Antioxidant Activity in the Early Stage of the Maillard Reaction

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ABSTRACT

Seven amino acids, alanine, glycine, arginine, lysine, serine, aspartic acid and histidine were heated with glucose at 100°C and samples were taken at 5 minute intervals for 30 minutes. The resulting Maillard reaction mixtures were scanned from 200-600 nm. Antioxidant activity relative to carnosine was monitored by the Clark oxygen electrode in a lipoxygenase /linoleic acid system. The results were expressed as the protective index. Only arginine and histidine showed a significant increase in the protective index with heating time. The increase in the absorbance between 250 and 350 nm was very much greater for histidine and arginine than for the other amino acids.

Keywords: Lipid oxidation, antioxidant-Millard reaction products, oxygen electrode, lipoxygenase, amino acids.

INTRODUCTION

Lipid oxidation is one of the most important and complex deterioration reactions occurring in foods. The oxidation results initially in the formation of hydroperoxides by a free radical chain mechanism. The hydroperoxides are subjected to several further reaction forming secondary products such as aldehydes, ketones and other volatile compounds which cause rancidity and deterioration of the sensory properties of many food products (Dugan, 1976; Kochhar, 1993).

Synthetic antioxidants such as butylated hydroxy anisole (BHA) and buylated hydroxy toluene (BHT) are often used in the food industry to retard autoxidation processes. However, the possibility that synthetic substances might have deleterious effects on humans have resulted in pronounced activity in the field of natural antioxidants (Johnson and hewgill, 1961; Branen, 1975; Barlow, 1990; Pratt and Hudson, 1990, Kochhar, 1993).

Natural antioxidants in food may arise from endogenous compounds in one or more components of the food, food additives isolated from natural sources and substances formed during processing (Pratt, 1992).

The Maillard reaction between amino acids and reducing sugars, is very common in food processing and many compounds are produced during this reaction (Wedzicha, 1984).

Antioxidant activity has been detected in these complex mixtures. A number of workers have effectively antioxidant activity in the preparation of MRP in model systems and in food. Kirigaya *et al.* (1969), investigated the antioxidative effect of Maillard reaction product (MRP) from various amino acids with various reducing sugars (pH 6.5, 100°C for 5 hrs). of these, products obtained from D-glucose and L-arginine and L- histidine or L-cysteine had the strongest antioxidant activity. Tomito (1971a), reported that the MRP from a mixture of amino acid and glucose (120°C for 1 hrs) have stronger antioxidant activity than the amino acids alone. In addition, the product obtained from tryptophan and glucose was noted to be the most effective.

Lingnert and Eriksson (1980), found that the antioxidative effect of MRP is strongly affected by choice of sugar and amino acids tested, the basic ones from the most antioxidative MRP. Tanaka *et al.* (1988), reported that the antioxidative effect of MRP obtained from refluxing L-histidine and glucose, increases as a function of reaction time higher initial pH.

Elizalde *et al.* (1991), reported that the volatile MRP affected the oxidative stability of soybean oil.

Chiu et al. (1991), found that the MRP obtained from tryptophan and glucose effectively inhibited the oxidation of sardine lipids and had a synergistic effect with tocopherol. Tomita (1971b), suggested that the colourless low molecular weight substances may play a role to some extent in the antioxidant activity. Maleki (1973)observed that a significant antioxidant effect is reached at an early stage where browning has not yet occurred. Lee *et al.* (1975), showed that colourless intermediates of the Maillard reaction probably contribute to the antioxidative effect of MRP. Hwang and Kim (1973), showed that most of the effective antioxidant compounds formed during the Maillard reaction could not be the brown coloured pigments. Evidence from cooking processes (Einerson and Reineceius, 1977; Karastogiannidou and Ryley, 1994) has indicated that measurable antioxidant activity is produced without obvious browning and has been attributed by the authors to the Maillard reaction.

