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# Role of sugar–amino acid interaction products (MRPs) as antioxidants in a methyl linoleate model system

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**Abstract**

Effects of early Maillard reaction products (MRPs) as antioxidants in methyl linoleate model system, during refrigerated storage at 5 °C for 6 days, have been evaluated. MRPs were prepared by reacting glucose with five different amino acids – glycine, methionine, tryptophan, aspartic acid and lysine – using two different concentrations and time intervals (30 mM/1 h, 30 mM/2 h, 60 mM/1 h and 60 mM/2 h). The preformed MRPs were incorporated, after studying pH and optical density profile, in a model system comprised of methyl linoleate and microcrystalline cellulose. Antioxidative characteristics were evaluated by estimating the thiobarbituric acid-reactive substance value (TBARS) and peroxide value (PV) and monitoring the gas chromatographic degradation pattern of methyl linoleate immediately after heating, as well as after storage at 5 °C for 2, 4 and 6 days. Analysis of variance revealed highly significant differences among TBARS, PV and the % of methyl linoleate degradation ($p < 0.01$) values, both initially and during storage in glucose + lysine and glucose + glycine with respect to control. Other treatments, such as glucose + methionine and glucose + tryptophan exhibited moderately significant differences ($p < 0.05$). But glucose + aspartic acid was not significantly different ($p > 0.05$) Significant differences were observed between 60 mM/2 h and 30 mM/1 h of MRPs with glucose + lysine and glucose + glycine ($p < 0.01$). Out of the five combinations tried, glucose + lysine and glucose + glycine exhibited good antioxidative potential at 60 mM/2 h. The % of antioxidant activity was in the order 60 mM/2 h > 60 mM/1 h > 30 mM/2 h > 30 mM/1 h for the treatments glucose + lysine > glucose + glycine > glucose + tryptophan > glucose + methionine > glucose + aspartic acid, respectively.


*Keywords:* Maillard reaction products; Antioxidants; TBARS; Glucose; Amino acids; Methyl linoleate

## 1. Introduction

Lipid oxidation is one of the primary causes of deterioration in food systems during cooking and storage, leading to the development of off-flavour, loss of colour and texture, decrease in nutritive value and the production of potentially toxic compounds (Buckeley, Morrissey, & Gray, 1995; Gray, Gomma, & Buckley, 1996; Morrissey, Sheehy, Galvin, Kerry, & Buckeley, 1998). The substrate for the reaction is generally unsaturated fatty acids, resulting in an autocatalytic process on oxidation (Simic & Taylor, 1987). To avoid or delay this auto-oxidation process, antioxidants have been utilised, with the practice being carried out successfully for over fifty years (Cuverlier, Berset, & Richard, 1994). Synthetic antioxidants, such as butylated hydroxy anisole, butylated hydroxy toluene and tertiary butyl hydroxy quinoline are commonly used as food preservatives (Verhagen, Deerenberg, Marxten Hoor, Henderson, & Kleingans, 1990) and are thus consumed in appreciable quantities by human beings (Nunn, Verhagen, & Kleinjans, 1991). However, the use of such compounds has been related to health risks resulting in strict regulations of their use in foods (Hettiarchchy, Glenn, Ganaesbandam, & Johnson, 1996). According to Ames (1983) and

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Baardseth (1989), they can have carcinogenic effects in living organisms. Because of the growing concern for the potential health hazard of synthetic antioxidants (Jones, 1992), there is renewed interest in the use of naturally occurring substances.

Maillard reaction products, generated through a reaction between amino acids or peptides with reducing sugars, are known to have antioxidative effects in food systems (Kirigaya, Kato, & Fujimake, 1968; Lingnert & Eriksson, 1980; Yamaguchi, Koyama, & Fujimaki, 1981). Some of the early MRPs are known to have antioxidative properties (Bailey, Shinlee, & Dupy, 1987; Lingnert & Eriksson, 1981). Amadori rearrangement products, the key intermediate products in the Maillard reaction are known to generate reactive oxygen, especially in the presence of transition metals (Kashimur & Mortia, 1984; Kawakishi, Okawa, & Uchida, 1990; Sakuri, Sugioka, & Nakano, 1990 and Wolff, 1993). The Maillard reaction has been found to evolve stable free radicals as well, that may interact with lipid free radicals, causing an inhibition of the lipid oxidation.

