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PRO-OXIDANT AND ANTI-OXIDANT ACTIVITY OF MONOFRUCTOSE GLYCINE AND MONOFRUCTOSE HISTIDINE

Benkhayal F.A.[1], A.H. AL-Saadi[2], and M.H. AL-Saadi[2]

[1.] **Department of Food Science and Technology, Faculty of Agriculture, University of Omar El-Mukhtar, Libya.**

[2.] **Department of Biology, Faculty of Science, University of Omar El-Mukhtar, Libya.**

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ABSTRACT: Amadori products of monofructose glycine and monofructose histidine were prepared and purified on gram scale. Crystals of both compounds were characterized by TLC, elemental analysis, ^{13}CNMR and FAB spectroscopy. The – NH_2 group of histidine is the only nitrogen involved in the production of Amadori product, and the imidazole ring is active and might be participating in the antioxidant activity.

Monofructose glycine gave pro-oxidant activity while glycine at the same concentration (15 mM) gave an anti-oxidant effect. It is possible that the amino group is important for the anti-oxidant activity. However, monofructose histidine gave an anti-oxidant effect greater than that of citric acid (15 mM) when the diene formation was measured. On the other hand, histidine at the same concentration also gave an anti-oxidant effect, but less so than its Amadori products. It is possible that the incorporation of sugar moiety enhances this effect. The molecular model of monofructose histidine shows its ability to chelate metals other than histidine. The ligating groups which can co-ordinate with metals are the amidazole nitrogen and the two hydroxyl groups at C_3, C_4 of the sugar moiety.

Key words: Amadori products, monofructose glycine, monofructose histidine, anti-oxidant.

INTRODUCTION

The constant need to increase the shelf life of foodstuffs means that the deterious effect caused by biological processes, physical and chemical changes in food must be controlled better. In order to overcome autoxidation of lipids, antioxidants are often incorporated into the foods susceptible to lipid oxidation (Kochhar and Meara, 1975; Kochhar, 1993). Several synthetic anti-oxidants are available which effectively control lipid oxidation e.g. butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate (PG). However, consumers have become increasingly concerned about the safety of these synthetic compounds (Barlow, 1990).

The trend in recent years, therefore, has been to use as food additives natural food constituents, showing antioxidant properties. As an example, variety of herbs, spices and various vegetable extracts have been reported to be used either commercially (Schuler, 1990) or to be potentially applicable to food stuffs (Pratt and Hudson, 1990). In this respect, the use of Maillard reaction products (MRPs) is particularly interesting as an alternative to synthetic antioxidants. Indeed, the Maillard reaction is amongst the most important reactions occurring in foods during processing and antioxidative compounds may be formed upon heating (Kitts and Jing, 2004 a and b).

Extensive research has been carried out on the antioxidative effects of MRPs over the last 45 years. Most of these studies concentrated on the later stages of the reaction after the brown pigments is formed. However, evidence from cooking processes and some modle system studies indicated that measurable antioxidant activity is produced without obvious browning (Kirigaya *et al.*, 1969; Einerson and Reineceius, 1977, Labe *et al.*, 1999).

The present study deals with the antioxidative effects of pure Amadori compounds (monofructose glycine "MFG" and monofructose histidine " MFH").

MATERIALS AND METHODS

Preparation of Amadori Compounds (1-amino-1-deoxy-D-fructose derivatives) from Glycine and Histidine

The Amadori compounds of glucose with glycine and histidine were prepared using the synthesis reaction described by Mossine *et al.*, (1994) with slight modification. Glucose (36 gr) and sodium bisulfite (0.2 gr) were suspended in a mixture of methanol (60 ml)- glycerol (30 ml) and refluxed on an oil bath for 30 minutes. Glycine (6.75 gr) or histidine (13.968 gr) and glacial acetic acid (8 ml) were then added and refluxed continued for 8 hr in the case of glycine and 2 hr in the histidine reaction. The resulting brown syrup was diluted with one volume of water, placed on a column of Dowex 50 WF 8880 and washed with ethanol – water (1:1, 500ml) and water (700 ml), thus removing all the free sugar and colour, the column was then eluted with 0.1M ammonium hydroxide and fractions (100 ml for glycine, 20 ml for histidine), were collected and evaporated to 25 ml and 10 ml, respectively using a rotary evaporator (at 20°C). All fractions were analysed by thin layer chromatography to detect the presence of Amadori compound. Fractions containing Amadori compounds and amino acids were poded and evaporated to small volume and rechromatographed on the Dowex column. In each case, all fractions containing pure Amadori compound were combined and evaporated using a rotary evaporator until a syrup was obtained. The syrup was dissolved in methanol water (3:1) and absolute methanol was added dropwise until the solution become turbid, the resulting mixture was allowed to stand at room temperature for 3 days, during which time crystallization occurred. The crystals were filtered off, washed with methanol-water (3:1) and dried in vacuo.

