JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Maillard Reaction Products Inhibit Oxidation of Human Low-Density Lipoproteins in Vitro

RALF DITTRICH,[†] FAROUKH EL-MASSRY,[‡] KATRIN KUNZ,[†] FRANCESCO RINALDI,[§,||] CARLO C. PEICH,[||] MATTHIAS W. BECKMANN,[†] AND MONIKA PISCHETSRIEDER*,[||]

Department of Obstetrics and Gynecology, University Erlangen-Nuremberg, Universitätsstr. 21-23, 91054 Erlangen, Germany, Flavor and Aroma Department, National Research Center, Cairo, Egypt, Institute of Pharmacy and Food Chemistry, University of Erlangen-Nuremberg, Schuhstr. 19, 91052 Erlangen, Germany.

Dietary intake of antioxidants has been associated with a reduced risk of cardiovascular diseases, which is very likely caused by their capability of prevent oxidation of low-density lipoproteins (LDL). During food processing and storage, substances with antioxidative properties are formed by Maillard reactions. In this study, the activity of Maillard products to inhibit copper-induced oxidation of human LDL in vitro was investigated. D-Glucose was heated with an equimolar amount of glycine, L-lysine, or L-arginine, for 1 h under reflux. The increase of the antioxidative activity (AOA) of the Maillard mixtures was highly significant compared to the control mixtures. Additionally, two defined Maillard products with amino reductone structure were tested. 3-Hydroxy-4-(morpholino)-3-buten-2-one (**1**) and amino hexose reductone (**2**) showed a significant and dose dependent AOA. Compound **1** was about half as active as ascorbic acid, which served as positive control. Thus, it can be concluded that Maillard products, particularly those with amino reductone structure, have the strong potential to inhibit LDL oxidation.

KEYWORDS: Amino reductones; Maillard reaction; LDL oxidation; low-density lipoprotein; amino hexose reductone

INTRODUCTION

Antioxidative compounds, which occur naturally in many food stuffs, are important for food technology, because they inhibit lipid oxidation, and as a result, prolong shelf life of the products (*1*, *2*). In addition to antioxidative vitamins, phenolic compounds from spices (*1*), tea (*3*), fruits and berries, (*4*) or wine (*5*) are very active. More recently, it was suggested that the intake of antioxidative food components is also beneficial for health. There is substantial evidence that antioxidative food components have a protective role against coronary heart diseases by inhibition of low-density lipoprotein (LDL) oxidation in vivo (*6*). Oxidized LDL plays a major role during the development of atherosclerotic plaques, and as a result, of cardiovascular diseases (*7*). During food processing and storage, natural antioxidants are considerably degraded. On the other hand, chemical reactions among food components lead to the formation of secondary antioxidants (*8*). Important candidates for secondary food antioxidants are Maillard products. During processing, cooking, or storage of foods, amino acids or proteins react with carbohydrates (Maillard reaction), leading to a complex mixture of reaction products (*9*). These compounds are very important for the quality of food, because they contribute to the development of flavor or browning and to changes in the nutritional value, mutagenicity, and toxicology (*10*−*12*). One of the first reports about the antioxidative effect of Maillard products was released in 1954 by Franzke and Iwainsky (*13*). Since then, antioxidant activities of Maillard products have been studied extensively, using different test systems (*14*−*16*). In most cases, crude Maillard mixtures or uncharacterized melanoidines were tested, whereas only in a few cases was antioxidative activity related to structurally defined Maillard products (*17*−*19*). The protective role of Maillard products against oxidative damage gained particular interest, because they considerably prolong the shelf life of processed food (*20*). Furthermore, Maillard products could be used as natural antioxidants to be added to food susceptible to oxidative damage (*21*). However, hardly anything is known about a potentially beneficial role of reducing Maillard products in vivo (*22*). It can be assumed that, similar to natural products, secondary food components are resorbed and thus protect for example LDL from oxidative damage.

In the present study, the ability of Maillard products to inhibit LDL oxidation in vitro was investigated. First, Maillard mixtures of D-glucose and three different amino acids and finally two

* To whom correspondence should be addressed. Tel: ++49-9131-8524102. Fax: ++49-9131-8522587. E-mail: pischetsrieder@lmchemie.uni-erlangen.de.
[†] Department of Obstetrics and Gynecology, University Erlangen-Nuremberg.
[‡] National Research Center, Cairo.
[§] Current address: European Patent Office, Munich.
[||] Institute of Pharmacy and Food Chemistry, University of Erlangen-Nuremberg.

