A Multiple-Variable Approach to Study Corn Oil Oxidation

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The induction time for oxidation (Γ_i) and the rate constant for bimolecular peroxide decomposition (k_2) were determined in refined corn oil with (CW) and without (CWO) antioxidant (ter-butil hydroxy-quinolein 0.0014%, citric acid 0.0012%), and their magnitude and variability were described through multiple regression. Γ_i in both CW and CWO was mainly described ($R^2 > 0.93$) through linear and quadratic interactions between the temperature (T°) and the initial peroxide concentration. Although the antioxidant increased Γ_i, carbonyls were produced in earlier stages (*i.e.*, $<\Gamma_i$) and lower T° than in CWO. These carbonyls were produced through a bimolecular peroxide decomposition. The equations for k_2 ($R^2 > 0.96$) indicated a faster decomposition of peroxide in CWO than in CW; nevertheless, as a function of T°, k_2 increased at a slower rate in CWO than in CW.

KEY WORDS: Antioxidants, carbonyl, oil oxidation, oxidation kinetics, peroxide.

Lipid autoxidation and the products of peroxide decomposition (*i.e.*, carbonyls) are important factors that influence fat/oil quality and stability. Lipid peroxides and some of their breakdown products interact with proteins, membranes and enzymes and affect vital cell functions (1) as well as food wholesomeness, shelf life and overall quality. Autoxidation of fat/oil proceeds *via* free-radical mechanism (2,3). The reactions involved are dependent on several factors including temperature, oxygen concentration (4,5), fatty acid composition (3,6) and the presence of prooxidants or antioxidants (6–10) intentionally added or naturally present in the oil/fat.

The shelf life of an oil may be defined as the period of time before deterioration becomes noticeable. The oxidation of oil is described through the oxidation curve (*i.e.*, a plot of peroxide value *vs.* time of oxidation). The time before the onset of autoxidation, the induction period (Γ_i), has been closely associated with oil shelf life (2). The appearance of off-flavors (*i.e.*, carbonyls) derived from peroxide decomposition (11–13) normally follows the rapid increase in peroxide concentration observed after the Γ_i. In the oil industry, the extension of Γ_i (*e.g.*, shelf life) is achieved mainly through the use of chain-breaking antioxidants, which scavenge the free radicals, thereby breaking the chain of autocatalytic reactions and therefore limiting peroxide production. However, there is evidence that, in the presence of antioxidants, oxidation takes place due to a bimolecular decomposition of peroxides, which provides the free radicals needed to activate oxidation (2,14). This indicates that some carbonyls might be produced before Γ_i. This last point would have implications in the organoleptic quality and shelf life of oils. Besides, antioxidants influence the direction of cleavage of peroxides (13). Therefore, in addition to the beneficial effect of antioxidants on Γ_i, food technologists need to consider their effect on the rate constant for bimolecular decomposition of peroxides (k_2) (*i.e.*, carbonyl production). There is no information regarding the variables that establish the magnitudes of Γ_i and k_2.

The objective of this research was to study, through multiple-variable regression analysis, the variables that describe the behavior and magnitude of Γ_i and k_2 in refined corn oil with and without antioxidant. An accelerated-aging model system with activated carbon (1% w/w) was utilized in this study.

EXPERIMENTAL PROCEDURES

Oil samples. Several lots of refined corn oil with (CW) and *without* (CWO) antioxidant (ter-butil hydroxy-quinolein 0.0014%, citric acid 0.0012%) were obtained from local industries (La Gloria and Productos de Maíz, Guadalajara, Jal., Mexico). The lots of oil were stored in amber bottles under nitrogen atmosphere and refrigeration (4°C). At the moment of experimentation (*i.e.*, determination of the oxidation curve), the oils were analyzed for iodine index, saponification value, percentage of free fatty acids (as oleic acid), percentage of total conjugated fatty acids (bi-, tri- and tetra-conjugated) and peroxide value (P_0) by following the procedures described by the Association of Official Analytical Chemists (15). Carotenoids were determined by spectrophotometry at 417 nm (16). The concentration of unsaturated carbonyls was determined by reaction with 2,4-dinitrophenyl hydrazine following the procedure described by Henick *et al.* (17) as modified by Fioriti (18); this modification significantly reduces the peroxide interference in the determination of carbonyls (18).