Thin Layer Chromatography of Amadori Compounds

The products obtained from the ion exchange chromatography were analyzed by TLC on silica gel No. 60f254. Elution was performed with a mixture of butanol, acetic acid and water (2: 1:1). The samples and standard glycine or histidine solution were spotted on TLC plates and these were placed in the solvent tank. After development, the plates were left to dry and sprayed with ninhydrin (0.2gm/100ml. acetone). Spots were developed by heating at 110°C for 5 min in an oven.

Preparation of Amadori Compound Derived from NcB-Z- Histidine

Glucose (1.08 gr) and sodium bisulfite (0.057 gr) were suspended

in a mixture of methanol (20 ml) – glycerol (10 ml) and refluxed using an oil bath for 30 minutes. NcB-Z- histidine (0.868 gr) and glacial acetic acid (2.66 ml) were then added and reflux resumed for 6 hr. Samples were taken at intervals to check for the formation of Amadori compounds.

Diene Test

The formation of peroxides was measured according to the procedure described by Torel *et al.* (1986) as follows. An emulsion of linoleic acid (2.5mM) in phosphate buffer (pH7.5) and tween 20 (0.5%) was prepared by stirring. The reaction mixture (40 ml.) was placed in conical flasks (250 ml) together with citric acid, glycine, histidine, MFG and MFH. A control, using water in place of MRPs was investigated under the same condition. The flasks were incubated at 20 ± 2°C in the dark for 200 hr. To measure the formation of conjugated dienes, a 1 in 20 dilution was made and the absorbance of the resulting solution was measured at 234 nm.

β- Carotene Bleaching Test

The antioxidative effect was measured using the β-carotene bleaching test, following the procedure described by Pratt (1976). A solution was prepared by adding linoleic acid (20 mg) and tween (200 mg) to 2 ml of β-carotene (0.02%) in chloroform. The chloroform was removed on a rotary evaporator and residue take up in 50 ml of oxygenated water. An aliquot (5 ml) of tines emulsion was incubated with a test solution (2 ml) or water (2 ml, for the control) in stoppred tubes in a water bath at 50 °C. the tubes were removed at intervals and the absorbance at 470 *nm* measured until the control sample had been bleached.

RESULTS AND DISCUSSION

The Amadori intermediate in glycine/glucose and histidine/ glucose reaction were synthesized and gave a reasonably good yield (0.9 – 1.0 gr) and (1.5 – 2 gr) respectively. TLC was used to identify the fractions containing Amadori products and to detect the contaminated fractions which were rechromatographed (Fig 1a – 1b and Fig 2a – 2b).

The pure crystals obtained in both cases MFG and MFH were subjected to elemental analysis. There was good agreement between the calculated and the experimental values Table (1).

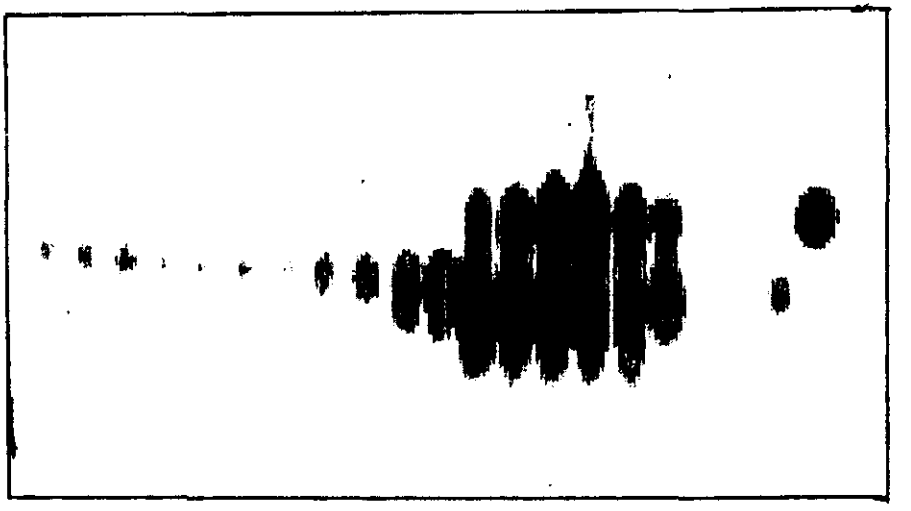

Figure 1a: Separation of monofructoseglycine by ion exchange chromatography (first column)

