JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antioxidant Properties of Malt Model Systems

THOMAS S. SAMARAS, MICHAEL H. GORDON, AND JENNIFER M. AMES*

School of Food Biosciences, The University of Reading, Whiteknights, P.O. Box 226, Reading, RG6 6AP, United Kingdom

The aim of this study was to investigate the effect of kilning and roasting temperatures on antioxidant activity of malt model systems prepared from combinations of glucose, proline, and ferulic acid. Model systems (initial $a_w = 0.09$, 6% moisture) were heated at 60 °C for up to 24 h, at 90 °C for up to 120 min, and at 220 °C for up to 15 min. The antioxidant activity of the glucose−proline−ferulic acid model system increased significantly on heating at 60 °C for 24 h or at 90 °C for 120 min. In contrast, the glucose−proline, ferulic acid−glucose, and ferulic acid−proline systems presented either nonsignificantly increased or unchanged antioxidant activity. The antioxidant activity of both the glucose−proline−ferulic acid and glucose−proline model systems increased significantly after heating at 220 °C for 10 min, followed by a significant decrease at 15 min. The data suggest that (1) at 60 °C, ferulic acid reacts with Maillard reaction products, resulting in a significant increase in antioxidant activity; (2) at 90 °C, the antioxidant activity of the glucose−proline−ferulic system comes from both ferulic acid and Maillard reaction products; and (3) at 220 °C, the major contributors to antioxidant activity in glucose−proline−ferulic acid and glucose−proline systems are glucose−proline reaction products.

KEYWORDS: Antioxidant activity; ferulic acid; ABTS$^{\bullet+}$ assay; ORAC assay; capillary electrophoresis; Maillard reaction

INTRODUCTION

Lipid oxidation leads to the deterioration of food quality due to the production of compounds, e.g., *trans*-2-nonenal, hexanal (*1*), that give stale flavors. Antioxidants delay stale flavor development, increasing flavor stability and extending the shelf life of foods. In addition, antioxidants, such as phenolic compounds (*2*) and Maillard reaction products (MRPs) (*3*), may have health benefits, including protection against cancer and cardiovascular disease.

The antioxidant activity of heat-processed foods, e.g., malt, tomato juice, coffee, comes from MRPs, e.g., reductones, melanoidins, and from components, e.g., phenolic compounds, present in their raw materials (barley, tomato, green coffee) (*4*−*6*). However, the role of phenolic compounds in the development of antioxidant activity in heated sugar−amino compound systems is unknown (*7*).

In an attempt to better understand the antioxidant activity of specific components, many researchers have employed the use of model systems (*8*−*12*). This approach has the benefit of simplicity, since the interaction of antioxidants with other food components is avoided. Moreover, the conditions for the development of antioxidant activity are controlled.

The development of antioxidant activity in heated carbohydrate−amino acid systems has been extensively studied (*9*, *13*−*15*). Different carbohydrate−amino acid combinations and different reaction conditions, e.g., heating temperature and time, have been employed. However, the development of antioxidant activity in systems comprising phenolic compounds and carbohydrates and/or amino acids has only been considered recently (*16*).

Therefore, the objectives of this study were to assess the effect of heating at temperatures relevant to kilning and roasting on the total antioxidant activity of model malt systems comprised of glucose, proline, and ferulic acid; to investigate the effect of heating on the fate of the reaction precursors; and to interpret changes in antioxidant activity in terms of changes in composition.

MATERIALS AND METHODS

Chemicals and Reagents. All chemicals and reagents were the purest grade available. L-Proline, ferulic acid (4-hydroxy-3-methoxycinnamic acid), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, sodium dihydrogen orthophosphate dehydrate, dipotassium hydrogen orthophosphate, sodium nitrate, capsules containing peroxidase and glucose oxidase, *o*-dianisidine dihydrochloride, glucose standard solution (5.56 mmol/L), and anhydrous D-(+)-glucose were obtained from Sigma (Poole, UK). Microcrystalline cellulose was obtained from Merck (Damstadt, Germany). Disodium fluorescein and sodium tetraborate decahydrate were from Fluka (Gillingham, UK). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was from Aldrich (Gillingham, UK). High-purity water was produced in-house using a Purite (High Wycombe, UK) Labwater R050 unit and was used throughout.

* To whom correspondence should be addressed. Tel: +44(0)118 931 8730. Fax: +44(0)118 931 0080. E-mail: j.m.ames@reading.ac.uk.

10.1021/jf0501600 CCC: $30.25
Published on Web 04/28/2005

Table 1. Composition of Model Malt-like Systems

model system[a]	proline (mg)	glucose (mg)	ferulic acid (mg)
Pr–Gl–Fe–Ce	253.8	309	17
Pr–Gl–Ce	253.8	309	–
Pr–Fe–Ce	253.8	–	17
Gl–Fe–Ce	–	309	17
Fe–Ce	–	–	17

[a] Pr, proline; Gl, glucose; Fe, ferulic acid; Ce, cellulose (used to give a total mass of 20 g).

Preparation of Model Systems. Model systems (20 g) consisting of five different combinations of glucose, proline, ferulic acid, and cellulose (as the matrix) were prepared as indicated in Table 1. Glucose, proline, ferulic acid, and cellulose were weighed on a five-figure decimal place balance. The amount of cellulose was adjusted for each model system so that the total mass in each case was 20 g. The solids were weighed separately and mixed with 40 mL of potassium phosphate buffer (15 mM, pH 5.4) and 20 mL of water. Each mixture was agitated to obtain homogeneity and samples were frozen at −20 °C and then freeze-dried for 3 days. Each mixture was transferred to a preweighed Petri dish, which was weighed again before placing in a vacuum desiccator containing saturated sodium nitrate solution at 20 °C. Samples were agitated daily to ensure equilibration and remained in the desiccator until constant mass was reached (2−3 days), giving samples with a moisture content of ∼6% ($a_w = 0.09$). The equilibrated samples were removed and divided into four equal portions (5 g each). One portion remained unheated and the other three were transferred to glass Petri dishes, distributed evenly in a thin layer, and heated in a preheated oven. Three different temperature−time combinations were used: 60 °C for 8, 16, and 24 h; 90 °C for 60, 90, and 120 min; and 220 °C for 5, 10, and 15 min. After removal from the oven, each sample was transferred to a labeled centrifuge tube and 15 mL of water was added. Tubes were centrifuged at 2012*g* for 5 min and the supernatant was 0.2-μm filtered. Filtrates were stored at −20 °C for <7 days prior to analysis. All the model systems were prepared in triplicate.

