Progress of the Maillard Reaction and Antioxidant Action of Maillard Reaction Products in Preheated Model Systems during Storage

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The progress of the Maillard reaction and the effect of Maillard reaction products (MRPs) on lipid oxidation in preheated model systems containing pregelatinized starch, glucose, lysine, and soybean oil have been studied during storage. The samples, either containing all components or excluding one or more of them, were heated at 100 °C for 90 min and then stored for up to 180 days at 25 °C. Browning indices and lipid oxidation were measured, and the results showed that, in samples containing oil, the Maillard reaction had a significant rate also at room temperature and confirmed the ability of MRPs to retard peroxide formation. Under the conditions adopted the rate of the Maillard reaction was increased by the presence of the oil and its oxidation products. The antioxidant action of the MRPs was also evaluated using a peroxide scavenging test based on crocin bleaching. The results demonstrated that antioxidant activity developed with increased browning of the samples.

Keywords: *Maillard reaction; antioxidant action; lipid oxidation; storage*

INTRODUCTION

The Maillard reaction has been studied in great detail because of its role in modification of food properties such as color, flavors, nutritional value, and stability during processing and storage. In fact, in many heated foods amines react with carbonyl compounds, mainly reducing sugars, leading to the formation of a complex series of compounds called Maillard reaction products (MRPs) (Griffith and Johnson, 1957; Eichner, 1981; Bailey and Um, 1992; Whitfield, 1992).

The first stage of the reaction is well documented; however, the subsequent reactions of the Amadori rearrangement products (ARPs) are still mainly unknown. This reaction step is very important because it can be initiated under mild conditions. Food products stored for a long time at room temperature may eventually turn brown due to the polymerization of the degradation products of ARPs. Alternatively, at high-temperature conditions such as baking and roasting, ARPs can be formed within hours and degrade or react with other food components to produce the characteristic aroma and the brown color (Yaylayan and Huyghues-Despointes, 1994).

It has also been observed that Maillard reaction products are able to decrease the lipid oxidation rate. The antioxidative activity of MRPs was first observed by Franzke and Iwainsky (1954); later the formation of antioxidant Maillard reaction products from model systems was extensively studied (Griffith and Johnson, 1957; Eichner, 1981; Bailey and Um, 1991; Elizalde et al., 1991, 1992; Whitfield, 1992; Dalla Rosa et al., 1992; Severini and Lerici, 1995; Munari et al., 1995; Bressa et al., 1996; Wijewickreme and Kitts, 1997; Alaiz et al., 1995, 1997, 1999). Several mechanisms are involved in the antioxidant activity of MRPs; however, the compounds accounting for this effect and the mechanisms of antioxidant action have not been completely identified.

In addition to reducing sugars, other carbonyl compounds including lipid peroxidation products are also able to react with amino groups, producing brown macromolecular pigments with properties similar to those of melanoidins (Labuza et al., 1971; Arnoldi et al., 1987; Whitfield, 1992; Browdy and Harris, 1997; Hidalgo et al., 1999). Recently the antioxidant effect of the products formed by the reaction of the oxidized lipids with amino acids and protein has been highlighted (Alaiz et al., 1995; Zamora et al., 1997; Alaiz et al., 1999). This protective effect seems to be general for most of the heterocyclic derivatives produced in oxidized lipid/amino acid browning reactions, and these products have been used to protect vegetable oils against oxidation (Alaiz et al., 1995, 1997)

Very few data are available on the interaction between the Maillard reaction and lipid oxidation when they take place simultaneously in a food system during storage after heating.

In this paper the progress of the Maillard reaction, the effect of MRPs on the kinetics of lipid oxidation, and the influence of oil on nonenzymatic browning kinetics in model systems containing pregelatinized starch, glucose, lysine, and soybean oil were studied during storage. In the experimental procedure the model systems were considered with all components or excluding one or more of them to highlight the role of the single ingredients.

