Synergies Between Plant Antioxidant Blends in Preventing Peroxidation Reactions in Model and Food Oil Systems

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ABSTRACT: A study was conducted to investigate the oxidative behavior of various mixtures of rosemary, sage, and citric acid in a linoleic acid model system by oxygen consumption measurement and in a palm olein system by differential scanning calorimetry (DSC) analysis. Response surface methodology was used to optimize the use of the mixtures. Results showed that rosemary and sage were two important factors for the protective index (PI). The two antioxidants were highly significantly ($P < 0.001$) in influencing PI values. There was a significant ($P < 0.01$) synergistic effect between rosemary and sage on PI values. Citric acid was also found to be significant ($P < 0.05$) for PI. With respect to onset time (T_o), all three antioxidants were significant ($P < 0.05$). However, no significant interaction among antioxidants was found for T_o. Mathematical models for both PI and T_o could be developed with confidence. The R^2 values for PI and T_o were 0.992 and 0.926, respectively. A combination of 0.078% rosemary, 0.067% sage and 0.037% citric acid was the optimal combination for PI, whereas a combination of 0.068% rosemary, 0.075% sage, and 0.039% citric acid was required to reach the optimal T_o value.

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KEY WORDS: Citric acid, differential scanning calorimetry, oxidative behavior, oxygen consumption measurement, response surface methodology, rosemary, sage.

Many lipids are particularly labile when exposed to a combination of heat, air, and light. Under conditions of heating or frying, the acceleration of both thermal and oxidative decomposition reactions occurs (1,2). Excessive heating of oils or fats can result in the formation of compounds, such as enzyme inhibitors (3), that possess antinutritional properties and in accelerated loss of antioxidant vitamins, such as vitamin E (4,5), leading to growth depression and histologic changes in gastrointestinal tissues (6,7). Moreover, oxidized lipid reportedly enhances peroxidation of membrane macromolecules (8), thus contributing to mutagenicity (9), genotoxicity (10), and angiotoxicity (11). These cellular aberrations are likely implicated in reports linking oxidized oils to growth retardation (12), colon carcinogenesis (13), and reproductive disorders (14). Besides potentially adverse health effects of thermally oxidized oil, lipid oxidation influences the acceptability of the fried product (15). Thus, antioxidants are required (16) to retard against undesirable changes in oil during storage and frying operations.

Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylated hydroxyquinone are antioxidants commonly used in cooking oils. However, with the safety concern that have been identified for these synthetic antioxidants (17), considerable interest in the use of natural antioxidants for frying purpose has developed.

Rosemary and sage are two plant-derived antioxidants with potential to replace synthetic ones in food processing (18). Both antioxidants have good antioxidative characteristics in foods (19–21). These natural antioxidants are also effective in stabilizing frying oils and have very good thermal resistance (12,22). Rosemary is more effective than BHA and BHT in inhibiting oil oxidation (23). Recently, Irwandi and Che Man (24) found that together with citric acid, rosemary and sage added to palm olein effectively retarded oil deterioration during deep-fat frying of potato chips. Citric acid was used in the study as a synergist.

The objective of the present study was to investigate the oxidative behavior of various mixtures of rosemary, sage, and citric acid in a palm olein system and in a linoleic acid model system.

MATERIALS AND METHODS

Materials. Refined, bleached and deodorized (RBD) palm olein was obtained from a local refinery in Selangor, Malaysia. Oleoresin rosemary (OR; Herbalox Brand, Type O) and oleoresin sage (OS; Herbalox seasoning, Type S-O) extracts were kindly donated by Kalsec Inc., Kalamazoo, MI (Gulf Chemical Sdn. Bhd., Selangor, Malaysia). Citric acid (CA) was purchased from a local supplier in Selangor, Malaysia. All other chemicals used in this study were of analytical grade.

Experimental design. Response surface methodology (RSM) was used to investigate the oxidative effectiveness of OR, OS, and CA and their different combinations. Echip soft-

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ware (Echip Inc., Hockessin, DE) (25), an RSM-based computer program, was used to design initial experiments, calculate multiple regression equations, and provide statistical evaluations. RSM uses an experimental design, such as central composite design, to fit a model by least squares analysis. Initial concentrations of OR and OS were varied from 0 to 0.1% each, and CA was varied from 0 to 0.05%, according to Irwandi and Che Man (24). Fifteen combinations of the three antioxidants (Table 1) established from the Echip software were tested to evaluate efficacy in a linoleic acid emulsion system by an oxygen consumption measurement and in a palm olein system by a differential scanning calorimetric (DSC) method. Experiments were performed in triplicate.

