Antioxidant Activity in the Early Stages of the Maillard Reaction
(Research Note)

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ABSTRACT
Seven amino acid, alanine, glycine, arginine, lysine, serine, aspartic acid and histidine were heated with glucose at 100 °C and samples were taken 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 absorbance between 250 and 350 nm was very much greater for histidine and arginine than for the other amino acid.

KEYWORDS: Lipid oxidation, antioxidant - Maillard 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 subject to several further reactions 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 antioxidant such as Butylated Hydroxy Anisole (BHA) and Butylated Hydroxy Toluene (BHT) are often used in the food industry to retard oxidation processes. However, the possibility that synthetic substances might have deleterious effects on humans has resulted in pronounced activity in the field of natural antioxidants (Johnson and Hewgill, 1961; Brainen, 1975; Barlow, 1990; Pratt, 1990; Kochhar, 1993).

Natural antioxidants in food may form endogenous compounds in one or more components of the food, food additives isolated from natural sources and substances formed during (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 (Hodge, 1953; Wedzicha, 1984). Antioxidant activity has been detected in these complex mixtures. A number of workers have effectively formed antioxidant activity in the preparation of MRP in model systems and in food. Kirigay et al.(1969), investigated the antioxidative effect of 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 had the strongest antioxidant activity. Tomita (1971a), reported that the MRP from mixture of amino acid and glucose (120 °C for 1 hr) 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 form the most antioxidative MRP. Tanaka et al.(1988), reported

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that the antioxidative effect of MRP obtained from Refluxing L-Histidine and glucose, increases as a function of reaction time and higher initial pH.

Elizade et al. (1991) reported that the volatile MRP affect 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 Tocoplerol. Tomita (1971b), suggested that the colourless, low molecular weight substance may play a role to some extent in the antioxidant activity. Malike (1973) observed that a significant antioxidant effect is reached at early stage where browning has not yet occurred. Lee et al.(1975) showed that colourless intermediates of the Millard reaction probably contribute to the antioxidative effect of MRP. Hwang and Kim (1973), showed that most of the effective antioxidant compounds formed during Maillard reaction could not be brown coloured pigment. Evidence from cooking processes (Einerson and Reineccius, 1977; Karastogiannidou and Ryley, 1994) have indicated that measurable antioxidants 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.

Material and Method

I. Reagents

Tris (Hydroxy Methyl) amino ethane, Sodium Dithionite, Linoleic acid, L-Serine, L-lysine, Glycine, L-Histidine, L-Aspartic acid, L-arginine, 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 made 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 the reaction between amino acids (Glycine, Serine, Alanine, Histidine, aspartic acid, Arginine, Lysine and Glucose) was studied. The standard procedure for MRP synthesis 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. 20 ml of the reaction mixture were taken and placed in 100 ml tubes and stoppered tightly. The tubes were then heated in a water bath at 100 °C. tubes were taken at 5 minutes interval for 30 minute heating period of the reaction. The crude reaction mixture was used without any further fractionation for antioxidant activity. Prior to spectral scanning, the solutions were suitably diluted.

IV. Oxygen Electrode Assay Procedure

A procedure originally described for measurement of lipoxgenase activity (Grossman and Zaklet, 1970) was slightly modified used in the present work. The oxygen electrode was calibrated with water containing sodium dithionire (Zero oxygen) and water saturated with air at 25 °C (240 nmoles / ml of O2 at 25 °C). The reaction mixture contained 900 µl of 10 mM linoleic acid, 1590 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 lipoxgenase solution which contained approximately 100.000 units of activity per ml.

The activity was fixed by using the Sigma method for lipoxgenase 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 Protective Index (PI) defined as the time required for 90% reduction of the oxygen concentration in the antioxidant system divided by 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 maillard solution at 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 lycine / sugar solution increased regularly with heating time. The magnatuted of the effect was very small, the antioxidant test used in this study was enzyme
catalysed. The possible mechanisms suggested for the antioxidant activity of traditional antioxidant butylated hydroxyanisol (PHA), Propyl Gallate (PG) and Nordihydroguaiaretic Acid (NDGA) in lipoxygenase catalysed system 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 activity in lipoxygenase catalysed system.

Under the condition of this experiment, a slightly 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 acid were characterized in terms of their absorbance in the range 200-600 nm. The result 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.

At zero heating time, only one absorption 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 between 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 solution tested, the rate of increase of the peaks high correlate with the rate of increase of PI. i.e. the peak light (300nm) after 30 minutes is very small, greater for histidine and arginine to for the other amino acids.

Further work will involve isolating the Amadori compounds and utilizing a wider range of antioxidant testing system to accumulate evidence to assist in the interpretation of the mechanism involved both in the development of antioxidant in the early stage of the Maillard reaction and the antioxidant reaction mechanism.

**Conclusion**

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

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