Antioxidative Action of Maillard Reaction Volatiles: Influence of Maillard Solution Browning Level

B.E. Elizalde[1], **F. Bressa** and **M. Dalla Rosa***
Istituto di Tecnologie Alimentari, Università degli Studi Via Marangoni 97—33100 Udine, Italy

Maillard reaction volatile compounds were prepared by heating a glucose-glycine solution. The antioxidant effect of the volatiles was tested in soybean oil (SBO) thermoxidation. Volatile compounds were transferred from the heated Maillard solution to the oil with a gas-tight syringe after removing the same volume of oil headspace (air). Standard accelerated oxidation was performed by heating the SBO at 90°C. Antioxidant activity was evaluated through peroxide value and headspace gas chromatographic analysis of oil volatile compounds. Furthermore, some indices, such as protection factor and oxidation kinetic rate, were used to measure the antioxidant effect. Maillard volatile effectiveness was related to browning level of glucose-glycine solution. The maximum antioxidant effect was obtained with volatiles from 12–18 hr of heating the glucose-glycine solution. This result is related to the quantity of Maillard volatiles transferred into the oil atmosphere and to the reduction power of the browned Maillard solution.

KEY WORDS: Browning level, lipid oxidation, Maillard reaction volatiles, soybean oil.

Many authors have reported on the inhibiting action of Maillard reaction products (MRP) on lipid oxidation (1–8). The antioxidant activity of the volatiles produced by Maillard reaction volatiles (MRV), obtained by heating a glucose-glycine solution, were studied previously (9). It was found that MRV, similar to the MRP, slowed the oxidative degradation of soybean oil by increasing the oxidation induction period and by decreasing the oxidation rate constant. The use of MRV has been suggested for packaging fatty food. More information is needed about the health significance of those products.

No research has been published on the relationship between the antioxidant effect of MRV and the level of the Maillard reaction. Published MRP results are contradictory. Tanaka *et al.* (7), Kirigaya *et al.* (10) and Yamaguchi *et al.* (11) found that the higher the browning level of Maillard solution, the more antioxidative effect resulted. Lingnert and Eriksson (12,13), Beckel and Waller (14) and Waller *et al.* (15) reported that the maximum antioxidant activity of MRP was achieved after about 20 hr of sugar-amino acid heating time. However, other studies showed that MRP produced in the early stages of the Maillard reaction had a strong antioxidant activity (4–6,16). The conflicting reports regarding stages of the browning and their antioxidant effect might be due to the different research conditions (8) and to the fact that compounds formed by Maillard reaction can exhibit antioxidative properties with different modes of action (17).

The present study was conducted to establish how the heating time of a glucose-glycine solution affects the ability of MRV to act as antioxidants during thermoxidation of soybean oil.

EXPERIMENTAL PROCEDURES

Lipid model systems and Maillard reaction volatiles (MRV). A commercial edible soybean oil (SBO) purchased from a local market was used as a lipid model system. MRV were obtained by heating a solution of 1.71 M glucose and 2.02 M glycine (RPE-ACS reagent, Carlo Erba, Milan, Italy) in an air-circulating oven at 90°C for different times (3, 6, 12, 18 and 24 hr). For each heating time, seven samples of glucose-glycine solution (5 mL) were placed into 20-mL capacity vials and sealed hermetically. The pH of the reaction mixture was adjusted to pH 6.0 with 1N NaOH (RPE-ACS reagent, Carlo Erba) before starting the reaction.

The following analyses of heated Maillard solutions were performed—pH, measured with a Beckman pH meter (Beckman Instruments, Fullerton, CA); optical density at 420 mn, as a browning index according to Lerici *et al.* (18), with a Varian Model DMS 80 spectrophotometer (Palo Alto, CA); reduction power, evaluated by the rate of oxygen consumption as previously described (9); and gas chromatographic head space analysis of CO_2 (expressed as % by weight) and organic Maillard volatile compounds (MRV), according to the methodologies described in previous work (9). For example, 15 mL of heated Maillard solution headspace was withdrawn with a gas-tight syringe and introduced into a 20-mL capacity vial containing 5 mL of soybean oil (SBO), after removing the same volume of oil headspace (air). A control sample (blank) of a 20-mL vial containing 5 mL of SBO alone, equilibrated in the presence of air, was also analyzed for each oxidative run.

Evaluation of antioxidant activity. Control and MRV-treated SBO samples were heated in a circulating air electric oven at 90°C and tested at regular time intervals for determination by peroxide value (PV) and headspace gas chromatographic (HSGC) analysis of volatile compounds, as reported earlier (9).