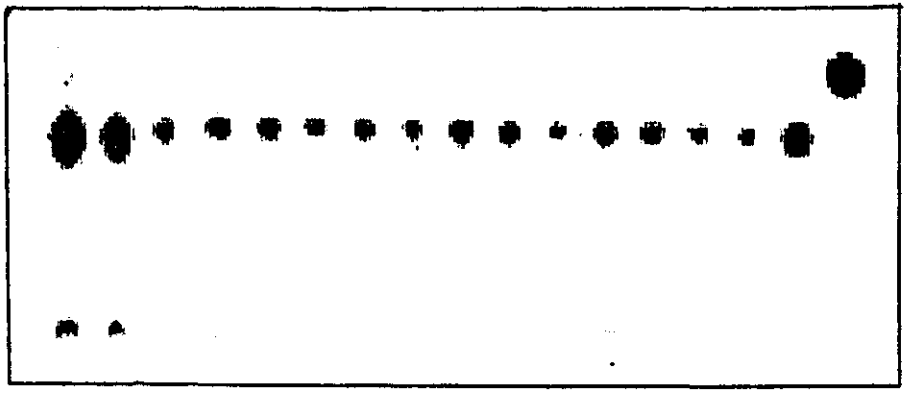

Figure 1b: Purification of monofructoseglycine by ion exchange chromatography (second column)

Figure 2a: Separation of monofructosehistidine by ion exchange chromatography (first column)

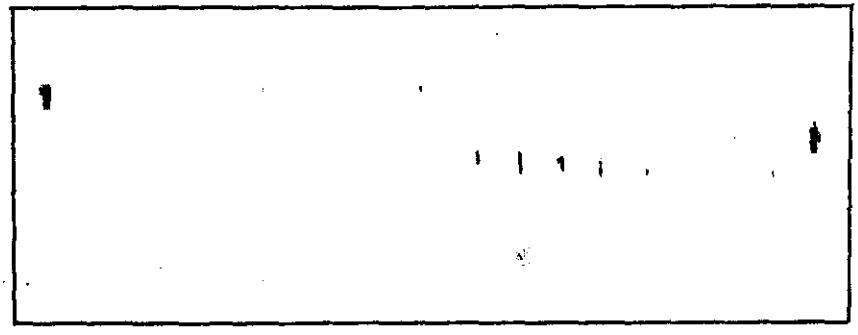

Figure 2b: Purification of monofructosehistidine by ion exchange chromatography (second column)

Table 1: Elemental analysis of MFG and MFH

Elemental analysis	Calculated for $C_8H_{15}N0_7$	Found %	Calculated for $C_{12}H_{19}N_3O_7$	Found %
C	40.50	40.35	42.98	42.40
H	6.30	6.55	6.13	6.20
N	5.90	5.60	12.53	12.15

The structures (Fig. 3a – 3b and Fig.4a - 4b) show the recorded spectra of both Amadori products which were corresponded well with reported data when comparing the spectra of β-pyranose forms. Whilst these compounds are confomationally unstable and in solution (D20) they are present as an equilibrium mixture of four forms, the predominant form being the β-pyranose (64%).

The Molecular weight of the two Amadori compounds were determined using Fast Atom Bombardment (FAB) mass spectrometry. The FAB mass spectrum of monofructoseglycine shows a molecular mass of 238 (M^+1) (Fig. 5), whereas the FAB mas spectrum of Monofructose-histidine shows a molecular mass of 318 (Fig. 6).

An attempt was made to determine whether the α-amino group in histidine was involved in the production of Amadori compound or the involvement of other nitrogen in the imidazole ring. When histidine which had its α-amino group blocked (N-CBZ-histidine) was allowed to react with glucose, there was no formation of Amadori production. This indicates that the nitrogen in the imidazole may still be available for antioxidant activity (Fig. 7).