UV−Visible Spectrophotometry. The absorbance of triplicate samples was measured at 420 nm in a 1-cm glass cuvette using a Perkin−Elmer (Beaconsfield, UK) Lambda 5 UV/Vis scanning spectrophotometer equipped with UV Winlab software and connected to a thermostatically controlled (25 °C) chamber and an automatic sample positioner.

Quantification of Glucose. The AOAC official method (969.39) was used to determine glucose in the filtrates. One capsule containing 500 units of glucose oxidase and 100 units of peroxidase was dissolved in 100 mL of water. *o*-Dianisidine dihydrochloride (50 mg) was dissolved in 20 mL of water and mixed with enzyme solution to give the combined enzyme colored reagent solution. Blank, glucose standard, or sample (0.2 mL) was added into 2 mL of combined enzyme color reagent solution, and the contents were mixed thoroughly. All mixtures were incubated at room temperature (18−26 °C) for 45 min. Exposure to daylight was avoided by covering cuvettes with aluminum foil. At the end of the incubation period, the absorbance of the samples was read at 540 nm. Readings were completed within 30 min. A standard calibration curve was constructed and used to quantify glucose in the model system filtrates.

Quantification of Ferulic Acid and Proline and Formation of New Compounds. Ferulic acid and proline were quantified, and the formation of new compounds was monitored by capillary electrophoresis (CE). The CE system (Agilent, Bracknell, UK) was equipped with a diode-array detector, an autosampler, and ChemStation software for data collection, data analysis, and system control. The fused silica capillary was 48.5 cm total length (40 cm to the detector), 50 μm i.d. Separations were performed at 25 °C using a voltage of 20 kV (ferulic acid) or 25 kV (proline). Samples were injected at 50 mBar for 5 s. Separations were monitored at 200 nm (ferulic acid and proline) and at 200, 280, 325, and 420 nm (new compounds). Spectra were collected between 190 and 600 nm. The capillary was conditioned before use as follows: 30 min flush with NaOH (1 M) followed by a 20 min flush with NaOH (0.1 M) and 15 min with water. Finally, the capillary was flushed for 15 min with running buffer, which was 50 mM borate, pH 9.5 (for ferulic acid) or 20 mM phosphate, pH 2.5 (for proline). Two injections were carried out for each replicate sample. Between runs, the capillary was flushed with 0.1 M NaOH for 3 min and running buffer for 3 min. Ferulic acid and proline were quantified by reference to standard calibration curves. The appearance of peaks was monitored with heating time in the electrophoretograms obtained using both running buffers.

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) Radical Cation ($ABTS^{\bullet+}$) Scavenging Activity. The antioxidant activity of the samples was determined by the $ABTS^{\bullet+}$ decolorization assay (*17*) as previously reported (*16*). A standard calibration curve was constructed by plotting percentage inhibition against concentration of ferulic acid. Antioxidant activities of the filtrates were calculated in ferulic acid equivalents using the calibration curve. Triplicate determinations were performed for the standards and each replicate sample was analyzed once.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC assay developed by Ou et al. (*18*) was used to determine the antioxidant activity of samples.

Statistical Analysis. Statistical analysis was performed using Minitab 13.1 for Windows. One-way analysis of variance (ANOVA) was used to determine if there were any statistically significant differences between the mean values. Tukey's test was then used to determine which mean values were different.

RESULTS AND DISCUSSION

Choice of Reactants and Heating Conditions. The imino acid proline (Pr) was selected as it is the most abundant amino acid or similar compound in both green and finished malt (*19*). Glucose (Gl) was selected as the carbohydrate because it is ubiquitous in foods and, being a reducing sugar, participates in the Maillard reaction. Ferulic acid (Fe) is the most abundant phenolic acid in green and finished malt (*19*). The levels of all of the reactants in the model systems were chosen to maintain the proline:glucose:ferulic acid ratio in green malt. The concentration of each reactant is about 5-fold higher than in green malt. Higher concentrations were used to facilitate analysis. Microcrystalline cellulose (Ce) was used as the matrix for the model systems because it is relatively inert. The initial a_w chosen was 0.09. This corresponds to a moisture content of ∼ 6% and was chosen because Chandra et al. (*4*) clearly demonstrated that at this moisture level the antioxidant effect of malts increased sharply. Three different combinations of temperature and time were chosen because of their relevance to standard and speciality malt production. Standard malts, such as pale and lager malt, are produced by kilning at 50−85 °C over 28−32 h (*19*). Temperatures of 80−100 °C are applied for up to 170 min for cara malt production (*20*, *21*). Colored malts are roasted at temperatures of up to 240 °C (*20*, *22*).

Visible Absorption Data for the Model Systems. Color development, measured by absorption at a single wavelength in the range 420−490 nm, is the most frequently used parameter to monitor the extent of browning due to the Maillard reaction (*23*). Absorbance measurements at 420 nm for all systems are depicted in Figure 1.

For systems Pr−Gl−Fe−Ce and Pr−Gl−Ce heated at 60 °C, absorbance readings increased significantly between 16 and 24 h of heating, implying that color generation was only significant over the last 8 h of heating. The lowest absorbance values were found for the 90 °C model systems, but relatively little color had developed after 120 min (the maximum time of heating at this temperature), indicating that the least reaction had occurred under these conditions. A gradual increase in absorbance was

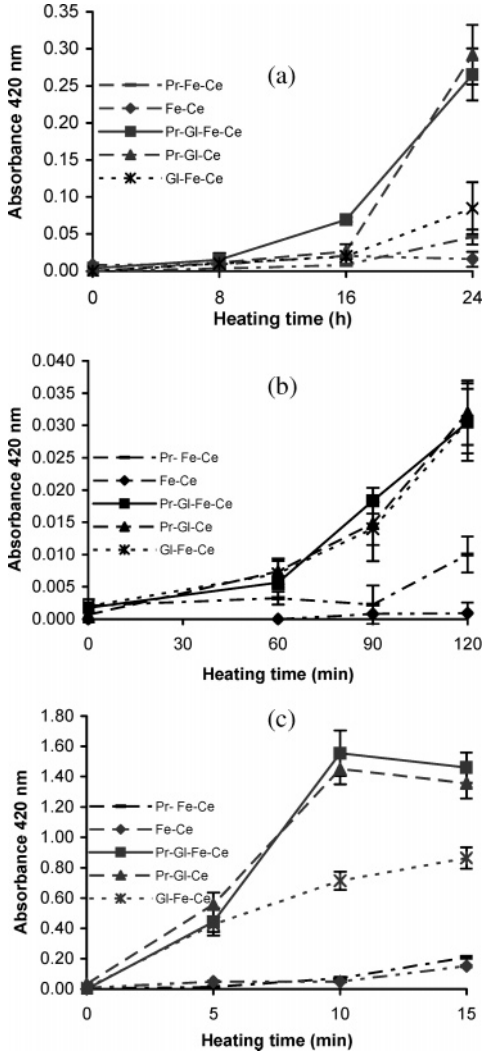


Figure 1. Absorbance at 420 nm of filtrates of model systems. Model systems were heated at (**a**) 60 °C, (**b**) 90 °C, and (**c**) 220 °C. The error bars represent the standard deviation of mean values for triplicate samples. Ce, cellulose; Fe, ferulic acid; Pr, proline; Gl, glucose.

