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VISIBLE DETECTION OF THE COMPOUNDS GENERATED BY MAILLARD SYSTEMS IN MILD CONDITIONS

Alina Mihaela ELISEI

Department of Biochemistry, *Dunarea de Jos* University of Galati, Faculty of Food Science and Engineering, 111, Domeasca St., Tel./Fax: +40 236 460165

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Abstract

Researches concerning the spectrophometrical detection for the maximum of visible absorbance of a reaction mixture of methylglyoxal and cysteine in relation with previous results concerning the type of compounds released by this kind of Maillard system were done. Yellow-brownished pigments were generated immediately in the case of cysteine. Other amino acids tested in the presence of methylglyoxal remained colourless. The absorbtion spectra reached a maximum at 360 nm. The nature of the coloured compound remained unidentified at the moment. In addition, the influence of the solvent used on the variation of optical density by time was studied.

Key words: cysteine, methylglyoxal, yellow-brown pigment, optical density

Rezumat

Au fost efectuate cercetari cu privire la detectarea spectrofotometrica in domeniul vizibil a maximului de densitate optica a unui amestec de cisteina si metilglioxal in relatie cu rezultatele raportate anterior referitor la natura compusilor produsi de acest tip de system Maillard. In cazul cisteinei in sistem a fost generata imediat o coloratie portocalie spre brun. Amestecurile de reactie continand alti aminoacizi in prezenta metilglioxalului au ramas incolore. Maximul de absorbtie a fost atins la lungimea de unda de 360 nm. Natura compusului colorat in portocaliu brun nu a fost identificata deocamdata. Totodata, a fost studiata influenta solventului asupra variatiei densitatii optice in timp.

1. Introduction

The Maillard reaction between amino and carbonyl compounds is a nonenzymatic browning reaction. It occurs in foods during processing and cooking, even during storage. Recently methylglyoxal has been shown to be generated from the Maillard reaction. Previous work has shown that methylglyoxal-cysteine system is important in generating flavours in hydroalcoholic solution under mild condition (room temperature, acidic *p*H 3.5), (Elisei, 2005).

In addition, the Maillard reaction is responsible for much of the colour which develops in many foods on thermal processing. It results in the formation of complex mixtures of coloured and colourless Maillard reaction products (MRPs) which possess a wide range of polarities and molecular weights, making their analysis difficult (Ames, 1998). Although this reaction contributes to the formation of brown color, flavours, antioxidants, and mutagens, it has been reported to be responsible for the nutritive loss of proteins and the formation of mutagenic products (Hayase and Kato, 1994). In the beginning of the reaction, the formation of characteristic brightly colored pigments, varying with amino acids, was reported in model systems with single amino acids and sugars (Gomyo, 1989). The structure of the blue pigment formed in Xyl-Gly reaction system was identified to be an intermediate of the brown pigment. The contribution of the brightly colored pigments to the radical-scavenging activity is not clear, although the brown pigments have been reported to have radical-scavenging activity (Hayase, 1989; Yen, 1995; Wijewickreme, 1999). If the brightly colored pigments have radical-scavenging activity, they can be used as safe and functional food additives. In addition, the early-stage Maillard reaction products may be contained in most

Corresponding author: alina.elisei@ugal.ro

foods. Therefore, it is important to analyse the nature of the brightly colored pigments released in the model systems due to the type Maillard reaction. This paper studied the behaviour of cysteine in the presence of methylglyoxal concerning the maximum value recorded for the optical density. Significative differences were found between cysteine and others amino acids tested. Moreover we have studied the variation of the absorbance according to the storage time.

2. Materials and Methods

Materials

Amino acids (cysteine, lysine, leucine, threonine, asparagine) and carbonyls (methylglyoxal) were purchased from Sigma Aldrich Chemical Co.

Inorganic reagents and solvents were all commercial products of analytical grade. The mixture of carbonyl compound and amino acid in an aqueous ethanolic solution (12% volume), was prepared under stoichiometric conditions (20 mM) and adjusted to pH 3.5 with H_3PO_4 (1/3) and 1 M NaOH (Pripis-Nicolau, 2000). The solutions were stored at 25ºC at dark during a 15 day period.