The pure Amadori compounds were tested for their antioxidant activity using the β-carotene oxidation test and conjugated diene test. The concentration used was 15mM and the comparisons with the behaviour of the parent amino acids and citric acid were performed at the same concentration. The MFG showed a pro-oxidant effect in each test (Fig. 8 and Fig. 9). However, glycine alone at the same concentration (15 mM) showed an antioxidant effect. The reason could be that glycine has chelating properties through the α-amino group and the carboxyl group. When the α-amino is involved in

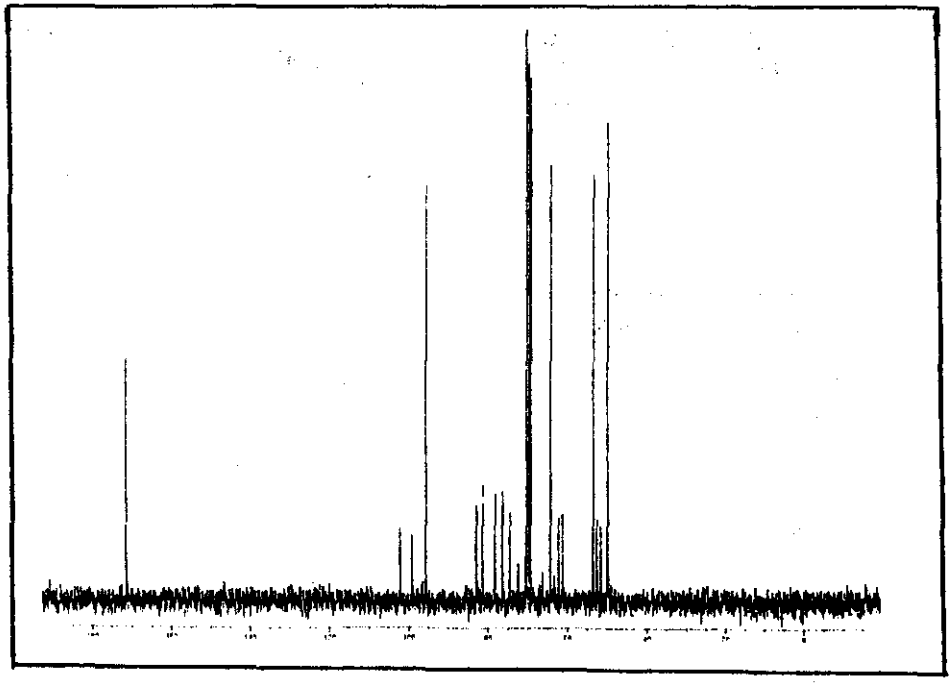

Figure 3a: ^{13}C-NMR of monofructosclycine

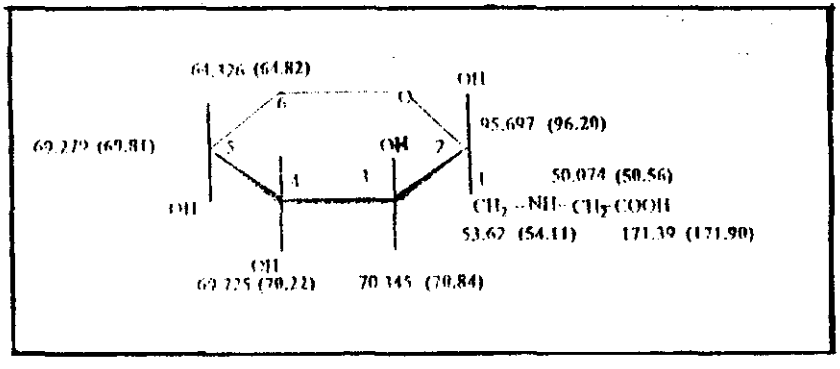

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Figure 3b: Values of ^{13}C-NMR of monofructoseglycine with reported data (Mossine *et al.*, 1994)