Based on results from both analyses, mathematical models or equations were developed in this study for optimization purposes:

$$\text{response} = \beta_0 + \beta_1[\text{OR}] + \beta_2[\text{OS}] + \beta_3[\text{CA}] + \beta_{12}[\text{OR}][\text{OC}] + \beta_{13}[\text{OR}][\text{CA}] + \beta_{23}[\text{OS}][\text{CA}] + \beta_1^2[\text{OR}]^2 + \beta_2^2[\text{OS}]^2 + \beta_3^2[\text{CA}]^2 \quad [1]$$

where β_0 = intercept, $\beta_{1,2,3}$ = coefficient for each antioxidant at the first-order form, $\beta_{12,13,23}$ = coefficient for each interaction among antioxidants, $\beta_1{}^2{}_{,2}{}^2{}_{,3}{}^2$ = coefficient for each antioxidant at the second-order form,[OR] = concentration of oleoresin rosemary extract in oil, [OS]= concentration of oleoresin sage extract in oil, and [CA] = concentration of citric acid in oil.

Oxygen consumption measurement. Oxygen depletion in a linoleic acid emulsion system with added ferrous (Fe^{2+}) ions, and in the absence and presence of natural antioxidant mixtures, was measured according to the methods of McGookin and Augustin (26), Lingnert *et al.* (27), and Wijewickreme and Kitts (28) using a YSI Model 5300 biological oxygen monitor (Yellow Springs, OH). Mixtures of natural antioxidants shown in Table 1 were first dissolved in a linoleic acid emulsion (1.5 g of linoleic acid mixed with 0.1 g of Tween 20 in 40 mL of 0.1 M potassium phosphate buffer, pH 6.8). One milliliter of this mixture was then mixed with 5 mL of buffer (0.1 M potassium phosphate buffer, pH 6.8), and 0.6 mL of 2 mM $FeSO_4$ dissolved in 0.1 M potassium phosphate buffer (pH 6.8). The reaction mixture was pumped into a jacketed reaction vessel at room temperature which contained an oxygen electrode. Oxygen depletion was recorded immediately after the reaction mixture was introduced into the vessel. Both antioxidant and prooxidant activities of natural antioxidant mixtures were expressed in terms of protective index (PI), defined as

$$\text{PI} = \frac{\text{time for 50\% } O_2 \text{ depletion with test compound}}{\text{time for 50\% } O_2 \text{ depletion without test compound}} \quad [2]$$

where PI < 1 denotes prooxidant activity, PI = 1 denotes no activity, and PI > 1 denotes antioxidant activity (27).

DSC analysis. Oxidative stability of oil was determined by using a PerkinElmer differential scanning calorimeter DSC-7 (Norwalk, CT), according to the method of Gupta and Jaworski (29) with minor modifications. The equipment was calibrated with pure indium, and the baseline was obtained with an empty open aluminum pan as a reference. An oil sample of 5.0 ± 0.5 mg was weighed in an open aluminum pan and placed in the sample chamber. The isothermal temperature was programmed at 150°C and purified oxygen (99.8%) was passed through the sample enclosure at 50 mL/min. The onset time (T_o) of the oxidation reaction corresponded closely to the intersection of the extrapolated baseline and the tangent line (leading edge) of the exotherm (Fig. 1).

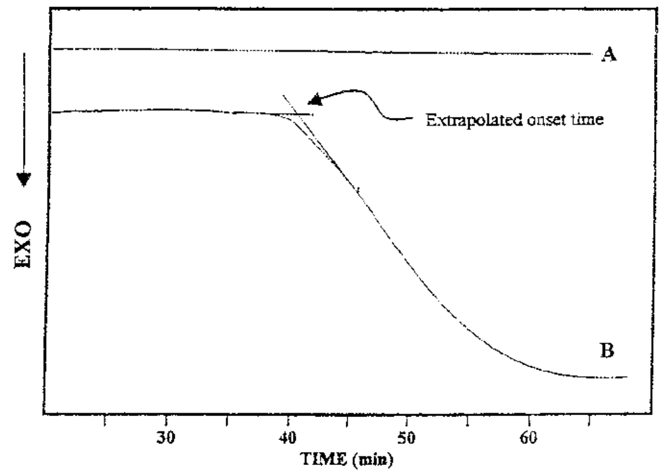

FIG. 1. Differential scanning calorimetric (DSC) oxidation curve of refined, bleached, and deodorized (RBD) palm olein without antioxidant. (A) Isothermal curve at 150°C with nitrogen (99.999%) flowed at 50 mL/min; and (B) isothermal curve at 150°C with oxygen (99.8%) flowed at 50 mL/min.

