

All of the other treatments listed in Table IV were essentially ineffective in reducing the contents of CB-1A by more than 50 to 75%, which may be considered negligible.

Summary

Results are reported of a series of experimental treatments of flaked castor bean meats and pomaces directed toward total detoxification of the ricin and total destruction or inactivation of the allergenic property.

The treatments comprised cooking of the flaked raw meats or pomaces under various conditions of moisture and temperature, with chemicals such as sodium hydroxide, hydrochloric acid, sodium hypochlorite, formaldehyde, ammonia, ammonium sulfate, potassium permanganate, and urea, and with selected combinations of these. Also tried were aerobic fermentation, enzymatic digestion, and simple heating of pomaces at elevated temperatures.

The five most promising deallergenization treatments and the corresponding percentage reductions in allergen content as measured by the precipitin test were as follows: dry heating of pomace to 401°F., 100%; moist-cooking of flaked meats with 2% NaOH and 10% HCHO, possibly 100%; moist-cooking with 0.9% HCl and 3% HCHO, possibly 100%; moist-cooking with 2% NaOH at 20 psig. pressure, possibly 100%; moist-cooking with 1% NaOH, 98.4%. For the first four treatments the Schultz-Dale test indicated possible reductions of 100, 99.9, 99.9, and 100%, respectively. While the latter method is generally considered to be reasonably accurate, final tests would have to be conducted with naturally sensitive human subjects.

The data also showed that the ricin component can be completely detoxified by a mild moist-cooking of the flaked meats, either with or without added alkali.

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The Formation of Polarographically Reducible Substances in Autoxidizing Lard¹

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THE FIRST APPLICATION of polarography to autoxidizing fat by Lewis and Quackenbush (7) served to demonstrate that more than one type of "peroxide" is included in the iodimetric determination of peroxide value. Subsequent studies by Swern *et al.* (12) were made with hydroperoxides and related compounds thought to occur in autoxidizing fats, and a method was presented by Willits *et al.* (13) and Ricciuti *et al.* (9) for the quantitative determination of hydroperoxides in oleate and linoleate. Evidence for distinct nonhydroperoxide type compounds has been found by Swern *et al.* (10) in methyl oleate and by Kalbag *et al.* (4) in soybean oil. In our laboratory it was observed that the addition of ethyl cellulose to the solvent greatly improved polarographic differentiation of the peroxidic compounds in a fat.

The present paper extends the investigation of re-

ducible autoxidation products in lard and the effects of temperature, pro-oxidants, and antioxidants on their formation.

Experimental

Apparatus. A Sargent Model XXI recording polarograph was used with a water-jacketed cell (30 ± 0.2°C.), which had an internal mercury pool anode with the approximate surface area of 6.6 sq. cm. The capillary in the solvent system with open circuit had a drop time *t* of 3.80 seconds. The value *M* was 1.85 mg./second; therefore $m^{2/3} t^{1/6}$ (5) was 1.88 $mg.^{2/3} sec.^{-1/2}$.

For the autoxidizing apparatus a bottomless Erlenmeyer flask (1 liter) was ground to fit tightly to a heavy glass plate. The sample was placed in a 100-mm. Petri dish on the plate and a Pyrex gas dispersion tube with coarse fritted disc (30 mm. in diameter) stoppered into the neck just above the sample. This apparatus was placed in a forced-air oven with

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temperatures controlled at $\pm .2^\circ\text{C}$. Air was forced through concentrated sulfuric acid, then through calcium chloride, and through the gas dispersion tube to flow over the surface of the sample at a rate of 50–60 ml./minute.

Materials. The solvent used was an equal-volume mixture of absolute methanol (Baker) and benzene (Merck, thiophene-free) with 0.3 M. lithium chloride and 0.01% ethyl cellulose (Hercules Powder Company, ethoxy content 48.7%, viscosity 42 cps.) and was allowed to stand over-night before use. The lithium chloride was recrystallized from methanol before use. Deodorized bleached lard (Swift and Company) was used in all experiments. Triple-distilled mercury, A. R. grade, was used as the cathode and anode in the polarographic cell assembly.

In the autoxidation studies commercial compounds were used as obtained, except for catechol, which was recrystallized from alcohol. The grades and sources were: ascorbic acid, U.S.P., Merck; DL- α -tocopherol, U.S.P., Merck; citric acid, U.S.P., Mallinckrodt; phosphoric acid, 85%, A.R., Mallinckrodt; chlorophyll, concentrated extract, Fritsche Bros, Inc.; and catechol, white label, Eastman.