10.1021/jf026172s CCC: $25.00
Published on Web 05/17/2003

structurally defined Maillard products with aminoreductone structure were tested.

MATERIALS AND METHODS

Preparation of the Maillard Mixtures. The reaction mixtures consisted of D-glucose (1M) and L-lysine, L-arginine, or glycine (1M) dissolved in water. The pH was adjusted to 7.1 with diluted phosphoric acid or sodium hydroxide, and the mixtures were heated under reflux for 1h. After cooling, the mixtures were directly used for LDL-assay. D-Glucose or the amino acids were heated alone under the same conditions and tested in the same way as the Maillard mixtures. Additionally, heated D-glucose and heated amino acid solutions were mixed in a ratio of 1:1 prior to the LDL assay (control mixtures).

Isolation or Synthesis of the Maillard Products. Piperidino hexose reductone (**2**) (*23*, *24*) and 3-hydroxy-4-(morpholino)-3-buten-2-ones (**1**) (*18*) were prepared according to methods described previously. Briefly, for the synthesis of the latter, 1-bromo-2,3-butanedione (1 M) and morpholine (2 M) were dissolved separately in tetrahydrofuran and cooled on ice. The solution of morpholine was added dropwise to the 1-bromo-2,3-butanedione solution while stirring, and the mixture was reacted for 30 min at room temperature and for 30 min at 50 °C. The product was obtained as lightly yellow crystals after column chromatography on silica gel with ethyl acetate/diisopropyl ether (4:1) as eluent. The purity of **1** was checked by CHN analysis (Anal. Calcd for $C_8H_{13}NO_3$: C, 56.13; H, 7.65; N, 8.18. Found: C, 56,16; H, 7.66; N, 8.06.). UV (CH_3OH) λ_{max} 321 nm (log $\epsilon = 4.3$) with negligible absorption in the visible range. Compound **2**: UV (water, pH 6) λ_{max} 308.5 nm with negligible absorption in the visible range (*25*).

Inhibition of Human LDL Oxidation. The assay was performed as described before (*26*). In brief description, LDL was prepared from the blood of healthy volunteers after an overnight fast of ≥ 12h by centrifugation in a density gradient. All experiments described here were performed with the same representative LDL preparation from one individual (male, 42 years, mixed western diet, no supplementation with antioxidants or medication; the clinical parameters of lipid metabolism were in the normal range). Similar results were obtained with LDL preparations from other volunteers. Prior to use, LDL was diluted with oxygen-saturated PBS pH 7.4 to a final concentration of 0.08 g/L LDL-cholesterol. The mixtures were added without further treatment in a final dilution of 1:1000. The test substances were diluted in water, and 0.5, 1, or 2 μl of these solutions were added to 1 mL of the LDL test solution to obtain the indicated concentrations. LDL oxidation was initiated by addition of a freshly prepared aqueous $CuSO_4$ solution (final concentration 5 μM). LDL oxidation was spectrophotometricly monitored by measuring the change in absorbance at 234 nm. Absorbance readings were made every 5 min at 37 °C until there was no further increase in the formation of conjugated dienes. Antioxidative activity was measured as duration of the lag phase and related to the lag phase of a negative control consisting only of LDL and $CuSO_4$. The lag phase was determined as described before in detail (*27*). In brief description, it was taken from the graph $y = OD_{234}$ and $x =$ time and described by the time point where the linear extrapolation of the propagation phase intersects $y = y$ (t_0). The experiments were performed in multiplicates as indicated in the legends of the figures.

Statistical Analyses. A paired Student's *t* test was used for statistical evaluation of significant differences between two groups with $p < 0.01$.

RESULTS

Maillard mixtures were prepared using equimolar amounts of D-glucose and the amino acids glycine, L-arginine, and L-lysine. The mixtures were added to human LDL in a final dilution of 1:1000, and oxidation was induced by the addition of Cu^{2+}. Because all tests were carried out in aqueous solution, ascorbic acid was used as positive control in a final concentration of 5 μM. Solutions of D-glucose or the amino acids alone, which were treated in the same way as the Maillard mixtures, were also tested. In some experiments, the heated glucose solution and the heated amino acid solution were mixed prior to LDL assay (control mixtures). Some characteristic properties of the test solutions are summarized in **Table 1**. LDL-oxidation was recorded by measuring UV-absorbance of conjugated diens, which are formed during its oxidation. Antioxidant activity (AOA) was determined by the capability of a substance or a mixture to prolong the lag phase compared to the negative control, consisting only of LDL and $CuSO_4$. The lag phase of the negative control was 58.1 min ± 3.8 (mean ± S. E. M.)