TABLE 1
Combinations of Oleoresin Rosemary Extract, Sage Extract, and Citric Acid

Trial no.	Rosemary (%)	Sage (%)	Citric acid (%)
1	0	0.1	0.05
2	0.1	0.05	0
3	0	0.1	0
4	0	0	0.05
5	0.1	0.1	0.05
6	0.05	0.1	0.025
7	0	0.05	0.025
8	0.05	0.05	0.05
9	0	0	0
10	0.1	0	0.025
11	0.1	0.1	0.025
12	0.05	0.1	0
13	0.05	0	0
14	0.1	0.05	0.05
15	0	0.05	0

RESULTS AND DISCUSSION

Assessment of antioxidant activity by oxygen consumption

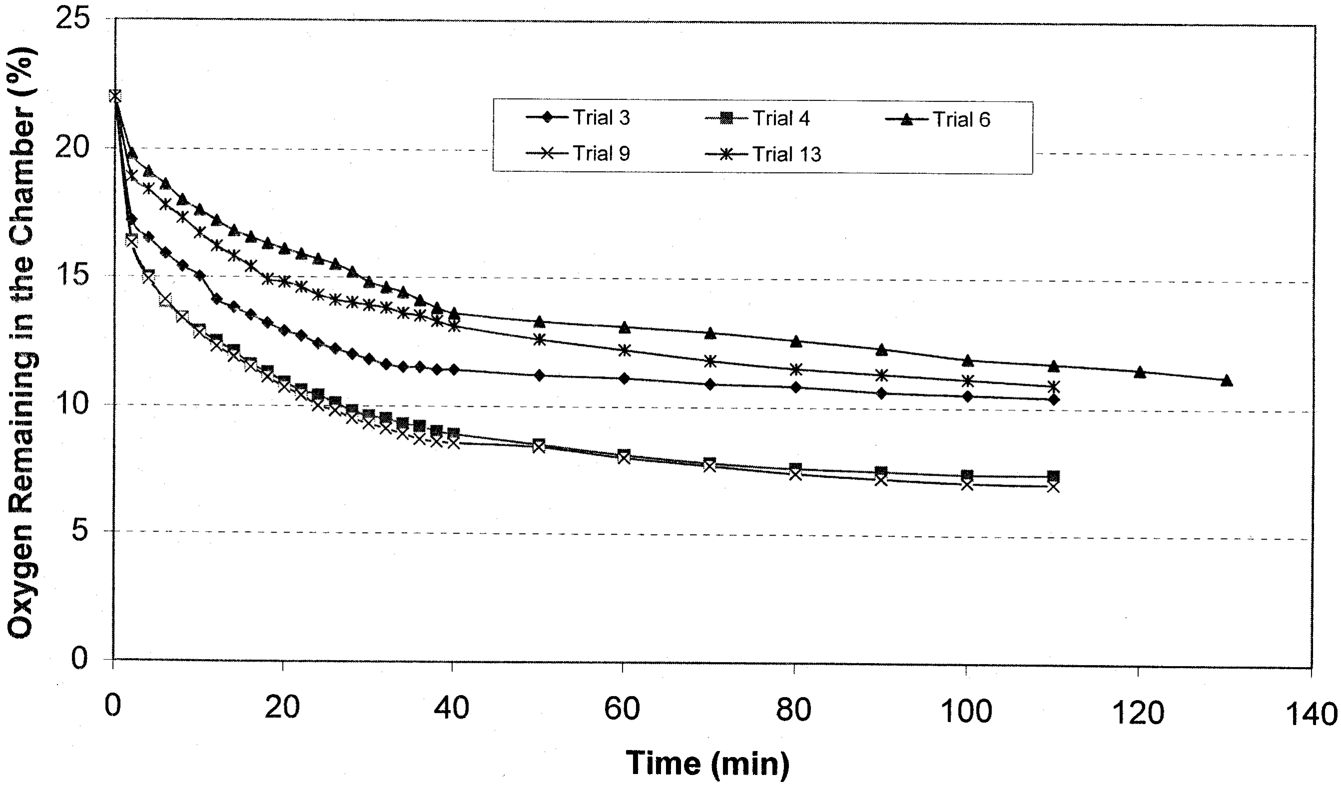

FIG. 2. Percentage oxygen consumption by natural antioxidant mixtures in a model linoleic acid emulsion system. Trial 3: 0.1% sage, 0% rosemary and citric acid. Trial 4: 0.05% citric acid, 0% rosemary and sage; Trial 6: 0.05% rosemary, 0.1% sage, 0.025% citric acid; Trial 9: 0% rosemary, sage, and citric acid; Trial 13: 0.05% rosemary, 0% sage and citric acid.

measurement. The percentage of oxygen remaining in the reaction chamber over a period of time, in the absence and presence of natural antioxidants, was recorded as a measure of their PI values. Figure 2 shows percentage oxygen consumption by four selected natural antioxidant mixtures and the control in a linoleic acid emulsion system. The percentage of oxygen remaining in the vessel declined more rapidly in the control emulsion (trial sample no. 9) than in the emulsions containing antioxidant mixtures. No mixture sample with prooxidant activity was observed. The control sample reached 50% oxygen in the chamber in 20 min. Sample no. 6 took the longest time, or 126 min, to reach 50% oxygen in the chamber, meaning that this sample had the highest level of antioxidant activity.

PI values were calculated for 15 natural antioxidant mixtures by employing an iron-supplemented linoleic acid model emulsion system, in which iron ions act as a promoter of lipid oxidation reactions. These values (Table 2 were used to distinguish between antioxidant and prooxidant activities of the various mixture samples. Trial samples 6 and 7 had antioxidant activities more than six times higher than the control. Sample no. 4, where only citric acid was added to the emulsion, showed the lowest antioxidant activity.