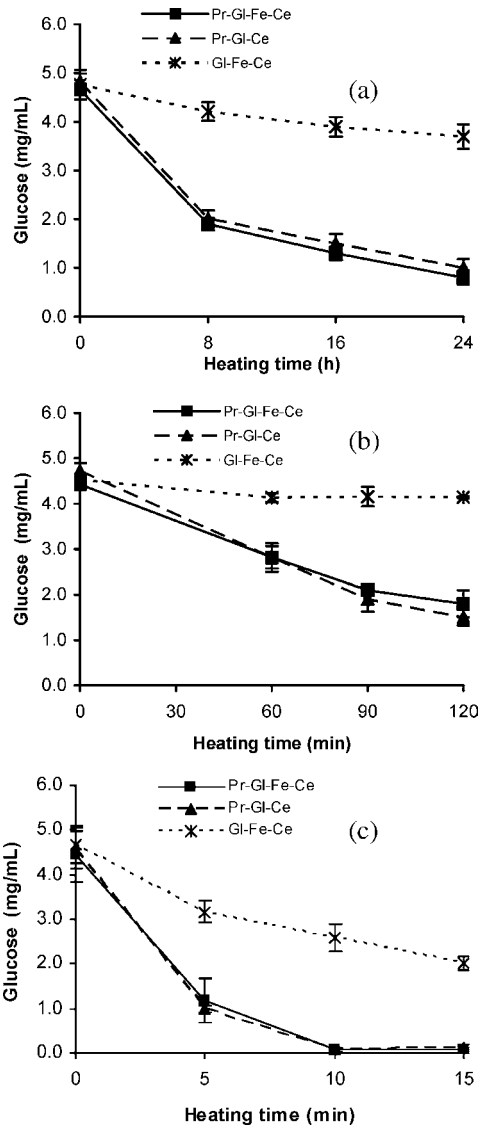


Figure 2. Concentration (mg/mL) of glucose in filtrates of model systems. Model systems were heated at (**a**) 60 °C, (**b**) 90 °C, and (**c**) 220 °C. The error bars represent the standard deviation of the mean value for triplicate samples. Ce, cellulose; Fe, ferulic acid; Pr, proline; Gl, glucose.

observed for Pr−Gl−Fe−Ce, Pr−Gl−Ce, and Gl−Fe−Ce heated at 90 °C.

The highest absorbance values were obtained for the 220 °C systems containing both proline and glucose, i.e., Pr−Gl−Fe−Ce and Pr−Gl−Ce. In comparison, significantly lower ($p < 0.05$) absorbance readings were observed for Gl−Fe−Ce at 10 and 15 min of heating, suggesting that the Maillard reaction was a more important source of color than sugar caramelization. The observed increase in absorbance in the visible region with time of heating is in line with the data reported for previous studies on Maillard model systems (*9*, *24*−*26*).

Quantification of Glucose and Proline. The concentration of glucose and proline in the filtrates is shown in Figures 2 and 3, respectively. Glucose in Gl−Fe−Ce and proline in Pr−Fe−Ce decreased nonsignificantly ($p > 0.05$) on heating at 60 °C for 24 h and 90 °C for 120 min. In contrast, significant decreases ($p < 0.05$) for both systems occurred on heating at 220 °C for 15 min. For Gl−Fe−Ce, this is consistent with sugar caramelization requiring high temperatures, e.g., 220 °C. For Pr−Fe−Ce, the reduction in proline concentration is due to its thermal degradation (*27*).

Proline and glucose in Pr−Gl−Fe−Ce and Pr−Gl−Ce decreased significantly on heating at all three temperatures and this is attributed to their participation in the Maillard reaction. The final concentration of both components was close to zero after 15 min of heating at 220 °C.

Quantification of Ferulic Acid. The concentration of ferulic acid in the filtrates of each model system, heated at 60, 90, and 220 °C, is shown in Figure 4. After heating at 60 °C for 24 h, the concentration of ferulic acid decreased significantly ($p < 0.05$) for Pr−Gl−Fe−Ce, but no loss of ferulic acid was observed in any of the other systems. This suggests that, at 60 °C, ferulic acid reacts with glucose−proline reaction intermediates. At 90 °C, no significant difference ($p > 0.05$) in the concentration of ferulic acid was observed for any of the model systems, although a nonsignificant decrease was observed for Pr−Gl−Fe−Ce. At 220 °C, the concentration of ferulic acid decreased in a similar manner over the first 5 min of heating for all model systems. After that point the concentration of ferulic acid decreased in a similar way for all systems except Pr−Gl−Fe−Ce, for which the rate of loss was faster. The data suggest not only that ferulic acid degraded on heating at 220 °C but that it may also have participated in reactions with proline−glucose reaction intermediates in Pr−Gl−Fe−Ce, leading to a more rapid rate of loss.

