Influence of Reaction Conditions on the Oxidative Behavior of Model Maillard Reaction Products

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Model nondialyzable Maillard reaction products (MRPs) were synthesized by reacting D-glucose or D-fructose with L-lysine under four different initial reaction parameters (pH, water activity, reaction time, and temperature) and evaluated for potential antioxidant or prooxidant activity and genotoxic properties. All model Glu−Lys and Fru−Lys MRPs showed various degrees of antioxidant activity when evaluated in a metal-free model linoleic acid emulsion system using thiobarbituric acid (TBA) as an end point measure of lipid oxidation. The oxidative behavior of the same MRPs, when determined by an oxygen electrode in the presence of copper ions, consisted of both prooxidant and antioxidant activities for specific MRPs. Using an *in vitro* DNA model system, both Glu−Lys and Fru−Lys MRP mixtures induced significant ($p \leq 0.05$) strand breakage in PM2 bacteriophage DNA at high concentrations (>0.001%, w/v). In general, Fru−Lys MRPs exhibited more prooxidant and genotoxic activity compared to Glu−Lys MRPs synthesized under identical conditions. The results demonstrate the importance of using a number of different *in vitro* model systems in defining the characteristic oxidative behavior of different MRPs synthesized under specifically different conditions.

Keywords: *Maillard reaction; antioxidant activity; prooxidant activity; DNA nicking*

INTRODUCTION

The nonenzymatic interaction between reducing sugars with amino acids, peptides, or proteins in foods has been referred to as the Maillard browning reaction (MR). MR is known to produce a multitude of intermediates (Huyghues-Despointes and Yaylayan, 1996), which are collectively referred to as Maillard reaction products (MRPs) (Hodge, 1953). The formation of MRPs is greatly influenced by both the source of reactants and the reactant conditions (Cammerer and Kroh, 1995; Wijewickreme *et al.*, 1997), and even fixed reactant and reaction conditions are also known to produce a variety of MRPs (Namiki, 1988).

MRPs have been shown to possess antioxidant activity by a number of investigators (Griffith and Johnson, 1957; Lingert *et al.*, 1979; Alaiz *et al.*, 1996). Incorporation of preformed MRPs into food systems directly (Lingert and Eriksson, 1981; Bedinghaus and Ockerman, 1995) or the application of food processing practices to derive MRPs within the food (Chiou, 1992; Smith and Alfawaz, 1995) can result in improved oxidative stability of some foods (Bressa *et al.*, 1996). In many processed carbohydrate-rich foods (e.g., bakery products, caramel, and coffee), MRPs are derived by thermal decomposition of reducing sugar−amino acid compounds. Therefore, MRPs could be considered as a group of compounds that are naturally present in foods. Knowing the reaction conditions that generate MRPs with specific antioxidant activity could therefore be valuable for use in food processing strategies.

The objective of the present study was to investigate and compare the antioxidant activity of different model MRPs synthesized by reacting D-glucose or D-fructose with L-lysine at various reaction time, pH, water activity (a_w), and temperature combinations using a battery of antioxidant assay methods. Due to the fact that certain compounds that act as antioxidants in lipid model systems also exhibit DNA breaking activity (Aruoma *et al.*, 1990), the different model MRPs synthesized in this study were analyzed for genotoxicity, in addition to their oxidative behavior in lipid model systems. The lipid model system employed in the present study comprised a linoleic acid (C18:2*n*−6) emulsion system, and the DNA model system employed comprised a double-stranded supercoiled PM2 bacteriophage DNA. The reaction conditions for the synthesis of MRPs were selected to simulate typical thermal processes conducted in many normal cooking situations. MRPs with a high molecular weight range were chosen for this study, as such compounds, if added to foods as antioxidant additives, are not likely to be available for absorption to the same degree as low molecular weight MRPs (Nair *et al.*, 1981).