Warmed over flavour (WOF) development during chilled storage of pre-cooked meat has been of continuing interest in recent years in relation to improving the quality of ready-to-eat meals and other convenience foods. It is generally accepted that membrane phospholipids are largely responsible for the development of WOF (Igene & Pearson, 1979; St. Angelo et al., 1987). Effects of some spices and MRPs in controlling rancidity development in pre-cooked refrigerated meat and meat products have been reported by Jayathilakan, Vasundhara, and Kumudavally (1997).

The present study was conducted to evaluate the early MRPs formed from the reaction between glucose and neutral (glycine), sulphur-containing (methionine), aromatic (tryptophan), acidic (aspartic) and basic (lysine) amino acids with respect to a methyl linoleate (a primary substrate for lipid oxidation in meat) model system and also to standardise the preparation as well as application procedures for the early MRPs as antioxidants.

## 2. Materials and methods

### 2.1. Materials/chemicals

Methyl linoleate, tetraethoxy propane, glycine, methionine, tryptophan, aspartic acid and lysine were procured from Sigma chemical Co., USA, while all other chemicals were of Analar grade procured from the BDH Company.

### 2.2. Preparation of MRPs

MRPs were prepared by refluxing 30 and 60 mM concentrations of glucose in the presence of similar concentrations of glycine, methionine, tryptophan, aspartic acid and lysine in 100 ml of water for 1 and 2 h over a sand bath maintained at 100 to 110 °C. Losses in water content were periodically restored for maintaining the final volume. Four sets of each combination of 30 mM/1 h, 30 mM/2 h, 60 mM/1 h and 60 mM/2 h (of glucose and amino acids) were prepared for storage studies at 5 °C with a methyl linoleate model system.

### 2.3. Preparation of methyl linoleate model system

Chloroform solution (25 ml), containing a weighed amount of (250 mg) methyl linoleate, was mixed with 5 g microcrystalline cellulose in a 250 ml capacity RB flask. Chloroform was removed through gentle evaporation under vacuum. Preformed MRPs from all the combinations were mixed thoroughly. The flasks were heated on a boiling water bath for 35 min and cooled. One set of samples, along with control, was taken immediately for analysis of TBARS, peroxide value and gas chromatographic profile, while the other 3 sets were kept at 5 °C to monitor the storage changes in the model system. All the experiments were repeated 5 times and the values were expressed as means ± SD.

### 2.4. Sample codes

These were: 30 mM/0 h, A; 30 mM/1 h, $A_1$; 30 mM/2 h, $A_2$; 60 mM/0 h, B; 60 mM/1 h, $B_1$ and 60 mM/2 h, $B_2$ and Control, CT; glucose + glycine, $T_1$; glucose + tryptophan, $T_2$; glucose + methionine, $T_3$; glucose + aspartic acid, $T_4$ and glucose + lysine, $T_5$.

### 2.5. pH measurement

The pH of $T_1$, $T_2$, $T_3$, $T_4$ and $T_5$ under the different concentration/time combinations of MRPs – A, $A_1$, $A_2$, B, $B_1$ and $B_2$-was measured initially and after heating with the help of a Eutech instrument (Cyberscan pH 510 pH/mv/temp).

### 2.6. Optical density measurement

Optical density measurements for all the concentrations of MRPs (A, $A_1$, $A_2$, B, $B_1$ and $B_2$) under different treatments ($T_1$, $T_2$, $T_3$, $T_4$ and $T_5$) were carried out using a Chemito UV visible spectrophotometer model 160, Chemito Instruments, India. Non-enzymatic browning index was measured at 420 nm.

### 2.7. Oxidative activity of methyl linoleate

The intensity of the oxidative activity of the methyl linoleate was evaluated by:

(i) Peroxide value, estimated colorimetrically in reaction with ammonium thiocyanate ($NH_4CNS$) (Osawa & Namiki, 1981).

(ii) TBARS values expressed as malonaldehyde content, and estimated colorimetrically in reaction with 2-thiobarbituric acid (Tarladgis, Watts, & Younathan, 1960).

