Effect of Maillard Reaction Volatile Products on Lipid Oxidation

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Maillard reaction volatile compounds (MRV), prepared by heating a glucose-glycine solution, were tested as antioxidants in soybean oil (SBO) thermoxidation. The volatiles were transferred into the oil by stripping with a stream of Nitrogen and substituting the atmosphere above the oil with air containing MRV. Standard accelerated oxidation was performed by heating the SBO. Peroxide value measurement and headspace gas chromatographic analysis were carried out on all the samples. The MRV antioxidant activity was evaluated by determining the effect of the induction period and the kinetic rate constant of peroxide and oxidation volatiles formation. The MRV showed a significant antioxidant activity. The effectiveness was variable depending on MRV transfer method to the oil, and the Maillard reaction extent was related to the browning level of glucose/glycine solution. It was found that the maximum effect of MRV prolonged about three times the induction period and reduced the kinetic rate constant by half in relation to the control sample. MRV affects oxidative stability of soybean oil by lengthening the induction period as well as by decreasing the rate of oxidation at the propagation state and reducing the formation of hexanal.

KEY WORDS: Lipid oxidation, Maillard reaction volatiles, soybean oil.

Lipid oxidation is a major problem in food technology. Although many synthetic antioxidants presently used by the food industry are effective in preventing rancidity, their safety has recently been questioned (1). Thus, the interest toward utilization of natural food constituents with antioxidative properties is increasing. Maillard reaction products (MRP) are widespread in processed foods, and have received much attention as antioxidants (2-26). Despite the number of studies carried out on antioxidant properties of MRP, no work has been published yet on the antioxidant effects of volatile Maillard reaction products.

While melanoidins are the ultimate products of the Maillard reaction, many compounds of low molecular weight, which are very important in flavor and off-flavor production, are formed. Their detection in the headspace of food can be used as a "fingerprint" to determine if Maillard reaction occurred (27). Hundreds of volatile products of the Maillard reaction have been identified in a variety of real and model food systems (28) and may be classified into three groups (29,30): i) Sugar dehydration/fragmentation products—furans, pyrones, cyclopentenes, carbonyls and acids; ii) amino acid degradation products—aldehydes and sulfur compounds; and iii) volatiles produced by further reactions—pyrroles, pyridines, imidazoles, pyrazines, oxasoles, thiazoles and aldol condensation products.

Because of their structure, many of these compounds are lipolitic, and they could present reducing and chelating properties and act as hydrogen donors or electron traps. Thus they could show antioxidant activity. Moreover, carbon dioxide can be produced by Strecker degradation in the Maillard reaction (31). From a toxicological point of view, Wilson (32) tested some flavor products resulting from heating protein-amino acid systems. Those products were claimed to be noncarcinogenic when fed to rats and mice. Omura *et al.* (33) isolated some mutagenic volatile substances, such as the 2-methylthiazolidine, produced by heating a glucose-amino acid system. Stich *et al.* (34) also found no mutagenic activity of pyrazine and some of its alkyl derivatives, although those compounds were highly active in inducing chromosomal aberrations in Chinese hamster ovary cells. However, it is now generally accepted that the mutagenity of Maillard reaction compounds is not extremely potent, and no definitive conclusions are available about the health significance of those products (35).

The main purpose of this investigation was to evaluate the antioxidant effect of Maillard reaction volatiles (MRV) from browning glucose-glycine mixtures on soybean oil, which we considered as a lipid model system. We looked at the possibility of using MRV to modify the atmosphere around foods that are susceptible to oxidative deterioration and to prolong their shelf life. Another purpose was to gain some information about the action mechanism of MRV by using two different oxidation assessment methods—peroxide value determination to evaluate primary oxidation products and sample headspace gas chromatographic analysis for evaluation of some secondary oxidation products.