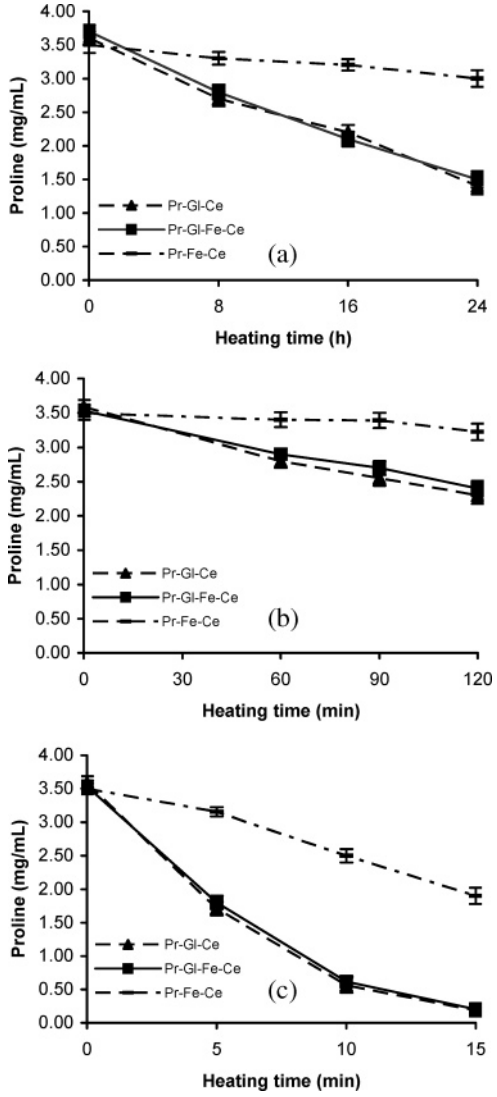


Figure 3. Concentration (mg/mL) of proline in filtrates of model systems. Model systems were heated (**a**) 60 °C, (**b**) 90 °C, and (**c**) 220 °C. The error bars represent the standard deviation of the mean value for triplicate samples. Ce, cellulose; Fe, ferulic acid; Pr, proline; Gl, glucose.

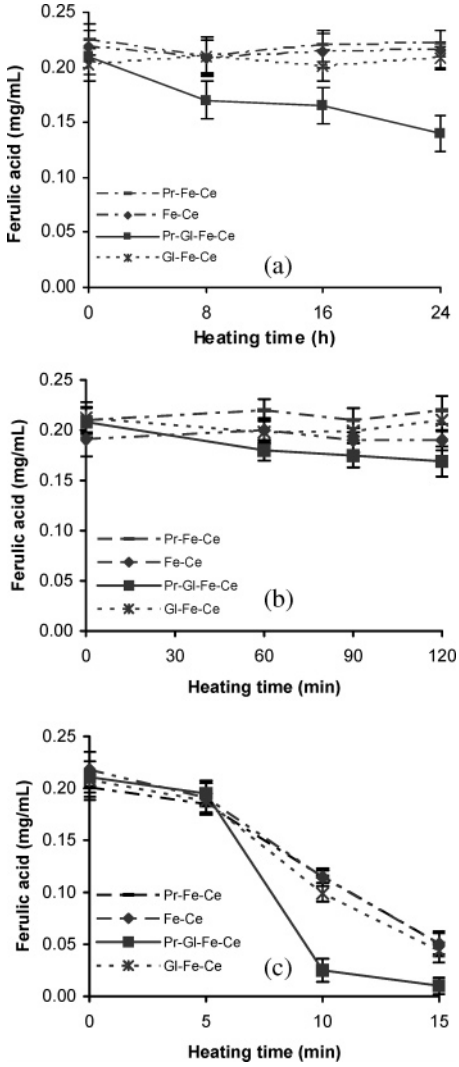


Figure 4. Concentration (mg/mL) of ferulic acid in filtrates of model systems. Model systems were heated at (**a**) 60 °C, (**b**) 90 °C, and (**c**) 220 °C. The error bars represent the standard deviation of the mean value for triplicate samples. Ce, cellulose; Fe, ferulic acid; Pr, proline; Gl, glucose.

Formation of New Compounds. The electrophoretograms obtained using borate buffer (50 mM, pH 9.5) of the 60 °C/24 h Pr−Gl−Ce filtrates revealed six new peaks (Figure 5a,b). Five of them were detected only in the 24 h samples, while the sixth (peak 4) was detected even after 8 h (Figure 6). All these peaks representing MRPs were also detected in the Pr−Gl−Fe−Ce filtrates after heating at 60 °C (Figure 5c,d). Again, the area of peak 4 increased with heating time (Figure 6). A further eight peaks, in addition to those observed for Pr−Gl−Ce, were detected in the 60 °C/24 h Pr−Gl−Fe−Ce filtrates (Figure 5b,d). They are associated with the presence of ferulic acid, glucose, and proline, since they were also absent from the Fe−Ce samples and provide further evidence that ferulic acid can react with reaction intermediates when proline is heated with glucose. Their spectra possess a λ_{max} between 300 and 325 nm with tailing to 375 nm. In comparison, ferulic acid gave two λ_{max} (at 225 and 325 nm) and tailed off to 375 nm, suggesting that the unidentified peaks may contain ferulic acid reaction products. Fe−Ce, Pr−Fe−Ce, and Gl−Fe−Ce gave no new peaks after heating at 60 °C.

The 90 °C systems received the mildest heat treatment. Only one peak (4) appeared in the electrophoretograms of the filtrates of Pr−Gl−Fe−Ce and Pr−Gl−Ce (Figure 6), and no new peaks were detected for any of the other samples.

Peak 4 appeared in the electrophoretograms of the filtrates of Pr−Gl−Fe−Ce and Pr−Gl−Ce after 5 min of heating at 220°C, but disappeared after heating for 10 min. The electrophoretograms of the filtrates of the 220 °C/10 min samples showed five new peaks for both systems. Four of them were reduced in size and the fifth one disappeared after heating for 15 min at 220 °C, presumably due to thermal degradation. The spectrum of one of the peaks has two shoulders (at 220 and 320 nm) and tails off to 350 nm. This spectrum is identical to one we obtained in our earlier malt work (*28*). Two other peaks had similar spectra, with shoulders at 205 and 280 nm and at 200 and 280 nm, respectively, with tailing to 300 nm.

Melanoidins migrate as a broad hump when analyzed by CE (*6*, *30*). In the current study, a broad hump migrated between 4 and 8 min, for the filtrates of Pr−Gl−Ce and Pr−Gl−Fe−Ce heated at 220 °C for 10 min. The broad hump disappeared over the last 5 min of heating at 220 °C, and this is attributed to thermal degradation.