### 2.8. Gas chromatographic determination of methyl linoleate degradation in the presence of MRPs

Methyl linoleate degradation was determined by GC analysis using a Chemito, Model 8610 HT Gas Chromatograph fitted with a $10 \times 1/16''$ SS column packed with 'Famex', with a flame ionisation detector and computerised integrator Chemito 5000 data processor. The analysis was carried out isothermally at 190 °C. Injector and detector port temperatures were maintained at 220 and 230 °C, respectively. The flow rate of carrier gas (nitrogen) was maintained at 45 ml/min and that of hydrogen at 40 ml/min.

### 2.9. Statistical analysis

Data obtained were analysed for statistical significance using analysis of variance (ANOVA) and the significance was established at $p < 0.01$.

## 3. Results and discussion

### 3.1. Evaluation of pH in glucose and amino acid combinations

The pH values of all combinations of glucose and amino acids (A, $A_1$, $A_2$, B, $B_1$ and $B_2$), both before and after heating (after the formation of early MRPs), are depicted in Fig. 1. The initial values, for all the combinations, were slightly different, having a lower pH value for $T_4$ of B (5.66) and higher (6.68) for $T_5$. During heating, the pH values increased slightly in all cases ($p < 0.01$). $T_1$ and $T_5$ of $B_2$ showed pH values of 6.70 and 6.90, respectively, which were the highest for all the combinations and, in proportion to that, exhibited good antioxidant potential in the methyl linoleate model system. The influence of higher pH of MRPs on the lipid stability has been previously reported by Lingnert and Eriksson (1981).

### 3.2. Optical density/browning index measurement

Optical density measurements for $T_1$, $T_2$, $T_3$, $T_4$ and $T_5$ of A, $A_1$, $A_2$, B, $B_1$ and $B_2$, before and after heating, are shown in Fig. 2. Initially, all the treatments gave a nil reading which increased significantly ($p < 0.01$) during heating, with highest values of 0.09 and 0.065 for $T_5$ and $T_1$ of $B_2$, respectively. In the case of $T_4$, it was not significantly different ($p > 0.01$). Since these are early Maillard reactions involving Amadori-rearranged products, very little browning was observed in $T_1$ and $T_5$ of $B_2$. The increase in optical density values corresponds to its antioxidant activity, as reported by earlier workers (Kirigaya, Kato, & Fujimaki, 1971). Fractionation of MRPs (Yamaguchi & Fujimaki, 1973) showed that the low molecular weight products formed during the early part of the reaction, were slightly browned but, however, contributed significantly to the overall antioxidative effect. Furthermore, Maleki (1973) conducted storage experiments on a model system containing vegetable oils and reported the antioxidative effect of MRPs where actual browning had not occurred.

### 3.3. Effect of MRPs on the peroxide value of methyl linoleate

Peroxide values and the degrees of oxidative lipid deterioration, for all the different treatments CT, $T_1$, $T_2$, $T_3$, $T_4$ and $T_5$ of $A_1$, $A_2$, $B_1$ and $B_2$, in the methyl linoleate model system during storage at 5 °C are shown in Tables 1 and 2. The values for control increased significantly. ($p < 0.01$), from 0.318 to 0.620 mg/kg, after 6 days of storage, while the corresponding increase in PV was less in all the treated samples. The increase was minimum in the case of $B_2$ of $T_1$ and $T_5$, indicating greater capability of retarding the peroxidation for these MRPs. The values for $T_5$ (0.098 mg/kg) and $T_1$ (0.128 mg/kg), when compared with CT (0.620 mg/kg), were significantly ($p < 0.01$) different. From the data reported in Tables 1 and 2, it could be clearly seen that $T_5$ and $T_1$ exhibited good antioxidative characteristics by inhibiting the lipid peroxidation under all concentration/time conditions during storage. The antioxidative properties of early MRPs have already been reported (Bailey et al., 1987; Lingnert & Eriksson, 1981; Namiki & Hayashi, 1975). MRPs were reported to have the capability of forming stable free radicals thus causing the inhibition of lipid oxidation.