The antioxidant action of MRPs was also evaluated using a peroxide scavenging test based on crocin bleaching.

MATERIALS AND METHODS

Model System Preparation. For model system preparation the following ingredients were used: glucose (RPE-ACS, Carlo Erba, Italy), L-(+)-lysine-HCL (RPE-ACS, Carlo Erba,

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10.1021/jf000278a CCC: $19.00 © 2000 American Chemical Society
Published on Web 07/25/2000

Table 1. Composition of Model Systems

component	model A (g kg^{-1})	model B (g kg^{-1})	model C (g kg^{-1})
gelatinized starch	320	500	520
water	300	300	300
glucose	160	160	
lysine	40	40	
soybean oil	180		180

Milan, Italy), pregelatinized starch, distilled water, and commercial soybean oil.

The formulation of model systems was chosen to simulate, in a simplified way, a bakery product. Starch was used as a flour substitute, and pregelatinization was adopted to avoid changes in the water holding capacity of the formulations during heating.

To obtain the pregelatinized starch, 200 g of corn starch (Carlo Erba, Milan, Italy) was dispersed in 4 L of distilled water, and the suspension was boiled for 10 min and then cooled in an ice−water bath. The starch suspension was frozen at −30 °C (Alaska, model EF 600, Bologna, Italy) and freeze-dried in a pilot plant drier (Edwards Alto Vuoto, Mini fast 1700, Milan, Italy), at a shelf temperature of 35 °C.

The ingredients were mixed (Ronic Ritmix, Imetec, Milan, Italy) to obtain the mixtures reported in Table 1.

Aliquots (9 g) of the different model systems were placed in 20 mL glass vials that were then hermetically sealed with butyl septa and metallic caps. Samples were heated at 100 °C, in a forced air circulation oven (Thermocenter, Salvis, Milan, Italy) for 90 min. After heat treatment, samples were cooled in an ice−water bath and stored for up to 180 days at 25 °C.

Analytical Methods. *Evaluation of Antioxidant Activity.* The procedure proposed by Bressa et al. (1996) was adopted.

To obtain liquid extracts, 5 g samples were suspended in 20 mL of a 0.4 M KCl aqueous solution (Carlo Erba**,** Milan, Italy) and homogenized using a Politron (PT 3000, Kinematica AG, Littau, Switzerland) for 1 min. Suspensions were centrifuged for 5 min at 4330*g* at 0 °C (refrigerated centrifuge ALC, RCF Meter, 4233R), and the aqueous phase was filtered on paper (Whatman no. 4) and diluted with 10 mL of 0.4 M KCI. Samples were frozen at − 18 °C for not more than 15 h in hermetically sealed containers. Storage for periods exceeding 24 h led to a significant loss of the antioxidant activity. For kinetic analysis, the extracts were dried and weighed.

According to the procedure adopted, the ability of a compound or a mixture of compounds to quench peroxyl radicals is measured by analyzing the first-order rates of crocin bleaching due to the presence of peroxyl radicals. The presence of an antioxidant slowed the rate of bleaching. The rate of bleaching of crocin was followed at 443 nm using a spectrophotometer (Uvikon 860, Kontron Instruments, Milano, Italy).

Analyses were carried out at 50 °C in 2 mL of incubation medium containing 0.1 mol/L phosphate buffer, pH 7.0, 9.5 μmol/L crocin (Sigma Chemical Co., St. Louis, MO), and increasing amounts of Trolox (Aldrich, Milwaukee, WI) or sample extracts. The reaction was started by adding 40 μL of a 97.7 mmol/L ABAP (Wako Chemicals Co., Osaka, Japan) aqueous solution. Triplicate measurements were made for each sample.

The ability of the sample extracts to slow the crocin bleaching rate was measured in terms of Trolox molar concentration. Thus, as reported by Tubaro et al. (1996) and Bressa et al. (1996), all the dry matter of the extracts was assumed to have antioxidant properties, and to calculate the antioxidant molar concentrations, the molecular weight of Trolox (MW = 250.29) was used.