Table 1. Characterization of the Maillard Mixtures

Maillard mixture or control	pH after reaction[a]	OD_{360} (dilution)	OD_{420} (dilution)
glycine control	7.0	0.028 (undil)	0.022 (undil)
lysine control	7.3	0.270 (undil)	0.056 (undil)
arginine control	7.9	0.036 (undil)	0.008 (undil)
glucose control	6.9	0.51 (undil)	0.20 (undil)
glycine + glucose MP	4.9	0.49 (1:10)	0.23 (1:10)
lysine + glucose MP	4.9	0.52 (1:10)	0.22 (1:10)
arginine + glucose MP	7.2	1.1 (1:20)	0.55 (1:20)

[a] The pH was adjusted to pH 7.1 prior to reaction.

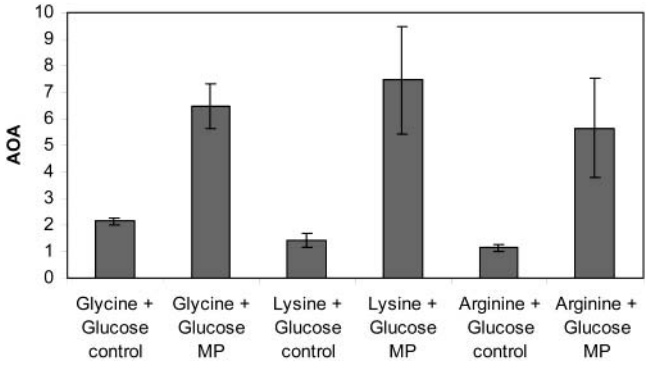


Figure 1. Effect of Maillard reaction mixtures on the copper-induced oxidation of human low-density lipoprotein. The AOA is expressed as the increase of lag time produced by the test solution compared to the negative control. D-Glucose was heated with glycine, L-lysine, or L-arginine for 1 h under reflux (MP = Maillard reaction mixture; means ± standard deviation; $n = 6$). For all samples, a highly significant increase of AOA compared to the respective control mixtures was measured.

As shown in **Figure 1**, all mixtures showed a highly significant increase in AOA compared to the control mixtures. AOA increased from 2.14 ± 0.1 to 6.46 ± 0.8 (glycine), from 1.41 ± 0.3 to 7.46 ± 2.0 (L-lysine), and from 1.13 ± 0.1 to 5.65 ± 1.9 (L-arginine), with $p < 0.01$. The effect was slightly, but not significantly, dependent on the amino acid that was used for preparing the Maillard mixtures.

The tested Maillard solutions are heterogeneous mixtures of different compounds that are formed in various concentrations. A study to identify the most active components in the mixtures is currently under progress. Additionally, two compounds were synthesized and tested for AOA toward LDL oxidation: 3-hydroxy-4-(morpholino)-3-buten-2-one (**1**), a C_4-aminoreductone, and amino hexose reductone (**2**) (**Scheme 1**). In model Maillard reaction mixtures, both compounds are formed as major reaction products (*18*, *28*). Furthermore, they both possess amino reductone structures, so that they are very likely candidates for antioxidative components in Maillard mixtures. Both compounds showed a significantly increased AOA compared to the control, which clearly was dose dependent: For **1**, AOA was 1.68 ± 0.5 at 2.5 μM, 2.45 ± 0.5 at 5 μM, and 3.17 ± 0.9 at 10 μM. For **2**, AOA was 1.33 ± 0.09 at 10 μM, 2.18 ± 0.3 at 20 μM and 3.2 ± 0.3 at 40 μM (**Figures 2** and **3**). At a given concentration (10 μM), the AOA of **1** was more than 2-fold