The effect of various natural antioxidants on PI values is given in Table 3. Rosemary and sage significantly ($P < 0.001$) influenced the PI value. These two antioxidants are significant to PI not only at the first-order form (Eq. 1) but also at the second-order ($P < 0.01$). The presence of citric acid alone in the emulsion also had a significant effect on the PI at a P level of 0.05. The interaction effect between rosemary and sage on PI values was likewise significant ($P < 0.01$). A mathematical model to predict PI values, as shown in Table 3, was developed with very high confidence, with R^2 of 0.992. In RSM methodology, R^2 values greater than 0.75 adequately

TABLE 2
Protective Index (PI) and Onset Time (T_o) of 15 Different Antioxidant-Treated Samples

Trial no.	PI	T_o (min)
1	4.5	52.9
2	5.5	56.0
3	3.8	50.9
4	1.3	44.5
5	5.8	71.2
6	6.1	75.5
7	3.9	49.6
8	5.7	74.6
9	1	39.9
10	4.8	51.1
11	5.3	79.2
12	5.0	57.9
13	4.0	48.5
14	6.0	75.5
15	3.6	46.7

TABLE 3
Effect of Natural Antioxidant Treatments as Reflcted by Regression Coefficients and R^2 for PI and T_o

Coefficients[a]	PI[b]	T_o[b]
β_0 (intercept)	5.95	71.86
β_1	21.41***	135.67*
β_2	17.75***	147.92*
β_3	9.38*	232.15*
β_{12}	−237.47**	1153.14
β_{13}	41.33	2321.85
β_{23}	178.40	453.73
β_1^2	−387.97**	−3928.42
β_2^2	−364.76**	−1835.91*
β_3^2	−482.53	−8431.92
R^2	0.992	0.926

[a]Subscripts: 1 = oleoresin rosemary extract; 2 = sage extract; 3 = citric acid.
[b]Significance levels: ***$P \leq 0.001$; **$P < 0.01$;; *$P < 0.05$. See Table 2 for abbreviations.

predict the effect of some parameters on a response (30,31).

A contour map for predicting PI values, with natural antioxidants as independent variables, is given in Figure 3. A combination of 0.078% rosemary, 0.067% sage, and 0.037% citric acid produced an optimal PI.