Lipid Oxidation. The peroxide value in the oil fraction, extracted from model systems using 20 mL of ethyl ether (Carlo Erba, Milan, Italy), was determined and expressed as mEq of active oxygen/kg of oil, according to AOAC (1995).

Hydroxymethylfurfural (HMF). HMF was determined using a high-performance liquid chromatograph (Jasco 880PU, Tokyo, Japan) equipped with a UV−vis detector (Jasco 875UV, Tokyo, Japan) operating at 284 nm. The analysis was carried out using a 250 × 4 mm reversed-phase column (Lichrosorb RP 18, Merck, Dorset, U.K.); pure water at a flow rate of 0.3 mL/min was used as the mobile phase. To obtain liquid extracts for HPLC analysis, the same procedure used for antioxidant activity was adopted.

Glucose. The HPLC method described by Nicoli et al. (1991) was used to determine residual glucose. A high-performance liquid chromatograph (Jasco 880PU, Tokyo, Japan) equipped with a manual 10 μL loop injector, a refractive index detector (RI-3, Varian, Sunnyvale, CA), and a recorder (Varian 4290, Sunnyvale, CA) was used. The analysis was carried out using a 250 × 4 mm Lichrosorb NH_2 Hibar RT 250-4 prepacked column (Merck, Dorset, U.K.), using acetonitrile−water (80:20) as the mobile phase at a flow rate of 1.5 mL/min.

For HPLC analysis 5 g of samples were suspended in 30 mL of distilled water and homogenized using a Politron (PT 3000, Kinematica AG, Littau, Switzerland) for 1 min. Samples were centrifuged for 5 min at 4330*g* at 0 °C (refrigerated centrifuge ALC, RCF Meter, 4233R), and the aqueous phase was filtered on paper (Whatman no. 4).

Lactose (Carlo Erba, Milan, Italy) (2×10^{-2} M) was used as the internal standard. The glucose concentration was calculated using the response factors determined from standard solutions.

Headspace Gas Chromatographic (GC) Analysis of Carbon Dioxide. The carbon dioxide in the headspace of different model systems considered was detected by GC analysis, using a Fisons model 8540 gas chromatograph (Fisons, Milan, Italy). The instrument was equipped with a hot wire detector (HWD) and a 2 m × 2 mm i.d. glass packed column, filled with Porapack Q 80−100 mesh (Supelchem, Milan, Italy). The operating conditions were column temperature 100 °C, detector oven temperature 180 °C, injector temperature 110 °C, filament temperature 230 °C, and carrier gas flow (He) 40 mL/min. A 0.2 mL headspace volume was injected using a Hamilton model 1750 CEST syringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) assembled on an HS 250 automatic sampler (Carlo Erba, Milan, Italy). Gas chromatographic traces and peak areas were evaluated with a Fisons model DP 700 integrator (Fisons, Milan, Italy).

Color Measurements. The color determination was made with a tristimulus colorimeter (Chromameter CR 200 II Reflectance, Minolta, Osaka CO Ltd., Japan) equipped with an illuminant C (CIE standard 6774 K) and a microprocessor for the statistical analysis of data. The instrument was calibrated before each series of measurements using a white tile reflector plate ($L^* = 95.3$; $a^* = -1.0$; $b^* = 0.8$). Chromaticity results are expressed in L^* (lightness) and a^* and b^* (chromaticity coordinates) (Mastrocola and Lerici, 1991).

Before each analysis the oil fraction was removed from the samples to avoid interference.

Data Analysis. All the data reported are the averages of at least three replications. Statistical comparison among different groups was made using ANOVA (Statistica for windows 5.1, Statsoft, Tulsa, OK). The coefficients of variation, calculated for all the measurements, taken as the ratio of the standard deviation and the mean value, were lower than 7% for color, 6% for HMF, 2% for glucose, and 5% for CO_2, for peroxide values and antioxidant activity. Correlation equations and correlation coefficients were calculated from linear regression of color L^* and a^* and headspace CO_2 concentration values vs antioxidant activity.