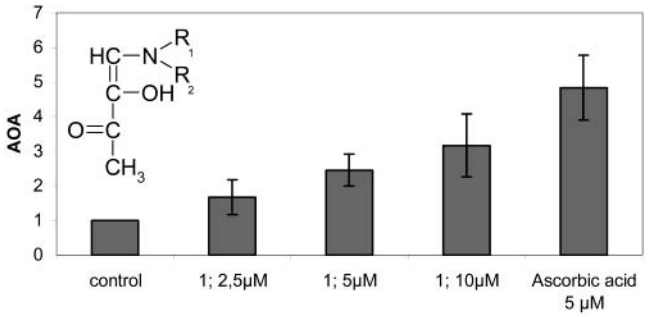


Figure 2. Dose dependent increase of the AOA of the Maillard product **1** (NR_1R_2 = morpholino). The antioxidative activity is expressed as the increase of lag time of copper-induced low-density lipoprotein oxidation compared to the negative control (means ± standard deviation; $n = 18$ for **1** and 5 for ascorbic acid). For all samples, a highly significant increase compared to the control was measured.

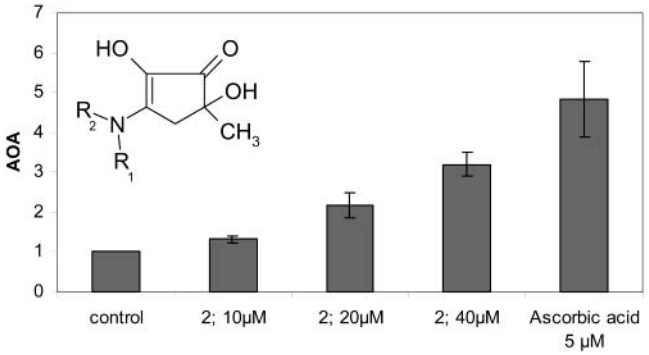
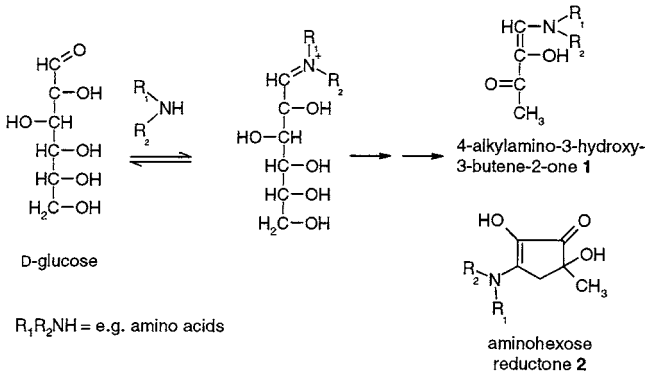


Figure 3. Dose dependent increase of the antioxidative activity (AOA) of the Maillard product **2** (NR_1R_2 = piperidino). The antioxidative activity is expressed as the increase of lag time of copper-induced low-density lipoprotein oxidation compared to the negative control (means ± standard deviation; $n = 8$ for **2** and $n = 5$ for ascorbic acid). For all samples, a highly significant increase compared to the control was measured.

Scheme 1. Formation of the Aminoreductones 4-Alkylamino-3-hydroxy-3-butene-2-one and Aminohexose Reductone during the Maillard Reaction of D-Glucose



higher than the AOA of **2**. Compared to ascorbic acid (5 μM), which is known as a very potent antioxidant in food, **1** showed about half of the AOA at the same concentration. AOA of **2** at 5 μM was not significantly above the control.

DISCUSSION

LDL oxidation was induced by the addition of Cu^{2+}, and AOA was measured by the capability of a test substance to prolong the lag phase of the reaction. LDL oxidation was monitored by UV-absorbance at 234 nm, resulting from the diene band of oxidized polyunsaturated fatty acids. This system is widely used as an in vitro model to evaluate a possible protective activity of food components against cardiovascular diseases (*29*), because there is strong evidence that oxidized LDL is a major factor in the pathogenesis of atherosclerosis (*30*).

In this study, it was shown for the first time that products that are formed by the Maillard reaction between D-glucose and amino acids can inhibit oxidation of human LDL in vitro. Maillard mixtures derived from L-lysine, which can be modified at the N^{α}- as well as at the N^{ϵ}-amino group, from glycine, and from L-arginine were tested. Although it must be expected that reaction of N^{α}- and N^{ϵ}-amino group leads to similar products, the yields of the Maillard products from the amino acids may be different due to differences in the pK_s values of the amino groups and the presence of a second amino group in the molecule. In contrast, the guanidinium group of arginine produces different structures as primary amines, often with imidazolone or pyrimidine moiety (*10*). However, in our experiments, only slight, but not significant, differences were found between the reaction mixtures derived from various amino acids.

