

Hydroxyl Scavenging Activity of Glucose, Fructose, and Ribose-Lysine Model Maillard Products

A. N. Wijewickreme, Z. Krejpcio, and D. D. Kitts

ABSTRACT

Nondialyzable model Maillard reaction products (MRPs) were synthesized by reacting L-lysine with D-glucose, D-fructose, and D-ribose under different conditions of reaction time, temperature, pH, and water activity (a_w). Five experiments common to all three models yielding greater than 0.5g of MRPs were assessed for antioxidant activity. All MRPs exhibited detectable, but variable, non site-specific hydroxyl radical ($\cdot\text{OH}$) scavenging activity (30–90%) in the deoxyribose assay. MRPs derived from a ribose-lysine study showed the highest $\cdot\text{OH}$ scavenging activity (80–90%) in both deoxyribose and DNA nicking assays. All MRPs at higher concentrations (0.2 mg/mL) decreased lipid peroxidation in linoleic acid emulsions. Relative effectiveness of different MRPs to scavenge free radicals can vary with reaction conditions and substrates.

Key Words: Maillard products, free radicals, hydroxyl radical, nondialyzable, non site-specific

INTRODUCTION

THE EFFECTS OF ACTIVE OXYGEN AND FREE RADICALS IN PRODUCING tissue damage in human disorders is becoming increasingly well recognized (Halliwell et al., 1993). Active oxygen as superoxide ($\text{O}_2^{\cdot-}$, hydrogen peroxide (H_2O_2), or hydroxyl radical ($\cdot\text{OH}$) is a by-product of normal metabolism. It represents a potential toxic hazard to various biological molecules leading to tissue damage, cell injury or death (Yen and Chen, 1995; Yuan and Kitts, 1997). Formation of $\cdot\text{OH}$ or highly oxidizing species from H_2O_2 have been associated with transition metal ions such as iron and copper and reducing agents (at low concentrations) such as $\text{O}_2^{\cdot-}$ and ascorbate (Aruoma et al., 1987). The damage induced by free radicals is often prevented by scavengers. Some of these are glucose, mannitol, formate, thiourea, butan-1-ol, ethanol, plant phenols, α -tocopherol, and β -carotene (Byers and Peri, 1992; Guo et al., 1997).

Maillard reaction products (MRPs) are well known to exhibit antioxidant activities in both model lipid (McGookin and Augustin, 1991; Wijewickreme and Kitts, 1997) and food (Bedinghaus and Ockerman, 1995; Wijewickreme and Kitts, 1998a; b) systems. Although many antioxidant activity measurements were associated with determining peroxyl radical scavenging activity of MRPs, very few studies have reported the affinity of different model MRPs to specifically scavenge $\cdot\text{OH}$ *in vitro*. Our objective was to assess the non site-specific $\cdot\text{OH}$ scavenging activity of 3 model MRPs (glucose-lysine, fructose-lysine, ribose-lysine) by employing both chemical (deoxyribose) and biological (DNA nicking) *in vitro* assays. Further, the effectiveness of MRPs to control lipid peroxidation in an

aqueous medium was compared with a nonaqueous lipid (linoleic acid emulsion) system.

MATERIALS & METHODS

Materials

All chemicals and reagents were of highest purity available. D-glucose, D-fructose, D-ribose, L-lysine, 2-butylatedhydroxy anisole (BHA), trichloroacetic acid (TCA), trizma base, ethidium bromide, bromophenol blue, xylene cyanole FF, ficoll, electrophoresis grade agarose, di-sodium ethylenediaminetetraacetic acid (2Na.EDTA), ferric chloride, ferrous sulphate, deoxyribose, L-ascorbic acid, 2-thiobarbituric acid (2-TBA), sodium acetate, chelex 100, and pBR322 plasmid DNA from *Escherichia coli* strain RRI were purchased from Sigma Chem. Co. (St. Louis, MO). Sodium dihydrogen orthophosphate, di-sodium hydrogen orthophosphate, and sodium hydroxide were purchased from BDH Chem. Co. (Detroit, MI). Hydrogen peroxide (H_2O_2) was obtained from Fisher Scientific Co. (Fair Lawn, NJ). Deionized double distilled water and all buffers were further purified by treating with chelex-100. All glassware was submerged overnight in 2N HCl and rinsed thoroughly with deionized, distilled water before use.

Model MRPs

MRPs were synthesized according to the method described by Wijewickreme et al. (1997). L-lysine (800 mM) was heated with the following sugars: D-glucose, D-fructose, or D-ribose (800 mM) in a hot air oven (Blue M, Blue Island, IL) set at a prescribed temperature for a designated time period. Following heating, round bottom flasks containing brown solutions were rapidly cooled on ice, dialyzed (6–8 kD) against 20–30 changes of deionized distilled water for 7 days, and the non-dialyzable fraction was lyophilized. The prepared lyophilized powders were termed as *Glu-Lys*, *Fru-Lys*, and *Rib-Lys* MRPs. Five sets of conditions common to all 3 models yielding >0.5g of MRPs were assessed for antioxidant activity. Except *Rib-Lys* MRPs, the other MRPs of *Glu-Lys* and *Fru-Lys* reactions were identical to those studied in earlier reports (Wijewickreme and Kitts, 1997).