### 3.4. Evaluation of TBARS values of methyl linoleate with MRPs

Changes in TBARS (measure of lipid oxidation) in the methyl linoleate model system consisting of MRPs ($T_1$, $T_2$, $T_3$, $T_4$ and $T_5$) during storage for $A_1$, $A_2$, $B_1$ and $B_2$ were evaluated and the trends in the results are depicted in the representative Fig. 3. Among the four different concentration/time groups, $B_2$ exhibited lower TBARS values during storage with all the treatments. Out of the five treatments, $T_5$ and $T_1$ showed reduced values of TBARS (0.213 and 0.281 mg/kg) for linoleate after 6 days of storage compared with CT, (1.14 mg/kg) which was significantly ($p < 0.01$) higher, thus showing good antioxidative characteristics of these treatments. Even though there was a slight decrease in the TBARS

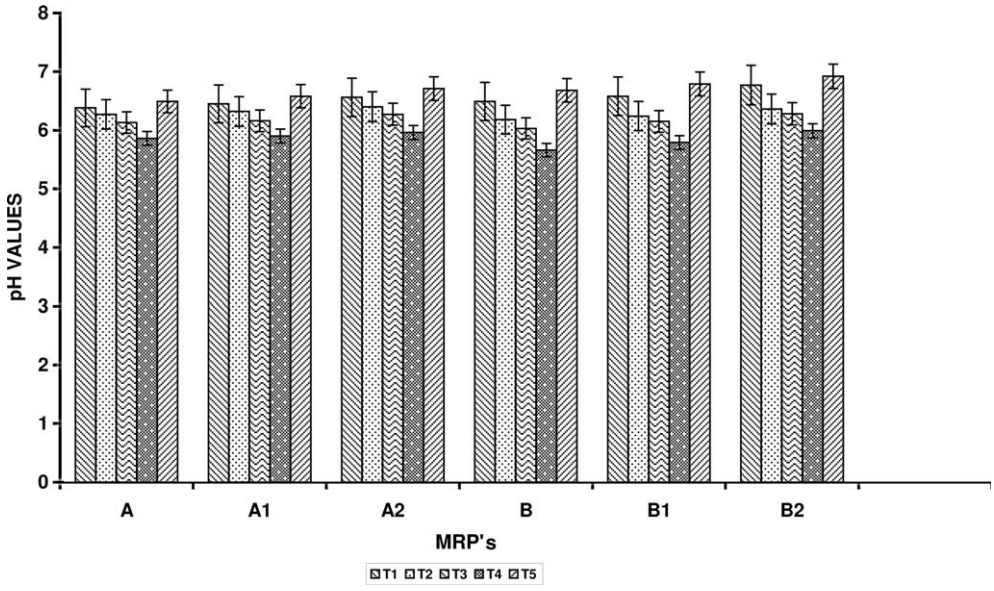


Fig. 1. pH values (means ± SD) for different combinations of MRPs ($n = 5$).

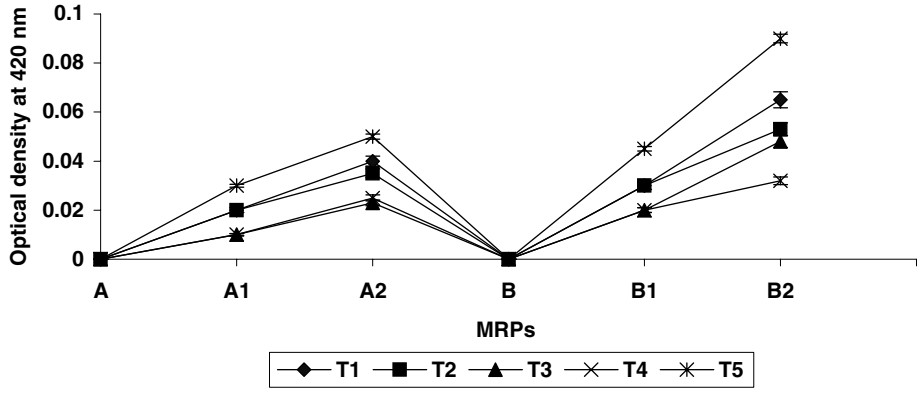


Fig. 2. Optical density values (means ± SD) of different combinations of MRPs ($n = 5$).