Antioxidant Activity. Two radical scavenging methods, the $ABTS^{\bullet+}$ and the ORAC assays, were employed to assess the

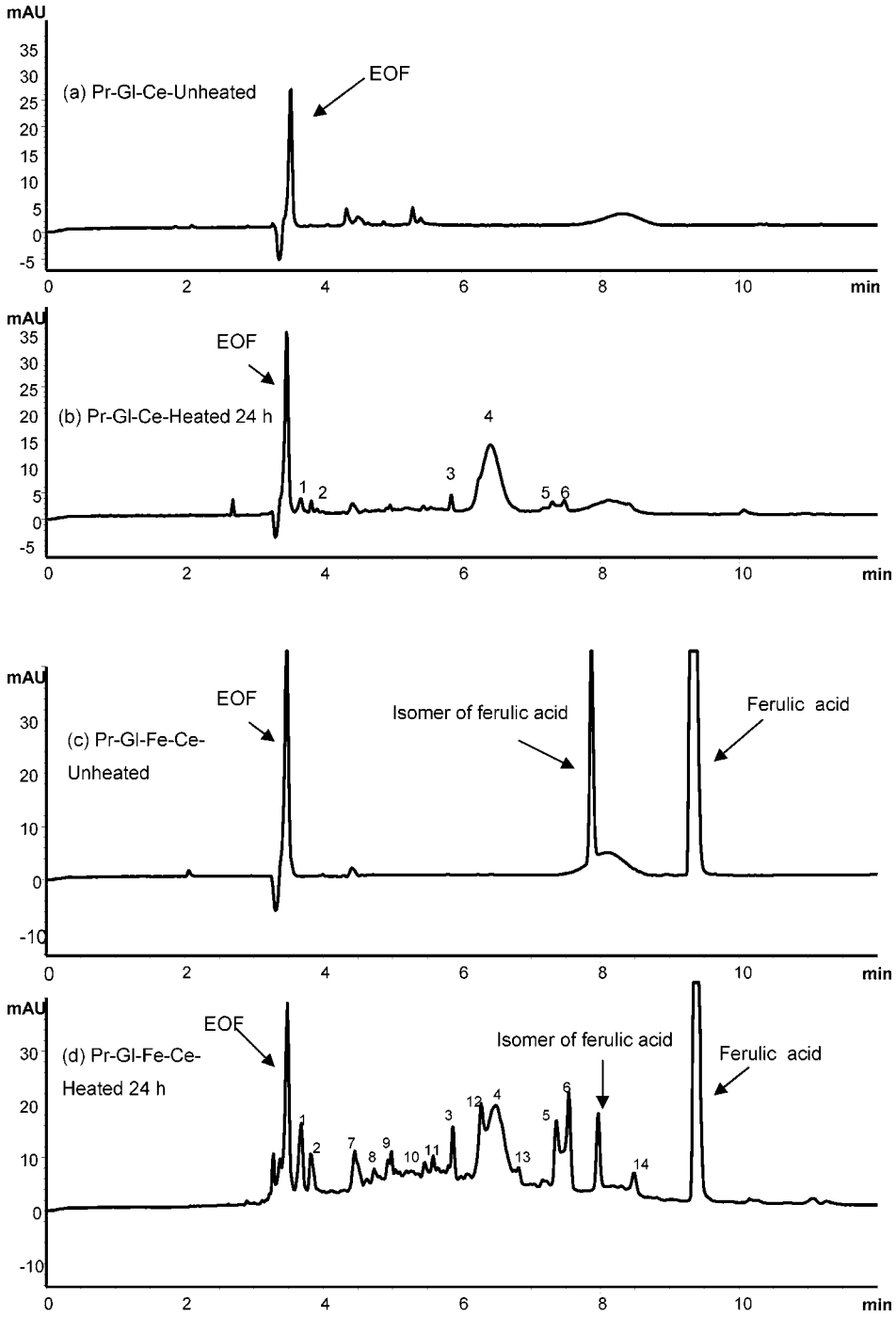


Figure 5. Electrophoretograms (detection at 200 nm) obtained for model systems (**a**) unheated Pr–Gl–Ce, (**b**) Pr–Gl–Ce heated at 60 °C for 24 h, (**c**) unheated Pr–Gl–Fe–Ce, and (**d**) Pr–Gl–Fe–Ce heated at 60 °C for 24 h. Ce, cellulose; Fe, ferulic acid; Pr, proline; Gl, glucose; EOF, electro-osmotic flow.

antioxidant activity of samples. Trolox is the standard compound normally used for the $ABTS^{\bullet+}$ assay, but other compounds, e.g., phenolic compounds (*16*), can be used instead. In this study, ferulic acid was used as the standard.

The antioxidant activity of model systems as measured by the $ABTS^{\bullet+}$ and the ORAC assays are depicted in Figure 7. All the unheated ferulic acid-containing model systems gave filtrates that did not differ significantly in antioxidant activity. In contrast, unheated filtrates from Pr−Gl−Ce presented significantly less antioxidant activity. At 60 and 90 °C the antioxidant activity of Pr−Gl−Fe−Ce increased linearly throughout heating, while there was no significant effect ($p > 0.05$) on the antioxidant activity of the other model systems containing ferulic acid. A gradual increase in the antioxidant activity of Pr−Gl−Ce was observed. At 220 °C, the antioxidant activity of Pr−Gl−Fe−Ce and Pr−Gl−Ce showed similar behavior, increasing significantly after 10 min at 220 °C, followed by a loss of antioxidant activity to the level present in the unheated Pr−Gl−Fe−Ce sample. The remaining model systems showed almost no change in antioxidant activity over 15 min of heating at 220 °C.

The same behavior was observed for all model systems by both antioxidant assays. A linear relationship was observed between the data obtained for samples using all three temperatures by using the $ABTS^{\bullet+}$ and ORAC assays, suggesting the ability of the components of the model system to scavenge both

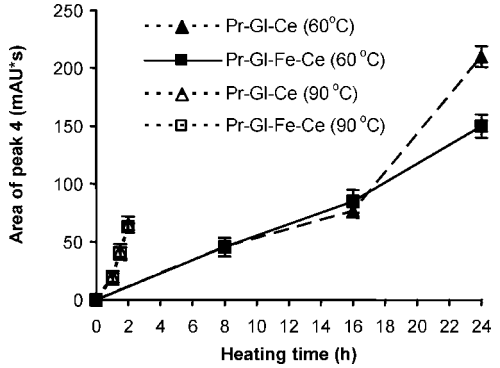


Figure 6. Increase in area of peak 4 during heating at 60 and 90 °C for systems Pr–Gl–Ce and Pr–Gl–Fe–Ce. The error bars represent the standard deviation of the mean value for triplicate samples. Ce, cellulose; Fe, ferulic acid; Pr, proline; Gl, glucose.

the $ABTS^{\bullet+}$ and peroxyl ($ROO^{\bullet}$) radicals. Higher levels of antioxidant activity are measured by the $ABTS^{\bullet+}$ assay compared to the ORAC assay. This indicates that compounds formed on heating of model systems are better scavengers of the $ABTS^{\bullet+}$ radical than of the peroxyl radical ($ROO^{\bullet}$).