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MATERIALS AND METHODS

L-Lysine, D-glucose, D-fructose, linoleic acid, butylated hydroxytoluene (BHT), 1,1,3,3-tetraethoxypropane, 2-thiobarbituric acid (2-TBA), sodium azide, and Chelex 100 were purchased from Sigma Chemical Co. (St. Louis, MO). Analytical grade glycerol, sulfuric acid, and cupric sulfate ($CuSO_4 \cdot 5H_2O$) were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Potassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate, and sodium hydroxide were obtained from BDH Chemical Co. (Toronto, ON). The dialysis tubing [molecular weight cutoff (MWCO) = 6000−8000] was obtained from Spectrum Scientific Co. (Houston, TX). C_{18} Sep-Pak cartridges were obtained from Waters Associates (Milford, MA). Difco Laboratories (Detroit, MI) was the source of Tween 80. Distilled water used to prepare model MRP mixtures was further purified by a Barnstead E-pure system and used throughout the study.

Production of Model MRP Mixtures. MRP mixtures were prepared by heating 0.8 M L-lysine with 0.8 M D-glucose or 0.8 M D-fructose according to the method given by Wijewickreme *et al.* (1997). Four variable reaction parameter (reaction time, temperature, initial a_w, and initial pH) com-

S0021-8561(97)00040-X CCC: $14.00

binations were used in the production of MRPs. Those combinations were randomly acquired using the random centroid optimization program (Dou *et al.*, 1993). Water activity of the reaction mixtures was adjusted according to the method of Eichner and Karel (1972), and the initial pH was adjusted with 5 M sodium hydroxide. Reaction solutions were heated for a designated time period in a Perma View hot air oven set at a given ambient temperature level. Following heating, solutions were rapidly cooled on ice, dialyzed (MWCO = 6000−8000) against 20−30 changes of deionized distilled water at 4 °C, and lyophilized.

Assessment of Antioxidant Activity of MRP Mixtures. *(a) Oxygen Consumption Measurements.* Oxygen depletion in a linoleic acid emulsion system with added cupric (Cu^{2+}) ions, and in the absence or presence of MRP mixtures, was measured according to the methods of McGookin and Augustin (1991) and Lingert *et al.* (1979), using a YSI Model 5300 biological oxygen monitor (Yellow Springs, OH). The reaction mixture consisted of 1.5 mL of linoleic acid emulsion [1.5 g of linoleic acid mixed with 0.4 g of Tween 80 in 40 mL of 0.1 M potassium phosphate buffer (pH 6.8)], 15.3 mL of buffer (0.1 M potassium phosphate buffer, pH 6.8), 0.6 mL of MRP solution (3 mg/mL buffer), and 0.6 mL of 2 mM $CuSO_4$ dissolved in 0.1 M potassium phosphate buffer (pH 6.8). The reaction mixture was pumped into a jacketed reaction vessel containing an oxygen electrode at room temperature. Oxygen depletion was recorded immediately after the reaction mixture was introduced into the vessel. For the measurement of oxygen depletion in the absence of antioxidant compounds, the experiments were conducted in an identical manner with the exception that 0.6 mL of buffer replaced the MRP solutions. Both antioxidant and prooxidant activity of MRP mixtures were expressed in terms of a protective index (PI), defined as

$$\mathrm{PI} = \frac{\text{time for 50\% } O_2 \text{ depletion with test compound}}{\text{time for 50\% } O_2 \text{ depletion without test compound}} \quad (1)$$

where PI < 1 denotes prooxidant activity, PI = 1 denotes no activity, and PI > 1 denotes antioxidant activity (Lingert *et al.*, 1979).

(b) Measurement of Thiobarbituric Acid Reactive Substances (TBARS). MRP mixtures (2 mg) were incubated with 5 mL of linoleic acid emulsion [1.5 g of linoleic acid and 0.4 g of Tween 80 in 200 mL of 0.1 M potassium phosphate buffer (pH 6.8)] and 5 mL of deionized distilled water for 48 h at 60 °C. Following incubation, the solution was diluted 10 times with 25 mM Tris buffer (pH 7.4) containing 0.02% sodium azide and passed through a C_{18} Sep-Pak cartridge. Delipidated extract was assayed for the MDA content using the TBA method described by Buege and Aust (1978). One milliliter of TBA reagent containing 0.02% freshly prepared BHA was added to 2 mL of delipidated homogenate, in test tubes with marble caps, and immersed in a boiling water bath for 15 min. After cooling, absorbance readings of the reaction solutions were made at 532 nm using a Shimadzu UV−vis spectrophotometer (Tekscience, ON). Quantification of malonaldehyde (MDA) content in the samples was made from a standard curve prepared from 1,1,3,3-tetraethoxypropane in 1% sulfuric acid. All results were expressed as percent antioxidant activity (%AO), defined as

$$\%\mathrm{AO} = \frac{(\text{TBA value of the control} - \text{TBA value of the test sample}) \times 100}{\text{TBA value of the control}} \quad (2)$$