Oxidation curves. Oil oxidation was accelerated through the use of 1% of activated carbon. The same lot of activated vegetal carbon (Clarificantes Mexicanos; Santa Clara, Estado de México, Mexico) was used in all the experiments after sieving (U.S. standard, −14/+24), washing (mixing for 24 h in two volumes of deionized water), filtering and drying (100°C for 24 h).

Within each group of lots, of either CW or CWO, the oxidation temperatures (T°) to be studied (50, 60, 70, 80, 90 and 95°C) were distributed in a complete randomized experimental design. The same T° of oxidation was duplicated in the design. The oil oxidation was performed in a rotary evaporator (Büchi RE 110; Büchi Laboratoriums-Technik AG, Flawil, Switzerland). The proportions of oil and activated carbon were weighed in the evaporator flask (1 L) to obtain 200 g of final weight. The mixture with either CWO or CW was constantly stirred during 8 h and 12 h, respectively, and aliquots of the mixture were obtained at 1-h intervals. The aliquots were centrifuged (5000 rpm/15 min), and the oil was analyzed for peroxide and unsaturated carbonyl. The logarithm of peroxide concentration as a function of oxidation time was plotted in a log-log graph, and the equation in the linear portion of the curve was determined through linear regression (Fig. 1). The Γ_i at each tempeature was calculated with these equations ($r > 0.99$) by extrapolation of the time corresponding to the peroxide concentration just before the onset of oxidation (*i.e.*, Γ_i) (Fig. 1). Additionally, the rate constant of bimolecular peroxide decomposition in

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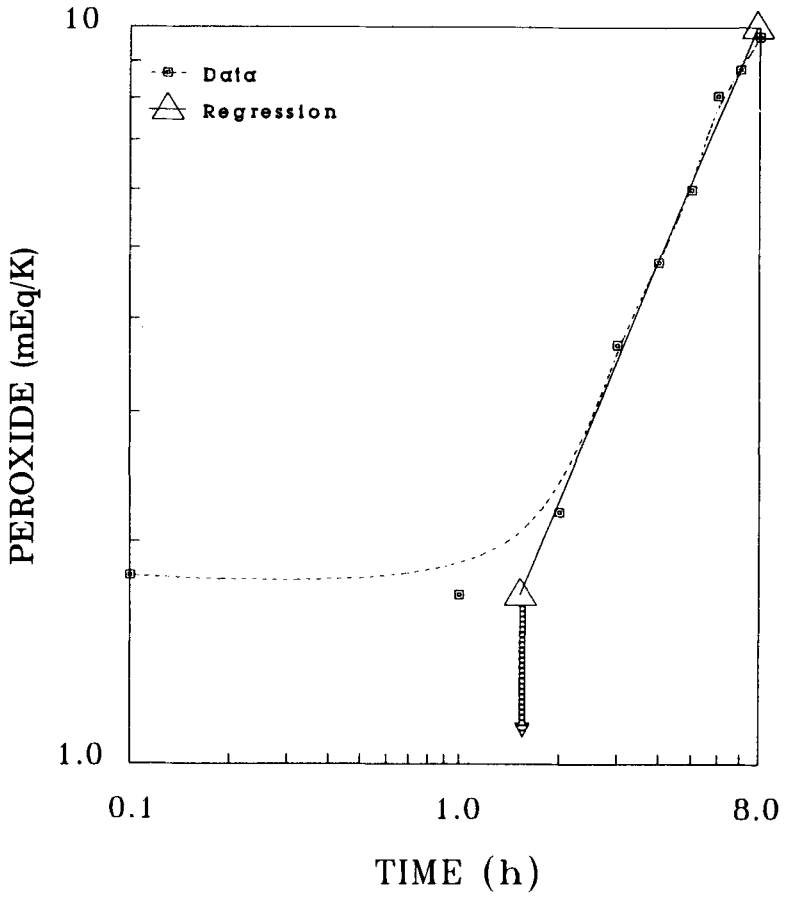

