Antioxidant Effect of Maillard Reaction Products: Application to a Butter Cookie of a Competition Kinetics Analysis

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The generation of a chain-breaking antioxidant capacity has been analyzed in butter cookies, as a function of the cooking time. A kinetics approach was used, by which the antioxidant capacity of aqueous extracts of cookies was measured in terms of equivalence by weight of a reference antioxidant. A diazo compound was used to generate at constant rate peroxyl radicals, and the reporting reaction was the bleaching of crocin in the presence of these radicals. Results indicated that during the first 20−30 min of cooking, when browning takes place, an antioxidant capacity accounting for up to 5 g of Trolox is produced in 100 g of dried aqueous extracts of the cookies. This result supports the concept that functionally relevant antioxidants are generated by Maillard reaction.

Keywords: *Maillard reaction; lipid oxidation; free radical scavenger; bakery product*

INTRODUCTION

During heat treatment of a food product, amino groups, usually of protein amino acids, react with the aldehydic moiety of sugars, thus leading to a complex series of compounds accounting for browning; these compounds are called Maillard reaction products (MRP) (Bailey and Um, 1992; Eiserich et al., 1992; Elizalde et al., 1992). On the other hand, in fat-containing foods lipid oxidation may take place (even when the fat content is relatively low), producing rancidity and thus, eventually, shortening the shelf life of the product. Most frequently, in the presence of available substrates, both reactions take place at the same time, induced by processing and storage, and one can influence each other. The kinetics of MRP production is affected by the presence of compounds different from actual substrates, namely sugars and amino acids, and specific products are generated in the presence of lipids (Whitfield, 1992; Arnoldi et al., 1990). On the other hand, MRP have been suggested to decrease the lipid oxidation rate, thus highlighting an interesting relationship by which the browning of the food, and thus the process conditions, could affect not only the organoleptic properties but also the oxidative resistance of the food (Lingnert and Hall, 1986).

Products isolated from solutions of sugars and amino acids, in which the Maillard reaction was induced by heating to mimic food processing, have been shown to depress the rate of lipid oxidation (Lingnert and Eriksson, 1980, 1981; Lingnert and Hall, 1986; Tanaka et al., 1988). However, the compounds accounting for this effect have not been identified and the mechanism of antioxidant effect is still under debate (Eichner, 1981; Kim and Harris, 1989; Park and Kim, 1983). The browning reaction could limit the availability of either oxygen, which is a reactant for peroxidative reactions (Lingnert and Waller, 1983; Anese et al., 1994), or metal catalysts which, when complexed by MRP, are prevented from decomposing lipid hydroperoxides, in turn initiating peroxidative chain reactions (Yamagushi et al., 1981). More recently it has been shown that MRP generated in a simple system account for a relevant antioxidant effect due to scavenging of peroxyl radicals (Tubaro et al., 1996). This chain-breaking antioxidant effect was kinetically evaluated in terms of vitamin E equivalents produced upon warming of a solution of glucose and glycine.

The clear-cut and quantitative evidence that a similar antioxidant effect takes place in a real food is obviously more difficult to achieve. The most empirical and direct approach, i.e. the demonstration that the lipid oxidation rate is actually lower in the presence of MRP, is based on the reactivity between MRP and the aldehydic lipid oxidation products generally used as markers of the ongoing oxidation (Gabrieli, 1992; Bot, 1993).

In this paper the issue of the production of chain-breaking antioxidants in a food was addressed using the kinetic procedure previously set up for simple molecules. On an aqueous extract of a bakery product (butter cookie), the time course of the production of molecules playing an antioxidant effect was evaluated by analyzing the kinetics of interaction with a peroxyl radical.

MATERIALS AND METHODS

Preparation of Model Systems and Heat Treatment. A model system (model A) was made to simulate a butter cookie formulation by mixing 70.25 g of pure wheat starch (ADEA, Padova, Italy) with 3.4 g of anhydrous glucose (Carlo Erba RPE-ACS, Milan, Italy), 1.35 g of L-lysine (Sigma, Italy), 21 g of concentrated butter (PREALPI, Varese, Italy), and 4 g of distilled water. In the same way, two reference systems were prepared without glucose or lysine (respectively models B and C).

Small round paper containers 3 cm wide and 2 cm high were filled completely with a small amount of each dough (10 ± 0.5 g) and cooked at 150 °C for 0, 10, 20, 30, or 40 min.

Preparation of the Extracts. Five grams of biscuit was suspended in 20 mL of a 0.4 M KCl aqueous solution (Carlo Erba) and homogenized using a Polytron (Kinematica AG, PT

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0021-8561/96/1444-0692$12.00/0

3000) for 1 min. Samples were centrifuged for 5 min at 5000 rpm at 0 °C (refrigerated centrifuge ALC, R.C.F. Meter, 4233R), and the aqueous phase was filtered on paper (Whatman No. 4) and diluted with 10 mL of 0.4 M KCl. 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.