(c) Assessment of DNA Breaking Activity of Model MRP Mixtures. The effect of MRP mixtures on DNA strand breakage was determined to investigate the potential genotoxic behavior of model MRP mixtures. PM2 bacteriophage DNA was extracted according to the method given by Espejo and Canelo (1968) and checked for purity by agarose gel electrophoresis before use. Buffers and deionized distilled water used for the experiments were treated with Chelex 100 to remove all adventitious metal ions present. Glassware was soaked in 1 N HCl for 24 h and rinsed thoroughly with deionized distilled water before use. All reactions were conducted in potassium phosphate buffer (pH 7.4, 50 mM) under ambient oxygen pressure. To detect the possible genotoxic effect of MRP mixtures on DNA at different MRP concentration levels, 2 μL of phage DNA was mixed with 2 μL of buffer and 2 μL each of MRP solutions (10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1}%, w/v) in a 500 μL Eppendorf tube. The final volume of the reaction mixture was brought to 10 μL with deionized distilled water, and the incubation was conducted for 1 h at 37 °C. At the end of the incubation, 2 μL of loading dye (0.25% bromophenol blue, 0.25% xylene cyanol FF, and 15% Ficoll in water) was added to the incubated reaction mixture, and 10 μL of this mixture was loaded onto a 0.7% (w/v) agarose gel. Electrophoresis was conducted at 60 V in tris acetate ethylenediaminetetraacetic acid (TAE) buffer (pH 7.4, 40 mM tris acetate, 1 mM EDTA) for 90 min. Following electrophoresis, the gel was stained with ethidium bromide (0.5 μg/mL deionized distilled water) for 20 min and destained with deionized distilled water for another 20 min. DNA bands were visualized under UV light and photographed. Photograph negatives were scanned by a Bio-Rad imaging densitometer using the Bio-Rad Molecular Analyst/PC image analysis program (version 1.0) to quantitate DNA breakage as percentages of supercoiled (S), nicked circular (NC), and linear (L) forms.

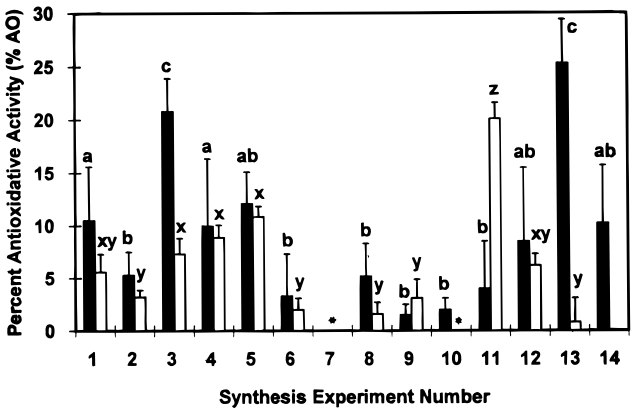

Figure 1. Percent antioxidant activity of crude Glu−Lys and Fru−Lys MRP mixtures as measured by the TBA method. Values represent mean ± SD. Letters a−c denote the Tukey test results obtained for comparisons among Glu−Lys MRP mixtures. Letters x−z denote the Tukey test results obtained for comparisons among Fru−Lys MRP mixtures. Experiment numbers with different letters are significantly different ($p \leq 0.05$). Asterisks indicate experiments with nondialyzable yields < 10 mg/100 mL. ■, Glu−Lys MRP mixtures; □, Fru−Lys MRP mixtures.

Statistical Analysis. One-way analysis of variance (ANOVA) followed by Tukey multiple-range test (Systat Inc., Evanston, IL) was used in the data analysis. The level of confidence required for significance was selected at $p \leq 0.05$. Each experiment was replicated three times, with internal controls.