FIG. 1. Oxidation curve at 70°C of refined corn oil without antioxidant and 1% (w/w) of activated carbon. The legend shows the original data and the regression line that was used to calculate the induction time for oxidation. The induction time is indicated with an arrow.

reciprocal time units (k_2) was calculated from the slope of a plot of the natural log of the peroxide concentration *vs.* time (4).

Statistical analysis. Discriminatory multiple regression analysis was used to develop equations that described the variability in Γ_i and k_2, by utilizing as independent variables the chemical characteristics of the oil, the oxidation T°, their quadratic effects and their linear and quadratic interactions. Likewise, a categorical variable that considered the absence (CWO) or presence (CW) of antioxidant was included in the set of independent variables; a categorical variable just assumes a limited number of discrete values usually utilized in nominal scales to classify data with similar characteristics (19).

The statistical analysis considered each lot of oil as an independent experimental unit. The reason for this relied on the fact that in this study the only controllable variable was temperature. The chemical characteristics of the oil were random variables that were considered as independent factors in the Γ_i and k_2 analysis. Therefore, no true replication existed. The bases and description of the statistical methodology have been described in detail elsewhere (10).

RESULTS AND DISCUSSION

The conventional accelerated aging techniques are generally performed at temperatures above 95°C. With chemiluminescence studies in sunflower oil, Cash *et al.* (2) showed that the activation energy associated with Γ_i increased at temperatures below 90°C. In the same way, Timms and Roupas (20) reported an increase in the activation energy for refined vegetable oils oxidized at temperatures below 60°C. This implies that the shelf life at room temperature is overestimated with the currently used accelerated aging techniques. However, induction times at temperatures below 60–70°C become too long for practical determination. Cash *et al.* (2,14), using alumina as an active substrate to accelerate lipid and oil oxidation, shortened Γ_i and enabled study of the effects of antioxidants on the kinetics of oxidation.

In this research, 1% of carbon has been used as an active substrate. On the basis of previous results (10) and considering the magnitudes of iodine index and saponification value observed for the corn oil (106.8 ± 9.6 and 166.8 ± 27, respectively), peroxide production ought to be accelerated at 1% of carbon dosage. This was confirmed in preliminary experiments (data not shown) in which different carbon concentrations (0–5%) were utilized in the oxidation of refined corn oil at 95°C. The same concentrations of silica (60G; Merck, Mexico City, Mexico) were also tested under similar conditions; just the 1% activated carbon dosage decreased Γ_i significantly.

As expected, the Γ_i's were smaller in the absence of antioxidant than in its presence. Typical oxidation curves for CW and CWO at 90° and 70°C are shown in Figure 2.