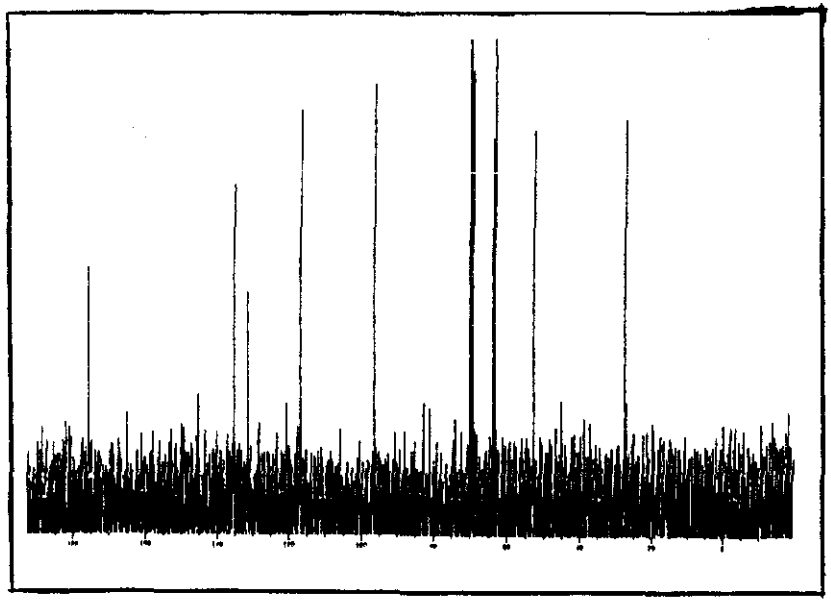

Figure 4a: ^{13}C-NMR of monofructosehistidine

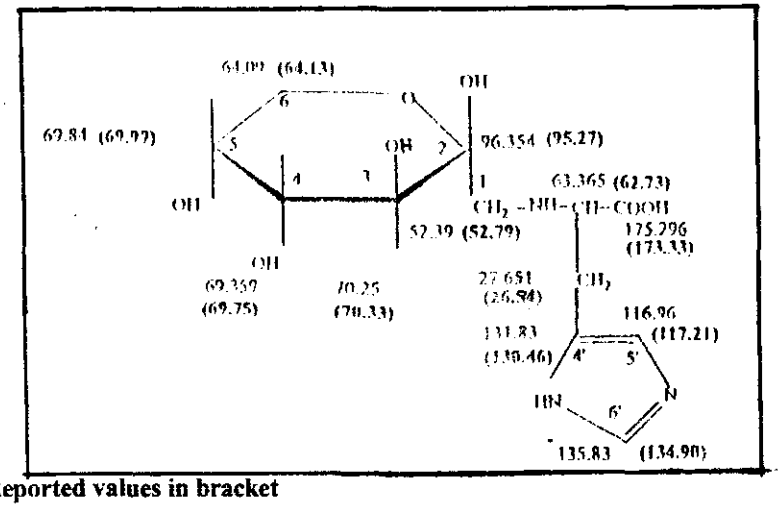

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Figure 4b: Values of ^{13}C-NMR of monofructosehistidine with reported data (Roper *et al.*, 1983)