RESULTS

Assessment of Antioxidant Activity by TBA Method. The relative susceptibilities of a model linoleic acid emulsion system to lipid oxidation containing different Glu−Lys or Fru−Lys MRP mixtures are presented in Figure 1. All nondialyzable water soluble products derived from different experimental conditions used to synthesize both Glu−Lys and Fru−Lys MRP mixtures reduced lipid peroxidation, albeit to different extents, as assessed by the TBA method. It is noteworthy that many of the MRPs derived from different Fru−Lys experiments produced relatively low (e.g. <5%) antioxidant activity compared to MRPs generated from counterpart Glu−Lys MRP synthesis experiments. For

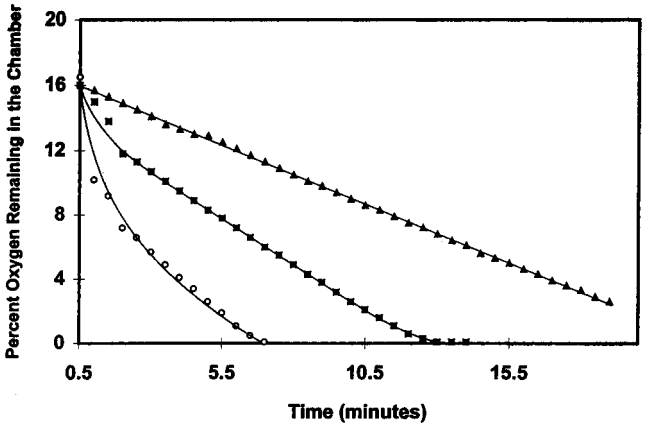

Figure 2. Percent oxygen consumption by nondialyzable MRP mixtures in a model linoleic acid emulsion (MLE) system: ■, control; ▲, MLE with added Fru−Lys MRP mixture 3; ○, MLE with added Fru−Lys MRP mixture 14.

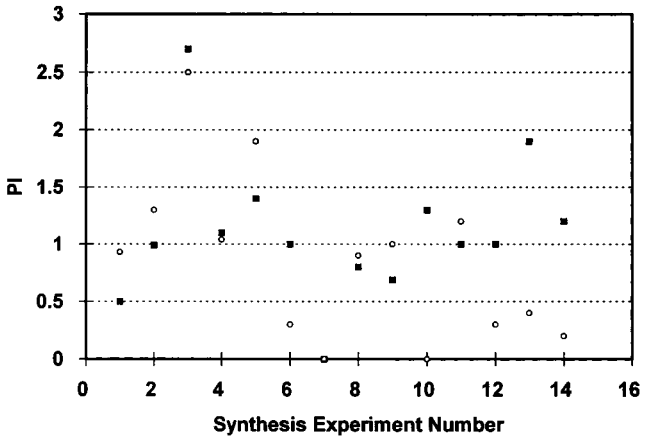

Figure 3. PI values calculated for crude Fru−Lys and Glu−Lys MRP mixtures synthesized by each MRP synthesis experiment: ○, Fru−Lys MRP mixture; ■, Glu−Lys MRP mixture.

example, while Glu−Lys MRP mixtures derived from synthesis experiments 3 and 13 specifically exhibited antioxidant activities as high as 26 and 22%, respectively, similarly synthesized Fru−Lys MRP mixtures exhibited antioxidant activities of only 7 and 2%, respectively.

Assessment of Antioxidant Activity by Oxygen Consumption Measurement. In the present study, oxygen consumption measurements were also undertaken as a chemical measure for evaluating lipid oxidation reactions. The percentage of oxygen remaining in the reaction chamber over a period of time, in the absence and presence of MRPs, was recorded as a measure of calculating PI values of MRP compounds. As shown in Figure 2, the percentage of oxygen remaining in the vessel depleted rapidly in the control emulsion compared to the emulsions containing certain MRP mixtures. Specific MRP mixtures (e.g. Glu−Lys MRP mixtures derived from experiments 1, 8, and 9 and Fru−Lys MRP mixtures derived from experiments 1, 6, 8, 12, 13, and 14), however, accelerated the rate of oxygen consumption in the vessel (i.e. PI < 1), thereby indicating a potential prooxidant activity not observed using the TBA assay method.