Three types of solvent were tested for dissolving the reagents:
1. 12% (v/v) hidroalcoholic solution in ethanol and 4g/l tartric acid;
2. 12% (v/v) hidroalcoholic solution in ethanol;
3. Phosphate-citrate buffer system with an initial *p*H of 3.5

Absorption spectra measurements

The visible absorption spectra of reaction solution were measured in the range 200 nm to 600 nm with a UV-VIS JASCO V-530 (Japan) spectrophotometer connected to with a computer HP Vectra VL 24/50. In all measurements, appropriate dilutions have been made, adapted to the spectrophotometer's measuring limits. Three ml quartz cuvettes, with a 1cm optical pathway were used.

3. Results and discussion

UV-VIS absorption spectra were realized for the study of amino acid - methylglyoxal systems. Table 1 presents the values of the wavelength (λ) that correspond to the maximum absorbance value reached by the analyzed systems. All experiments were done in 12% (v/v) hidroalcoholic solution in ethanol and 4g/l tartric acid.

Table 1. λ values of the analyzed systems

System	λ (nm)
Methylglyoxal (MG)	278
Cysteine +MG	360
Lysine +MG	279
Leucine+MG	279
Threonine+MG	279
Asparagine+MG	279
2-Methylpyrazine	267
2,5- Dimethylpyrazine	276
2,6-Dimethylpyrazine	275
Trimethylpyrazine	277

The absorption spectra were featured for each probe conserved at a temperature 25ºC. The spectra show a maximum of absorption at 360 nm wavelenght. Simultaneously absorbtion spectra of all components of the system: cysteine, methylglyoxal, ethanol were built as reference, as well as the absorption spectra of pyrazines previously identified in the system (Elisei, 2004). Additionally, the maximum of the absorption of the basic system of study, cysteine/methylglyoxal was compared to the maximum obtained for other amino acids coupled separately with methylglyoxal, maintaining the other parameters of the system at constant values for all analyzed situations. Figure 1 shows a few significant results.

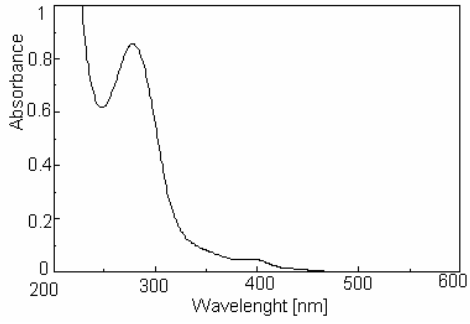
3.1. Experiments done with 12% (v/v) hidroalcoholic solution in ethanol and 4 g/l tartric acid

The absorption spectrum of the hidroalcoholic solution without reagent led to a very low maximum of absorption at 400 nm, which is found in most of the built spectra. It has been established that this one belongs to ethanol.

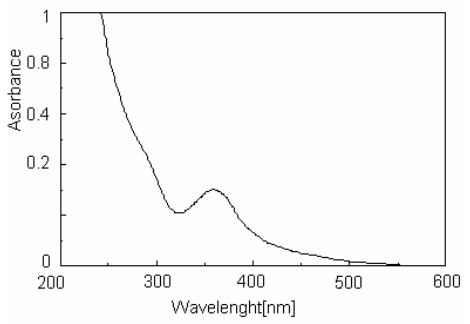
The hidroalcoholic solution of cysteine did not show any maximum of absorption, while for the methylglyoxal hydroalcoholic solution, the maximum of absorption is obtained at 278 nm.

We concluded that under was mild conditions (3.5 *p*H and 25ºC) only cysteine lead the reaction towards yellow-brown compounds responsible for

the absorption at 360 nm. The nature of these compounds is still unidentified. The same reaction does not take place in other mixtures of amino acids/ methylglyoxal that remained colourless even after 8 days of storage. We assumed that in these systems the methylglyoxal was unconsumed, as long as the maximum of absorbance was reached at the same wavelenght for both situations: the hidroalcoholic solution of methylglyoxal (as reference) or mixture of amino acid (other than cysteine) with methylglyoxal.



a)



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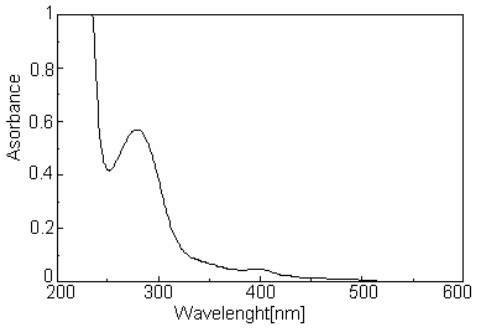

c)

Figure 1. Spectra of the 12% (v/v) hidroalcoholic solution in ethanol of a): methylglyoxal; b) cysteine and methylglyoxal; c) lysine and methylglyoxal

Figure 2 shows the variation of the optical density of the cysteine/ methylglyoxal solution during 15 days of reaction.