Table 1
Peroxide value (mg/kg)[a,b] of methyl linoleate model system during storage at 5 °C

| Sample | 30 mM/1 h | | | | 30 mM/2 h | | | |
|---|---|---|---|---|---|---|---|---|
| | Storage period (days) | | | | Storage period (days) | | | |
| | 0 | 2 | 4 | 6 | 0 | 2 | 4 | 6 |
| CT | 0.318 ± 0.019[a] | 0.409 ± 0.020[a] | 0.516 ± 0.023[a] | 0.620 ± 0.018[a] | 0.318 ± 0.019[a] | 0.409 ± 0.020[a] | 0.516 ± 0.023[a] | 0.620 ± 0.018[a] |
| $T_1$ | 0.146 ± 0.011[b] | 0.176 ± 0.009[b] | 0.186 ± 0.013[b] | 0.210 ± 0.007[b] | 0.141 ± 0.009[b] | 0.167 ± 0.014[b] | 0.177 ± 0.016[b] | 0.162 ± 0.009[b] |
| $T_2$ | 0.216 ± 0.009[c] | 0.285 ± 0.016[c] | 0.359 ± 0.018[c] | 0.399 ± 0.008[c] | 0.209 ± 0.012[c] | 0.260 ± 0.006[c] | 0.333 ± 0.009[c] | 0.373 ± 0.018[c] |
| $T_3$ | 0.223 ± 0.013[c] | 0.290 ± 0.009[c] | 0.379 ± 0.016[c] | 0.470 ± 0.011[c] | 0.225 ± 0.015[c] | 0.275 ± 0.018[c] | 0.365 ± 0.009[c] | 0.409 ± 0.008[c] |
| $T_4$ | 0.301 ± 0.012[a] | 0.391 ± 0.020[a] | 0.499 ± 0.021[a] | 0.599 ± 0.018[a] | 0.300 ± 0.024[a] | 0.380 ± 0.013[a] | 0.488 ± 0.018[a] | 0.580 ± 0.010[a] |
| $T_5$ | 0.125 ± 0.009[b] | 0.132 ± 0.006[b] | 0.142 ± 0.003[b] | 0.162 ± 0.018[b] | 0.111 ± 0.013[b] | 0.128 ± 0.016[b] | 0.123 ± 0.008[b] | 0.120 ± 0.009[b] |

Values are shown as means ± SD ($n = 5$).
Within a column, values superscripted with different letters are significantly different. ab, ($p < 0.01$) ac and bc ($p < 0.05$).
Values with same letters are not significantly different ($p > 0.05$).

values for $T_4$ compared with the control, the values were not significantly ($p > 0.05$) different. Other treatments, such as $T_2$ and $T_3$, exhibited moderate antioxidative potential in controlling the rancidity development during storage. The TBARS values with respect to control were significant at $p < 0.05$. From the TBARS values, it was

Table 2
Peroxide value (mg/kg)[a,b] of methyl linoleate model system during storage at 5 °C

| Sample | 60 mM/1 h | | | | 60 mM/2 h | | | |
|---|---|---|---|---|---|---|---|---|
| | Storage period (days) | | | | Storage period (days) | | | |
| | 0 | 2 | 4 | 6 | 0 | 2 | 4 | 6 |
| CT | 0.318 ± 0.019[a] | 0.409 ± 0.020[a] | 0.516 ± 0.023[a] | 0.620 ± 0.018[a] | 0.318 ± 0.019[a] | 0.409 ± 0.020[a] | 0.516 ± 0.023[a] | 0.620 ± 0.018[a] |
| $T_1$ | 0.129 ± 0.008[b] | 0.148 ± 0.009[b] | 0.155 ± 0.018[b] | 0.158 ± 0.020[b] | 0.100 ± 0.006[b] | 0.107 ± 0.011[b] | 0.125 ± 0.009[b] | 0.128 ± 0.013[b] |
| $T_2$ | 0.200 ± 0.009[c] | 0.250 ± 0.013[c] | 0.318 ± 0.018[c] | 0.338 ± 0.016[c] | 0.181 ± 0.019[c] | 0.200 ± 0.015[c] | 0.308 ± 0.018[c] | 0.335 ± 0.013[c] |
| $T_3$ | 0.211 ± 0.010[c] | 0.261 ± 0.018[c] | 0.353 ± 0.020[c] | 0.391 ± 0.018[c] | 0.200 ± 0.015[c] | 0.250 ± 0.021[c] | 0.342 ± 0.019[c] | 0.371 ± 0.025[c] |
| $T_4$ | 0.290 ± 0.016[a] | 0.379 ± 0.024[a] | 0.480 ± 0.030[a] | 0.576 ± 0.025[a] | 0.282 ± 0.018[a] | 0.370 ± 0.026[a] | 0.471 ± 0.019[a] | 0.570 ± 0.025[a] |
| $T_5$ | 0.100 ± 0.009[b] | 0.103 ± 0.008[b] | 0.110 ± 0.016[b] | 0.115 ± 0.008[b] | 0.092 ± 0.006[b] | 0.092 ± 0.06[b] | 0.098 ± 0.007[b] | 0.098 ± 0.007[b] |