The antioxidant activity was evaluated through the following indices: i) The ratio of induction period of antioxidant-treated sample to induction period of control sample (protection factor) reported as PF according to Parmar and Sharma (19). The induction time was computed from the equation:

$$t = (a_1 - a_2)/(k_2 - k_1)$$

where t, induction time; a_1, intercept of the regression line during lag time; a_2, intercept of the regression line during linear index increase; k_1, slope of the regression line during lag time; and k_2, slope of the regression line during linear index increase.

ii) The ratio of the kinetic rate constants (OKR) of the linear parts of oxidation curves (related to PV, total peak area and hexanal peak area *vs.* time) of treated sample to that of control sample (k of treated sample/k of control sample).

[1]Research fellow of Consejo Nacional de Investigaciones Cientificas y Tecnicas de la Republica Argentina.
*To whom correspondence should be addressed.

iii) The percent decrease of PV, total peak area and hexanal peak are at 24 hr of oxidative heating in respect to the control.

RESULTS AND DISCUSSION

Optical density, % CO_2 and headspace volatile total peak area are plotted in Figure 1 as a function of heating time of the glucose-glycine solution. It is evident that % CO_2 in the Maillard solution sample headspace and the optical density increased continuously during heating, while volatile compounds (MRV) production showed a maximum at about 12 hr of heating.

MRV transfer from Maillard solution sample headspace to the SBO atmosphere led to the results plotted in Figure 2, where % CO_2 and total peak area of transferred volatiles to the SBO headspace as a function of Maillard solution heating time are reported. The amount of transferred volatiles was around 10% of the initial volatile content in the Maillard samples headspace, while the CO_2 level remained at about 50%.

Thermal oxidation of SBO samples with MRV-modified atmosphere and the changes of PV showed that treated samples, in comparison with the untreated control, gave a reduction of both the rate of PV increase and the maximum PV (Fig. 3a). The behavior of PV increase appeared to be similar in treated and untreated samples, reaching maximum PV at 40 hr of oxidative heating.

Similarly, in Figure 3b, data relative to oxidative volatile formation are reported as a function of SBO heating time. The maximum effect of MRV is reached after 12 hr heating of the glucose-glycine solution. After this time, the effectiveness of MRV remained almost constant for peroxide formation, while the reduction of the oxidative volatile formation decreased quickly. Hexanal formation (Fig. 3c) was slowed by MRV, resulting in the highest level of antioxidative effect between 12 and 18 hr Maillard heating time. Also, after this period of Maillard heating time hexanal formation decreased the MRV effectiveness quickly.

The mechanisms of the MRV antioxidant action were considered in a previous study (9), and MRV served both as oxygen scavenger and as radical chain breaker in the volatile formation. To obtain further information on antioxidant mechanisms, the relationship between peroxide and oxidative volatile formation was investigated. The regression of the percent reduction of total volatile peak area *vs.* the decreasing percentage of PV at 48 hr of thermal oxidation of SBO was found to be:

$$\%\ \text{Total peak area reduction} = (0.877 * \%\ \text{reduction PV}) - 0.0477$$

The determination coefficient was $R^2 = 0.66$, with a significance of $p \leq 0.1$. Thus, only 66% of the reduction of the volatile formation can be considered as an effect of the inhibition of the preceding decrease in the peroxide formation.

Considering hexanal the most important volatile product (by weight) formed from oxidation of linoleic acid, the correlation equation was

$$\%\ \text{Hexanal area reduction} = (1.36 * \%\ \text{reduction PV}) - 4.68$$

with $R^2 = 0.816$ and $p \leq 0.05$. There is a good correlation between reduction in peroxide and hexanal formation, and it appears that MRV could be a peroxide destroyer as well as an oxygen scavenger, thus inhibiting the peroxide formation.

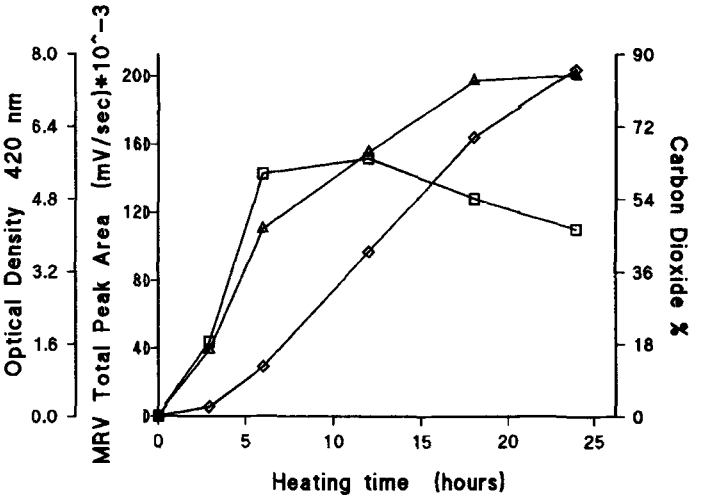

FIG. 1. Changes in browning index: OD at 420 nm (◇), headspace CO_2, % (△), and headspace MRV content (total peak area) (□) of glucose-glycine solution.