Competition Kinetics Test. The kinetics procedure has been previously described for analyzing the antioxidant capacity of single molecules or extracts (Bors et al., 1984; Tubaro et al., 1995). In brief, the specific absorbance of the carotenoid crocin decreases with a rate V_0 following interaction with peroxyl radicals ($ROO^{\cdot}$) and the bleaching ($-\Delta A_0/\Delta t$) slows down in the presence of an antioxidant, competing for the same radical with a rate V_a, to a new value ($-\Delta A_a/\Delta t$). The competition kinetics follows the equation

$$\Delta A_a/\Delta t = V = V_0K_c[C]/(K_c[C] + K_a[A]) \quad (1)$$

where ΔA_0 = absorbance variation in absence of antioxidants, ΔA_a = absorbance variation in presence of antioxidants, V_0 = bleaching rate in absence of antioxidants, V = bleaching rate in presence of antioxidants, $K_c = K_1[ROO^{\cdot}]$, $K_a = K_2[ROO^{\cdot}]$, [C] = concentration of crocin, [A] = concentration of antioxidant, K_1 = rate constant for the reaction between free radicals and crocin, and K_2 = rate constant for the reaction between free radicals and antioxidant.

Thus, by transforming

$$\Delta A_0/\Delta A_a = V_0/V = (K_c[C] + K_a[A])/K_c[C] = 1 + K_a/K_c[A]/[C] \quad (2)$$

The slope of the straight line (K_a/K_c) resulted by fitting the experimental data obtained in the presence of different concentrations of the antioxidant indicates its relative capacity to interact with $ROO^{\cdot}$.

When the overall antioxidant capacity of complex mixtures is analyzed, a theoretical value $K_a[A]$ is defined, by the above equation, as the sum of the antioxidant capacity of each individual free radical scavenger present. By considering the whole amount of the sample as Trolox (a hydrosoluble analogue of vitamin E), its "theoretical" concentration [pseudo-Trolox] is used to plot the results of crocin bleaching. Thus, the slope of the fitting of the kinetics equation data describes the antioxidant capacity of the sample in terms of the ratio between the rate constants:

$$V_0/V = 1 + (K_{\text{pseudo-Trolox}}/K_c)([\text{pseudo-Trolox}]/[C]) \quad (3)$$

$K_{\text{pseudo-Trolox}}$ represents the rate constant for the interaction of pseudo-Trolox with peroxyl radicals.

Finally, since

$$K_a[A] = K_{\text{pseudo-Trolox}}[\text{pseudo-Trolox}] = K_{\text{Trolox}}[\text{Trolox}] \quad (4)$$

and since K_{Trolox} is known from independent measurements, the ratio [pseudo-Trolox]/[Trolox] can be calculated. This ratio indicates the relative antioxidant capacity in terms of weight of Trolox vs the sample.

Determination of the Antioxidative Activity of the Extracts. 2,2′-Azobis(2-amidinopropane) dihydrochloride (ABAP) was purchased from Wako Chemical Co., Japan, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) from Aldrich, Germany. Potassium hydrogen phosphate and potassium hydroxide were from Carlo Erba. Saffron (*Crocus sativus*) was obtained from Sigma, Italy. Crocin [8,8′-diapocarotenedioic acid bis(6-*O*-D-glucopyranosyl-D-glucopyranosyl) ester] was prepared from saffron by methanolic extraction after repeated washing with ethyl ether.

Tests were carried out at 40 °C in 2 mL of medium composed of 0.1 M phosphate buffer, pH 7, 0.4 M KCl, 10 μM crocin (from a 1 mM metanolic stock solution, $\epsilon = 1.33 \times 10^5$ M^{-1} $\lambda = 443$ nm), and increasing quantities of either MRP extract or the reference antioxidant (vitamin E hydrosoluble analogue Trolox from a 1.7 mM aqueous solution). The reaction was started by adding 30 μL of ABAP (from a 5 mM fresh aqueous solution). The rate of crocin bleaching was recorded at 443 nm using a spectrophotometer (Kontron Instruments, Uvikon 860) with six thermostatic cells. The linear bleaching rate from 3 to 12 min after the addition of the diazo compound was used for calculations.