EXPERIMENTAL PROCEDURES

Lipid model system and Maillard reaction volatiles (MRV). A commercial edible soybean oil (SBO) was used as the lipid model system. No stripping procedure was performed to separate the nontriglyceride components, so that the antioxidant activity of MRV was evaluated in comparison with a blank sample, as described below.

MRV were obtained by heating a stock solution of 1.71 M glucose (RPE-ACS reagent, Carlo Erba, Milan, Italy) and 2.02 M glycine (Carlo Erba RPE reagent) in an air circulating oven at 90°C for 24 hr. The heating of glucose-glycine solution was performed in two different types of containers according to the MRV-transferring method described below.

The pH of the reaction mixture was adjusted to pH 6.0 with 1N NaOH (RPE Carlo Erba reagent) before starting the reaction.

Maillard browning mixtures obtained at 24 hr had the following characteristics: pH 3.72; reduction power, as the rate of oxygen consumption: 6.76 μL/min. These data were obtained as described by Lingnert and Waller (36) and Pitotti *et al.* (37) by using a Clark oxygen electrode connected to a YSI 5300 oxygen monitor (Yellow Springs Instruments Co., Yellow Springs, OH).

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MRV transfer to the oil: Modifying of sample oil atmosphere with MRV. Glucose-glycine solution (5 mL) was placed into 20-mL vials and they were hermetically sealed. After heating, an aliquot of the headspace above the heated Maillard solution was withdrawn with a gas-tight syringe and was added to a 20-mL vial containing 5 mL of SBO. Prior to the MRV addition, 15 mL of oil headspace was withdrawn from the vial containing the oil sample and the same amount of Maillard solution headspace was added to obtain a MRV-modified atmosphere (MA). For each oil sample with modified atmosphere (MA), a vial containing the browning solution was prepared. For each oxidative run, a control sample (blank) of a 20-mL vial containing 5 mL of SBO alone, equilibrated in the presence of air, was also evaluated.

Stripping of MRV from Maillard reaction mixture by N_2 stream and bubbling into SBO. Glucose-glycine mixture (50 mL) was placed in a 250-mL cylindrical flask fitted with a Drechsel head which had a two-way teflon valve. During heating time the valves were closed to avoid any loss of volatiles. At the end of the heating period and after cooling to achieve room temperature, the Drechsel head flask was connected by teflon tubes to a N_2 cylinder and to a Drechsel head conical flask containing 50 mL of SBO. Stripping was started by opening the valves so that the volatiles were continuously transferred into the oil by bubbling N_2 into the Maillard solution.

The stripping-bubbling procedure was performed at an N_2 flow rate of 120 mL/min and a stripping time of 60 min. A control sample was prepared by bubbling N_2 directly into SBO, at the same time and flow rate conditions. Both control and MRV-treated samples were subdivided into 7 samples of 5 mL, distributed into 20-mL vials and sealed with butyl septa and metallic caps.

A stripping process was performed with an air stream (air for gas chromatography) under the following conditions: air flow rate 500 mL/min, stripping time 20 min. In this case the control sample was prepared by bubbling air directly into SBO.

Assessing oil oxidation. For accelerated thermoxidation, control and MRV-treated SBO samples were heated up to 120 hours in an electric circulating air oven at 90°C. At regular time intervals both control and MRV-treated samples were withdrawn from the oven, cooled to room temperature, and two replicates were analyzed for peroxide value and headspace gas chromatography.

Peroxide value (PV). This was expressed as meq of active oxygen on 1000 g of oil, by following the AOCS Official Method Cd 8-53 (38).

Headspace gas chromatographic (GC) analysis of volatile compounds. Because the linoleic acid content of SBO is about 55% (39), and hexanal formation is attributed to the autoxidation of 18:2 (40–42), the main contribution to flavor in SBO is probably due to this volatile (43–46). Other authors have reported the possibility of using headspace hexanal concentration as an oxidative index (47,48); it also has been used to test the antioxidant activity of MRP in model and real food systems (22, 49–51). Total peak area of organic volatile substances present in the headspace (the sum of the single areas of the gas chromatographic peaks expressed in mV/sec) and hexanal peak area (mV/sec) were used.