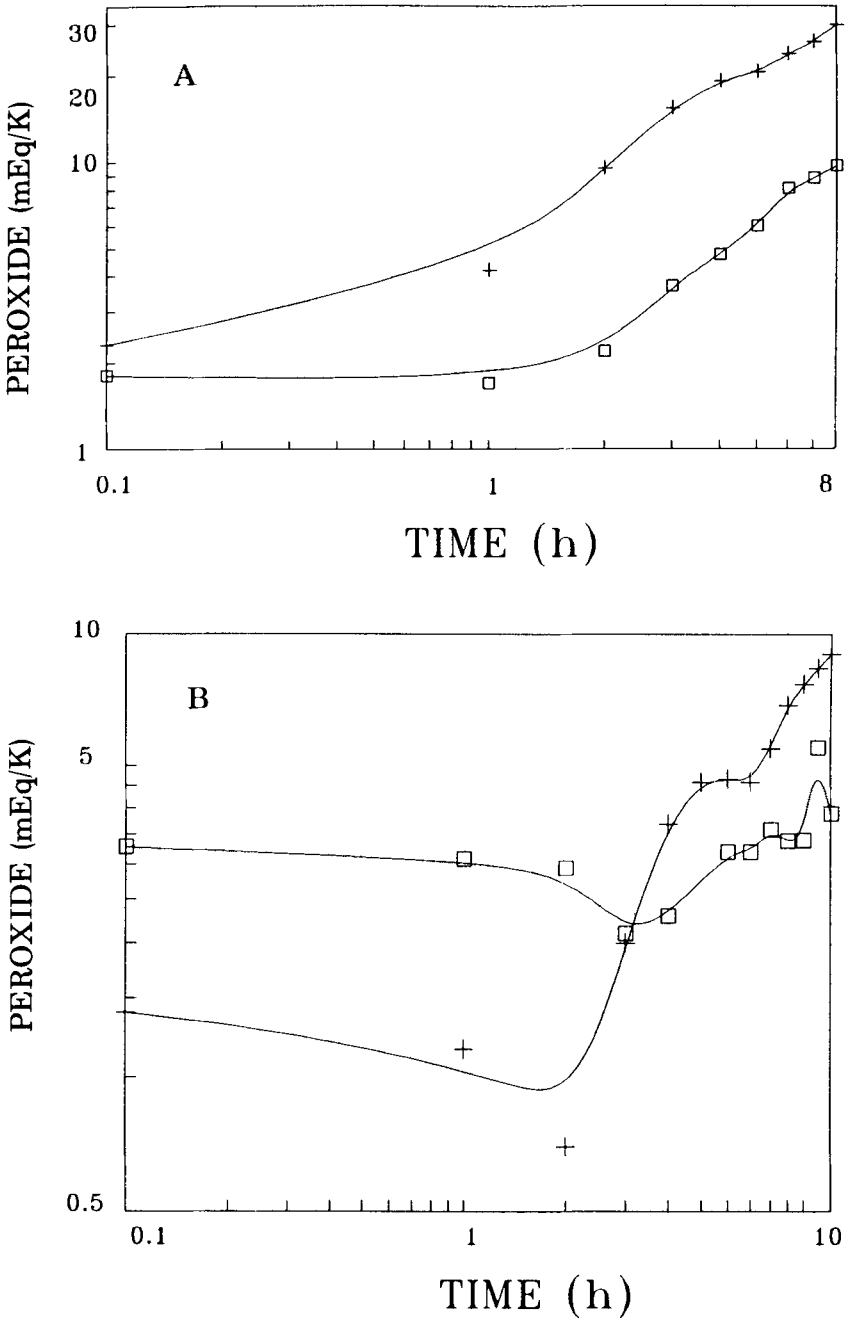

FIG. 2. Oxidation curves at 90° and 70°C of some lots of refined corn oil without (A) and with (B) antioxidant. Symbols: +, 90°C and □, 70°C.

With the exception of 95°C, the antioxidant produced a decrease in the peroxide concentration previous to the onset of oxidation; this reduction in peroxide concentration during the induction period is apparent in the oxidation curves shown in Figure 2B. In the presence of high antioxidant concentration (*i.e.*, early stages of oxidation) most of the free radicals react with the antioxidant, avoiding the production of peroxides and new free radicals (2,3). Therefore, the peroxides originally present in the oil were decomposed either through monomolecular or bimolecular reactions (2), generating in this way free radicals and/or carbonyl compounds. Cash *et al.* (2), on the basis of kinetic studies utilizing methyl linoleate and sunflower oil in the presence of antioxidant and using alumina as active substrate, had proposed a bimolecular peroxide decomposition for this stage of oxidation.