RESULTS AND DISCUSSION

The Hunter L^* and a^* values in the samples belonging to models A, B, and C raw, after the heating phase able to induce the Maillard reaction, and during storage are reported in Figure 1. As expected, sample C (without glucose and lysine) did not show any color change. On the contrary, for model systems A and B it was possible to appreciate a light browning due to the Maillard reaction (Ames and Nursten, 1989) already after the

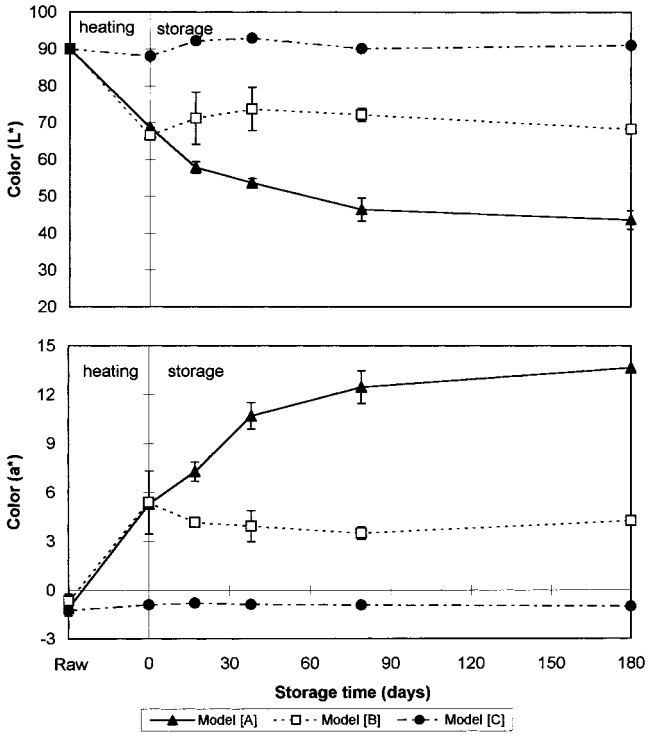

Figure 1. Color *L** and *a** in model systems raw, after heating, and during storage.

heating phase. During storage model systems containing glucose and lysine with (A) or without (B) oil showed different behavior. In fact, the presence of oil in model A induced a relevant increase of browning in the first 80 days of storage: on the contrary the product without the lipid phase did not show significant color changes. The higher browning rate in the presence of oil can be due to the participation of intermediate products of lipid oxidation in the Maillard reaction (Labuza et al., 1971; Arnoldi et al., 1987; Whithfield, 1992). In fact, Amadori compounds can react with other carbonyls to form highly reactive intermediates. Such carbonyl compounds can be formed prior to Amadori rearrangement directly from sugars or from the degradation of the Amadori products themselves in a chainlike reaction and from lipid oxidation (Yaylayan and Huyghues-Despointes, 1994). However, these last reactions are not favored under the same conditions; at low temperature, such as storage temperature, oxidized lipid/amino acid reactions will be more important for causing browning effects than carbohydrate/amino acid reactions, and these roles will be inverted at high temperatures (Alaiz et al., 1999). The low reactivity of sugars in the storage conditions that we adopted can be due to high "kinetic hindrance" of the substratum at medium/low temperatures (Karel and Saguy, 1991; Yaylayan and Despointes, 1994). In addition, the formation mechanism of most products identified in Maillard reactions are mainly rationalized by enolization reaction of the open chain forms of monosaccharides. However, these forms represent 1−7% of the total concentration at medium/low temperatures (Yaylayan, 1990; Yaylayan and Huyghues-Despointes, 1994).

The hypothesis of low participation of sugars in the Maillard reaction during storage at room temperature is confirmed by the data reported in Table 2. In fact, the glucose content of model systems A and B slightly decreased after heating of samples but did not show any further decrease during storage.