Maillard reaction mixtures are very heterogeneous and complex, and their exact composition remains mostly unknown. Although there are numerous studies that show that Maillard reaction products possess antioxidative activity, the AOA could seldom be assigned to defined structures. Volatile flavor compounds, such as 2-acetylpyrrole, HMF, or 1-methylpyrrole, that are formed by Maillard type reaction inhibited hexanal oxidation (*31*). However, it seems that these compounds are only formed in relatively small amounts in Maillard reaction mixtures (*32*). Monti et al. investigated the antioxidative efficiency of a lactose−lysine model system, and they were able to assign some of the detected activity to defined peaks in the HPLC chromatogram. The most active peaks were tentatively assigned to 5-hydroxymethylfurfural (HMF), galactosyliso-maltol, and pyrraline (*33*). The antioxidative activity of reductones and amino reductones is well established (*19*, *34*). Under certain reaction conditions, particularly after short-term incubation, aminoreductones are the main UV-absorbing Maillard reaction products (*18*, *35*, *36*). Using other test systems, it was shown that aminoreductones that can be formed by Maillard reaction have similar anti- or pro-oxidative properties to L-ascorbic acid (*18*). And indeed, Shimamura et al. suggested that a C6-aminoreducton is the main reducing substance in a Maillard mixture of lactose (*37*). Therefore, we have investigated the activity of two colorless aminoreductones formed by the Maillard reaction to inhibit LDL oxidation in vitro. Compound **1** is a major Maillard product of D-glucose with strong reducing properties (*18*). Compound **2**, which is formed in large amounts during the reaction of glucose with secondary amines, possesses a cyclic aminoreductone structure (*23*, *24*) (**Scheme 1**). Compound **1**, and to a lesser extent **2** showed strong dose dependent activity to prevent LDL oxidation in vitro. Because both compounds possess a very similar β-amino reductone structure, the differences in activity could be explained by steric or conformational differences or differences in the lipophilic properties, which results in another actual concentration at the oxidation site. Interestingly, a considerably higher activity of **1**, compared to a structurally similar C6-β-amino reductone, was also found when AOA was measured in other test systems (*17*). The exact mechanism of the antioxidative effect has not been clarified. It must be assumed, however, that amino reductones behave in a similar way to ascorbic acid (*38*), which rather reacts

with radicals and other reactive oxidative species than chelate the copper ions. Reductones and amino reductones possess highly reducing properties (*34*, *39*) and are not stable in the presence of metal ions (*40*). It is not clear yet if amino reductones of the types **1** and **2** are indeed responsible for the AOA observed in the test mixtures. Compound **2** is mainly formed from secondary amines and sugars, so it is unlikely that the substance is present in the tested mixtures from primary amines. Although HPLC chromatography of the test mixtures strongly indicate the presence of amino reductones of type **1**, (peak with a characteristic UV spectrum and maximum at 321 nm), quantification of these compounds in the mixtures was not performed.

This study shows clearly that Maillard reaction products can prevent oxidation of human LDL in vitro. In this test system, amino reductones -particularly those of type **1**- show a strong antioxidative activity. It remains to be investigated if the intake of certain Maillard products contribute to the nutrition-dependent increase of antioxidative capacity, and -as one consequence- reduction of LDL oxidation in vivo.

ABBREVIATIONS USED

AOA, antioxidative activity; LDL, low-density lipoprotein; PBS, phosphate buffered saline.

ACKNOWLEDGMENT

We thank Inge Hoffmann for excellent technical assistance.

LITERATURE CITED

(1) Henderson, D.; Slickman, A.; Henderson, S. Quantitative HPLC determination of antioxidative activity of capsaicin on the formation of lipid hydroperoxides of linoleic acid: a comparative study against BHT and Melatonin. *J. Agric. Food Chem.* **1999**, *47*, 2563−2570.

(2) Kähkönen, M.; Hopia, A.; Vuorela, H.; Rauha, J.; Pihlaja, K.; Kujala, T.; Heinonen, M. Antioxidative activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem*. **1999**, *47*, 3954−3962.

(3) Huang, S.; Frankel, E. Antioxidative activity of tea catechins in different lipid systems. *J. Agric. Food Chem*. **1997**, *45*, 3033−3038.