Within the interval of T° studied, in the absence of antioxidant, carbonyl production was observed after 2 to 4 h only at T° equal or greater than 80°C. In CW, with the exception of 50°C at which temperature no carbonyl production was detected, production of carbonyls started after 4 to 6 h of oxidation. Under the same conditions of temperature, Γ_i fluctuated in the CWO between 0.18 h and 2.25 h, and in the case of CW between 1.41 h and 8.19 h. As an example, Figure 3 shows the production of unsaturated carbonyls at 90° and 70°C in both CW and

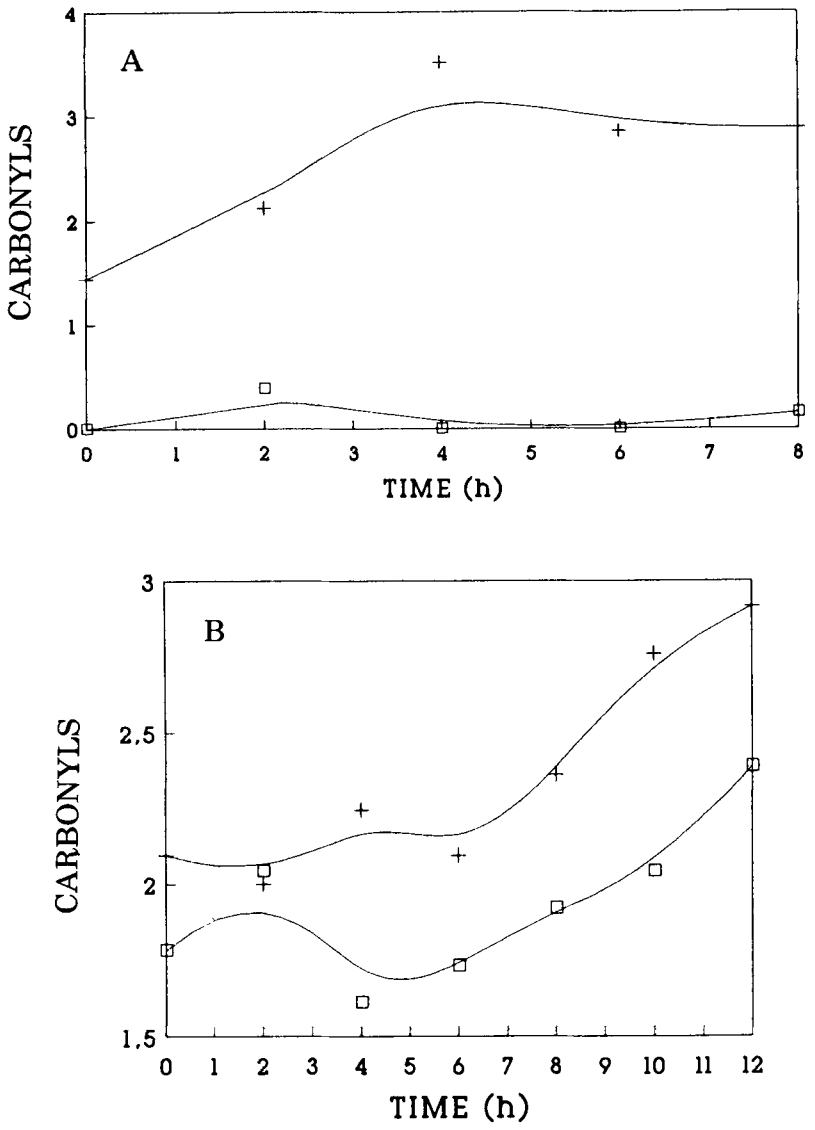

FIG. 3. Carbonyl production (mMol/g of oil) at 90° and 70°C in some lots of refined corn oil without (A) and with (B) antioxidant. Symbols: +, 90°C and □, 70°C.

CWO. Carbonyl compounds are associated with the development of off-flavors in fats, oils and food products (11–13). Therefore, because carbonyl production occurred at temperatures lower than in the absence of antioxidant and in general before Γ_i, the involvement of peroxide decomposition as a deteriorative mechanism in vegetable oil might be accentuated by the presence of antioxidants. These results agree with those obtained by Cash *et al.* (2); thus, although the antioxidant utilized in this study limited the development of propagation reactions (*i.e.*, increasing Γ_i), it did not have any effect on the reactions involved in peroxide decomposition. The fact that in CW the peroxide concentration did not drop to zero (Fig. 2B) during the induction stage indicated that a certain level of oxidation was still taking place. The antioxidant concentration might not be sufficient to scavenge all the free radicals originally present, in addition to the free radicals likely produced during peroxide decomposition. These observations might indicate that in the presence of antioxidant, peroxide decomposition operates as an initiation reaction (2). Therefore, in addition to the beneficial effect of antioxidants on Γ_i, food technologists need to consider their effect on the initiation and rate of peroxide decomposition (*i.e.*, carbonyl production).