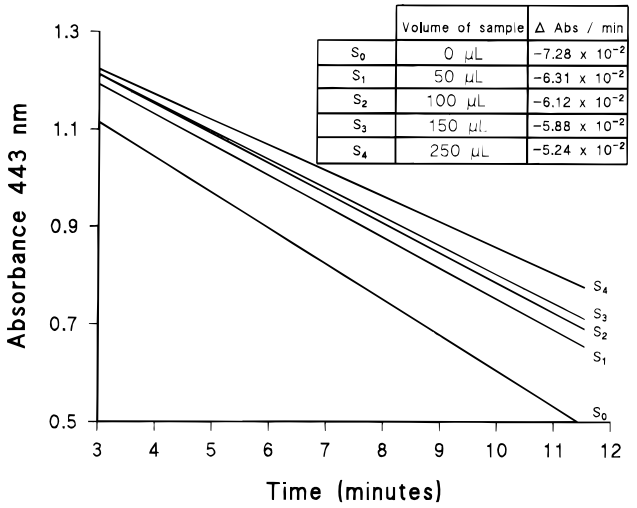

Figure 1. Crocin bleaching kinetics in the presence of increasing amounts of extracts obtained from a 20 min heat treatment of model system A.

Before each determination, possible spectroscopic interference of extract with the crocin bleaching was ruled out.

When necessary, extracts were diluted to maintain the range of absorption at an optimal level for spectrophotometric measurements, with an absorbance unity lower than 1.5 at the beginning of the reaction.

Determination of the Color of the Extracts. The color determination was made with a tristimulus colorimeter (Chromater CR 200 II Reflectance, Minolta, Japan) equipped with an illuminant C and a microprocessor for the statistical analysis of data. Results have been reported in the Hunter system as lightness L^* and chromatic coordinates a^* and b^* for each cooked system of different composition. The changes in lightness were continuously observed for the KCl extraction solutions of the complete model system during heat treatment. The reported data are the mean values of seven determinations.

RESULTS

The production of chain-breaking antioxidant capacity has been analyzed in butter cookies, as a function of the cooking time and, thus, of the browning related to the formation of MRP. A kinetics approach was used, by which the antioxidant capacity of aqueous extracts was measured in terms of equivalence by weight of a reference antioxidant. A diazo compound was used to generate at constant rate peroxyl radicals and the reporting reaction, the inhibition of which was measured, was the bleaching of crocin in the presence of peroxyl radicals.

Data reported in Figure 1 show that the KCl extract of butter cookies contains molecules able to inhibit crocin bleaching.

For the kinetics analysis, for which a molar concentration of the antioxidant is required, the whole dry matter of cookie extract was considered as Trolox; thus, the kinetics treatment provided data in terms of the ratio between the rate constants with peroxyl radicals of the pseudo-Trolox and crocin. As an example of a typical measurement, Figure 2 reports the kinetics

Table 1. Kinetics of Crocin Bleaching Inhibition, Statistical Data of the Kinetics Equation Plot, and Antioxidant Capacity of MRP Reported as MRP/Trolox Ratio[a]

samples	competition kinetics eq $V_0/V = (K_a/K_c)[A]/[C] + q$	K_a/K_c	r	MRP/Trolox[b]
Trolox	$y = 0.35x + 1.02$	0.35 ± 0.03	0.991	1
A				
0 min	$y = 1.02 \times 10^{-4}x + 1.02$	$(1.02 \pm 0.15) \times 10^{-4}$	0.977	undetectable
10 min	$y = 2.29 \times 10^{-3}x + 1.01$	$(2.29 \pm 0.14) \times 10^{-3}$	0.994	156
20 min	$y = 1.03 \times 10^{-2}x + 1.01$	$(1.03 \pm 0.02) \times 10^{-2}$	0.999	34
30 min	$y = 1.67 \times 10^{-2}x + 1.01$	$(1.67 \pm 0.06) \times 10^{-2}$	0.999	21
40 min	$y = 1.30 \times 10^{-2}x + 0.99$	$(1.30 \pm 0.06) \times 10^{-2}$	0.996	27
B				
40 min	$y = 2.34 \times 10^{-4}x + 0.98$	$(2.35 \pm 0.20) \times 10^{-4}$	0.988	undetectable
C				
40 min	$y = 6.30 \times 10^{-5}x + 0.95$	$(6.30 \pm 4.15) \times 10^{-5}$	0.659	undetectable

[a] Data refer to different model systems A, B, C. In the equations the term y is referred to V_0/V and x to [A]/[C]. [b] The ratio indicates the relative amount of MRP exhibiting the antioxidant effect of Trolox on a weight base. See text for details.