(4) Koga, T.; Moro, K.; Nakamori, K.; Yamakoshi, J.; Hosoyama, H.; Kataoka, S.; Ariga, T. Increase of antioxidative potential of rat plasma by oral administration of proanthocyanidin-rich extracts from grape seeds. *J. Agric. Food Chem*. **1999**, *47*, 1892−1897.

(5) Kondo, Y.; Ohnishi, M.; Kawaguchi, M. Detection of lipid peroxidation catalyzed by chelated iron and measurement of antioxidant activity in wine by a chemiluminescence analyzer. *J. Agric. Food Chem.* **1999**, *47*, 1781.

(6) Ames, B.; Shigenaga, M.; Hagen, T. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 7915−7922.

(7) Steinberg, D.; Parthasarathy, S.; Carew, T.; Khoo, J.; Witzum, J. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* **1989**, *320*, 915−924.

(8) Nicoli, M.; Anese, M.; Parpinel, M.; Franceschi, S; Lerici, C. Loss and/or formation of antioxidants during food processing and storage. *Cancer Lett*. **1997**, 71−74.

(9) Ledl, F.; Schleicher, E. New aspects of the Maillard reaction in foods and in the human body. *Angew. Chem., Int. Ed. Engl*. **1990**, *29*, 565−594.

(10) O'Brien, J.; Morrissey, P. A. Nutritional and toxicological aspects of the Maillard browning reaction in foods. *Crit. Rev. Food Sci. Nutr*. **1989**, *28*, 211−248.

(11) Danely, J. P. Maillard reactions: Nonenzymatic browning in food system with special reference to the development of flavour. *Adv. Food Res*. **1986**, *30*, 77−138.

(12) Namiki, M. Chemistry of Maillard reactions: Recent studies on the browning reaction mechanism and the development of antioxidants and mutagens. *Adv. Food Res*. **1988**, *32*, 115−184.

(13) Franzke, C.; Iwainsky, H. Zur antioxidativen Wirksamkeit der Melanoidine. *Dtsch. Lebensm. Rundsch*. **1954**, *50*, 251−254.

(14) Wijewickreme, A.; Kitts, D. Influence of reaction conditions on the oxidative behavior of model Maillard reaction products. *J. Agric. Food Chem*. **1997**, *45*, 4571−4576.

(15) Kawashima, K.; Itoh, H.; Chibata, I. Antioxidant activity of browning products prepared from low molecular carbonyl compounds and amino acids. *J. Agric. Food Chem.* **1976**, *25*, 202−204.

(16) Monti, S.; Ritieni, A.; Graziani, G.; Randazzo, G.; Mannina, L.; Segre, A.; Fogliano, V. LC/MS analysis and antioxidative efficiency of Maillard reaction products from a lactose-lysine model system. *J. Agric. Food Chem*. **1999**, *47*, 1506−1513.

(17) Pischetsrieder, M.; Rinaldi, F.; Gross, U.; Severin, T. Assessment of the antioxidative and prooxidative activities of two amino-reductones formed during the Maillard reaction: effects on the oxidation of β-carotene, N^{α}-acetylhistidine, and cis-alkenes. *J. Agric. Food Chem*. **1998**, *46*, 2945−2950.

(18) Ando, K.; Ogawa, K.; Li, X.; Kikugawa, K. Inhibition of iron ion-induced oxidative damage of erythrocyte membranes and low-density lipoprotein by a Maillard product, 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone (HEMF). *Biol. Pharm. Bull*. **2000**, *23*, 689−694.

(19) Lindenmeier, M.; Faist, V.; Hofmann, T. Structural and Functional Characterization of Pronyl-lysine, a novel protein modification in bread crust melanoidins showing in vitro antioxidative and phase I/II enzyme modulating activity. *J. Agric. Food Chem.* **2002**, *50*, 6997−7006.

(20) Bressa, F.; Tesson, N.; Dalla Rosa, M.; Sensidoni, A.; Tubaro, F. Antioxidant effect of Maillard reaction products: application to a butter cookie of a competitive kinetics analysis. *J. Agric. Food Chem*. **1996**, *44*, 692−695.

(21) Smith, S.; Alfawaz, M. Antioxidative activity of Maillard reaction products in cooked ground beef, sensory and TBA values. *J. Food Sci*. **1995**, *60*, 234−240.

(22) Daglia, M.; Papetti, A.; Gregotti, C.; Berte, F.; Gazzani, G. In vitro antioxidant and ex vivo protective activities of green and roasted coffee. *J. Agric. Food Chem.* **2000**, *48*, 1449−1454.