Monitoring effect of natural antioxidants by DSC. Cross (32) and Hassel (33) first reported the use of DSC for measuring oil stability. The tests were carried out isothermally with an oxygen purge. The end point was taken as the time at which a rapid exothermic reaction between oil and oxygen occurred. According to Hassel (33), the use of DSC could shorten the time needed to analyze the oxidative stability of oil samples from the 14 d of Schaal oven test (34) to less than 4 h.

In this study oxidation reactions produced DSC traces like that in Figure 1, line B. No exothermic peak was detected when the oil sample was scanned under nitrogen (Fig. 1, line A). The extrapolated onset time (T_o) was taken as a measure of the relative oxidative stability of the oil. DSC oxidation curves of 15 samples containing different antioxidant mixtures are shown in Figure 4. Addition to the oil of rosemary, sage, and citric acid reduced the oxidation, as evidenced by the longer T_o of antioxidant-treated samples. The control sample (sample no. 9) had the shortest T_o with 39.9 min, whereas the longest one was sample no. 11, 79.2 min. The T_o values for all treatment samples and control are given in Table 2.

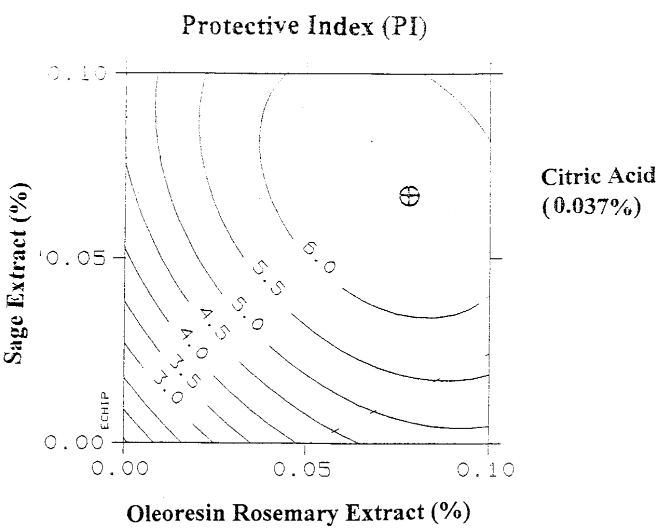

FIG. 3. Contour map of protective index (PI) of RBD palm olein samples treated with natural antioxidants. For abbreviation see Figure 1.

Table 3 also shows the correlations obtained between the 15 different combinations of the three antioxidants examined in this study and T_o. The equation for predicting the effect of natural antioxidants on the T_o values of RBD palm olein had an R^2 of 0.926. Tests on the estimates in Table 3 showed all three antioxidants significantly ($P < 0.05$) affected T_o values. The second-order form of sage also significantly ($P < 0.05$) influenced the values. However, no significant effects were found among the three antioxidants on T_o.

Contour maps for T_o values of natural antioxidant-treated RBD palm olein (Fig. 5) indicated that changes in T_o values produced the same pattern of relative change in the PI (Fig. 3). The use of moderate levels of all antioxidants produces an optimal point of the T_o. The optimum from this study was achieved by combining 0.068% rosemary, 0.075% sage and 0.039% citric acid.