Table 2. Glucose Content in Model Systems A and B Raw, after Heating, and after Storage for 180 Days at 25 °C

	glucose content[a] (g kg^{-1})		
model	raw	after heating	after 180 days of storage
A	160 ± 0.5[a]	152.2 ± 3.9[a]	150.8 ± 2.5[a]
B	160 ± 0.4[a]	152.3 ± 2.9[a]	151.6 ± 2.8[a]

[a] Values in a column with a common superscript are not significantly different ($p \geq 0.05$).

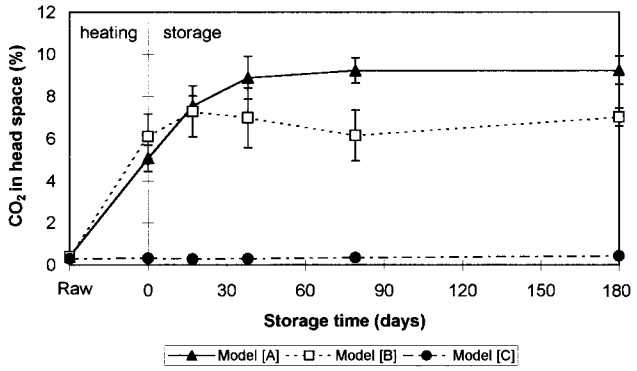

Figure 2. CO_2 content in the headspace of model systems raw, after heating, and during storage.

Table 3. HMF Content in Model Systems A, B, and C Raw, after Heating, and after Storage for 30 and 180 Days at 25 °C

	HMF content[a] (μg g^{-1})			
model	raw	after heating	after 30 days of storage	after 180 days of storage
A	nd	63.8 ± 7.1[a]	28.1 ± 7.1	16.2 ± 2.9
B	nd	69.23 ± 5.14[a]	62.46 ± 7.1	28.6 ± 3.14
C	nd	nd	nd	nd

[a] Values in a column with a common superscript are not significantly different ($p \geq 0.05$).

In Figure 2 the evolution of carbon dioxide in the different model systems considered is shown. As expected, significant changes of CO_2 content in the headspace of samples A and B during heating have been observed. In fact, in these models the presence of glucose and lysine allows the Maillard reaction to take place, and carbon dioxide can be considered an early indicator of the reaction because it derives from nucleophilic addition reactions between α-dicarbonyl intermediate with amino acids with subsequent decarboxylation to produce the so-called Strecker aldehyde. In general, the amount of carbon dioxide generated from a heat-treated system also showed good correlation with the quantity of both brown pigments and carbonyl compounds produced (Lerici et al., 1990). In the case of model A the amount of CO_2 detected in the gaseous phase significantly increased during the first period of storage, and this behavior confirmed that the Maillard reaction carried on also at low temperature probably for the participation of lipid oxidation products in the reaction.

Table 3 reports the changes of the HMF content in heat-treated and stored model systems. HMF is an intermediate product of the Maillard reaction formed from hexose derivatives after Amadori rearrangement. Under less acidic conditions HMF polymerizes quickly to a dark-colored insoluble material containing nitrogen (Yaylayan and Huyghues-Despointes, 1994). In our experimental conditions the concentration of HMF did

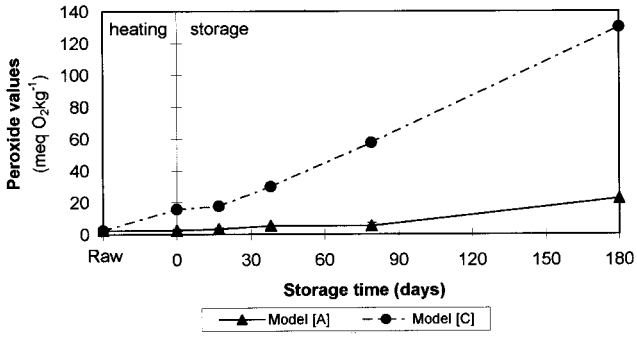

Figure 3. Peroxide values in model systems raw, after heating, and during storage.