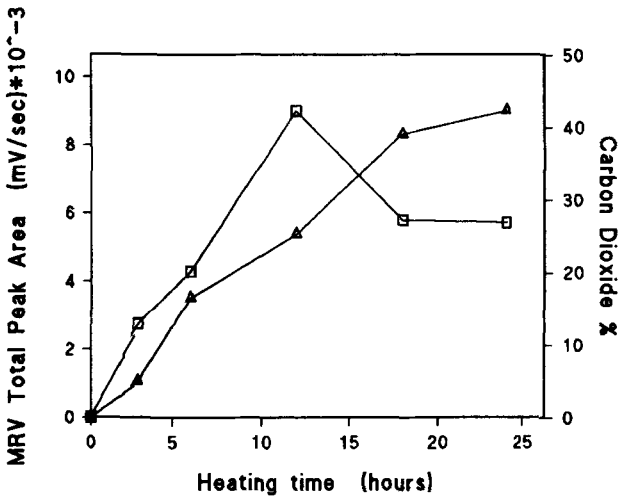

FIG. 2. SBO headspace content of transferred CO_2 (△) and MRV (□) from glucose-glycine solution.

The effect of MRV on oxidation induction time and oxidation kinetic rate are reported in Figure 4a for the ratios (PF) of the oxidation induction time (peroxide, hexanal and total volatile formation) of MRV-treated samples, in respect to the control, *vs.* Maillard heating time. Ability to prolong the induction time of peroxide formation (Fig. 3) is pronounced for MRV produced by heating of the glucose-glycine solution for 12 and 18 hr. The effect on the induction period of secondary oxidation production formation (considered here in terms of volatile compounds present in the SBO headspace) continued to increase with Maillard heating time up to 12 hr and then remained at the same level. The increase of the induction time for hex-