The PI values calculated for different MRP mixtures obtained from different synthesis experiments are given in Figure 3. In summary, seven antioxidant, two prooxidant, and four inactive MRP mixtures were

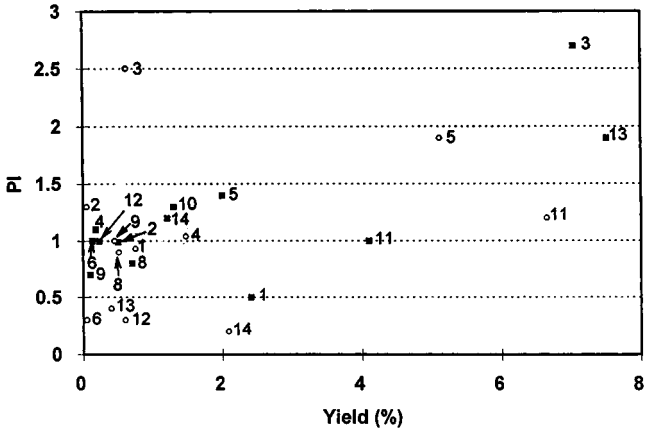

Figure 4. Relationship between the PI values and the yield of crude nondialyzable MRP mixtures: ○, Fru−Lys MRP mixtures; ■, Glu−Lys MRP mixtures. The numbers beside symbols denote the experiment number for each set of conditions used in MRP synthesis.

recovered from Glu−Lys synthesis experiments. In comparison, five antioxidant, five prooxidant, and three inactive MRP mixtures were recovered from Fru−Lys synthesis experiments. Among the different model MRP derivation experiments employing aldo and keto sugars, antioxidative activity was more predominantly found in MRPs derived from Glu−Lys reactions than counterpart Fru−Lys reactions.

PI values plotted against the yield of nondialyzable MRP mixtures derived from individual Glu−Lys and Fru−Lys synthesis experiments are presented in Figure 4. No relationship was found between the yield of nondialyzable Glu−Lys and Fru−Lys MRP mixtures and characteristic oxidative behavior. Rather, identical experimental conditions used to generate MRPs from both Glu−Lys and Fru−Lys reactions were individually unique in producing a relationship between antioxidant or prooxidant activity with the overall yield of nondialyzable material produced. For example, identical conditions employed in specific experiments 3 and 11, although producing contrasting comparative yields for Glu−Lys and Fru−Lys MRP mixtures, respectively, manifested similar antioxidant activities (Figure 4). In contrast, Fru−Lys experiment 5, which produced the highest yield among the Fru−Lys reactions, showed less antioxidant activity compared to Glu−Lys experiment 5. Similarly, MRP mixtures obtained for specific experiments 6, 12, and 14, respectively, resulted in antioxidant activity for the Glu−Lys MRP mixtures but prooxidant activity for Fru−Lys MRP mixtures.

***In Vitro* DNA Nicking Studies.** A dose response effect of model Glu−Lys and Fru−Lys MRP mixtures in breaking PM2 bacteriophage DNA strands, in the absence of metal ions, is shown in Figure 5. As shown in the figure, agarose gel electrophoresis effectively separated the three forms of DNA [i.e., double-stranded supercoiled (S), nicked circular (NC), and linear (L)] into three separate bands. The NC form of DNA migrated behind the S form, whereas the L form migrated between the S and NC forms. Thus, quantification of the three bands using densitometry gave an indication on the extent of DNA strand breakage.