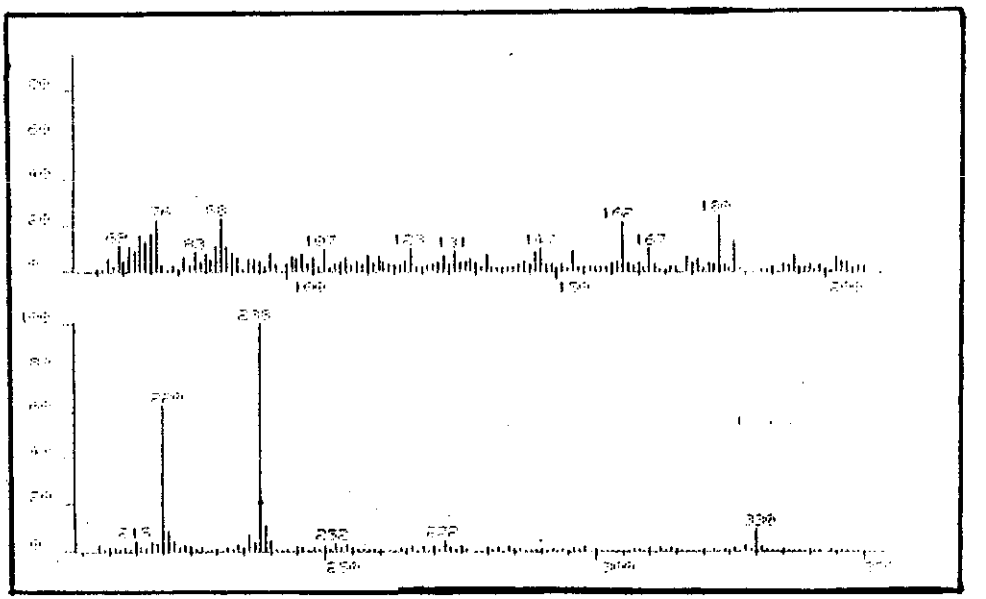

Figure 5: FAB mass spectrum of monofructoseglycine

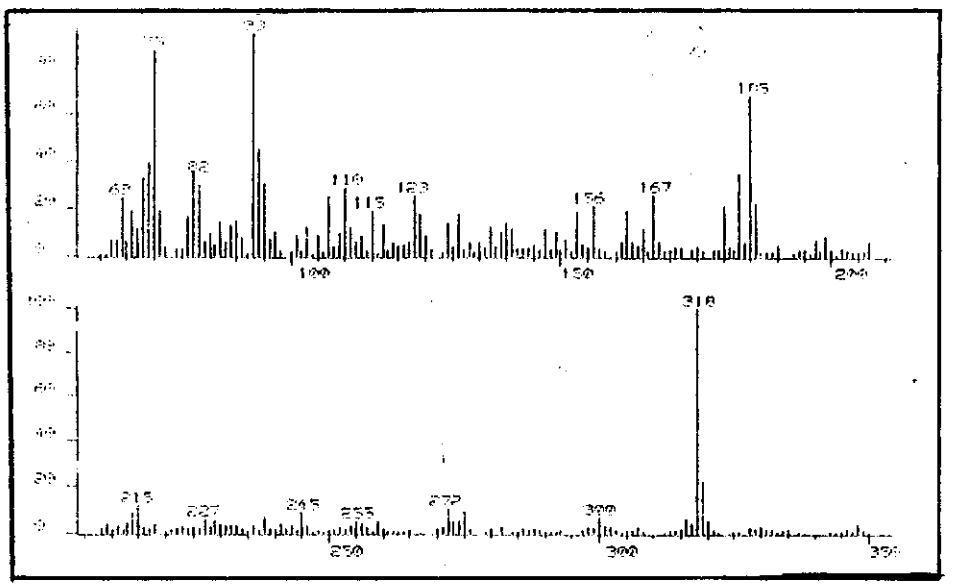

Figure 6: FAB mass spectrum of monofructosehistidine

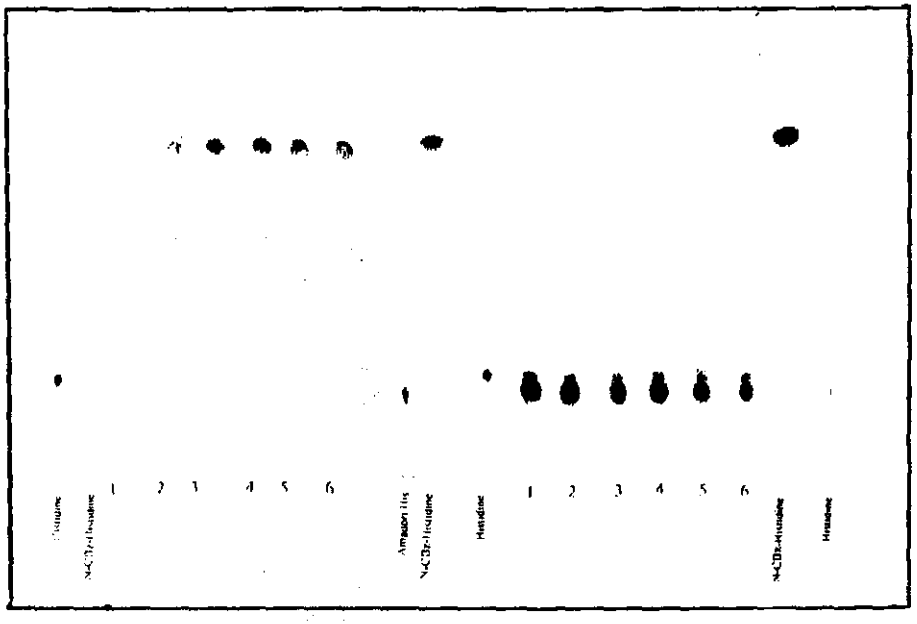

Figure 7: Synthesis of Amadori compound N-CBZ-histidine/glucose and histidine/glucose heated for one to six hours

