The Effect of 12, Oxo-*cis*, 9-octadecenoic Acid on the Autoxidation of Stripped Corn Oil[1]

V. R. BHALERAO, M. G. KOKATNUR, and F. A. KUMMEROW, Department of Food Technology, University of Illinois, Urbana, Illinois

The effect of 12, oxo-*cis*, 9-octadecenoic acid (keto acid) on the autoxidation of stripped corn oil at 37°C. has been studied. The stripped corn oil containing 50 μg. of tocopherol per g. was prepared by Distillation Products Industries by removal of approximately 10% of the corn oil in a molecular still. Addition of 2.5% of the keto acid resulted in a higher rate of oxidation. As the concentration of keto acid was increased from 0 to 10%, peroxides increased rapidly, resulting in a shorter induction period.

The antioxidants BHA, BHT, propyl gallate, NDGA, Ethoxyquin, and DPPD were found to lengthen the induction period for stripped corn oil in the presence of keto acid. DPPD was found to be by far the most effective and α-tocopherol acetate had no antioxidant activity in presence of keto acid.

It was observed that keto acid oxidized very rapidly with the formation of peroxides and probable breakdown to free radicals. These free radicals then accelerated the autoxidation of stripped corn oil through a chain reaction mechanism. Heptanoic, suberic, and azelaic acids were identified among the breakdown products of keto acid.

IT IS NOW RECOGNIZED that autoxidation of a fat or a fatty acid involves a free radical chain process (1,2). Tappel (3) has recently shown that the addition of a small amount of preformed linoleate peroxide increased the oxidation rate of an unsaturated fatty acid. Bolland *et al.* (4) have used benzoyl peroxide as an initiator of autoxidation of ethyl linoleate at 45°C. More recently Bickel and Kooyman (5) showed that the thermal decomposition of 2,3,3,3-tetraphenylbutane in the presence of oxygen at 60°C. provided free radicals to initiate the oxidation of 9,10-dihydroanthracene. In a recent investigation (6) it was observed that the presence of keto acid (12,oxo-*cis*, 9-octadecenoic acid) as a dietary component caused rapid development of encephalomalacia in chicks. It was postulated that the keto acid acted as a pro-oxidant and enhanced rapid development of peroxides in the dietary fat (7). Studies were, therefore, undertaken to determine the effect of keto acid on the autoxidation of stripped corn oil and the effectiveness of some antioxidants in the prevention of oxidation in the presence of keto acid.

Experimental

Materials. The 12,oxo-*cis*,9-octadecenoic acid (keto acid) was prepared from castor oil according to the method of Nichols and Schipper (8). Melting point, refractive index, saponification value, and iodine value were determined by official methods (9). The % carbonyl compound was determined according to the method of Bryant and Smith (10). The physical and chemical constants of the synthesized keto acid (Table I) agreed closely with the theoretical values and the melting point agreed with that reported in the literature (8).

Antioxidants Used. The antioxidants used in the present series of experiments included α-tocopherol acetate, propyl gallate, 2,tertiarybutyl-4,methoxy phenol (BHA), 2,6-ditertiary butyl-4-methyl phenol (BHT), nordihydroguairetic acid (NDGA), diphenyl-paraphenylene-diamine (DPPD), and 6,ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (Ethoxyquin).

TABLE I

Physical and Chemical Constants of 12,oxo-*cis*,9-octadecenoic Acid

Constants	Found	Theoretical	Literature(8)
Melting point (°C.)	39.2	–	39–40
Ref. index 40°C	1.4602	–	–
Iodine value	85.1	85.8	–
Sap. value	189.7	189.5	–
% Carbonyl compound	100.4	100.0	–

Substrate Used. The antioxidant activity of various compounds with or without keto acid was compared at 37°C. using stripped corn oil as a substrate. The stripped corn oil was prepared by Distillation Products Industries, by removal of approximately 10% of the fat in a molecular still. The volatile fraction containing substantially all the naturally occurring tocopherols was rejected. The resulting stripped corn oil was estimated to contain 50 μg. of tocopherol per g.

Preparation of Samples for Autoxidation. Twenty g. of corn oil was placed in a shallow dish (inside diameter 59 mm.) and the antioxidant was added as a solution in 5 ml. ethyl ether at a level of 1 μ mole per g. of the substrate. Keto acid was added to the fat directly. Five ml. of ether without keto acid or antioxidant was added to 20 g. of an oil to serve as a blank in each series of experiments. After mixing, the solvent was thoroughly removed on a hot water bath, and the dishes transferred to a constant temperature room maintained at 37°C.