The results of this study (Figures 5 and 6) clearly demonstrated that an increased addition of MRPs to DNA enhanced the degree of strand breakage irrespective of the synthesis reaction conditions used. This could be visualized by a loss in the supercoiled form and by an increase in the extent of nicked circular form. The

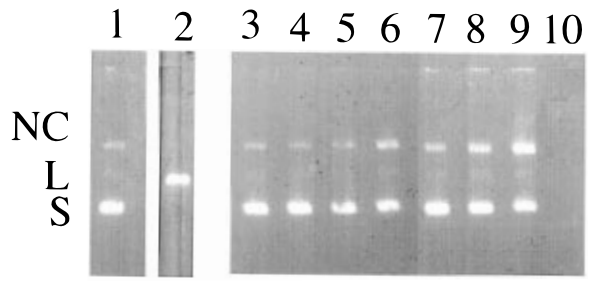

Figure 5. Concentration-dependent PM2 DNA nicking observed for two MRP mixtures: lane 1, supercoiled PM2 DNA; lane 2, supercoiled PM2 DNA treated with *Msp*I; lanes 3−6, DNA nicking patterns observed with Glu−Lys MRP mixture 3; lanes 7−10, DNA nicking patterns observed with Fru−Lys MRP mixture 11; lanes 3 and 7, PM2 DNA + 0.0001% (w/v) MRP mixture; lanes 4 and 8, PM2 DNA + 0.001% (w/v) MRP mixture; lanes 5 and 9, PM2 DNA + 0.01% (w/v) MRP mixture; lanes 6 and 10, PM2 DNA + 0.1% (w/v) MRP mixture. S, supercoiled DNA; NC, nicked circular DNA; L, linear DNA.

concentration of linear form of DNA was found to be retained at a constant level. However, with certain added MRPs (0.1%, w/v), a broad smear migrating between supercoiled and nicked circular forms was observed. This was an indication of the occurrence of extensive secondary strand breakage (degraded DNA). In some cases DNA fragmentation was so strong as to leave an empty lane on the gel (Figure 5, lane 10).

The percentage of original supercoiled DNA left at the end of each incubation with different MRP concentrations is given in Figure 6a. The relative efficacy of different model MRP mixtures to break supercoiled DNA to either nicked circular or degraded form was shown to be specific to the individual synthesis conditions used in different experiments that produced both Glu−Lys and Fru−Lys model MRP mixtures. A greater breakage of DNA generally occurred when Fru−Lys MRP mixtures were used. The exposure of supercoiled DNA to low concentrations of MRP mixtures [e.g. 10^{-3} and 10^{-2}% (w/v)] produced similar DNA strand breakage (≈1−10%) for both Glu−Lys and Fru−Lys MRPs across all experiments. Increasing the concentration of MRP mixtures to 10^{-1}% (w/v) not only increased the DNA breakage for both model MRP mixtures but also increased the spread of results obtained between common experiments within different Glu−Lys and Fru−Lys reactants. For example, at 10^{-1}% (w/v), MRP mixtures derived from Fru−Lys experiments 3−5, 12, and 14 caused maximal supercoiled DNA breakage compared to similarly synthesized Glu−Lys MRP mixtures. As a result, with an increase in the concentration of added MRPs, the percentage of nicked circular (Figure 6b) and degraded (Figure 6c) forms of DNA increased. It is noteworthy that while breakage of DNA by Glu−Lys MRP mixtures remained relatively constant for all synthesis experiments, considerable variation in DNA breakage was observed for different Fru−Lys MRP mixtures.

DISCUSSION

Measurement of Antioxidant Activity by Oxygen Consumption Measurements. Despite the known antioxidant activity of model MRPs reported by others (Bedinghaus and Ockerman, 1995; Lingert *et al.*, 1979), few studies have characterized the variable antioxidant activity of different MRP mixtures derived under specific synthesis conditions. The PI values calculated in the present study, employing a copper-supplemented linoleic acid model emulsion system, in which copper ions act as a promoter of lipid oxidation reactions, were useful to distinguish between antioxidant and prooxidant activities of model MRPs. The finding that many of the Fru−Lys MRP mixtures in the oxygen electrode experiment showed prooxidant activity compared to similarly synthesized Glu−Lys MRP mixtures indicated that those two sugars not only react at different rates but also to different extents in generating characteristic intermediates with variable oxidative behavior. Due to the complexity of the reactions involved in MR, and the uncertainty of common melanoidin end point formation, the exact structures of intermediate and final products responsible for the observed oxidative behavior could not be fully determined. In a parallel study conducted with the same MRP mixtures, it was determined that MRPs derived from Fru−Lys experiments possessed a relatively lesser number of copper binding sites compared to their Glu−Lys counterparts (Wijewickreme *et al.*, 1997). It is noteworthy that specific Fru−Lys experiments 6, 13, and 14, which exhibited pronounced prooxidant activity in the oxygen consumption method herein, were also observed to possess no copper binding activity. Therefore, relative metal chelating affinity of MRP mixtures could have a critical role in ascertaining the characteristic oxidative behavior of the differently synthesized MRP compounds.