Non site-specific $\cdot\text{OH}$ assay

Non site-specific $\cdot\text{OH}$ radical scavenging activity of MRPs was measured according to the method given by Halliwell et al. (1987). Solutions of FeCl_3 and ascorbate were prepared in deaerated water immediately before use. Final reaction solution (1 mL) consisted of aliquots (0–200 μL) of MRPs (0.2 mg/mL), FeCl_3 (100 μmol), EDTA (100 μmol), H_2O_2 (1 mmol), deoxyribose (3.6 mmol), and L-ascorbic acid (100 μmol) in potassium phosphate buffer. The reaction mixture was incubated for 1h at 37°C and heated further in a boiling water bath for 15 min following the addition of 1 mL each of TCA (10%) and 2-TBA (0.5% 2-TBA in 0.025M NaOH containing 0.02% BHA). After cooling, color development was measured at 532 nm. All readings were corrected for any interference from brown color of the MRPs.

DNA nicking assay

All experiments were conducted in potassium phosphate buffer

Authors Wijewickreme and Kitts are with Food Science, Univ. of British Columbia, 6650 N.W. Marine Drive, Vancouver, BC, Canada. Author Krejpcio is with the Dept. of Food Hygiene & Human Nutrition, Agriculture Univ., Poznan, Poland. Address inquiries to Dr. D. D. Kitts.

(pH 7.4, 50 mM) at atmospheric pressure. To assess the non site-specific $\cdot\text{OH}$ scavenging activity of model MRPs, 2 μL each of MRPs (0.02 mg/mL), EDTA (30 mM), buffer (50 mM, pH 7.4), hydrogen peroxide (30 mM), ferrous sulphate (16 mM), and DNA (0.1 $\mu\text{g}/\mu\text{L}$) were mixed in a 500 μL microcentrifuge tube. The molar ratio of ferrous sulphate/EDTA was kept at 0.53 (Repine et al., 1981). The final volume of the reaction mixture was brought to 12 μL with deionized distilled water and incubated for 1h at 37°C. Following incubation, 2 μL of loading dye (0.25% bromophenol blue, 0.25% xylene cyanol FF, and 15% ficoll in water) was added and 12 μL of this mixture was loaded onto an agarose gel well. Electrophoresis was conducted at 60 volts in Tris-Acetate-EDTA (TAE) buffer (0.04M tris-acetate and 0.001M EDTA, pH 7.4). The agarose gel was stained with ethidium bromide (0.5 $\mu\text{g}/\text{mL}$ deionized distilled water) for 20 min. DNA bands were visualized under UV light and photographed with a Bio-Rad polaroid camera using type 665 positive films.

Reducing activity of MRPs

Reducing activity of model MRPs were measured according to the method of Yen and Chen (1995). Samples (1 mL) of MRPs or ascorbic acid (0.02 mg/mL), were mixed with 2.5 mL of phosphate buffer (pH 7.4; 20 mM) and 2.5 mL of potassium ferricyanide (1%, w/v). Samples were incubated at 50°C for 20 min. Then 0.5 mL of ferric chloride (0.1%, w/v) and 2.5 mL of deionized distilled water was added to 2.5 mL of reaction mixture. The absorbance was read at 700 nm against a control without test compounds.

Ammoniumthiocyanate assay

The ammoniumthiocyanate assay was a modification of Asamari et al. (1996). A linoleic acid pre-emulsion was made by vortex mixing 3 mL of linoleic acid with 3 mL of Tween 20 in 200 mL of 30% (v/v) ethanol. MRPs (1 mL) dissolved in water (0.002–0.2 mg/mL) were added to 10 mL of pre-emulsion, and the final volume of the mixture was adjusted to 25 mL with deionized distilled water. The mixed solutions were incubated in conical flasks at 40°C. Aliquots (100 μL) from the incubated mixture were removed at several intervals and tested for lipid peroxidation products. The assay was conducted by adding 5 mL of 75% ethanol, 0.1 mL of ammonium thiocyanate solution (30% w/v), and 0.1 mL of ferrous chloride (0.1% w/v) to 100 μL of sample. The absorbance of the reaction mixture was measured at 500 nm against 75% ethanol contained in a reference cell. The anti-/pro-oxidant activity of MRPs were calculated as the absorbance difference at A_{500} nm between emulsions with and without MRPs. A negative value demonstrated an antioxidant activity of MRPs, and a positive value demonstrated a prooxidant activity.

RESULTS & DISCUSSION

SOME REACTION CONDITIONS USED FOR THE GENERATION OF NON-dialyzable MRPs gave yields >0.5 g (Table 1). The relatively mild reaction conditions consisted of short reaction times and low temperatures typical of standard cooking conditions. Only 5 of the 14 experiments produced detectable (>0.5 g) MRP yields; thus products from these experiments were evaluated for possible antioxidant activity.