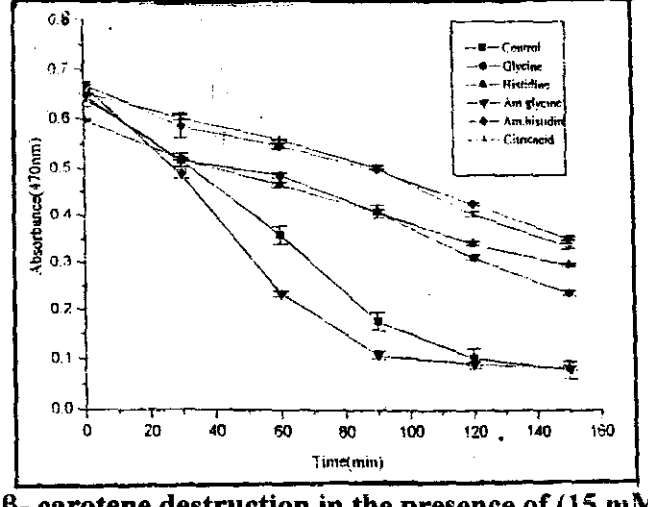

Figure 8: β- carotene destruction in the presence of (15 mM) amino acids and their Amadori products

All the data are means of three replicates. Vertical bars = ± SD

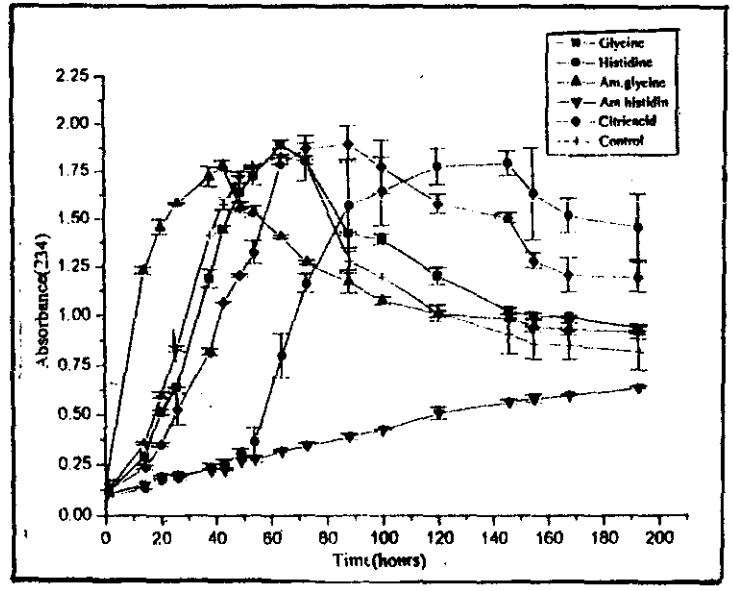

Figure 9: Effect of amino acids (glycine and histidine) and their Amadori products (15 mM) on auto-oxidation measured using the diene test

All the data are means of three replicates. Vertical bars = ± SD

the Amadori structure, the amino acid loses this effect. This result is consistent with the work done by Chen and Nawar (1991). Who found that the α-amino group was important for the antioxidant activity of some amino acids, particularly mono-amino-mono-carboxylic acids. On the other hand, a marked antioxidant effect was obtained using MFH, relative to citric acid in diene test (Fig. 8), but had a similar effect to citric acid in β-carotene test (Fig. 9). The difference in this result could be due to the difference in the system used for measuring lipid oxidation. For instance, in the β-carotene test, β- carotene oxidation is consequential on lipid oxidation, where as in diene test only lipids is involved in the reaction. Another possibility is that the β-carotene test was carried out at 50°C and this could lead to the breakdown of Amadori compound to some degree, which, in turn, leads to lesser effect than when the diene test was used.

It is clear that the parent amino acids are very important for the preparation of their Amadori product. This fact stems from the structure of the amino acids. In histidine, the imidazole ring is still active and might be responsible for

their antioxidant activity of MFH as it is in the histidine alone. However, the incorporation of sugar moiety enhances this effect, even though the α-NH_2 was involved in the Amadori structure. This could be due to several hydroxyl groups in addition to the imidazole ring and the carboxyl group. These might have a proper geometric relation to each other and can chelate metals better than histidine.

An attempt was made using the Molecular Model of MFH and Histidine whether or not the two compounds were able to interact with metals from the point of view of their sterio chemistry.