A discriminatory multiple regressional analysis was performed to evaluate the variables that significantly affect the magnitude and variability of Γ_i and k_2 in both CW and CWO. The descriptive statistics of the variables utilized in this analysis are shown in Table 1. The best regressional models developed in this research provided $R^2 > 0.92$ ($P < 0.05$) (data not shown); the equations arising from these models are shown below (Equations 1 and 2 represent corn oil without antioxidant; Equations 3 and 4 represent corn oil with antioxidant):

$$\text{Ln}(\Gamma_i) = 2.62 - (5.43 \times 10^{-2})T^\circ + (1.22 \times 10^{-2})T^\circ \cdot PV_0 - (2.34 \times 10^{-3})T^\circ \cdot PV_0^2 + (2.06 \times 10^{-2})C_0 \quad [1]$$

$$k_2 = (8.9 \times 10^{-3})T^\circ + (4.20 \times 10^{-5})(T^\circ)^2 - (4.96 \times 10^{-2})PV_0 \quad [2]$$

TABLE 1

Descriptive Statistics of the Variables Utilized in the Multiple Regression Analysis to Describe the Variability of the Induction Period (Γ_i) and the Rate Constant for Bimolecular Peroxide Decomposition (k_2)

Variable	n[a]	Mean	Standard deviation	Minimum	Maximum
Temperature (°C)	6	74.17	17.44	50.00	95.00
P_0[b] (mEq/K of oil)	24	2.40	1.19	0.55	5.99
II_0[c]	3	106.79	9.55	97.28	116.38
SV_0[d]	3	166.81	26.93	144.53	196.74
C_0[e]	22	10.84	9.20	0.00	34.70
FFA_0[f] (% oleic acid)	3	0.12	0.03	0.09	0.16
CFA_0[g]	22	0.15	0.19	0.00	0.87
UC_0[h] (mMol/g of oil)	24	1.05	0.79	0.00	3.10
Γ_i (h)	23	2.28	1.87	0.18	8.19
k_2 (h^{-1})	23	0.37	0.13	0.08	0.50

[a]Number of replicates. Each replicate was done in duplicate.

[b]Peroxide value at zero time in the oxidation curve.

[c]Iodine index at zero time in the oxidation curve.

[d]Saponification value at zero time in the oxidation curve.

[e]Carotenoids at zero time in the oxidation curve.

[f]Free fatty acids at zero time in the oxidation curve.

[g]Total conjugated fatty acids at zero time in the oxidation curve.

[h]Unsaturated carbonyls at zero time in the oxidation curve.

$$\text{Ln}(\Gamma_i) = 2.62 - (8.01 \times 10^{-2})T^\circ + (5.34 \times 10^{-2})T^\circ \cdot PV_0 - (1.06 \times 10^{-3})T^\circ \cdot PV_0^2 \quad [3]$$

$$k_2 = (3.97 \times 10^{-3})T^\circ - (4.96 \times 10^{-2})PV_0 \quad [4]$$

where the natural logarithm of the induction period is $\text{Ln}(\Gamma_i)$ and the coefficients associated with the variables were all significant ($P < 0.085$); the variables are listed in the footnotes in Table 1. Figure 4 shows, at 6 ppm of carotenoids, the graphic behavior for Γ_i in both CW and CWO.