Immediately after adjusting the *p*H value of the system to 3.5, the recorded absorbance is only 0.88, which increases rapidly almost 4 times, so that after 5 hours of reaction, the value is 3.05. The maximum is obtained after 3 days of depositing (3.75) and remains practically constant for other 5 days and then hardly diminishes in the following week of study.

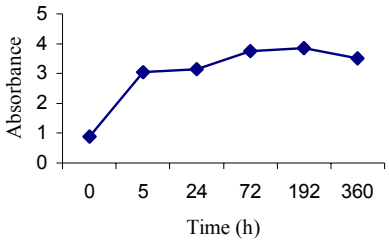

Figure 2. Evolution of the optical density at 360 nm of the cysteine/methylglyoxal reaction mixture (12% (v/v) hidroalcoholic solution in ethanol and 4 g/l tartric acid). Reaction was carried out at 25ºC in the dark during a 15 day period

3.2. Experiments done with 12% (v/v) hidroalcoholic solution in ethanol

The absorption spectra recorded at the established moments in time, in the case of reaction mixture cysteine/methylglyoxal disolved in 12% (v/v) hidroalcoholic solution in ethanol, showed maximums of absorption at the same wavelenght of 360 nm, as well as the system with tartric acid addition. The values obtained after the appropriate dilutions are shown in figure 3.

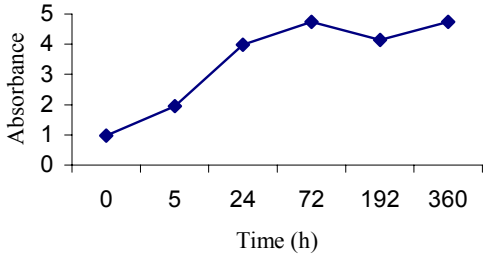

Figure 3. Evolution of the optical density at 360 nm in the cysteine/methylglyoxal reaction mixture (12% (v/v) hidroalcoholic solution in ethanol)

The optical density of the system increases spectacularly in the first 24 hours from 0.98 (after adjusting the *p*H to 3.5), to 3.98, it continues to

increase moderately to 72 hours of reaction up to 4.75, after which the absorbance maintains itself constant in the system.
The increase of the optical density is more emphatic in the system from which the tartric acid was excluded, in comparison to the previously presented system.

3.3. Experiments done with phosphate-citrate buffer system with an initial *p*H 3.5

For the entire tested period the optical density in the phosphate-citrate buffer system showed the lowest values of all analyzed systems, the maximum value recorded being 2.45 at 24 hours of reaction, between 24 and 72 hours the extinction of the system decreased to 1.7, after which it had a slight tendency of increasing, as showed in figure 4.

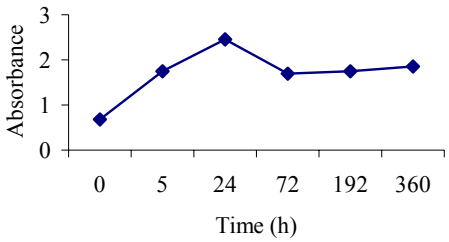

Figure 4. Evolution of the optical density of the 360 nm

4. Conclusions

Brightly coloured pigments were found in Maillard reaction under mild conditions. The color varied with the type of amino acid and with the solvent used as reaction medium. Regarding the colour of the solutions, different shades were obtained for the three different solvents: a) yellow-brown in the case of reaction mixture cysteine/methylglyoxal disolved in 12% (v/v) hidroalcoholic solution in ethanol and tartaric acid; 2. orange-brown for the same system without tartaric acid; 3. intense yellow in the phosphate-citrate buffer system.

In mild conditions (3.5 *p*H and 25ºC) only the hidroalcoholic mixture of cysteine/methylglyoxal developed yellow-brownished pigments specific to Maillard reactions. These pigments are responsible for the absorption at 360 nm.

The moment the methylglyoxal is added to cysteine, the colour is developed in the system. The same reaction does not take place in other mixtures of amino acids/ methylglyoxal that remained colourless even after 8 days of storage.

During the storage, the optical density increased in all situations no matter what was the solvent used in the three systems.
The maximum value of absorbance was recorded in the case of cysteine/methylglyoxal disolved in 12% (v/v) hidroalcoholic solution in ethanol without the addition of tartric acid.

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