The primary objective of this study was to quantify the antioxidant activity developed in the early stages of the reaction between amino acids and glucose prior to the development of colour.

MATERIALS AND METHODS

I. Reagents

Tris (hydroxyl methyl) amino ethane, sodium dithionite, linoleic acid, L-serine, L-lysine, L-glycine, L-histidine, L-aspartic acid, L-alanine, lipoxidase-type 1-B, (Sigma Co. Ltd.) were used.

II. Equipment

Absorbance measurements were made using a Cecil Spectrophotometer (CE 292). pH measurements were made using a combined glass electrode connected to a digital pH- Meter (Jenway pH M6). Oxygen electrode assay was performed using the Clark oxygen electrode (Rank Brothers Ltd).

III. Model study of the antioxidative effect of Maillard reaction product

The formation of antioxidant MRP from reaction amino acids (glycine, serine, alanine, histidine, aspartic acid, arginine, lysine) and glucose was studied. The standard procedure for MRP synthetic was as follows: a solution consisting of 0.5 M of each amino acid and 1 M glucose was prepared and adjusted to pH 5.5. 20ml of the reaction mixture were taken and placed in 100 ml tubes and stoppared tightly. The tubes were then heated in a water bath at 100°C. Tubes were removed from the bath at 5 minutes intervals during the 30 minute heating period. The crude reaction mixture was used without any further fractionation for antioxidant activity assay. Perior to spectral scanning, the solutions were suitably diluted.

IV. oxygen electrode assay procedure

A procedure originally described for measurement of lipoxygenase activity (Grossman and Zakut, 1979) was slightly modified for use in the present work. The oxygen electrode was calibrated with water containing sodium dithionite (Zero oxygen) and water saturated with air 25°C (240 nmoles/ml of O_2 at 25°C). The reaction mixture contained 900 µl Of 10 mM linoleic acid, 1950 µl buffer (pH= 7) and 500 µl MRP solution or 500 µl of buffer (for the control). The reaction was started by the addition of 10 µl of lipoxygenase solution which contained approximately 100.000 units of activity per ml.

The activity was fixed by using the Sigma method for lipoxygenase activity, also the activity level of the enzyme was selected to reduce the oxygen content of the control by 90% in 5 minutes. The results were expressed as productive index (PI) defined as the time required for 90% reduction of the oxygen concentration in the antioxidant system divided the corresponding time for the control. A solution of 1000 ppm of carnosine was treated in the same way as the MRP solution.

RESULTS AND DISCUSSION

Figure (1) shows the protective index (PI) of the Millard solution as a function of heating time, relative to a solution of carnosine (1000 ppm). Histidine was the only amino acid which showed a greater antioxidant effect than carnosine prior to heating with sugar. Only histidine and arginine showed a substantial increase in the (PI) with heating. In both cases a significant increase in (PI) occurred after 20 minutes of heating. Although, the (PI) of the lysine/sugar solution increased regularly with heating time, the magnitude of the

effect was very small. The antioxidant test used in this study was enzyme catalyzed. The possible mechanisms suggested for the antioxidant activity of traditional antioxidants butylated hydroxyannisol (BHA), propyl gallate (PG) and nondihydroguatiaretic acid (NDGA) in lipoxygenase catalyzed systems include the possibility that the enzyme itself is inhibited (Hsieh,, 1994). Many mechanisms have been suggested for the antioxidant activity of MRP (Gordon, 1990) but MRP have not formerly been assessed for antioxidant activity in a lipoxygenase catalyzed system.

Under the conditions of this experiment a slight decrease in the pH was observed during heating (Table 1). This was attributed to the loss of basic groups in the Maillard reaction.

Samples taken during the reaction between glucose and different amino acids were characterized in terms of their absorbance in the range 200-600 nm. The results shown in Figs. 2a, b, c, d, e, f and g of the amino acids glycine, alanine, serine, aspartic acid, lysine, arginine and histidine respectively.