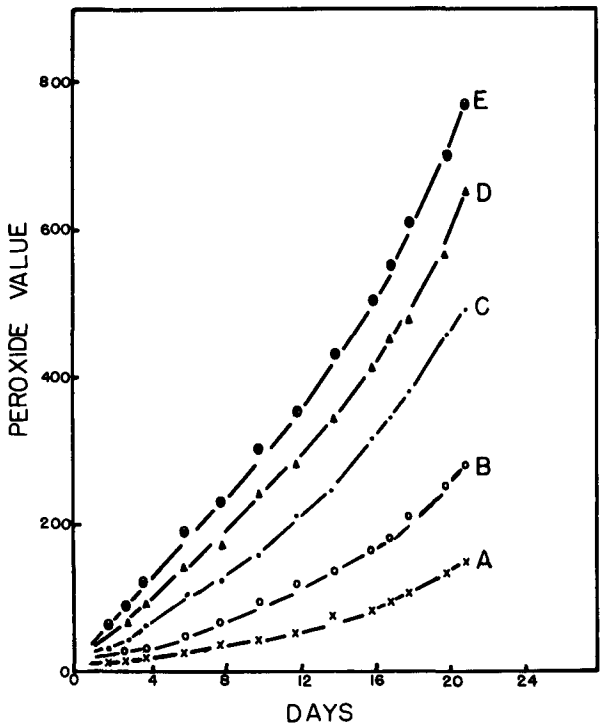

FIG. 1. Stability of stripped corn oil containing (A) none, (B) 2.5% keto acid, (C) 5% keto acid, (D) 7.5% keto acid, (E) 10% keto acid. The peroxide value was expressed as milliequivalents of peroxide per kg. of fat.

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Test for Antioxidant Activity. All the samples in each series of the experiment were run in duplicate and the antioxidant activity was compared on the basis of the average peroxide value of the duplicate samples. The peroxide value was determined according to the method of Lundberg *et al.* (2) every 24 hr. for the first 4 days and subsequently every alternate day till the experiment was terminated. The peroxide value was expressed as milliequivalents per kg. of fat.

Results

Influence of Keto Acid. The effect of addition of varying amounts of keto acid on the autoxidation of stripped corn oil at 37°C. is shown in Figure 1. The peroxide value of about 200 milliequivalents per kg. of fat was taken as a convenient point for the induction period. The data indicate that an increase in the concentration of keto acid from 0 to 10% caused an increase in the rate of oxidation and shortened the induction period.

Oxidation of Keto Acid. The keto acid may have supplied free radicals to catalyze fat oxidation. To test this postulation, 20 g. of pure crystalline keto acid was subjected to autoxidation at 37°C. It was observed that the peroxide value of the keto acid increased rapidly from 24 to 162 and 290 after 24 and 48 hr. respectively. The oxidation products obtained after 24 hr. were examined by gas-liquid chromatography using diethylene glycol succinate and Apizon columns. Suberic, azelaic, and heptanoic acids were identified on the gas chromatogram. The amount of these fatty acids was not quantitatively determined.

Influence of Antioxidants. In order to study the effect of antioxidants 500 mg. of keto acid was added to 20 g. of stripped corn oil. The effect of adding α-tocopherol acetate to corn oil in the presence and absence of keto acid is shown in Figure 2. The autoxidation was found to proceed at the same rate in the absence of keto acid, even when α-tocopherol acetate was added to the fat. It was further observed that the α-tocopherol acetate had no antioxidant activity in the presence of keto acid under the experimental conditions. It is well established that tocopherol esters are oxidized much more slowly than tocopherols. It is, therefore, possible that tocopherol acetate is not a very good antioxidant for stripped corn oil.

BHA, BHT, and propyl gallate were found to be good antioxidants for stripped corn oil (Figure 3). However, in the presence of keto acid, BHA and BHT were poorer than propyl gallate in their antioxygenic properties. The addition of NDGA, Ethoxyquin, and DPPD (Figure 4) also lowered the rate of oxidation of stripped corn oil. NDGA and Ethoxyquin were able to overcome the pro-oxidant properties of keto acid and their activity was similar to that of propyl gallate. DPPD was, however, found to be the most effective antioxidant in the presence of keto acid.

Discussion

The results indicated that keto acid can act as a pro-oxidant in stripped corn oil and that antioxidants differ in their antioxygenic properties in the presence of keto acid. It is believed that the oxidation of an unsaturated fat proceeds by way of a free radical chain reaction and antioxidants are themselves oxidized while fulfilling their chain-stopping function (4). The end of the induction period usually depends upon the disappearance of the antioxidant. The strong antioxidant property of DPPD appears to be due to the fact that the normal oxidation product-quinondianil- is itself a good antioxidant, which has been illustrated by Budowski and Bondi (11).