Note that this is an extension of our previous study, which examined the peroxy radical scavenging activity of *Glu-Lys* and *Fru-Lys* model MRPs (Wijewickreme and Kitts, 1997).

The elemental analysis, average empirical formulae, and reducing activity of the synthesized MRPs were determined (Table 2). A narrow range of reaction temperatures produced a wide range of MRP yields with slight variations in empirical formulae (under identical molar ratios of different sugar/amino acid reactants) and reducing activity. This demonstrates that Amadori re-arrangement reactions, although sensitive to temperature, can be modified by slight differences in initial medium pH or a_w . Studies have shown that the source and the molar ratio of reaction sugar to available nitrogen and reaction conditions influence the rate and structure of synthesized MRPs (Renn and Sathe, 1997; Wijewickreme et al., 1997). Since high activation energies are required for Amadori rearrangements (Labuza, 1994), reaction temperature and initial pH are important in the generation of MRPs in model systems (Baisier and Labuza, 1992; Renn and Sathe, 1997; Wijewickreme et al., 1997). A pH increase enhances the production of Amadori rearrangement products since alkaline pH favours the MRP precursor Schiff base formation (Ge and Lee, 1997). The higher percentage of nitrogen and lower percentage of carbon observed in *Fru-Lys* MRPs, compared to both *Glu-Lys* and *Rib-Lys* MRPs from counterpart experiments, confirmed the occurrence of a lower degree of polymerization during the production of *Fru-Lys* MRPs. These findings extend our previous study that demonstrated different sugar substrates yield different MRPs with a common amino acid, L-lysine (Wijewickreme et al., 1997).

The TBA-malonaldehyde adducts were produced by reacting deoxyribose with Fe^{3+} -EDTA, H_2O_2 , and ascorbic acid in an aqueous medium (Halliwell et al., 1987). In this assay, Fe^{3+} in the presence of a reducing agent, ascorbic acid, and H_2O_2 produces the highly reactive $\cdot\text{OH}$ which degrades deoxyribose into a complex mixture of adducts that produce color with TBA in an acidic medium (Fig. 1a). The presence of EDTA in the reaction mix enhances the formation of free $\cdot\text{OH}$ in solution since EDTA can chelate Fe^{3+} and keep it from attacking deoxyribose. Thus, $\cdot\text{OH}$ generated in this manner from Fenton reactants attack deoxyribose in a non site-specific manner (Aruoma, 1996). In our study, different MRPs produced characteristically different affinities towards inhibiting $\cdot\text{OH}$.

$\cdot\text{OH}$ inhibition

The percent $\cdot\text{OH}$ inhibition vs the yield of individual MRP synthesis experiments were compared (Fig. 1b). Results showed that the relative $\cdot\text{OH}$ scavenging activities of MRPs did not always correspond directly with the yield and varied considerably with source of the reacting sugar. The degree of suppressing $\cdot\text{OH}$ and associated yield of derived MRPs was closest for the ribose-lysine reaction. Good agreement was found between the number of carbon atoms in empirical formulae and the yield of MRPs. A relationship between the number of carbon atoms in MRP products and $\cdot\text{OH}$ scavenging activity could not be made for all sugar/lysine reactants. Similar reaction conditions produced variable MRP yields and affinities to inhibit $\cdot\text{OH}$

Table 1—Initial and final experimental conditions [reaction time, oven temperature, initial water activity (a_w), and initial pH] used and obtained in the preparation of *Glu-Lys*, *Fru-Lys*, and *Rib-Lys* nondialyzable model MRP mixtures and the yield gained at the end of the reaction

Expt. no.	Initial Study Conditions				Final Study Conditions						Yield (g) ^a		
	Time (min)	Oven temp ^b	pH	a_w	<i>Glu-Lys</i>		<i>Fru-Lys</i>		<i>Rib-Lys</i>		<i>Glu-Lys</i>	<i>Fru-Lys</i>	<i>Rib-Lys</i>
					pH	a_w	pH	a_w	pH	a_w			
1	65	110	6.37	0.58	3.20	0.56	3.20	0.56	2.66	0.59	2.41±0.004	0.75±0.003	1.49±0.002
2	119	127	6.14	0.74	3.14	0.71	3.12	0.68	2.77	0.61	7.03±0.002	0.55±0.001	4.04±0.001
3	107	157	8.51	0.57	3.62	0.53	3.75	0.51	4.03	0.70	2.00±0.003	5.11±0.002	4.45±0.021
4	43	159	8.57	0.62	3.54	0.59	3.51	0.61	4.53	0.74	4.10±0.003	6.65±0.003	0.64±0.016
5	71	129	8.41	0.78	3.86	0.74	3.84	0.73	4.79	0.73	7.50±0.002	0.50±0.003	2.11±0.027

^aYield in gram nondialyzable MRP per 100 mL of final reaction solution. Values represent mean \pm SEM (n=3).

^bAmbient temperature inside the oven (°C).