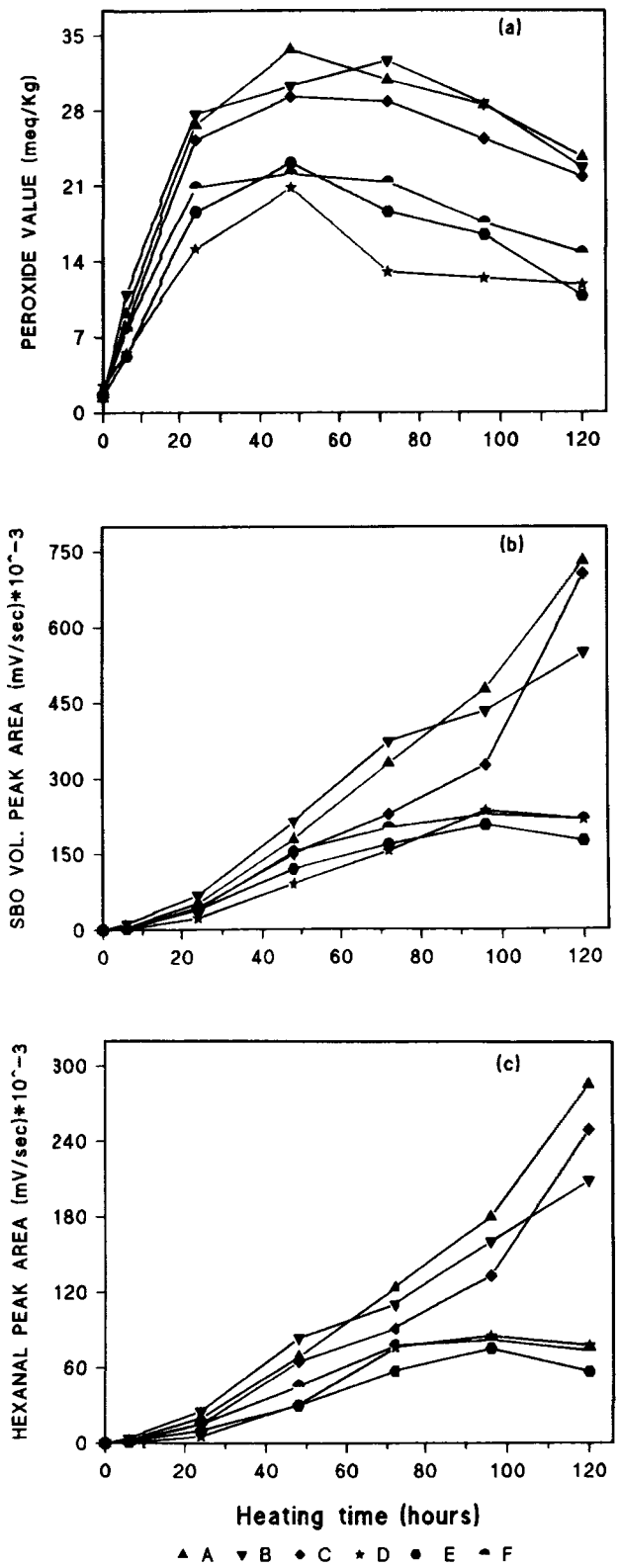

FIG. 3. Peroxide value (a), oxidation volatiles (b) and hexanal content (c) changes during thermal oxidation of control sample and MRV-treated samples of SBO. A, control; B, 3 hr MRV-treated sample; C, 6 hr MRV; D, 12 hr MRV; E, 18 hr MRV; and F, 24 hr MRV.

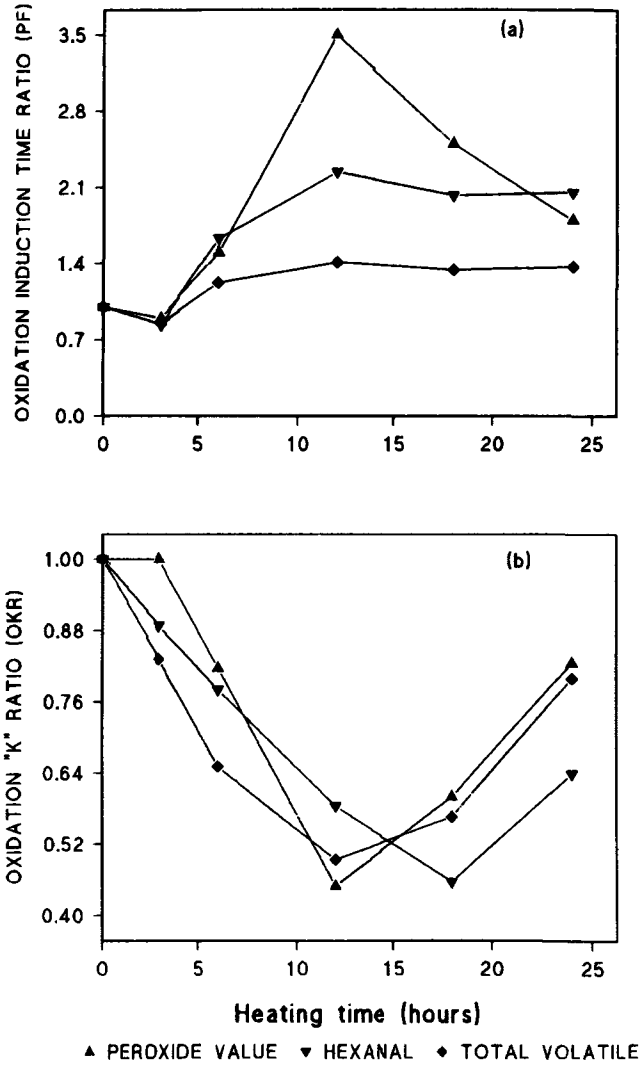

FIG. 4. Ratio of oxidation induction times (PF) (a) and ratio of kinetic rate constants (OKR) (b) between control and MRV-treated SBO samples as a function of Maillard reaction time of the glucose-glycine solution.

anal formation appeared higher than that for the total volatiles. This result confirmed that for soybean oil the principal oxidation volatile compound is hexanal. The effect of MRV pertains mainly to hexanal.

However, the higher PF values, compared with those of the volatile formation, did not imply a longer oxidation time for peroxide than for volatile production. In fact, as shown in Figures 3b and 3c, the induction times of volatile formation (for our experimental conditions) ranged approximately between 6 and 20 hr, while the computation of the induction time for peroxide formation gave values ranging from 20 to 70 min.