Antioxidant Activity of MRP Mixtures As Measured by the TBA Method. All model MRPs displayed some degree of antioxidant activity in the model lipid system that used TBAR as the end point measurement. Since these studies were conducted without copper-supplemented model systems, the effectiveness of different MRP mixtures to display antioxidant activity in this lipid emulsion system likely signifies an affinity of MRP mixtures to scavenge free radicals. Compounds that act as free radical scavengers are known to lessen the propagation step in the lipid autoxidation reactions. The affinity of glucose−glycine conjugate derived melanoidins to scavenge superoxide free radicals, under aerobic conditions, has been reported earlier by Hyase *et al.* (1990).

Genotoxicity of Model MRP Mixtures. The dose response effects of both model Glu−Lys and Fru−Lys MRP mixtures on the breakage of DNA strands support previous findings that have reported mutagenecity of browning mixtures (Shinohara *et al.*, 1980; Kitts *et al.*, 1993). Comparison of those results with the apparent yield of MRP mixtures generated in each experiment revealed little indication of a relationship between DNA breakage and the yield of material generated from individual MRP synthesis experiments. DNA strand cleavage could occur due to the metal-driven Fenton reaction (Aruoma *et al.*, 1990) or due to the presence of chemiluminescent compounds (Hiramoto *et al.*, 1993). The latter is suspected in our study, since precautions were taken to remove any adventitious metal ions present in the system. Moreover, Kurosaki *et al.* (1989) showed that the chemiluminescent activity and browning reactivity of sugars follow the same order, namely, aldopentoses (ribose, arabinose, xylose) > aldohexoses (glucose, galactose) > ketohexoses (fructose). One exception to this was found in their study with D-glucose, whereby, although D-glucose possessed higher browning reactivity relative to fructose, it showed low chemiluminescent activity (Kurosaki *et al.*, 1989). Thus, the greater DNA breaking activity observed predominantly with Fru−Lys MRPs compared to Glu−Lys MRPs