MRV evaluation. In a previous study (27), data about volatiles and CO_2 production of a heated glucose-glycine solution at the same conditions employed in this study were presented. Under the gas chromatographic conditions we used it was possible to detect the Maillard reaction volatiles together with hexanal and other oxidation compounds. The presence of MRV did not interfere with the gas chromatographic analysis of volatile oxidation products. Data of total peak areas for SBO samples treated with MRV were normalized by subtracting the value at zero time (due to the presence of Maillard volatiles) from the measured amounts.

Gas chromatographic analysis. For organic volatile compounds and CO_2 evaluation two different gas chromatographic (GC) units were used, equipped with a flame ionization detector (FID) and a thermal conductivity detector (TCD), respectively. Instrumental and operative conditions were as reported by Lerici *et al.* (27). Gas chromatographic traces and peak areas were evaluated with a CR1-B Shimadzu (Tokyo, Japan) electronic integrator.

Samples prior to GC analysis were kept at 20°C for 12 hr to reach equilibrium. A direct headspace procedure was used, withdrawing 1 mL of vial headspace above oil with a gas-tight syringe Pressure-Lok model A2 (Dynatech, Precision Sampling, Baton Rouge, LA) and injecting into the GC column. The hexanal peak was identified by comparing the retention time with a standard (SIGMA ACS reagent n.6162).

To evaluate volatile compounds and gases in the headspace, two different sets of samples were prepared—one set for TDC and one for FID GC analysis. Because of the good reproducibility of replicate analyses (coefficient of variation less than 10%), no internal standard was used.

Evaluation of antioxidant activity. The antioxidant activity was evaluated through the following indices: i) The ratio of induction period of the antioxidant-treated sample to induction period of the control sample (protection factor) was reported as PF according to Parmar and Sharma (52). The induction time was computed with 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. The higher the PF value, the stronger the ability to retard the lipid oxidation.

ii) The ratios of the kinetic rate constants (OKR) for the linear part of the oxidation curves (related to peroxide value, total peak area and hexanal peak are *vs.* time) of treated sample to those of the control sample (k of treated sample/k of control sample). The lower the OKR value, the stronger the action to decrease the oxidation rate.

iii) The percent of the decrease of peroxide value, total peak area and hexanal peak area at 24 hr of oxidative heating compared to the control.

RESULTS AND DISCUSSION

Peroxide values, total volatiles peak areas and hexanal peak areas as a function of oil heating time are reported in Figure 1 for control and MRV-modified oil samples.

Total volatile peak areas and hexanal peak areas can be used for monitoring the oxidation of SBO under our conditions because they continued to increase even for samples for which PV was decreasing, in agreement with Gordon and Williamson (53). Maximum values of variation coefficients (C.V.%) were found to be 0.7% for peroxide determination; 5% for total peak areas; and 10% for hexanal peak areas. Values of antioxidant indices are given in Table 1.

Data shown in Figure 1 and Table 1 indicate that modification of the sample atmosphere with MRV had a strong inhibiting action on oxidative reactions and affected the oxidation induction time slightly. Nevertheless, the MRV-modified samples showed a strong decrease in peroxide and volatile formulation throughout oil heating time, as evidenced by a significant reduction of the oxidation kinetic rate constants.