The antioxidant activity of heated Pr−Gl−Ce comes exclusively from the thermally generated products of the Maillard reaction between proline and glucose. Franke and Iwainsky (*8*) in 1954 were the first to discover an increase in antioxidant properties of MRPs with heating time. Since then, many researchers (e.g., refs *9*−*10*, *26*, *29*−*31*) have reported the increase in antioxidant activity of MRPs with heating time. The antioxidative effect of Pr−Gl−Fe−Ce may include contributions from ferulic acid, thermally generated MRPs, and products of reactions involving glucose, proline, and ferulic acid. A comparison of the antioxidant activity of Pr−Gl−Fe−Ce with the sum of the antioxidant activity of Pr−Gl−Ce and Fe−Ce heated at 60 and 90 °C illustrates that Pr−Gl−Fe−Ce possesses a significantly higher level of antioxidant activity than that accounted for by the sum of the values for Pr−Gl−Ce and Fe−Ce. Heating at 220 °C gave a different antioxidant activity profile for Pr−Gl−Fe−Ce, compared to the lower temperatures (60 and 90 °C). Furthermore, the antioxidant activity of this

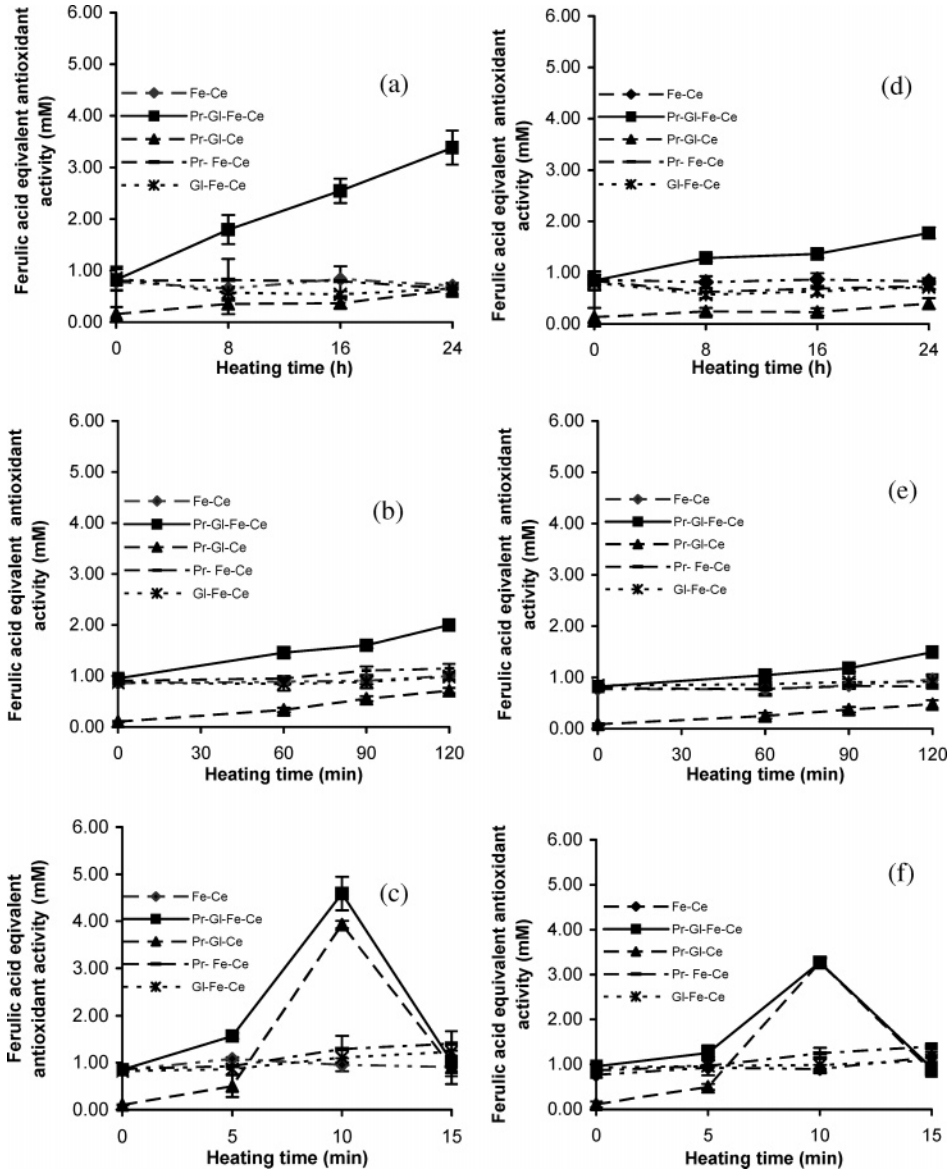


Figure 7. Ferulic acid equivalent antioxidant activity of model systems. Model systems were heated at (**a**) 60 °C, (**b**) 90 °C, and (**c**) 220 °C ($ABTS^{\bullet+}$ data) and at (**d**) 60 °C, (**e**) 90 °C, and (**f**) 220 °C (ORAC data). The error bars represent the standard deviation of mean values for triplicate samples. Ce, cellulose; Fe, ferulic acid; Pr, proline; Gl, glucose.

system is equal to the sum of the antioxidant activities of Pr−Gl−Ce and Fe−Ce. This indicates that the development of antioxidant activity of the mixture Pr−Gl−Fe−Ce comes mainly from the MRPs of proline and glucose, with ferulic acid or any of its degradation products making only a minor contribution, at most.

Since both antioxidant activity and color develop during the Maillard reaction, the relationship between antioxidant activity and absorbance at 420 nm was studied.