In the mechanism of autoxidation of linoleate (2, 12,13), the existence of a free radical of linoleate is indicated. Lundberg and Chipault (2) have observed

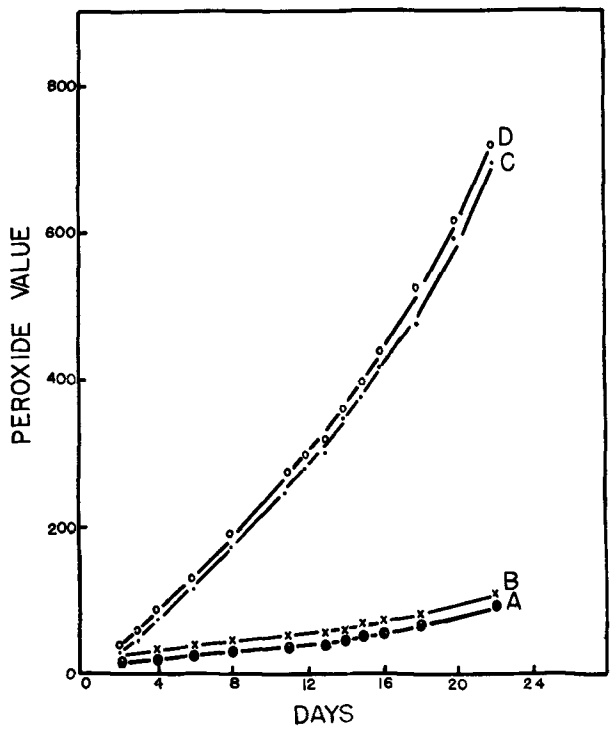

FIG. 2. Stability of stripped corn oil containing (A) α-tocopherol acetate, (B) none, (C) α-tocopherol acetate + 2.5% keto acid, and (D) 2.5% keto acid. The peroxide value was expressed as milliequivalents of peroxide per kg. of fat.

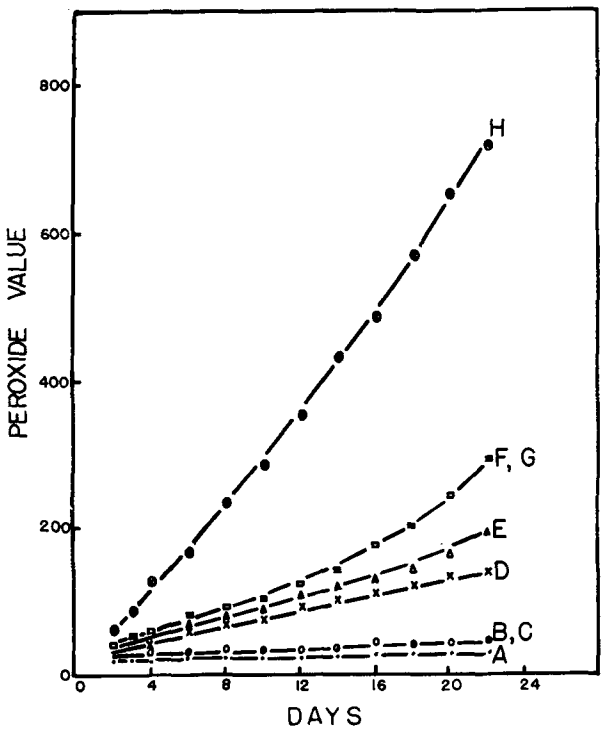

FIG. 3. Stability of stripped corn oil containing (A) propyl gallate, (B) BHA, (C) BHT, (D) none, (E) propyl gallate + 2.5% keto acid, (F) BHA + 2.5% keto acid, (G) BHT + 2.5% keto acid, and (H) 2.5% keto acid. The peroxide value was expressed as milliequivalents of peroxide per kg. of fat.