Table 2—Elemental analysis and average empirical formulae of *Glu-Lys*, *Fru-Lys*, and *Rib-Lys* Model MRPs

Type of MRP mixture	%C	%H	%O ^a	N%	Average Empirical Formula ^b	Reducing Activity
<i>Glu-Lys</i> MRP mixture 1	50.34	7.41	35.54	6.71	C _{8.75} H _{15.46} NO _{4.63}	16.57
<i>Glu-Lys</i> MRP mixture 2	51.87	6.74	35.03	6.36	C _{9.50} H _{14.83} NO _{4.82}	15.21
<i>Glu-Lys</i> MRP mixture 3	53.48	6.17	33.92	6.43	C _{9.70} H _{13.43} NO _{5.60}	17.34
<i>Glu-Lys</i> MRP mixture 4	48.58	6.75	38.01	6.66	C _{8.51} H _{14.12} NO _{5.00}	16.69
<i>Glu-Lys</i> MRP mixture 5	50.06	7.27	35.84	6.83	C _{8.56} H _{14.90} NO _{3.59}	14.59
<i>Fru-Lys</i> MRP mixture 1	48.41	7.15	36.87	7.57	C _{7.46} H _{13.22} NO _{4.26}	17.31
<i>Fru-Lys</i> MRP mixture 2	51.62	6.72	34.04	7.62	C _{7.95} H _{12.83} NO _{3.91}	15.39
<i>Fru-Lys</i> MRP mixture 3	53.21	6.42	33.69	7.76	C _{8.02} H _{13.29} NO _{4.36}	15.69
<i>Fru-Lys</i> MRP mixture 4	47.63	7.39	37.22	7.76	C _{7.16} H _{13.33} NO _{4.20}	17.37
<i>Fru-Lys</i> MRP mixture 5	50.39	7.60	33.66	8.35	C _{7.04} H _{12.75} NO _{3.53}	15.54
<i>Rib-Lys</i> MRP mixture 1	53.61	6.36	33.58	6.45	C _{9.70} H _{13.72} NO _{4.56}	15.69
<i>Rib-Lys</i> MRP mixture 2	55.00	6.30	31.81	6.89	C _{9.76} H _{12.76} NO _{4.06}	16.71
<i>Rib-Lys</i> MRP mixture 3	55.27	6.49	31.75	6.49	C _{10.00} H _{14.00} NO _{4.30}	16.65
<i>Rib-Lys</i> MRP mixture 4	51.95	6.72	33.33	8.00	C _{7.60} H _{11.70} NO _{3.65}	15.57
<i>Rib-Lys</i> MRP mixture 5	51.51	6.99	33.42	8.08	C _{7.40} H _{11.95} NO _{3.60}	16.01

^aCalculated from C, H, and N%.^bCalculated as N=1.^cCalculated on the basis of ascorbic acid as 100% at 0.2 mg/mL concentration.

for three different sugar/lysine MRP model systems in an aqueous test medium. The fact that MRPs vary in relative affinities to chelate metal ions (Gomyo and Horikoshi, 1976; Wijewickreme et al., 1997) may explain the relative affinity of different MRPs in suppressing Fenton reaction catalyzed prooxidant reactions (Yoshihiro et al. 1997; Wijewickreme and Kitts, 1998b).

DNA nicking

The efficiency of MRPs to protect DNA against breakage from Fenton reactants in an aqueous medium was also studied. The effects of *Glu-Lys*, *Fru-Lys*, and *Rib-Lys* model MRPs were studied from different synthesis experiments on pBR 322 plasmid DNA peroxidation (Fig. 2 a, b, c). The $\cdot\text{OH}$ generated by Fenton reactants attack DNA guanosine residues, resulting in strand breakage and transformation from native supercoiled (i.e., lane 1) to relaxed nicked circular or linear forms (i.e., lane 2). The 3 sugar-lysine model MRPs alone, did not break DNA (lanes 3,5,7,9 and 11 in Fig.2 a, b, and c, respectively) at 0.02 mg/mL, thus, supporting our previous findings (Wijewickreme and Kitts, 1997). However, model MRPs, synthesized from all three sugar-lysine mixtures, when in the presence of Fenton reactant agents, failed to prevent non-site specific $\cdot\text{OH}$ induced oxidative damage to DNA (lanes 4,6,8,10, and 12 in Fig.2 a, b, and c, respectively). The exception was that MRPs synthesized from experiment 3 of the *Rib-Lys* reaction (lane 8, Fig. 2c) protected DNA in nicked circular form, without allowing complete breakage to degraded form by Fenton agents. This result was similar to the observed strong affinity of *Rib-Lys* MRPs from experiment 3 to protect deoxyribose against oxidation (Fig. 1). Notwithstanding, the effectiveness of different model MRPs to prevent oxidative damage to DNA was relatively less than that observed using the deoxyribose assay.