Table 1. Change in the pH of glucose/Amino acid solutions during the heating periods.

Heating time (min)	Lysine	Serine	arginine	Glycine	Aspartic acid	Histidine	Alanine
0	5.50	5.50	5.50	5.50	5.50	5.50	5.50
5	5349	5.48	5.45	5.49	5.35	5.48	5.48
10	5.47	5.45	5.43	5.47	5.34	5.46	5.48
15	5.44	5.44	5.40	5.43	5.33	5.42	5.46
20	5.41	5.42	5.39	5.41	5.33	5.42	5.45
25	5.39	5.42	5.35	5.40	5.31	5.41	5.44
30	5.34	5.41	5.34	5.38	5.30	5.41	5.44

At zero heating time, only one absorbance peak at 227 nm was observed. However, in the samples heated at 5, 10, 15, 20, 25 and 30 mins, a second peak was observed 250-350 nm. This peak increased with increasing the reaction time. Although, the occurrence of absorbance peaks at 300 nm in apparent for all the Maillard solutions tested, the rate of increase of the peak heights correlate with the rate of increase of PI. i.e. the peak height (300 nm) after 30 minutes is very much greater for histidine and arginine than for the other amino acids.

Further work will involve isolating the Amadori compounds and utilizing a wider range of antioxidant testing systems to accumulate evidence to assist in the interpretation of the mechanisms involved both in the development of

antioxidant activity in the early stages of the Maillard reaction and the antioxidant reaction mechanisms.

CONCLUSION

This study has clearly demonstrated that significant antioxidant activity is generated before substantial colour development when histidine and arginine are heated with glucose. The development of antioxidant activity in the same heating period was negligible with lysine, alanine, serine and aspartic acid.

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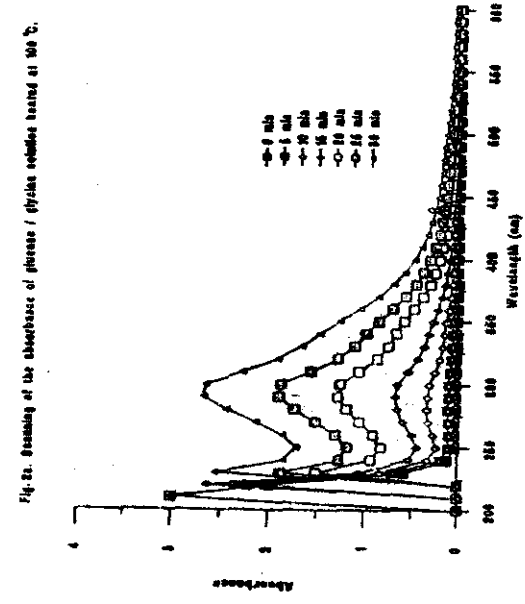