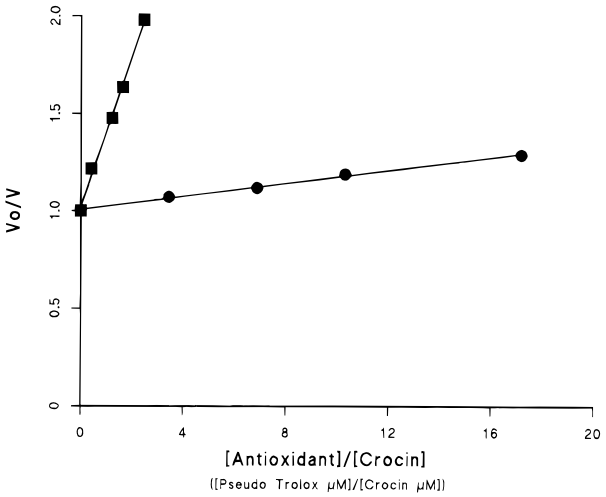

Figure 2. Kinetics analysis of Trolox (■) and of a cookie extract after 30 min of treatment (●). The antioxidant capacity of the dried cookie extracts was calculated in terms of pseudo-Trolox as described under Materials and Methods.

analysis of Trolox and the extract of cookies after 30 min of heat treatment. From the ratio between rate constants a simple calculation permits the definition of the antioxidant capacity in the cookie in terms of ratio (by weight) vs the reference antioxidant. After 30 min of thermal treatment, 21 g of dried cookie extract contains the antioxidant capacity of 1 g of Trolox.

The complete kinetics analysis and the time course of antioxidant capacity generation during cooking are reported in Table 1. Remarkably, after cooking, the controls without glucose or lysine as well as the complete sample at time zero did not show any significant effect. For practical reasons, the antioxidant capacity of samples producing a slope lower than 5×10^{-4} in the kinetics test is not measurable by this approach and was considered nil.

The generation of antioxidant capacity takes place during the first 20 min of cooking, reaching a plateau before 30 min. Notably this value remains constant, thus suggesting that the molecules bearing free radical scavenging capacity are rather stable.

In agreement with the attribution of the antioxidant capacity to the products of the Maillard reaction, the browning, measured as the decline in lightness of the aqueous extracts during heat treatment, increased in parallel up to 20 min and remained more or less constant during the subsequent 20 min (Table 2). Consistently in models B and C, in which either glucose or lysine was omitted and which failed to show any

Table 2. Lightness L^* of the Extracts Obtained from Model System A during Heat Treatment

model A	lightness L^a	model A	lightness L^a
0 min	78.01 ± 1.15	30 min	60.27 ± 2.99
10 min	64.98 ± 1.54	40 min	51.61 ± 1.53
20 min	55.57 ± 1.79		

[a] Mean values ± standard deviation.

Table 3. Influence of the Model System's Composition on the Evolution of the Colorimetric Parameters of Cookies during the Thermal Treatment

model[a]	ΔL^*	Δa^*	Δb^*
A	−21.02	+4.12	+15.19
B	−4.59	−0.12	+5.41
C	−3.52	−0.19	+1.72

[a] A, starch, lysine, glucose, butter, and water; B, starch, lysine, butter, and water; C, starch, glucose, butter, and water.

significant antioxidant capacity (Table 1), the lightness decrease was much less evident (Table 3).

DISCUSSION

This study was addressed to the quantitative evaluation of the antioxidant capacity of compounds developed during the heat treatment of a complex system undergoing a thermal treatment simulating the baking of a cookie.

The results indicate that under the adopted conditions and 30 min of cooking a substantial amount of antioxidant capacity is developed. By calculating the ratio between rate constants of a pure sample of Trolox and a cookie heat treated for 30 min, an equivalence between 21 g of aqueous extract and 1 g of Trolox is obtained. Notably, this amount has to be related to up to 500 g of cookies.

This value is rather high and could account for the observed effect of MRP on the oxidative stability and shelf life of processed food (Lingnert and Lundgren, 1980; Lingnert, 1980). However, to draw a conclusion on the actual prevention of rancidity in a food, other concepts must be taken in account. The proximity of antioxidant and lipid molecules undergoing oxidation in food could limit the actual efficiency of the antioxidant capacity as measured in a homogeneous solution. Moreover, the antioxidant effect of liposoluble MRP was not screened in this study. Eventually, an antioxidant mechanism different from free radical scavenging, brought about by MRP, such as the capacity to sequestrate metal ions or act as oxygen scavengers, could also play a relevant role.

Nevertheless, this study presents, for the first time in quantitative terms, evidence that the Maillard reac-

tion produces a dramatic increase of molecules having a free radical scavenging effect, and this must be relevant to food technology and the shelf life of the product as well, as it could have an impact on human health in light of the growing evidence of the protective effect of antioxidants (Addis and Warner, 1991) and the harmful effect of lipid oxidation products (Esterbauer, 1982).

ACKNOWLEDGMENT

We thank Fulvio Ursini for invaluable advice.

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Received for review July 13, 1995. Accepted December 19, 1995.[⊗] Research supported by National Council of Italy, Special Project RAISA, Subproject 4, Paper 2397.

JF950436B

[⊗] Abstract published in *Advance ACS Abstracts*, February 1, 1996.