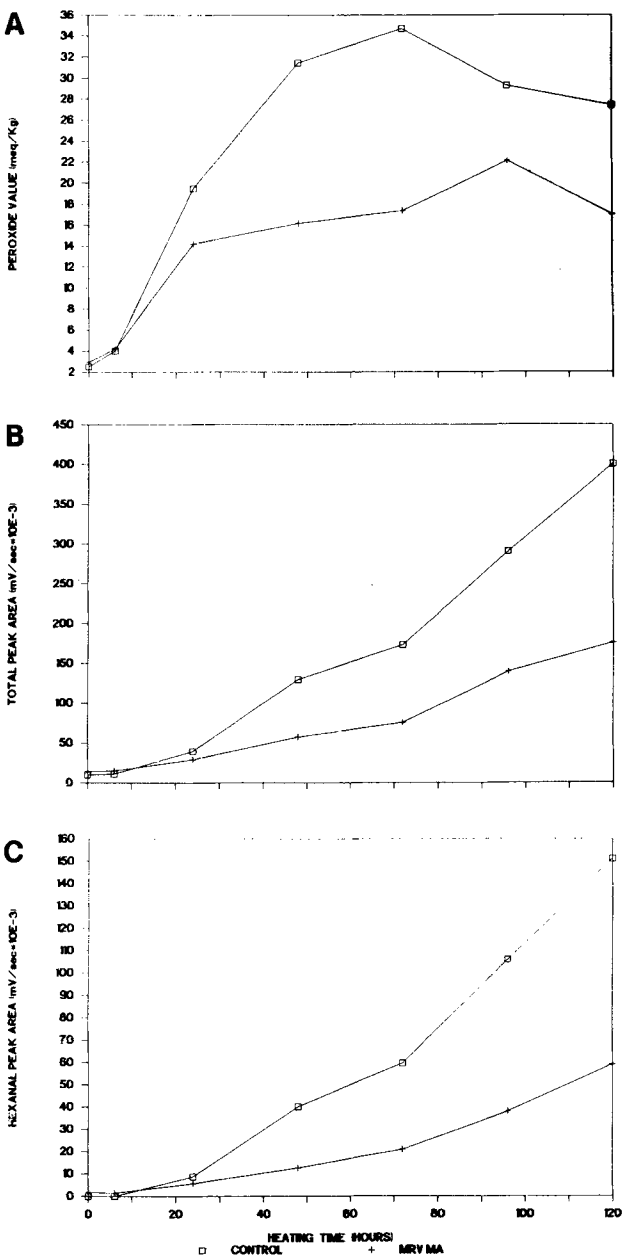

FIG. 1. Effect of atmosphere modification with MRV on peroxide value, total volatiles peak area and hexanal peak area changes during oxidative heating of SBO.

TABLE 1

Antioxidative Indices for Samples with MRV-Modified Atmosphere

Oxidative index	PF[a]	OKR[b]
Peroxide value	1.26	0.64
Total peak area	1.32	0.33
Hexanal peak area	1.23	0.38

[a]Protection factor, ratio of induction period of MRV-treated sample to the induction period of control sample.

[b]Ratio of the kinetic rate constant of the linear part of the oxidation curve of treated sample to that of control sample.

By substituting headspace of oil samples with MRV, the concentration of CO_2 increased from 0.5% to about 50% and a great enrichment of MRV in the sample headspace was found.

Because of the high level of CO_2 in the oil headspace before heating, the antioxidant effect could be attributed to the limited oxygen in the sample atmosphere, rather than to MRV action. Therefore, a stripping procedure to transfer the MRV from the browned glucose-glycine solution directly into the oil was performed. In this way, it was supposed that only the organic volatile compounds, mostly able to dissolve in oil because of their lipophily, could be trapped in the SBO, and that CO_2 would be stripped from the Maillard solution and bubbled out from the oil. In fact, CO_2 concentration in the oil headspace after bubbling reached only 0.3%, thus the level of transferred CO_2 from MRV to the oil can be considered close to zero.

In Figure 2 peroxide value, organic volatile substances and hexanal produced by oil oxidation of control and MRV-treated oil samples are plotted *vs.* oxidative heating time. Results show that MRV treatment slows down peroxide rate formation, even with a low CO_2 level. Again, the antioxidant effect of the MRV was evident.

