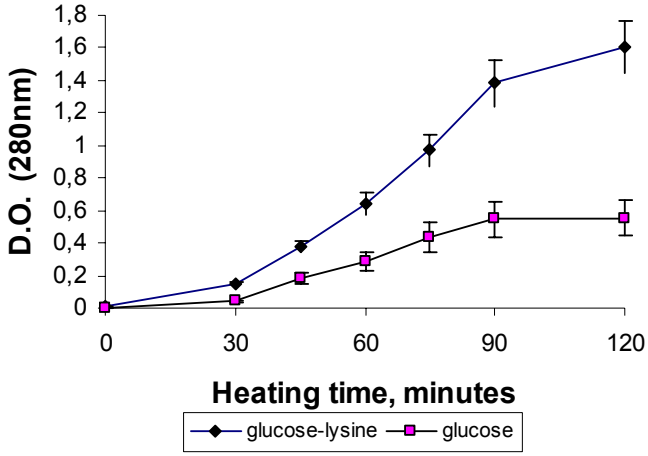

Fig. 1 – The absorbance values at 280 nm for glucose-lysine and glucose-water model systems.

In mesurements of DO values at 280nm the glucose lysine model system was diluted 100 times and glucose water was diluted 10 times.

The variation of optical density (DO) from figure 1 correlated with the results from table 1 shows that the increasing in absorption at 280nm of the glucose-lysine model system on heating time is partially due of the conjugated forms like furan derivatives occur in system. We can correlate the small DO values of glucose water system to a very small 5-HMF quantity.

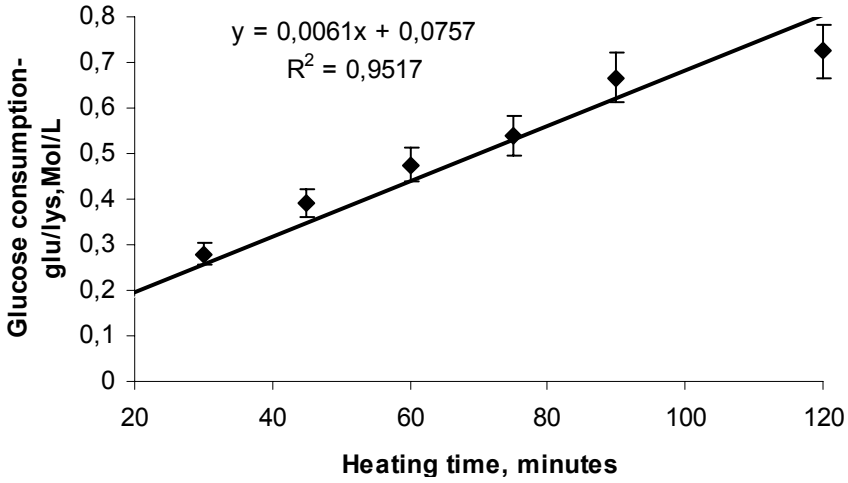

Fig. 2 – The glucose consumption in glucose-lysine system model.

The R- squared value on the chart from Figure 2 is around the unit value. So, at 200 degrees Celsius in the range of 0-120 minutes, there is a good liniarity between consumption of glucose and heating time of glucose lysine model system. At 90 and 120 minutes of thermical treatment is consumed 66% respectively 72,5% of glucose (Fig. 2). At these moments the quantity of 5-HMF is unexpectedly decreasing relating to the quantities obtained at 60, 75 and 120 minutes of heating of model systems (table 1). That fact is likely due of the multiple reactions which occur(condensation, polimerisation etc.) implying 5HMF which has a big reactivity in the given conditions.[10,11]

CONCLUSIONS

As a result of the systematic investigations made on the browning chemical non enzymatic effects in the Maillard reaction on glucose-lysine, a sufficient number of data have been gathered to allow the following conclusions:

The lysine reacts with the glucose resulting in considerable amounts of 5-HMF which further increase with longer heat treatment times and more acid reaction media (from pH 7 to pH 2 according to the data in).[12,13]

It is thus shown that in the glucose-lysine model the amount of 5-HMF is much higher than in the glucose solution alone at pH = 7. This makes obvious the importance of the chemical interactions between glucose and amino acid. Correlating the results from experiments a significant increasing in the amount of 5-HMF confirms the direct contribution of the sugars in the primary phase of Maillard reaction only in the presence of the α amino acid as protoning agent.

From investigation it was carried out the 5-HMF is an essential intermediary in the Maillard reaction to the formation of which participate hexoses and fragments of α-amino acids of side proteic catene or metabolic nitrogen compounds. The volatile and non-volatile reaction products are the result of chemical interaction of the hexoses, α-amino acids, peptides, proteins, byogene amines, vitamins with amino groups and others.

The results of this work proved that, by applying an apropriate analytical approach to the dynamics of 5-HMF as intermediary in Maillard reaction between hexoses and the primary amino a standard methodology can be conceived to control the preservation abilities of the food products rich in sugar and amino compounds.

Using HPLC method for quantification of 5-HMF, once precisely calibrated, it should be of great interest for controlling or adapting of process in order to ensure that a better nutritional quality for the food products was obtained.

REFERENCES

1. J. Hawthorn, “Foundations of Foods Science”, W. H. Freeman (Ed.), Oxford, 1981, p. 156-160.
2. S. Fors, “Annual Chemical Society Symposium Series”, G.R. Waller, M.S. Feather (Ed.), 1983, Vol. 215 p. 185-187.
3. M., Dogan, T., Sienkiewicz and R. A., *Proceedings volume, Euro Food Chem. XIII*, Germany, **2005**, p. 357-360.
4. H., Jing and S., Nakamura, *J. Med. Food*, **2005**, *8,* 291-298.
5. M. Cioroi, M. Leonte, T. Florea and C. R. Lerici, *Annals of Al. I. Cuza University, Iasi*, **1999**, *VII*, 65-70.
6. M. Cioroi, *Proceedings Volume, 19th European Colloquium on Heterocyclic Chemistry,* **2000,** p. 233-236.
7. H. S. Rupp and S.B. Turnipseed, *J. AOAC Intern.,* **2000**, *83*, 612-620
8. A. Theobald, A. Müller and E. Anklam, *J. Agric. Food Chem.*, **1998**, *46*, 1850–1854.
9. M. Mandoza, R. A. Olano and M. Villamie, *Food Chemistry*, **2002**, 79, 513-516.
10. I. Birlouez-Aragon and J. Leclerc, *Nahrung Food*, **2001**, *45,* 201-205.
11. P. Fernandez–Artigaz, E. Guerra–Hernandez, B. Garcia–Villanova, *J. Agric. Food Chem.* **2000**, *47*, 2872–2878.
12. M. Cioroi, M. Leonte, M. Munari, D. Mastrocola and C. R. Lerici, *Rev. Roum. Chim.*, **2000,** *45*, 153-157.
13. D. Mastrocola, M. Munari, M. Cioroi and C. R. Lerici, *J. Sci. Food Agric.,* **2000,** *80*, 684-690.