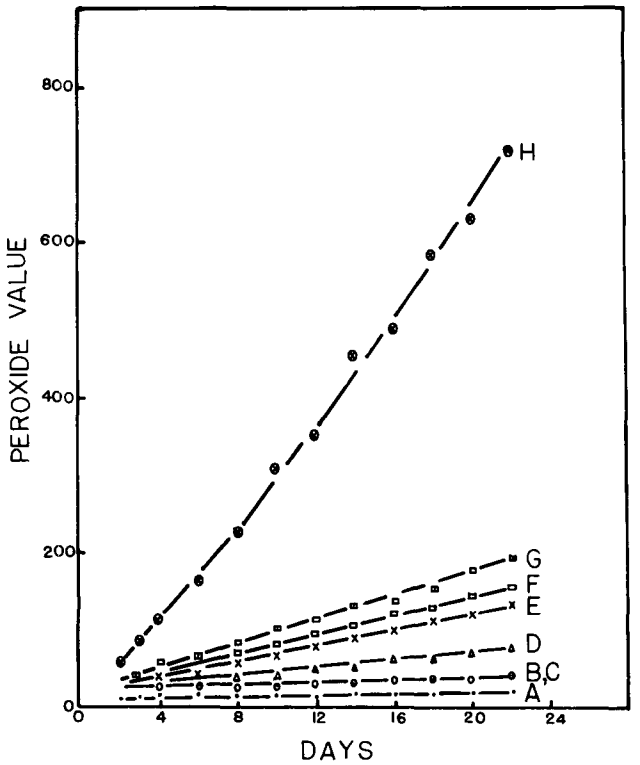

FIG. 4. Stability of stripped corn oil containing (A) DPPD, (B) Ethoxyquin, (C) NDGA, (D) DPPD + 2.5% keto acid, (E) none, (F) Ethoxyquin + 2.5% keto acid, (G) NDGA + 2.5% keto acid, and (H) 2.5% keto acid. The peroxide value was expressed as milliequivalents of peroxide per kg. of fat.

that methyl linoleate has a long lag period due to a lack of chain-starting free radicals in the system before oxidation commences at 40° or 60°C. Once a little hydroperoxide is formed it can break down by thermal decomposition to give free radical fragments. These free radicals can abstract hydrogen from the reactive methylene group of the unsaturated fatty acid to form a hydroperoxide of linoleate and another free radical. This mechanism represents the chain reaction by which linoleate is oxidized. Any substance which furnishes a hydrogen atom, and which in turn does not accept one from linoleate, would stop the chain reaction.

When keto acid is added to stripped corn oil, the oxidation proceeds very rapidly (Figure 1) indicating that keto acid probably supplies free radicals for the chain reaction. It was observed that keto acid itself oxidized rapidly with the formation of short chain fatty acids, namely suberic, azelaic, and heptanoic acids. This is in confirmation with the results reported by Ellis (14) who showed that autoxidation of keto acid at 50°C. yielded α,β-unsaturated keto acid and the scission products—suberic, azelaic, heptanoic, and octanoic acids. Some or all of these oxidation products serve as free radical fragments in the autoxidation of stripped corn oil.

Other evidence for the breakdown of keto acid peroxides was obtained by spectrophotometric studies of autoxidized keto acid. Fresh keto acid was found to have a strong spectral absorption at 282 mμ, which increased after oxidation. A scission of the -O-O- bond of peroxide with a simultaneous cleavage of an adjacent -C-C- bond would produce aldehyde, which would show an increase in the absorption in this range.

The pro-oxidant activity of keto acid may, therefore, be due to a rapid formation of keto acid peroxide with a subsequent release of free radical decomposition products capable of initiating a chain reaction. Therefore, increasing the initial concentration of keto acid in the system should cause an increase in the rate of oxidation and a decrease in the induction period. This postulation is in essential agreement with the experimental observation (Figure 1).

The results indicated that stripped corn oil plus 2.5% keto acid developed a peroxide value of about 250 at the end of 20 days in the first series of experiment. However, the same mixture in the last three experiments had a peroxide value of approximately 600 at the end of 20 days. This discrepancy was probably caused by the low peroxide value of 0 in the stripped corn oil used in the first series of the experiment, while the peroxide value of the stripped corn oil used in the other experiments was about 15 at the start of the experiment. The small amount of peroxides present might have accelerated the pro-oxidant property of keto acid resulting in a high peroxide value of the mixture at the end of 20 days. Even though there was a discrepancy in the peroxide value of stripped corn containing 2.5% of keto acid at the end of 20 days, the results definitely indicated that keto acid had a strong pro-oxidant activity.

On the basis of the data presented, a mechanism of unsaturated fat oxidation catalyzed by keto acid could be visualized. The first step involves the formation of keto acid peroxide followed by decomposition into free radicals. Although the nature of all the products resulting from breakdown of keto acid peroxide remain unknown, the experimental evidence indicates that heptanoic acid, suberic acid, and azelaic acid are formed.

The observed inefficiency of α-tocopherol acetate as an antioxidant in the presence of keto acid is apparently caused by rapid initiation of oxidation of stripped corn oil in the presence of keto acid at 37°C. as measured by peroxide value determinations. These antioxidants probably terminate chains by interaction with peroxide radicals with the formation of a stable product, thus preventing the accumulation of free radicals necessary for the initiation reaction.

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