Table 2 shows the values of protection factor (PF) and the kinetic rate constant ratio (OKR) computed from SBO peroxide values, total volatiles peak areas and hexanal peak areas during heating time. Data from Table 2 show that stripped MRV were effective in lengthening the induction time of peroxide and hexanal production. The effect on induction time of total volatiles formation was also evident.

For the purpose of a mechanism of antioxidant action, MRV can be considered as oxygen scavengers, thus they are in competition with oxidation radicals to form peroxides. The effect on lowering the development of secondary products, which are responsible for organoleptic changes, could be attributed both to less initial peroxide formation and to an inhibitory action on hydroperoxide decomposition to form rancid products. Some authors have shown that the antioxidant activity of MRP increases as a function of their reducing power (19,22,24), so that a loss of antioxidative effects was found in the presence of oxygen (32). In order to verify the sensitivity of MRV to oxygen and its effect on antioxidant activity, a stripping process with air instead of nitrogen was carried out. The changes of peroxide value *vs.* heating time are reported in Figure 3, showing a very low antioxidant effect of MRV

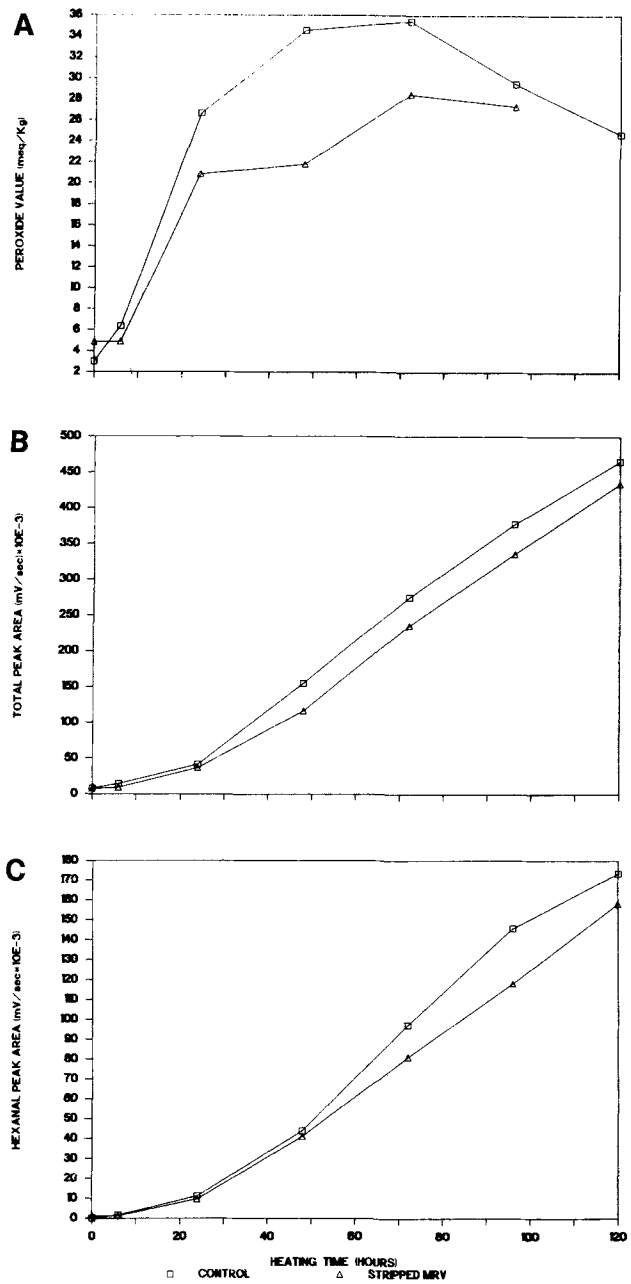

FIG. 2. Peroxide value, total volatiles peak area and hexanal peak area of control and MRV-treated SBO (N_2 stripping procedure) during heating time.