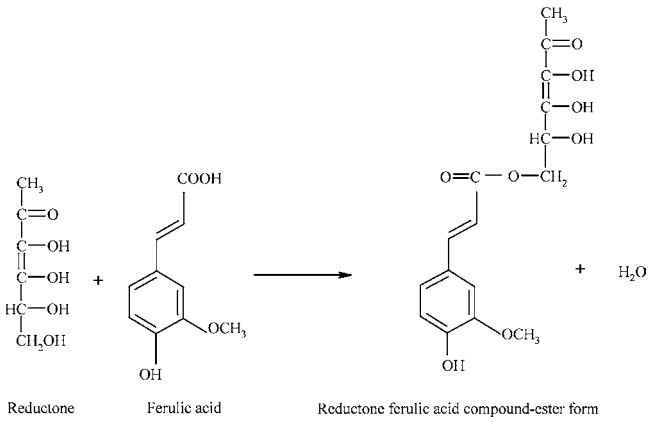
Model systems Pr−Gl−Fe−Ce and Pr−Gl−Ce heated at moderate temperatures (60 and 90 °C) showed antioxidant activity increasing with absorbance at 420 nm, and the relationship was exponential rather than linear. At 220 °C, a linear relationship was observed between antioxidant activity and absorbance for the first 10 min of heating for model system Pr−Gl−Fe−Ce. However, between 10 and 15 min of heating, the antioxidant activity decreased, while absorbance readings remained at the same level. Findings from the current study and the literature lead to the conclusion that both antioxidant activity and the color increase with heating time to a maximum level, after which point antioxidant activity decreases while color levels off. Thermally induced compounds produced at higher temperatures (*6*, *16*) and/or long heating times (*9*) may contribute to color development, but not to antioxidant activity. Also, some thermally induced compounds possessing antioxidant activity may degrade on prolonged heating, and this appears to be the case for the 220 °C/15 min system in the current study, since several unidentified components observed in the electrophoretograms for the 220 °C/10 min sample could not be detected when heating continued to 15 min.

The relationship between the antioxidant activity and the ferulic acid concentration was also studied. A strong negative ($r < 0$) correlation between antioxidant activity ($ABTS^{\bullet+}$ assay) and concentration of ferulic acid for model systems Pr−Gl−Ce and Pr−Gl−Fe−Ce was found at all three temperatures. The data presented for the 60 and 90 °C systems are in line with those from previous studies concerning food systems heated at moderate temperatures. Studies on wine heated at 55 °C (*32*) and on plum drying at 60 or 85 °C (*33*) have demonstrated a progressive increase in free radical scavenging activity with heating time while the content of phenolic compounds decreased. The stability of ferulic acid and the antioxidant activity of the other ferulic acid-containing systems heated at 60 and 90 °C implies that ferulic acid is the major contributor to the antioxidant activity. However, at 220 °C the concentration of ferulic acid in Pr−Fe−Ce, Gl−Fe−Ce, and Fe−Ce decreased significantly on heating, while the antioxidant activity was little changed. This can be explained by the formation of ferulic acid degradation products possessing antioxidant activity, e.g., 4-vinylguaiacol. 4-Vinylguaiacol possesses appreciable antioxidant activity (*34*) and was identified in increasing amounts with time of heating at 220 °C for all ferulic acid-containing systems. The concentration of 4-vinylguaiacol in the filtrates of Pr−Fe−Ce, Gl−Fe−Ce, and Fe−Ce was ∼0.01 mg/mL after 15 min.

The mild temperatures and long heating times used and the low absorbance values obtained on heating model systems Pr−Gl−Fe−Ce at 60 and 90 °C suggest that the most likely contributors to the antioxidant activity of these systems are LMM MRPs such as reductones. The antioxidant activity of reductones originates from their ability to react with peroxides or free radicals produced during fat oxidation with the consequent retardation of lipid oxidation (*26*).

The high temperature used (220 °C) and the high absorbance values (>1) obtained at 420 nm during heating systems Pr−Gl−Ce and Pr−Gl−Fe−Ce suggest that HMM MRPs (i.e., melanoidins) are the components most likely to be responsible for the antioxidant activity. The typical broad hump of melanoidins was detected in the electrophoretograms for systems Pr−Gl−Ce and Pr−Gl−Fe−Ce heated at 220 °C for 10 min. Since higher antioxidant activity was measured in these Maillard systems heated at 220 °C than at 60 and 90 °C, it may be that HMM MRPs from proline and glucose possess higher antioxidant activity than LMM MRPs. This would be in line with the findings of Yen and Tsai (*35*), who showed that melanoidins that are formed during the final stage of the Maillard reaction are stronger antioxidants than reductones that are formed during the intermediate stage.

Scheme 1. Hypothetical Direct Reaction of Ferulic Acid with Reductone



The effect of ferulic acid on the Maillard reaction between proline and glucose, leading to an additional increase in the antioxidant activity at 60 °C, may be attributed to the formation of reaction products by three possible routes, i.e., reaction of ferulic acid with reductones, a synergistic effect between ferulic acid and reductones, and inhibition of free radicals generated during the early stages of the Maillard reaction. Reaction of ferulic acid with reductones may lead to a ferulic acid−reductone reaction product that might possess much higher antioxidant activity than ferulic acid itself, due to the combined action of both ferulic acid and the reductone (Scheme 1). Alternatively, ferulic acid and reductones may act synergistically because reductones might reduce any radical formed from ferulic acid, thereby regenerating ferulic acid. Furthermore, free radicals have been shown to be responsible for the pro-oxidant activity (*5*). Djilas and Milic (*36*) investigated the effect of four phenolic acids (ellagic, gallic, syringic, and ferulic acid) on the formation of free radicals during the Maillard reaction. They found that the relative intensity of electron spin resonance (ESR) signals, corresponding to the concentration of free radicals, decreased with increasing concentration of the added phenolic acid. Therefore, it is possible that ferulic acid effectively inhibited the pro-oxidant activity of Maillard reaction intermediates in the current study, leaving MRPs with antioxidant activity to accumulate.

In conclusion, this study investigated the effect of the thermal processing, relevant to kilning and roasting of malt, on the total antioxidant activity of model systems prepared from combinations of glucose, proline, and ferulic acid. It shows for the first time that ferulic acid reacts with Maillard reaction intermediates formed from glucose and proline at kilning temperatures (60 °C), leading to higher antioxidant activity.

ACKNOWLEDGMENT

We thank the State Scholarships Foundation (IKY) of Greece for funding this work.

LITERATURE CITED

(1) Goupy, P.; Hughes, M.; Boivin, P.; Amiot, M. J. Antioxidant composition and activity of barley (*Hordeum vugare*) and malt extracts and of isolated phenolic compounds. *J. Sci. Food Agric.* **1999**, *79*, 1625−1634.

(2) Bonnely, S.; Peyrat-Maillard, M. N.; Rondini, L.; Masy, D.; Berset, C. Antioxidant activity of malt rootlet extracts. *J. Agric. Food Chem.* **2000**, *48*, 2785−2792.

(3) Aeschbacher, H. U. Anticarcinogenic effect of browning reaction products. In *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*; Finot, P. A., Aeschbacher, H. U., Hurrell, R. F., Liardon, R., Eds.; Birkhauser: Basel, Switzerland, 1990; pp 335−347.

(4) Chandra, G. S.; Buggey, L. A.; Peters, S.; Cann, C.; Liegeois, C. *Factors affecting the development of antioxidant properties of malts during the malting and roasting process*; Home Grown Cereals Association Project no. 242; HGCA Publication: London, U.K., 2001.