Γ_i in CW and CWO was mainly a function of linear and quadratic interactions between T° and P_0 ($P < 0.02$). The quadratic effect of peroxides on the induction period had a maximum in both CW and CWO at ≈2.7 mEq/K of oil (Fig. 4). A peroxide concentration lower than 2.7 mEq/K

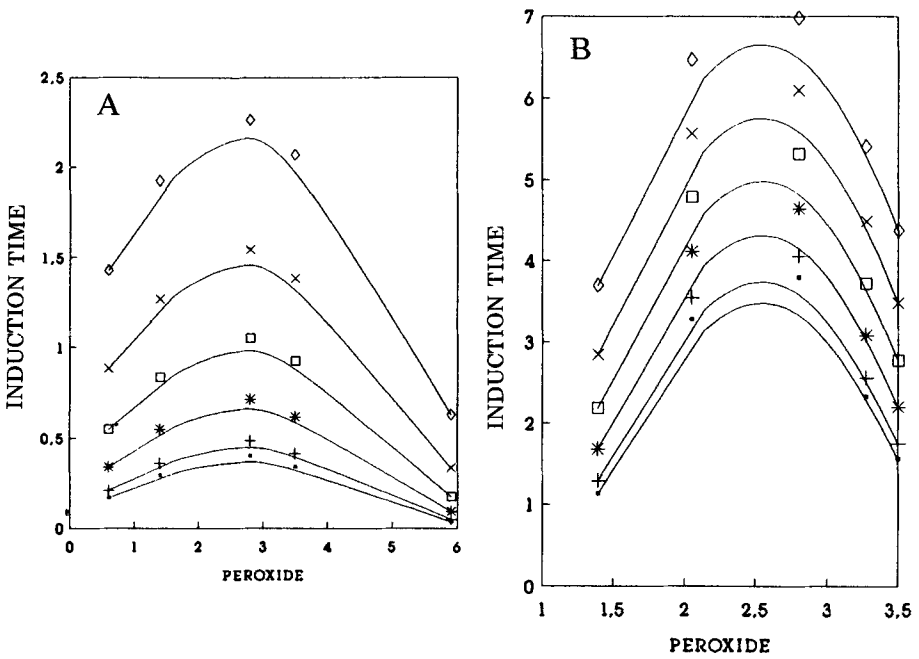

FIG. 4. Graphic behavior of the induction time (h) at different peroxide concentrations (mEq/K of oil) in refined corn oil without (A) and with (B) antioxidant. The induction time was calculated with the Equations 1 and 3 keeping the carotenoids concentration constant at 6 ppm. Symbols: •, 95°C; +, 90°C, *, 80°C; □, 70°C; ×, 60°C; open diamond, 50°C.