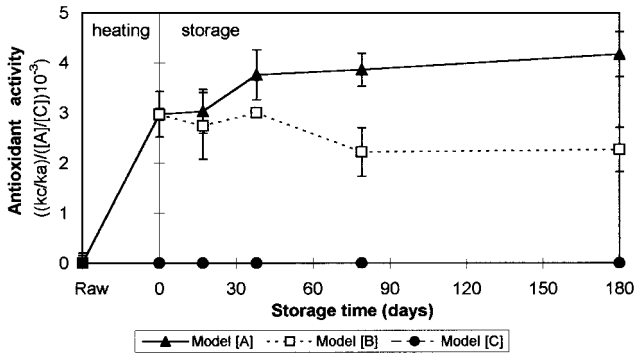

Figure 4. Antioxidant activity of model systems raw, after heating, and during storage.

not reach detectable levels for model C; on the contrary, after a similar increase during heating, models A and B showed a different evolution during the first phase of storage (0−30 days). In fact, the HMF content decreased more quickly for samples that showed a higher browning level during storage; instead its lowering appeared slower for samples belonging to model B. In every case, since both models A and B showed similar drops in HMF content (about 50%) between 30 and 180 days, it is possible to assume the loss is due to the reaction of HMF with lysine.

In Figure 3 the peroxide values of the lipid fraction of models A and C as a function of storage time are reported. The oxidative process, very evident in model C, is strongly inhibited in model A. These results suggest that the simultaneous induction of the Maillard reaction greatly affects the development of lipid oxidation, slowing the reaction and confirming the well-known antioxidant activity of the Maillard reaction products (Franke and Iwainsky, 1957; Elizalde et al., 1991; Dalla Rosa et al., 1992; Munari et al., 1995; Severini and Lerici, 1995, Alaiz et al., 1997, 1999).

In Figure 4 the antioxidant capacity of the model systems considered as a function of heating time is shown. From the data reported in Figure 4 it is evident that only samples A and B, in which nonenzymatic browning developed, generated a detectable antioxidant activity during heating. After the heating period, when the behavior of models A and B appeared very similar, the samples without oil did not show further formation of compound with antioxidant activity during storage. On the contrary, in model A the presence of a lipid phase, favoring the development of the Maillard reaction, was also able to increase the antioxidant activity of the system.

In every case, to draw a conclusion on the prevention of oxidative damages in foods, it should be considered that the proximity of antioxidant and lipid molecules undergoing oxidation could limit the efficiency of the measurement of antioxidant capacity in a complex matrix. Nevertheless, the crocin bleaching test allows the fact that the Maillard reaction produces a dramatic increase of molecules having a free radical scavenging effect to be highlighted in quantitative terms, and this could be relevant in food formulation, processing, and storage.

Table 4. Correlation Equations and Correlation Coefficients (r) of Antioxidant Activity as a Function of Color L^* and a^* Values and Headspace CO_2 Concentration

index (x value)	equation[a] (y = antioxidant activity)	r
color (L^*)	$y = -0.0952(\pm 0.0078)x + 8.855$	−0.950
color (a^*)	$y = 0.3155(\pm 0.0292)x + 0.6297$	0.938
CO_2	$y = 0.4299(\pm 0.0228)x + 0.0197$	0.978

[a] For all correlation equations $p \leq 0.001$.

In Table 4, the correlation equations and the correlation coefficients (r) of antioxidant activity as a function of color and headspace CO_2 concentration for all samples considered are reported. The high correlation coefficients suggest that, for a given food, in given processing and storage conditions, the determination of some appropriate indices could give useful indications of the antioxidant capacity of the product.

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Received for review March 1, 2000. Revised manuscript received May 22, 2000. Accepted May 22, 2000.

JF000278A