(23) Hodge, J.; Rist, C. The Amadori rearrangement under new conditions and its significance for nonenzymatic browning reactions. *J. Am. Chem. Soc.* **1953**, *75*, 316−322.

(24) Weygand, F.; Simon, H.; Bitterlich, W.; Hodge, J.; Fisher, B. Structure of piperidino-hexose-reductone. *Tetrahedron* **1958**, *6*, 123−138.

(25) Hodge, J.; Rist, C. The Amadori rearrangement under new conditions and its significance for nonenzymatic browning reactions. *J. Am. Chem. Soc.* **1953**, *57*, 316−322.

(26) Dittrich, R.; Müller, A.; Hensen, J.; Wildt, L. The inhibition of low density lipoprotein oxidation by 17-alpha estradiol. *Exp. Clin. Endocrinol. Diabetes* **1999**, *107*, 53−57.

(27) Esterbauer, H.; Striegl, G.; Puhl, H.; Rothender, M. Continuous monitoring of in vitro oxidation of human low-density lipoprotein. *Free Radical Res. Common* **1989**, *6*, 67−75.

(28) Pehr, H.; Manninger G.; Severin, T. Umsetzungen von D-Glucose mit Diethylammoniumtartrat: Isolierung von 3,5-Dihydroxy-6-methyl-2H-pyran-2-on sowie Nachweis cyclischer Aminoreduktone als Reduktonmethyläther. *Carbohydr. Res*. **1978**, *64*, 1−7.

(29) Vinson, J.; Dabbagh, Y.; Mamdouh, S.; Jang, J. Plant flavanoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. *J. Agric. Food Chem*. **1995**, *43*, 2800−2802.

(30) Lusis, A. Atherosclerosis. *Nature* **2000**, *407*, 233−241.

(31) Fuster, D. M.; Mitchell, A.; Ochi, H.; Shibamoto T. Antioxidative activities of heterocyclic compounds formed in brewed coffee. *J. Agric. Food Chem.* **2000**, *48*, 5600−5603.

(32) Chen, Y.; Ho, C.-T. Effects of carnosine on volatile generation from Maillard reaction of ribose and cysteine. *J. Agric. Food Chem*. **2002**, *50*, 2372−2376.

(33) Monti, S.; Ritieni, A.; Graziani, G.; Randazzo, G.; Mannina, L.; Segre, A.; Fogliano, V. LC/MS analysis and antioxidative efficiency of Maillard reaction products from a lactose-lysine model system. *J. Agric. Food Chem*. **1999**, *47*, 1506−1513.

(34) Pischetsrieder, M.; Severin, T. New aspects on the Maillard reaction − formation of aminoreductones from sugars and L-ascorbic acid. *Recent Res. Dev. Agric. Food Chem.* **1997**, *1*, 29−37.

(35) Pischetsrieder, M.; Schoetter, C.; Severin, T. Formation of an aminoreductone during the Maillard reaction of lactose with N^{α}-acetyllysine or proteins. *J. Agric. Food Chem.* **1998**, *46*, 928−931.

(36) Schoetter, C.; Pischetsrieder, M.; Lerche, H.; Severin, T. Formation of aminoreductones from maltose. *Tetrahedron Lett.* **1994**, *35*, 7369−7370.

(37) Shimamura, T.; Ukeda, H.; Sawamura, M. Reduction of tetrazolium salt XTT by aminoreductone formed during the Maillard reaction of lactose. *J. Agric. Food Chem.* **2000**, *48*, 6227−6229.

(38) Euler, H.; Eistert, B. In *Chemie und Biochemie der Reduktone und Reduktonate.* Ferdinand Enke, Ed. Verlag: Stuttgart, Germany, 1957.

(39) Kurata, T.; Otsuka, Y. Amino-reductones. Formation mechanisms and structural characteristics. In *Process-Induced Chemical Changes in Food.* Plenum Press: New York, 1998; pp 269−276.

(40) Davies, M.; Austin, J.; Partridge, D. In *Vitamin C: Its Chemistry and Biochemistry*. Royal Society of Chemistry: Cambridge, U.K. 1991; pp 130−135.

Received for review November 28, 2002. Revised manuscript received April 4, 2003. Accepted April 6, 2003. The project was supported by the "Fond der chemischen Industrie".

JF026172S