Values are shown as means ± SD ($n = 5$).
Within a column, values superscripted with different letters are significantly different. ab, ($p < 0.01$) ac and bc ($p < 0.05$).
Values with same letters are not significantly different.

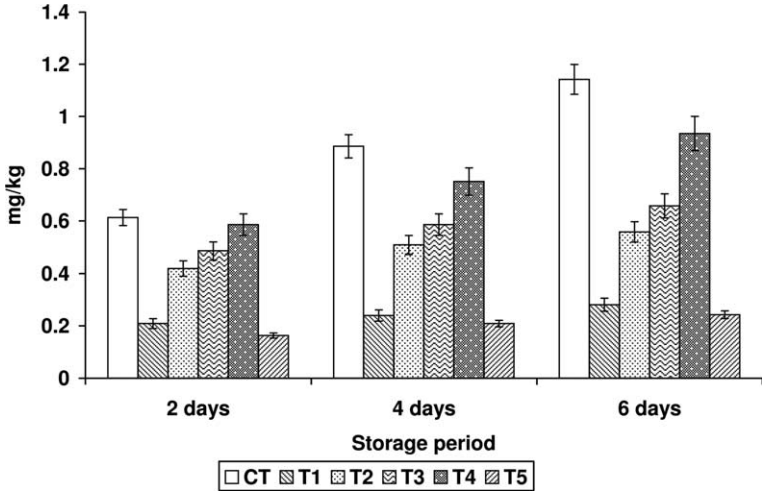


Fig. 3. TBARS values (means ± SD) of methyl linoleate system with MRPs during storage at 5 °C ($n = 5$).

revealed that the order of antioxidative potential of MRPs within the treatments was $T_5 > T_1 > T_2 > T_3 > T_4$ and $B_2 > B_1 > A_2 > A_1$. This is in accordance with the peroxide values. All these studies were also carried out for 90 mM/1 h and 90 mM/2 h batches. However, the results were found to be similar to those for 60 mM/1 h and 60 mM/2 h batches. The antioxidative potential of MRPs, in controlling the oxidative rancidity, has been reported by Chiou (1992). Bedihaus and Ockerman (1995) have reported the use of MRPs incorporation, prior to cooking, to control the lipid oxidation in fresh ground pork patties. Smith and Alfarvaz (1995) have also reported the positive effects of using MRPs as natural antioxidants in controlling the oxidative breakdown of the fat in cooked ground beef. The MRPs used in the present study were early reaction products, as indicated by their browning index and pH, and were found to exhibit desirable antioxidative effects in controlling warmed over flavour and rancidity development. The MRPs have been reported to retard the formation of secondary oxidation products formed mainly during storage at low temperature (Chiou, 1992).