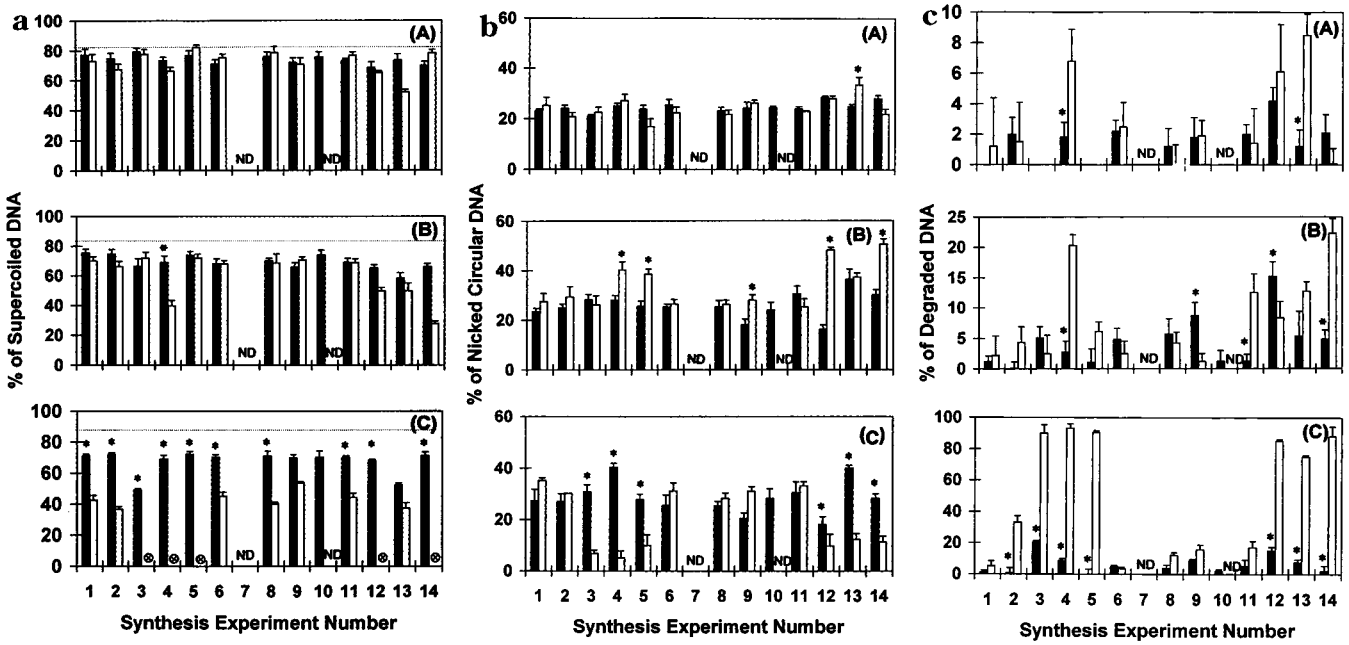

Figure 6. Percentage of supercoiled (a), nicked circular (b), and degraded (c) DNA remaining after PM2 phage DNA was incubated for 1 h at 37 °C with different concentrations of crude MRP mixtures derived from different synthesis experiments. Dotted lines represent initial percentage of DNA in control preparation. Values represent mean ± SD. Asterisks indicate significant difference ($p \leq 0.05$) with respect to similar Fru−Lys experiment. Cross-product indicates no remaining supercoiled DNA. ND, nondialyzable MRP not detected. (A) 0.001% (w/v) MRP; (B) 0.01% (w/v) MRP; (C) 0.1% (w/v) MRP. ■, Glu−Lys MRP mixture; □, Fru−Lys MRP mixture.

suggests that the precise chemical makeup of the complex mixture of compounds produced in different specific model MRP mixtures, although presently unknown, could be attributed to a difference in chemical composition of the synthesized products depending on the type of reducing sugar used to produce them. Moreover, Suarez *et al.* (1988) reported that Heyns products resulting from glycation with ketoses are highly reactive and facilitate a faster conversion to fluorophores compared to ketonic groups present in Amadori products. Therefore, ketose sugars seem to be more reactive in nonenzymatic glycation reactions. The results of the present study complement the above statement by showing the relatively greater genotoxic properties of Fru−Lys MRP mixtures compared to Glu−Lys MRP mixtures produced under similar reaction conditions. Finally, the greater DNA breaking activity noticed in our study with Fru−Lys MRP mixtures compared to Glu−Lys MRP mixtures could be attributed to characteristically different chemiluminescent compounds produced under different synthesis conditions.

CONCLUSIONS

Factors including pH, a_w, reaction time, temperature, and concentration of reactants have been regarded as important in influencing the composition of derived MRP mixtures (Pomeranz *et al.*, 1962). Results of the current study extend these findings to include that both the source of reactant sugar and the reaction conditions used in MRP synthesis will result in variable antioxidant and DNA strand breaking activity. An underlying characteristic of whether or not different MRPs exhibit antioxidant or prooxidant activity is likely explained both by the ability of MRPs to sequester transition metals that accelerate lipid oxidation reactions and by the possible scavenging of free radicals that are involved in the autoxidation propagation reactions. In general, Glu−Lys MRP mixtures gave rise to nondialyzable MRP mixtures possessing greater antioxidant activities with less genotoxic potential compared to counterpart Fru−Lys MRP mixtures. The present study, therefore, shows the importance of employing different *in vitro* antioxidant assay methods to evaluate the oxidative behavior of different Glu−Lys and Fru−Lys MRPs.

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Received for review January 13, 1997. Revised manuscript received June 2, 1997. Accepted September 4, 1997.[⊗] Financial support from the Natural Sciences and Engineering Research Council of Canada to D.D.K. is gratefully acknowledged.

JF970040V

[⊗] Abstract published in *Advance ACS Abstracts,* November 1, 1997.