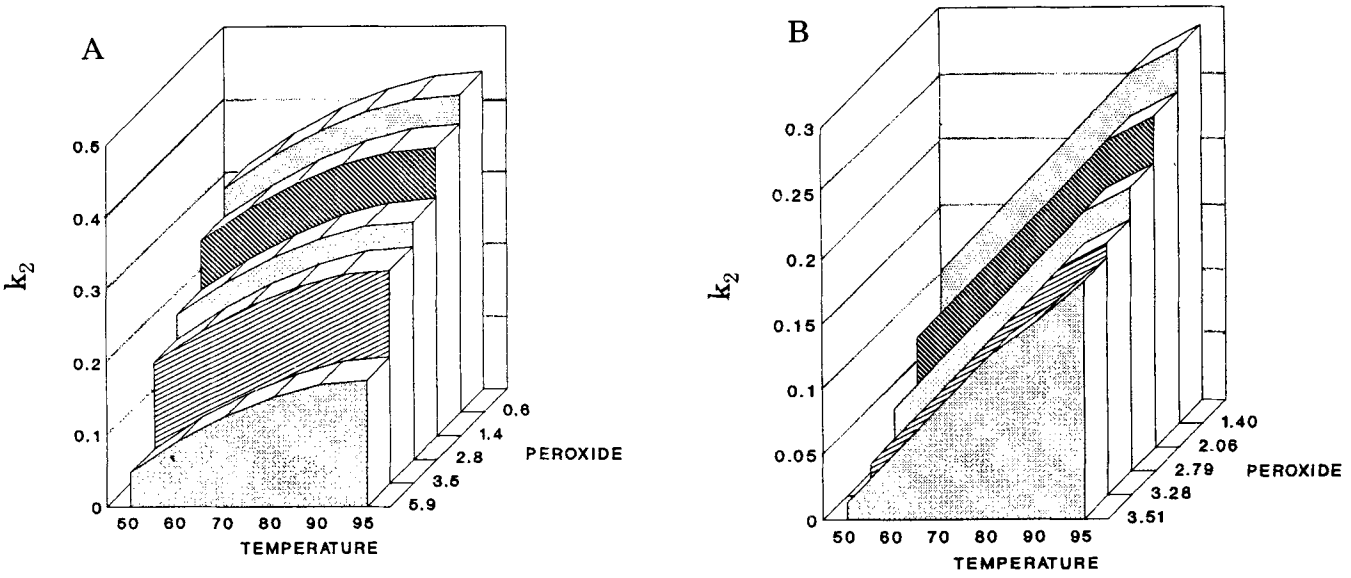

FIG. 5. Graphic behavior of the constant rate of bimolecular peroxide decomposition (h^{-1}) at different peroxide concentrations (mEq/K of oil) in refined corn oil without (A) and with (B) antioxidant. The constant rate of bimolecular peroxide decomposition was calculated with the Equations 2 and 4.

of oil might not be high enough to activate peroxide decomposition; within this interval of peroxide concentration (*i.e.,* 0 to 2.7 mEq/K) the magnitude of Γ_i would depend mainly on an inverse function of the unsaturation degree of the fatty acids (*i.e.,* susceptibility of oxidation) as well as their concentration in the oil. However, at $P_0 > 2.7$ mEq/K, peroxide decomposition might be activated and produce the free radicals needed to initiate the propagation reactions. Thus, Γ_i would be reduced as P_0 increased (Fig. 4). The fact that the Γ_i effect observed the same maximum in both the CW and CWO indicates that the antioxidant did not modify the concentration of peroxide needed to initiate peroxide decomposition. However, as the T° increased, the quadratic effect of P_0 on the induction period decreased more drastically in the absence of antioxidant than in its presence (Fig. 4), *i.e.,* within the interval of T° studied, peroxide decomposition is more involved in defining the magnitude of Γ_i in oil with antioxidant than in oil without antioxidant.

On the other side, the antioxidant effect of carotenoids (8,10) was evident in CWO in both the graphic behavior (data not shown) and the equation for Γ_i, *i.e.,* independent of T° and P_0, as the carotenoids concentration increased in the oil, the Γ_i increased exponentially. The antioxidant effect of the carotenoids was not significant in CW, probably because the antioxidants added to the oil overshadow their effect.

Current prediction of oil shelf life at room temperature assumes an Arrhenius-type relationship between Γ_i (usually determined at T° > 95°C) and the temperature. The equations presented in this paper are not suitable for shelf life predictions, mainly because the system utilized accelerated oil oxidation and the acceleration factor is unknown. However, the equations developed in this investigation showed that, in both CW and CWO, temperature was not the only significant factor in defining the magnitude and variability of Γ_i.

Figure 5 shows the graphic behavior of k_2 in both CW and CWO. While, in the absence of antioxidant, k_2 was a function of linear and quadratic effects of T° and a linear effect of P_0 ($P < 0.08$), in the presence of antioxidant, k_2 was a function of independent linear effects of P_0 ($P < 0.031$). The differences in the equations describing the behavior of k_2 indicated that the utilized antioxidant modified the mechanism of oxidation. Thus, although the magnitude of k_2 was greater in the absence of antioxidant than in its presence, peroxide decomposition (*i.e.,* carbonyl production) was evident just at T° greater or equal to 80°C and mainly at times longer than Γ_i (Figs. 2 and 3). In contrast, in the presence of antioxidant peroxide, decomposition was detected at T° > 50°C and, in general, at times shorter than Γ_i (Figs. 2 and 3).

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