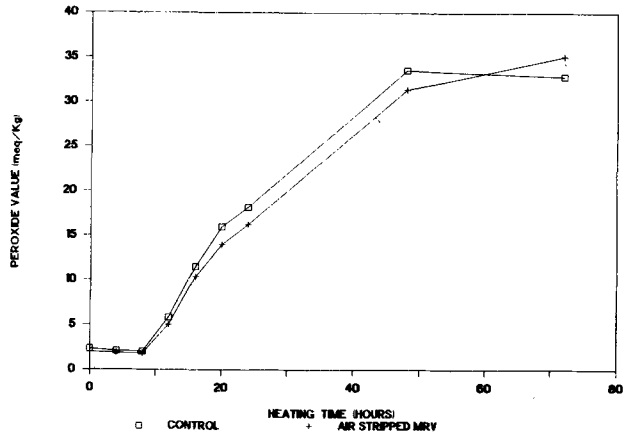

FIG. 3. Peroxide value changes of control and MRV-treated SBO samples (air stripping procedure) during heating time. Air flow rate, 500 mL/min; stripping time, 20 min.

TABLE 2

Antioxidative Indices for Samples of SBO Treated with MRV (N_2 Stripping Procedure)

Oxidative index	PF[a]	OKR[b]
Peroxide value	1.47	0.81
Total peak area	1.73	1.00
Hexanal peak area	1.91	0.88

[a] Protection factor, ratio of induction period of MRV-treated sample to the induction period of control sample.

[b] Ratio of the kinetic rate constant of linear part of oxidation curve of treated sample to that of control sample.

transferred into the oil with an air stream. This result seems to reveal that in the presence of an excess of oxygen MRV are readily oxidized and lose most of their antioxidant properties. This might confirm that the antioxidative effect of MRV is, in part, correlated with their reducing properties, thereby making the oxygen unavailable for lipid oxidation.

To obtain some information regarding the inhibiting action of MRV on hydroperoxide and hexanal formation, a correlation was performed between the MRV effect on the decrease of hexanal and peroxide production. The linear regression equation was: % decrease in hexanal peak area = 9.27 + (0.70 × % decrease in peroxide value). The determination coefficient was found to be $R^2 = 0.53$, with a significance of $p \leq 0.005$ and 13 d.f. The data show a good correlation between the two effects, although the determination coefficient demonstrates that only 53% of the effect of MRV to decrease rancid product formation is explained by the effect of MRV on reducing peroxide formation. This result seems to confirm that MRV acts as peroxide destroyers to decrease rancid product formation in addition to acting as oxygen scavengers, as was supposed above.

As was found for Maillard reaction products earlier, the volatiles produced by nonenzymic browning (NEB) of a sugar-amino acid solution slow the oxidative degradation of soybean oil, considered in this work as a model of fatty food. By modifying the sample atmosphere with MRV, a high level of CO_2 formed during the NEB reaction was found in the SBO sample headspace, and the effect on oxidation could be attributed to oxygen limitation. By using stripping-bubbling of MRV into the oil, the level of CO_2 in the SBO sample headspace was low, and a comparable antioxidant effect with MRV atmosphere modification was observed.

Results suggest that the effects of MRV on lipid oxidation could be due to inhibiting the peroxide formation as well as to reducing the production of rancid compounds arising from hydroperoxide decomposition.

However, the antioxidation mechanism of MRV is not yet well known, and investigations on the different actions of MRV obtained from glucose-glycine heated at different levels are now in progress in our laboratory.

Although the main purpose of the present work was to investigate the interaction between MRV and lipid oxidation, from an applicative viewpoint the results could provide some useful suggestions for packaging of fatty food where lipid oxidation could reduce the shelf life. Moreover, the use of MRV could by-pass some inconveniences (bitter taste, dark color and undesirable flavor) revealed by other authors (24) about the practical use of MRP as antioxidants.

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