Reducing activity

Reducing activity of products derived from model MRPs has also been reported to be a factor associated with $\cdot\text{OH}$ scavenging activity in xylose-lysine MRPs (Osawa et al., 1992; Yen and Hsieh, 1995). Certain phenolic compounds accelerate the production of $\cdot\text{OH}$ in Fenton induced reactions (Arumoa, 1993), due to reducing activity in the presence of transition metal ions. Ascorbic acid and Cu^{+2} exhibit a behaviour characterized by prooxidant activity at low concentrations and antioxidant activity at higher concentrations (Kaner et al., 1977; Mahoney and Graft, 1986). Differently synthesized MRPs used in our study had similar reducing activity (Table 2) but different affinities to inhibit $\cdot\text{OH}$, regardless of MRP yield. It is unlikely that reducing power was the only explanation for the characteristically different affinities of synthesized MRPs to inhibit Fenton induced $\cdot\text{OH}$ degradation of deoxyribose or DNA.

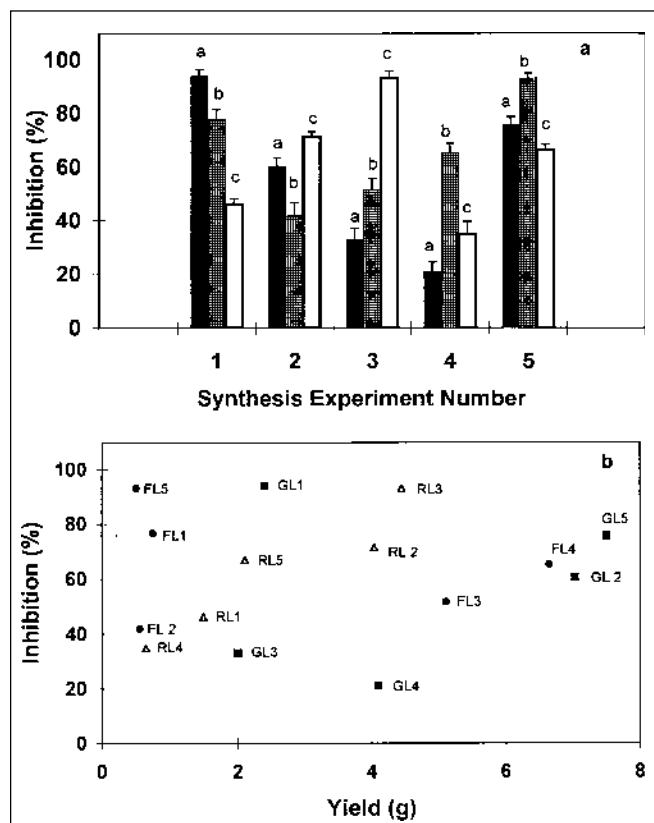


Fig. 1—(a) Non site-specific hydroxyl radical scavenging activity of nondialyzable *Glu-Lys*, *Fru-Lys*, and *Rib-Lys* model Maillard reaction products. MRP concentration=0.2 mg/mL. Values represent mean \pm SD. Different letters (a,b,c) denote that % inhibition values are significantly different among the three different models at a given synthesis experiment. ■ *Glu-Lys* MRPs; ▨ *Fru-Lys* MRPs; □ *Rib-Lys* MRPs. (b) Percent hydroxyl radical scavenging activity vs yield of different model MRP synthesis experiments. ■ *Glu-Lys* MRPs; ● *Fru-Lys* MRPs; △ *Rib-Lys* MRPs. GL 1-5=*Glu-Lys* synthesis experiment number; FL 1-5=*Fru-Lys* synthesis experiment number; RL 1-5=*Rib-Lys* synthesis experiment number.

Antioxidant activity

We employed a model lipid peroxidizing system consisting of linoleic acid substrate and Tween-20 emulsified in ethanol to evaluate antioxidant activity potential of different MRPs in a lipid medium. A similar approach has been reported from our laboratory to detect antioxidant activity of *Glu-Lys* and *Fru-Lys* MRPs, but using a different

endpoint measurement for lipid peroxidation (Wijewickreme and Kitts, 1997). In the present study, a time-dependent generation of peroxy radicals as measured by the thiocyanate method showed that *Glu-Lys* MRPs derived from experiments 1, 2, and 5 exhibited antioxidant activity (e.g. negative number) at all concentrations of MRP tested. Antioxidant behaviour of *Fru-Lys* MRPs was less than that of *Glu-Lys* and *Rib-Lys* MRPs in all synthesis experiments. The prooxidant activities in *Fru-Lys* MRP synthesis experiments 3 and 4 were quite distinct from the observed antioxidant activities obtained from *Glu-Lys* and *Rib-Lys* MRPs generated using identical conditions. These