The kinetic constant rate ratios (MRV-treated sample/control) regarding peroxide, hexanal and total volatile formation are plotted in Figure 4b *vs.* Maillard heating time. Data show that the effectiveness of MRV to reduce the oxidation kinetic rate increased with the Maillard heating level and appeared to be of the same order of magnitude, regardless of the oxidation index. For peroxide and total volatile formation, the maximum antioxidant

TABLE 1

Correlation Coefficients |r| Between the Parameters of Heated Maillard Solution and Percentage of Peroxide and Hexanal Formation Inhibition After 48 hr of Thermal Oxidation

Maillard heating parameters	Inhibition %	
	Peroxide	Hexanal
Heating time	.844*[a]	.754 n.s.[b]
Transferred MRV	.941***	.816*
Transferred CO_2	.867*	.808*
OD[c] (420 nm) of solution	.828*	.781 n.s.
Reduction power of solut.	.898*	.840*

[a]*p < 0.05, and ***p < 0.001.

[b]n.s., Not significant.

[c]OD, optical density.

effect was found for 12 hr of Maillard heating, whereas for hexanal the maximum effect was produced by MRV at 18 hr of glucose-glycine solution heating.

To better understand the relationship between the experimental antioxidant activity of MRV and the characteristics of the heated Maillard solution, a correlation analysis between percentage reduction of the oxidative indices and data related to the heating of the Maillard solution was performed. Statistical results are given in Table 1. The antioxidative effect of MRV was found to be mainly related to the quantity of MRV transferred to the oil atmosphere and with the reducing power (oxygen consumption) created by the browned solution. From these results it is possible to deduce that not only the level of the Maillard reaction affects the antioxidant activity, but also that at the longest heating time, while the glucose-glycine solution is continuously browning, the formation of reducing compounds and active volatiles slows down and that this affects the antioxidant action. This was more true for peroxide formation than for hexanal formation, confirming that MRV act not only as oxygen scavengers but also as oxidation product chain breakers (9).

ACKNOWLEDGMENT

Research supported by National Council of Italy, Special Project RAISA, Sub-project No. 4, Paper No. 210

REFERENCES

1. Yamaguchi, N., Y. Yokoo and Y. Koyama, *Nippon Shokuhin Kogyo Gakkaishi 11*:184 (1964).
2. Maleki, M., *Fette Seifen Anstrichm. 75*:10 (1973).
3. Yamaguchi, N., and M. Fujimaki, *Nippon Shokuhin Kogyogakkai Shi*:13 (1974).
4. Evans, C.D., H.A. Moser, P.M. Cooney and J.E. Hodge, *J. Am. Oil Chem. Soc. 35*:84 (1958).
5. Park, C.K., and D.H. Kim, *Ibid. 60*:22 (1983).
6. Eichner, K., *Prog. Fd. Nutr. Sci. 5*:441 (1981).
7. Tanaka, M., W.C. Kuei, Y. Nagashima and T. Taguchi, *Nihon Suisan Gakkaishi 54*:1409 (1988).
8. Kim, N.K., and D.H. Harris, in *Trends in Food Science*, edited by A.H. Ghee, L.W. Sze and F.C. Woo, Singapore Institute of Food Science and Technology, Singapore, 1989, pp. 19–24.
9. Elizalde, B.E., M. Dalla Rosa and C.R. Lerici, *J. Am. Oil Chem. Soc. 68*:758 (1991).
10. Kirigaya, N., H. Kato and M. Fugimaki, *Agric. Biol. Chem. 32*:287 (1968).
11. Yamaguchi, N., Y. Koyama and M. Fujimaki, *Prog. Fd. Nutr. Sci. 5*:429 (1981).
12. Lingnert, H., and C.E. Eriksson, *J. Food Proc. Pres. 4*:161 (1980).
13. Lingnert, H., and C.E. Eriksson, *Prog. Fd. Nutr. Sci. 5*:453 (1981).
14. Beckel, R.W., and G.R. Waller, *J. Food Sci. 48*:996 (1983).
15. Waller, G.R., R.W. Beckel and B.O. Adeleye, in *The Maillard Reaction in Foods and Nutrition*, edited by G.R. Waller and M.G. Feather, ACS Symposium Series 215, American Chemical Society, Washington, D.C., 1983, pp. 125–140.
16. Farlag, R.S., Y. Ghali and M.M. Rashed, *Can. Inst. Food Sci. Technol. J. 15*:179 (1982).
17. Beckel, R.W., and G.R. Waller, *J. Food Sci. 48*:996 (1983).
18. Lerici, C.R., D. Barbanti, M. Manzano and S. Cherubin, *Lebensmittel Wiss.u. Technol 23*:289 (1990).
19. Parmar, S.S., and R.S. Sharma, *Food Chem. 35*:99 (1990).

[Received August 3, 1991; accepted January 7, 1992]