### *3.5. Methyl linoleate degradation profile by gas liquid chromatography*

Studies carried out to assess the antioxidative characteristics of MRPs at various concentrations and times revealed that the 60 mM/2 h combination had comparatively better antioxidative potential in terms of PV and TBARS values (Tables 1 and 2 and Fig. 3). Methyl linoleate has been reported to be the primary substrate for oxidation in meat and hence its degradation was followed to monitor the onset of rancidity. Methyl linoleate concentration, selected in this study, was based on the nominal level of occurrence in the meat system. Immediately upon heating, slight differences were observed in different treatments with respect to loss of methyl linoleate compared with control and it varied from 5.29% to 8.75% ($p < 0.05$). But, on storage at 5 °C for 6 days, the loss of methyl linoleate in control (CT) increased to 8 times, followed by $T_4$, $T_3$ and $T_2$ during storage. Within the storage period for different treatments, $T_1$ and $T_5$ exhibited significant ($p < 0.01$) differences from CT. $T_2$ and $T_3$ exhibited moderate

Table 3
Methyl linoleate degradation (% loss[a,b]) in the presence of MRPs (60 mM/2 h) during storage at 5 °C

| Sample | Initial | 2 days | 4 days | 6 days |
|---|---|---|---|---|
| CT | 8.75 ± 1.26[a] | 28.46 ± 2.09[a] | 48.6 ± 3.25[a] | 68.3 ± 5.26[a] |
| $T_1$ | 5.68 ± 0.98[c] | 9.26 ± 1.08[b] | 12.5 ± 2.16[b] | 19.0 ± 1.89[b] |
| $T_2$ | 6.09 ± 1.39[c] | 20.4 ± 2.16[c] | 32.3 ± 3.46[c] | 48.9 ± 3.26[c] |
| $T_3$ | 8.11 ± 1.68[a] | 18.6 ± 0.99[c] | 29.3 ± 2.18[c] | 36.5 ± 4.09[c] |
| $T_4$ | 8.56 ± 2.19[a] | 25.3 ± 1.86[a] | 45.3 ± 3.98[a] | 62.6 ± 5.18[a] |
| $T_5$ | 5.59 ± 1.86[c] | 8.16 ± 0.85[b] | 11.0 ± 1.68[b] | 13.3 ± 1.26[b] |

CT = Control, $T_1$ = Glu + Gly, $T_2$ = Glu + Met, $T_3$ = Glu + Try, $T_4$ = Glu + Asp, $T_5$ = Glu + Lys.
Values are shown as means ± SD ($n = 5$).
Within a column, values superscripted with different letters are significantly different. ab, ($p < 0.01$) ac and bc ($p < 0.05$).
Values with same letters are not significantly different ($p > 0.05$).

differences from CT ($p < 0.05$), but there were no significant differences observed in the values of CT and $T_4$ ($p > 0.05$). In the GLC analysis, in addition to the undegraded methyl linoleate peak, there were a few additional peaks observed, probably corresponding to oxidation products and other chloroform-soluble volatile components. The latter were not quantified. The retardation effect was more pronounced with respect to oxidative breakdown of methyl linoleate in heated and stored samples (Table 3). $T_5$ and $T_1$ treatments, with the methyl linoleate model system, exhibited good stability characteristics during storage by controlling the extent of degradation. The percentage of degradation of methyl linoleate was in the order $CT > T_4 > T_3 > T_2 > T_1 > T_5$. These results are in accordance with the findings of the oxidative rancidity studies based on PV and TBARS presented in Table 2 and Fig. 3.

## 4. Conclusion

Early MRPs exhibited good antioxidative potential in a methyl linoleate model system. Out of the five different amino acids studied, lysine and glycine, in combination with glucose, produced antioxidative MRPs capable of inhibiting lipid peroxidation. Concentration, temperature and time for the preparation of the MRPs were an important for in assessing the retardation effects of MRPs on the oxidation of lipids. MRPs prepared at 60 mM concentration of glucose and lysine, for 2 h at 100–110 °C, exhibited the most antioxidative effect. Early MRPs, generated from basic amino acids, produced highest antioxidant potential, followed by neutral, aromatic, sulphur-containing and acidic amino acids (being the lowest). Since the studies were carried out in methyl linoleate, which is a primary substrate for evaluating the oxidation in meat, the most antioxidative MRPs of glucose + lysine and glucose + glycine could be employed as natural antioxidants for controlling the development of warmed over flavour as well as rancidity in precooked refrigerated stored meat and meat products.

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