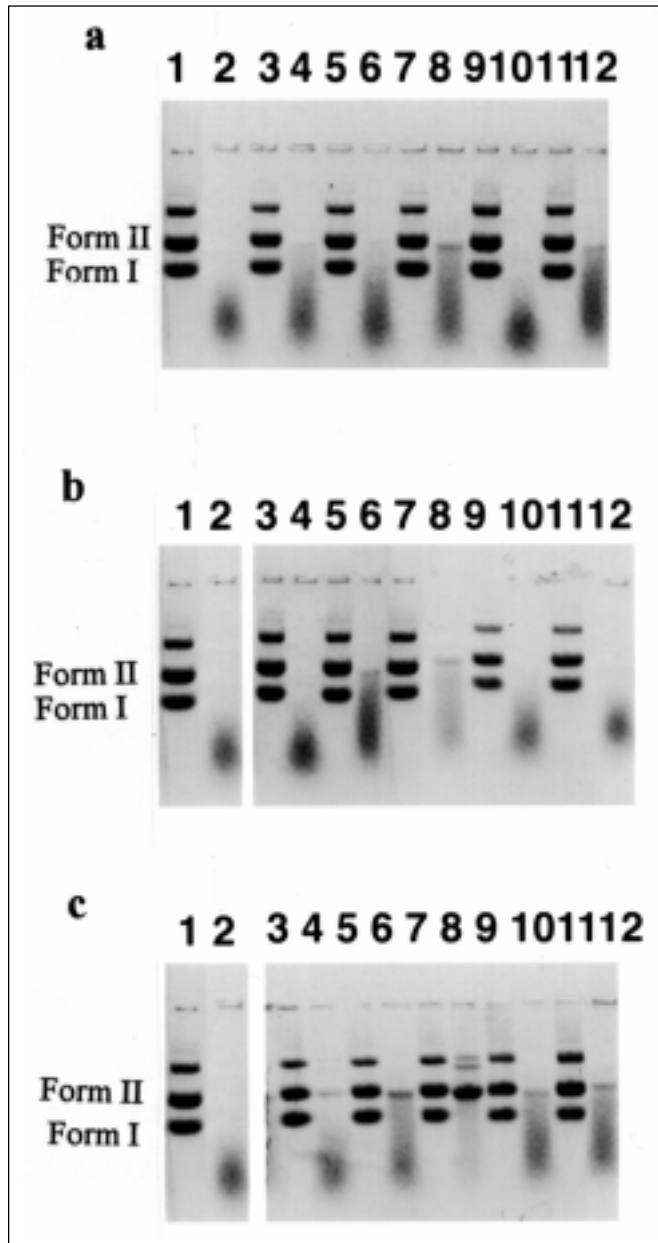


Fig. 2—Modulation of non site-specific DNA strand cleavage by (a) *Glu-Lys* MRPs; (b) *Fru-Lys* MRPs; and (c) *Rib-Lys* MRPs. Lanes 1 and 2 are common to all three figures. Lane 1, Original DNA; Lane 2, DNA (0.2 µg) + EDTA (30 mM) + H₂O₂ (30 mM) + FeSO₄ (16 mM) + phosphate buffer (50 mM, pH 7.5); Lanes 3, 5, 7, 9, and 11 in (a), (b), and (c) are DNA + MRPs (0.02 mg/mL) synthesized from experiment numbers 1, 2, 3, 4, and 5 of *Glu-Lys*, *Fru-Lys*, and *Rib-Lys*, respectively (without Fenton agents). Lanes 4, 6, 8, 10, and 12 in (a), (b), and (c) are DNA + MRPs (0.02 mg/mL) synthesized from experiment numbers 1, 2, 3, 4, and 5 of *Glu-Lys*, *Fru-Lys*, and *Rib-Lys*, respectively + Fenton agents (reactants used in Lane 2). Form I=supercoiled DNA; Form II=Nicked circular DNA.