A macromodel, V 3.0 model energy minimization program using Molecular Mechanics (MMs), was used. The molecular structure of MFH showed ability to form chelates, when copper was used as a metal. The possible legating groups are the imidazole nitrogen and the two hydroxyl groups at C_3 and C_4 of the sugar moiety as shown in Fig. (10). In addition, the diene test showed a clear inhibition of the formation of conjugated diene, this suggests that MFH could act as a hydrogen donor to the peroxy radical thus retarding the autoxidation of linoleic acid by chain radical mechanisms.

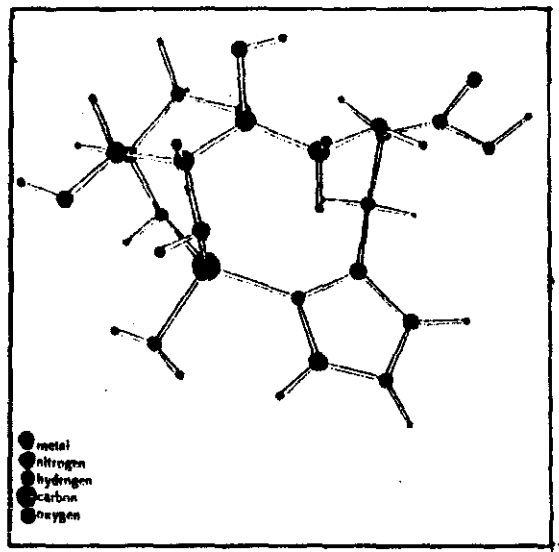

Figure 10: Molecular model of a chelate between monofructose histidine and copper

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تأثير مركبى أحادى الفركتوز جلايسين وأحادى الفركتوز هيستيدين على الأكسدة

فهيم عبد الكريم بن خيال[1] – على حمود السعدى[2] – محمد حمود السعدى[2]
[1] قسم علوم الأغذية والتكنولوجيا ، كلية الزراعة ، جامعة عمر المختار، ليبيا.
[2] قسم البيولوجى، كلية العلوم ، جامعة عمر المختار، ليبيا.

استهدفت الدراسة تخليق مركبى أحادى الفركتوز جلايسين (Monofructose glycine) وأحادى الفركتوز هيستيدين (Monofructose histidine)، بكميات على مستوى الجرامات. تم توصيف البللورات لمركبى الأمادورى والتأكد من نقاوتها وذلك باستخدام كروماتوجرافى الطبقة الرقيقة (TLC) ، التحليل العنصرى لها، الرنين المغناطيسى للكربون 13، وأيضاً طيف الكتلة لتحديد أوزانها الجزيئية باستخدام تقنية القذف الذرى السريع (FAB).

أظهرت النتائج أن مجموعة الألفا أمين (α-NH_2) لحمض الهيستيدين هى المجموعة الوحيدة التى تدخل فى تكوين مركبات الأمادورى (Amadori compounds) وهذا قد يعطى فكرة على مساهمة نتروجين حلقة الأميدازول فى حمض الهيستدين فى التأثير المضاد للأكسدة.

التأثير المضاد أو المحفز للأكسدة تم إختياره بإستخدام طريقة إنتاج الدايين (Diene test) وقصر لون البيتا كاروتين (β-Carotene bleaching test).

أعطى مركب أحادى الفركتوز جلايسين تأثير محفز للأكسدة عند نفس التركيز المستخدم (15mM) وهذا قد يدلل على: أن مجموعة الألفا أمين هامة فى التأثير المضاد للأكسدة والتى فقدت هذا التأثير عند دخولها فى تفاعلات ميلارد (Maillard)، وعلى العكس من ذلك فقد أعطى مركب أحادى الفركتوز هيستيدين تأثير مضاد للأكسدة أعلى من حمض الستريك وأيضاً حمض الهيستيدين عند نفس التركيز (15mM) وهذا واضح فى إختبار الدايين، ويرجع السبب فى ذلك إلى زيادة القدرة المخلبية لمركب أحادى الفركتوز هيستيدين عن طريق توفير مجموعتى هيدروكسيل عند الذرة رقم 3 ، 4 فى جزئ السكر فى مركب الأمادورى المختبر مع ذرة نيتروجين حلقة الإميدازول كما وضحه النموذج الجزيئى بإستخدام الحاسوب لمركب أحادى الفركتوز هيستدين وأيونات النحاس.