(5) Anese, M.; Manzocco, L.; Nicoli, M. C.; Lerici, C. R. Antioxidant properties of tomato juice as affected by heating. *J. Sci. Food Agric.* **1999**, *79*, 750−754.

(6) Del Castillo, M. D.; Ames, J. M.; Gordon, M. H. Effect of roasting on the antioxidant activity of coffee brews. *J. Agric. Food Chem.* **2002**, *50*, 3698−3703.

(7) Manzocco, L.; Calligaris, S.; Mastrocola, D.; Nicoli, M. C.; Lerici, C. R. Review of non enzymatic browning and antioxidant capacity of processed foods. *Trends Food Sci. Technol.* **2001**, *11*, 340−346.

(8) Franke, C.; Iwainsky, H. The antioxidant activity of melanoidins. *Dutch J. Food Chem.* **1954**, *50*, 251−254.

(9) Lingnert, H.; Eriksson, C. E. Antioxidative effect of MRPs. *Prog. Food Nutr. Sci.* **1981**, *5*, 453−466.

(10) Anese, M.; Nicoli, M. C.; Lerici, C. R. Influence of pH on the oxygen scavenging properties of heat-treated glucose-glycine systems. *Ital. J. Food Sci.* **1994**, *3*, 339−343.

(11) Shaker, E. S.; Ghazy, M. A.; Shibamoto, T. Antioxidative activity of volatile browning reaction products and related compounds in a hexanal/hexanoic acid system. *J. Agric. Food Chem.* **1995**, *43*, 1017−1022.

(12) Jing, H.; Kitts, D. D. Comparison of the antioxidant and cytotoxic properties of glucose-lysine and fructose-lysine Maillard reaction products. *Food Res. Int.* **2000**, *33*, 509−516.

(13) Kim, N. S.; Harris, N. D. Antioxidant effect of nonenzymatic browning reaction products on linoleic acid. In *Trends in Food Science;* Ghee, A. H., Sze, L. W., Woo, F. C., Eds.; Singapore Institute of Food Science and Technology: Singapore, 1987; pp 19−24.

(14) Severini, C.; Lerici, C. R. Interactions between Maillard reaction and lipid oxidation in model systems during high-temperature treatment. *Ital. J. Food Sci.* **1995**, *2*, 189−196.

(15) Morales, F. J.; Babbel, M. B. Antiradical efficiency of Maillard reaction mixtures in a hydrophilic media. *J. Agric. Food Chem.* **2002**, *50*, 2788−2792.

(16) Charurin, P.; Ames, J. M.; Del Castillo, M. D. Antioxidant activity of coffee model systems. *J. Agric. Food Chem.* **2002**, *50*, 3751−3756.

(17) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolourisation assay. *Free Radical Biol. Med.* **1999**, *26*, 1231−1237.

(18) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* **2001**, *49*, 4619−4626.

(19) Briggs, D. E. *Malts and Malting*; Blackie Academic and Professional: London, U.K., 1998.

(20) Bemment, D. W. Speciality malts. *Brewer* **1985**, *71*, 457−460.

(21) Blenkinsop, P. The manufacture, characteristics and uses of speciality malts. *Techn. Q.- Master Brew. Assoc. Am.* **1991**, *28*, 145−149.

(22) Jupp, D. H. *Coloured Malts*; Technical Note; French and Jupp Ltd Company Publication: Ware, U.K., 1998.

(23) Davies, C. G. A.; Wedzicha, B.; Gillard C. Kinetic model of the glucose-glycine reaction. *Food Chem.* **1997**, *60*, 323−329.

(24) Mundt, S.; Wedzicha, B. A kinetic model for the glucose-fructose-glycine browning reaction. *J. Agric. Food Chem.* **2003**, *51*, 3651−3655.

(25) Morales, F. J.; Perez, S. Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence. *Food Chem.* **2001**, *72*, 119−125.

(26) Eichner, K. Antioxidants effect of Maillard reaction intermediates. *Prog. Food Nutr. Sci.* **1981**, *5*, 441−451.

(27) Yaylayan, V.; Keyhani, A. Carbohydrate and amino acid degradation pathways in l-Methionine/D-[^{13}C] Glucose model systems. *J. Agric. Food Chem.* **2001**, *49*, 800−803.

(28) Royle, L.; Ames, J. M.; Castle, L.; Nursten, H. E. Analysis of malts by capillary electrophoresis. *J. Sci. Food Agric.* **2001**, *82*, 443−451.

(29) Park, C. K.; Kim, D. H. Relationship between fluorescence and antioxidant activity of ethanol extracts of a Maillard browning mixture. *J. Am. Oil Chem. Soc.* **1983**, *60*, 99−102.

(30) Yoshimura, Y.; Iijima, T.; Watanabe, T.; Nakazaura, H. Antioxidant effect of Maillard reaction products using glucose-glycine model system. *J. Agric. Food Chem.* **1997**, *45*, 4106−4109.

(31) Yen, G. C.; Hsieh, P. P. Antioxidative activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction products. *J. Sci. Food Agric.* **1995**, *67*, 415−420.

(32) Manzocco, L.; Mastrocola, D.; Nicoli, M. C. Chain-breaking and oxygen scavenging properties of wine as affected by some technological procedures. *Food Res. Int.* **1998**, *31* (9), 673−678.

(33) Piga, A.; Del Caro, A.; Corda, G. From plums to prunes: Influence of drying parameters on polyphenols and antioxidant activity. *J. Agric. Food Chem.* **2003**, *51*, 3675−3681.

(34) Walters, M. T.; Hughes, P. S.; Bamforth, C. W. The evaluation of natural antioxidants in beer and its raw materials. *Proc. Conv. Inst. Brew.* **1996**, *24*, 103−109.

(35) Yen, G. C.; Tsai, L. C. Antimutagenicity of a partially fractionated Maillard reaction products. *Food Chem.* **1993**, *47*, 11−15.

(36) Djilas, S. M.; Milic, B. L. Naturally occurring phenolic compounds as inhibitors of free radical formation in the Maillard reaction. In *Maillard Reactions in Chemistry, Food, and Health*; Labuza, T. P., Reineccius, G. A., Monier, V. M., O'Brien J., Baynes J. W., Eds.; The Royal Society of Chemistry: Cambridge, U.K., 1994; pp 75−80.

Received for review January 23, 2005. Revised manuscript received March 30, 2005. Accepted April 1, 2005.

JF0501600