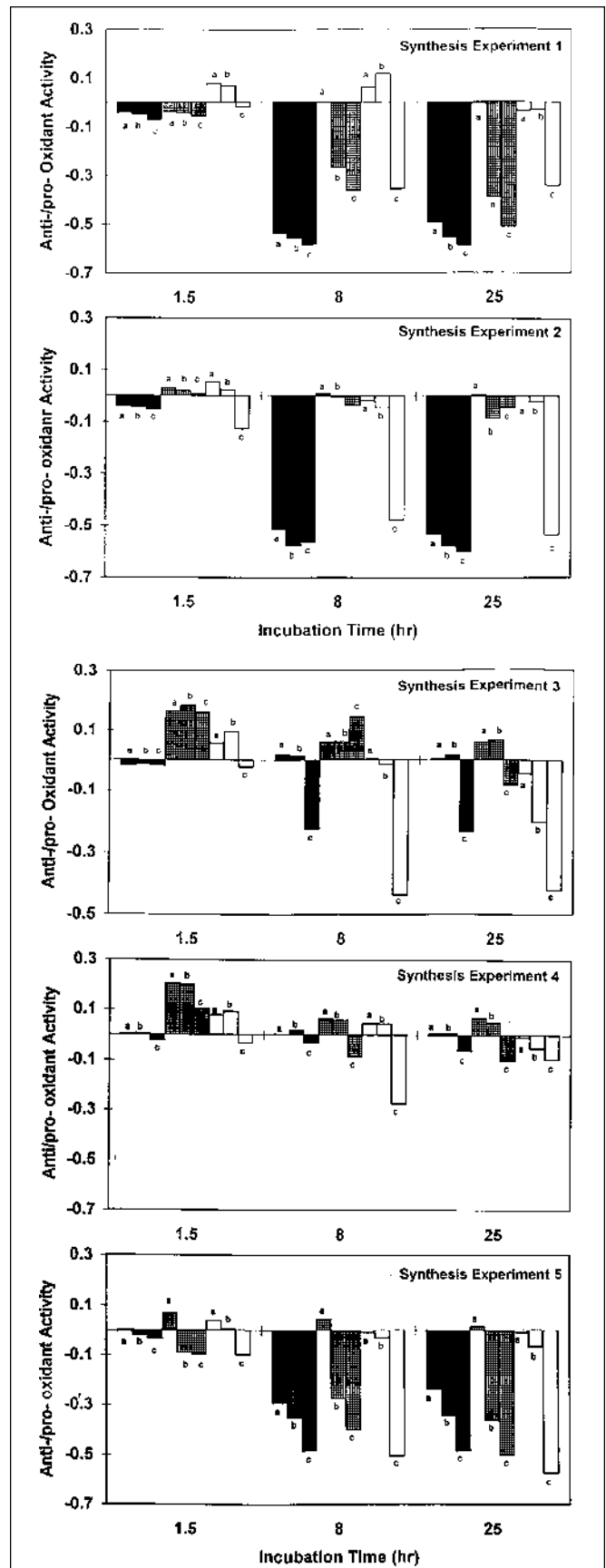


Fig. 3—Effectiveness of *Glu-Lys*, *Fru-Lys*, and *Rib-Lys* model MRPs to mitigate lipid peroxidation in a linoleic acid emulsion. (a) 0.002 mg/mL MRPs; (b) 0.02 mg/mL MRPs; (c) 0.2 mg/mL MRPs. ■ *Glu-Lys* MRPs; ▨ *Fru-Lys* MRPs; △ *Rib-Lys* MRPs.

findings extend our previous observations concluding that *Fru-Lys* MRPs, in general, had a greater tendency for prooxidant activity (Wijewickreme and Kitts, 1997) influenced by synthesis conditions. While all *Rib-Lys* MRPs showed prooxidant activity at both low MRP concentration and at an early stage in the lipid oxidation reaction, this oxidative behavior was lost with prolonged incubation of emulsion constituents. This effect was specifically noted with MRP synthesis experiments 1, 2 and 5 of *Rib-Lys* MRPs. A similar prooxidant-antioxidant behaviour was also observed in *Fru-Lys* MRP synthesis experiment 3, demonstrating different concentration thresholds for inhibition of lipid oxidation.

An association has been demonstrated between generation of $\cdot\text{OH}$ and subsequent formation of lipid oxidation products *in vitro* (Mahoney and Graf, 1986). Our findings confirmed this association involving both Fenton reactant induced deoxyribose and DNA oxidation by $\cdot\text{OH}$ in an aqueous medium and peroxy radical induced lipid oxidation in a lipid medium. Moreover, MRPs exhibiting at least 60% inhibition power against $\cdot\text{OH}$ in the deoxyribose assay also showed strong antioxidant activity in the lipid emulsion assay. Therefore, our findings demonstrated that unique, but slight, differences in composition of derived MRP constituents produced different expressions of oxidative behaviour. For example, all MRPs derived in synthesis experiment 4 produced relatively low $\cdot\text{OH}$ inhibition and antioxidant activity in lipid emulsions. Specifically, the MRP derived from *Rib-Lys* experiment 3 produced both strong $\cdot\text{OH}$ and peroxy radical inhibition affinity. Further studies are needed to ascertain the structure-activity relationships that are the basis of antioxidant activity of MRPs.

CONCLUSIONS

MODEL MRPS GENERATED FROM *GLU-LYS*, *FRU-LYS* AND *RIB-LYS* mixtures produced characteristic antioxidant and prooxidant behavior that varied with the source of reaction sugar and reaction conditions. Among the three models, *Fru-Lys* MRPs exhibited the greatest tendency for prooxidant behavior. *Rib-Lys* MRPs synthesized under conditions of 157°C; pH=8.51; $a_w=0.57$ and a reaction time of 107 min produced the greatest overall antioxidant activity common to three test systems, deoxyribose, DNA and lipid emulsion.