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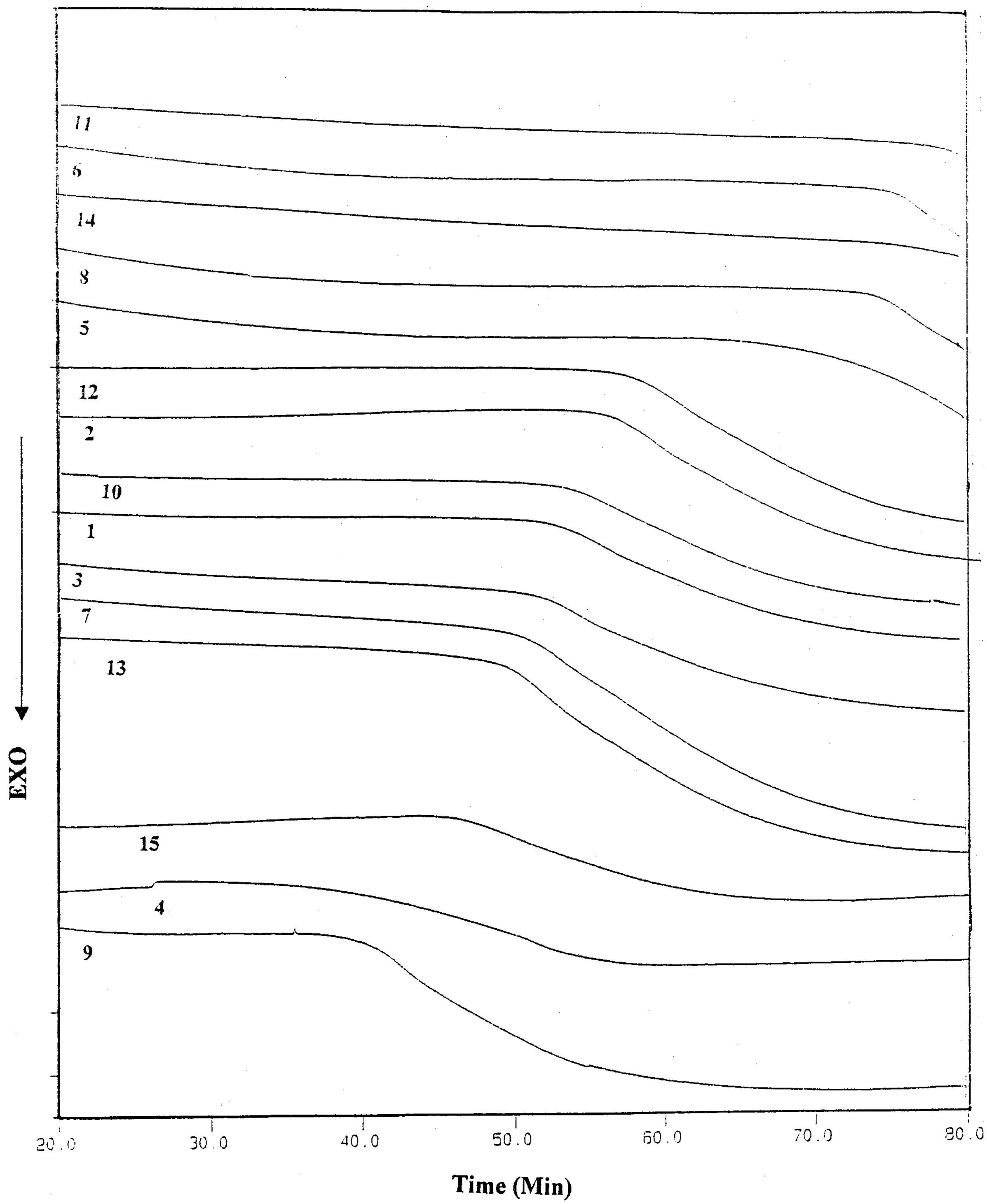

FIG. 4. DSC oxidation curves of RBD palm olein with natural antioxidant mixtures. For mixture descriptions see Table 1. For abbreviations see Figure 1.

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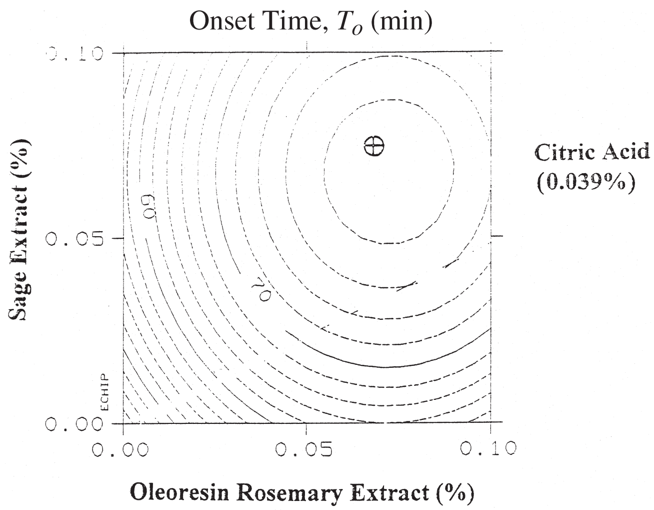

FIG. 5. Contour map of onset time (T_o) of RBD palm olein samples treated with natural antioxidants. For abbreviation see Figure 1.

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