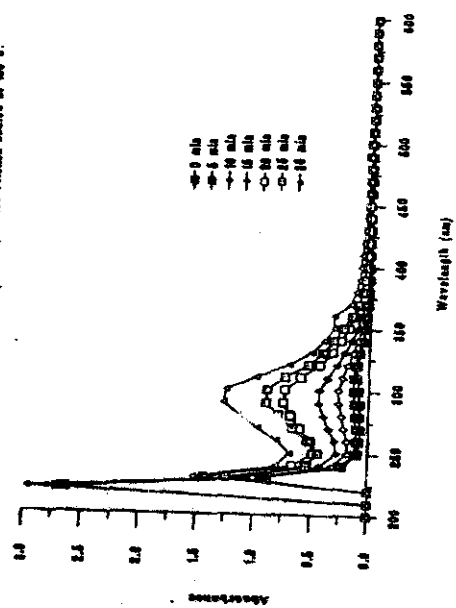

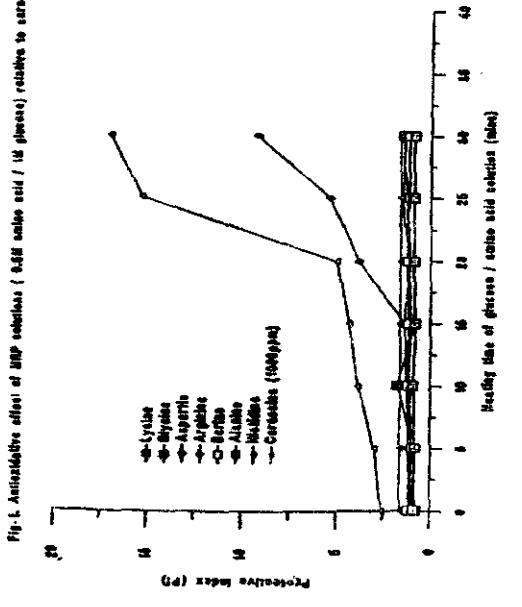

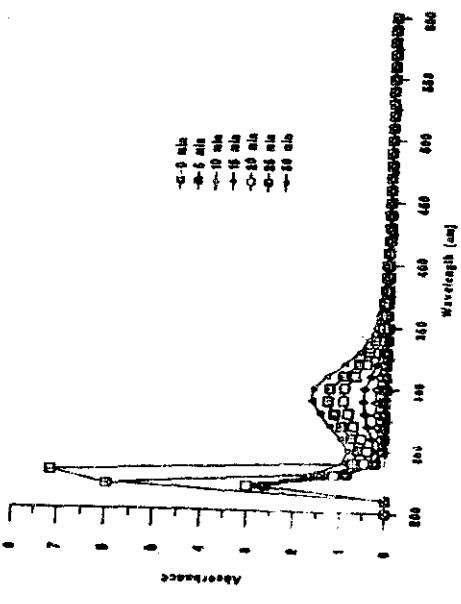

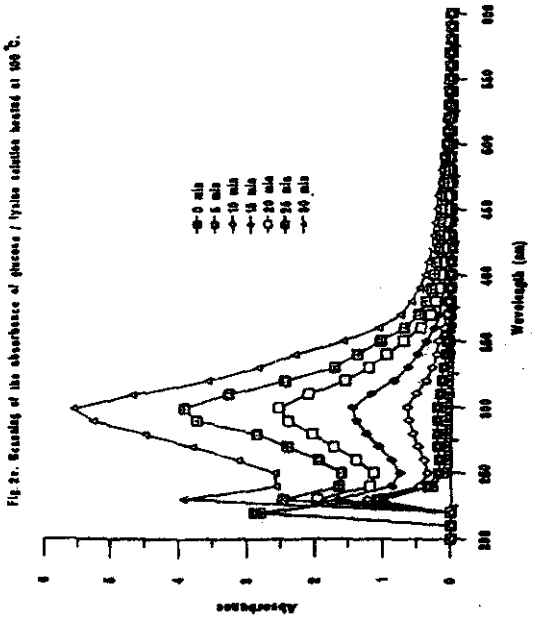

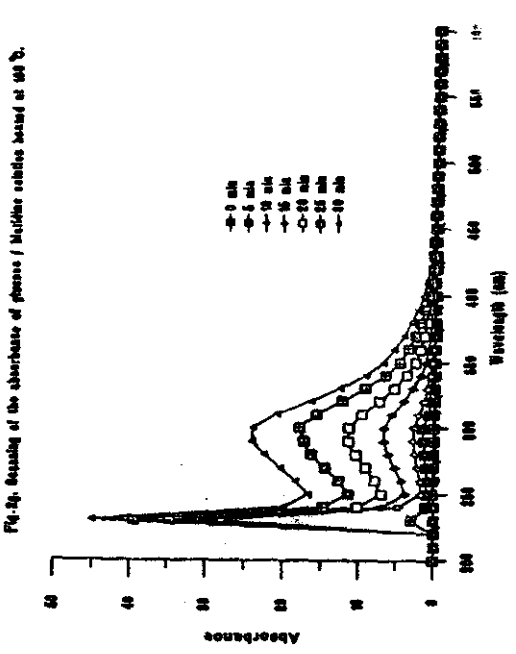

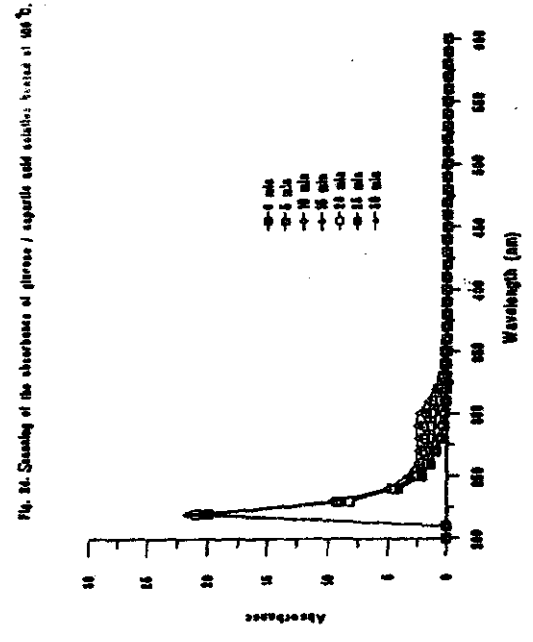

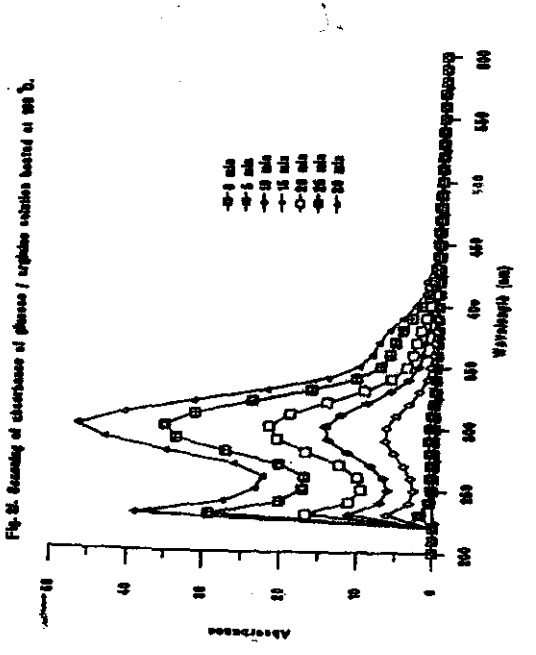

الملخص العربي

مضادات الأكسدة لنواتج تفاعل ميلارد في المراحل الأولى

فهيم عبد الكريم بن خيال

قسم علوم وتقنية الأغذية – كلية الزراعة – جامعة عمر المختار

سبعة أحماض أمينية وهي : الانين ، كلايسين ، أرجنين ، لايسين – سيرين ، حمض الاسبارتيك وهستدين . تم تسخين كل واحد منها مع الجلوكوز عند درجة حرارة ١٠٠°م . وقد سحبت عينات نواتج تفاعل ميلارد (MPRs) كل ٥ دقائق لمدة ٣٠ دقيقة وفحصت النواتج عند طول موجي من ٢٠٠ إلى ٦٠٠ نانوميتر ولختبر نشاطها المضاد للأكسدة مقارنة بالكارنوسين بواسطة قطب الأوكسجين (Clark oxygen electrode) في نظام الـ Lipoxygenase/linoleic acid حيث عبر عن النتائج بمعامل الحماية PI . وقد أظهر الارجنين والهستدين فقط زيادة جوهرية في معامل الحماية مع وقت التسخين كما أن الزيادة الامتصاصية بين ٢٥٠ و ٣٥٠ كانت كبيرة جداً للهستدين والأرجنين مقارنة بالأحماض الأمينية الأخرى .