REFERENCES

- Aruoma, O.I., Grootveld, M., and Halliwell, B. 1987. The role of iron in ascorbate-dependent deoxyribose degradation. Evidence consistent with a site specific hydroxyl radical generation caused by iron ions bound to the deoxyribose molecule. *J. Inorg. Biochem.* 29: 289-299.
- Aruoma, O.I. 1993. Free radicals in foods. *Chemistry in Britain* 29: 210-214.
- Aruoma, O.I. 1996. Assessment of potential prooxidant and antioxidant actions. *J. AOCS* 73: 1617-1625.
- Asamari, A.M., Addis, P.B., Epley, R.J., and Krick, T.P. 1996. Wild rice hull antioxidants. *J. Agric. Food Chem.*, 44: 126-130.
- Baisier, W.M., and Labuza, T.P. 1992. Maillard browning kinetics in a liquid model system. *J. Agric. Food Chem.* 40: 707-713.
- Bedinghaus, A.J. and Ockerman, H.W. 1995. Antioxidative Maillard reaction products from reducing sugars and free amino acids in cooked ground pork patties. *J. Food Sci.* 60: 992-995.
- Byers, T. and Perry, G. 1992. Dietary carotenes, vitamin C, and vitamin E as protecting antioxidants in human cancers. *Annu. Rev. Nutr.* 12: 139-159.
- Ge, S.J. and Lee, T.C. 1997. Kinetic significance of the Schiff base reversion in the early stage Maillard aqueous model system. *J. Agric. Food Chem.* 45: 1619-1623.
- Gomyo, T. and Horikoshi, M. 1976. On the interaction of melanoidins with metal ions. *Agric. Biol. Chem.* 40: 33-40.
- Guo, C., Cao, G., Sofic, E., and Prior, R.L. 1997. High performance liquid chromatography coupled with coulometric array detection of electroactive components in fruits and vegetables: Relationship to oxygen radical absorbance. *J. Agric. Food Chem.* 45: 1787-1796.
- Halliwell, B., Gutteridge, J.M.C., and Aruoma, O.I. 1987. The deoxy ribose method: A simple test tube assay for determination of rate constants for reaction of hydroxyl radicals. *Anal. Biochem.* 165: 215-219.
- Halliwell, B. and Chirico, S. 1993. Lipid peroxidation: Its mechanism, measurement, and significance. *Am. J. Clin. Nutri.* 57: 7158-7253.
- Kanner, J., Mandel, H., and Budowski, P. 1977. Prooxidant and antioxidant effects of ascorbic acid and metal salts in a β -carotene-linoleate model system. *J. Food Sci.* 42: 60-64.
- Labuza, T.P. 1994. Interpreting the complexity of the kinetics of Maillard reaction. In *Maillard Reaction in Chemistry, Food, and Health*, T.B. Labuza, G.A. Reineccius, J. O'Brien, and J. Baynes (Ed.), p. 176-181. The Royal Society of Chemistry, Cambridge, UK.
- Mahoney, J.R. and Graf, E. 1986. Role of α -tocopherol, ascorbic acid and EDTA as oxidants in model systems. *J. Food Sci.* 51: 1293-1296.
- McGookin, B.J. and Augustin, M-A. 1991. Antioxidant activity of Maillard reaction products from casein-sugar mixtures. *J. Dairy Res.* 58: 313-320.
- Osawa, T., Katsuzaki, H., Hagiwara, Y., Hagiwara, H., and Shibamoto, T. 1992. A novel antioxidant isolated from young green barley leaves. *J. Agric. Food Chem.* 40: 1135-1138.
- Renn, P.T. and Sathe, S.K. 1997. Effects of pH, temperature, and reactant molar ratio on L-leucine and D-glucose Maillard browning reaction in an aqueous system. *J. Agric. Food Chem.* 45: 3782-3787.
- Repine, J.E., Pfenninger, O.W., Talmage, D.W., Berger, E.M., and Pettjohn, D.E. 1981. Dimethyl sulfoxide prevents DNA nicking mediated by ionizing radiation or iron/hydrogen peroxide-generated hydroxyl radical. *Proc. Natl. Acad. Sci.* 78: 1001-1003.
- Wijewickreme, A.N., Kitts, D.D., and Durance, T.D. 1997. Reaction conditions influence the elementary composition and metal chelating affinity of non-dialyzable model Maillard reaction products. *J. Agric. Food Chem.* 45: 4577-4583.
- Wijewickreme, A.N. and Kitts, D.D. 1997. Influence of reaction conditions on the oxidative behaviour of model Maillard reaction products. *J. Agric. Food Chem.* 45: 4571-4576.
- Wijewickreme, A.N. and Kitts, D.D. 1998a. Oxidative reactions of model Maillard reaction products and α -tocopherol in a flour-lipid mixture. *J. Food Sci.* 63: 466-471.
- Wijewickreme, A.N. and Kitts, D.D. 1998b. Modulation of metal induced genotoxicity by Maillard reaction products isolated from coffee. *Food Chem Toxicol.* 36: 543-553.
- Yen, G.-C. and Chen, H.-Y. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* 43: 27-32.
- Yen, G.C. and Hsieh, P.P. 1995. Antioxidant activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction products. *J. Sci. Food Agric.* 67: 415-420.
- Yoshihiro, Y., Iijima, T., and Watanabe. 1997. Antioxidant effect of Maillard reaction products using glucose-glycine model system. *J. Agric. Food Chem.* 45: 4106-4109.
- Yuan, Y.V. and Kitts, D.D. 1997. Endogenous antioxidants: Role of antioxidant enzymes in biological systems. In *Natural Antioxidants: Chemistry, Health Effects and Applications*, F. Shahidi (Ed.), p. 258-270. AOAC Press, Champaign, IL.

Ms received 2/23/98; revised 11/25/98; accepted 12/14/98.

This work was supported by a NSERC-operating research grant to DDK.