Procedure. The lard (30 g.) was weighed into the Petri dish and placed in the autoxidizing apparatus. At various intervals samples were taken, weighed, and analyzed both polarographically and titrimetrically (11). In the polarographic procedure 5 ml. of the solvent system and a sample of autoxidized lard (0.05–0.08 g.) were placed in the polarographic cell, and oxygen-free nitrogen was bubbled through the solution for 10 min. One ml. of mercury was placed in the cell, and the nitrogen stream was continued into the solution for 2 min., then raised to pass over the surface of the solution during polarography. All polarographic results were calculated on a 1% by weight-volume basis (1 g. of autoxidized lard/100 ml. of solvent).

Results and Discussion

When polarograms obtained on autoxidized lard with ethylcellulose present as a maximum suppressor were compared to those obtained by the original Lewis and Quackenbush procedure (5), it was observed that the reduction wave at a half-wave potential of -0.15 volt was of about the same magnitude but the large reduction wave at a half-wave potential of -0.7 volt was resolved into two waves having half-wave potentials at -0.68 and -1.10 volts.

Deodorized bleached lard, during autoxidation at 55°C ., slowly developed a peroxide value of approximately 300 (Figure 1). Curves A, B, and C were plotted from diffusion current values obtained by polarographing samples at 12 points during the 300-hr. autoxidation period. Curve A, representing the diffusion current obtained at a half-wave potential of -0.15 volt, approached a maximum in about 200 hrs. The Curves B and C, representing diffusion currents obtained at half-wave potentials of -0.68 and -1.10 volts, respectively, showed the same general trend as the peroxide values.

Copper ions (1.5 p.p.m.), added to the lard, decreased to 170 hrs. the time that was needed for Curve A to reach a maximum. A peroxide value of 300 was reached approximately 100 hrs. sooner in the copper sample than in the control sample. Both the peroxide value and Curve B increased slightly after 200 hrs. while Curve C reached a plateau at 150 hrs.

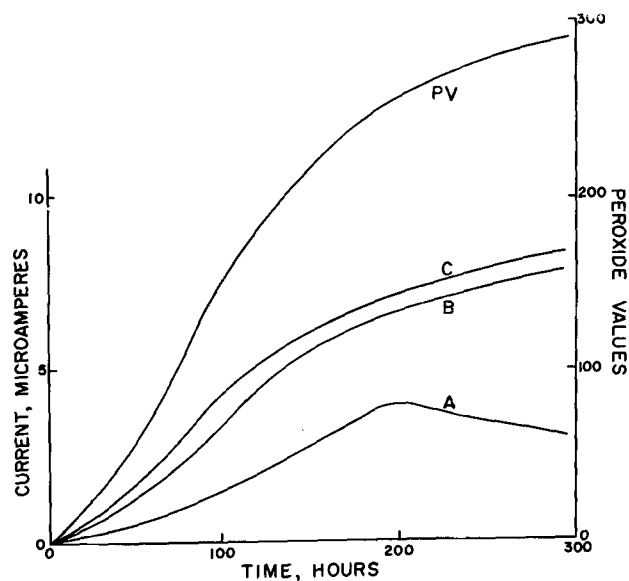


Fig. 1. Comparison of peroxide values with diffusion current for lard during autoxidation at 55°C .

A is ia at $E_{1/2} = -0.15$ v.; B is ia at $E_{1/2} = -0.68$ v.; C is ia at $E_{1/2} = -1.10$ v.; PV is peroxide value (m. mols. O_2/kg .)

Higher temperature (100°C .) increased the rates of both peroxide formation and decomposition in lard (Figure 2). Curve A reached a maximum in about 20 hrs. while the peroxide value reached a maximum of 125 in about 30 hrs. Curves B and C reached their maxima in about 35 hrs. In a few cases a small fourth wave, D, was observed at -1.6 volts after the maximum peroxide value had been reached. Since emphasis in this study was on early autoxidation products, little attention was given to this wave. Copper showed a much greater pro-oxidant effect at the higher temperature as Curve A reached a maximum in 9 hrs. Curves B and C, which reached their maxima approximately 13 hrs. earlier than the control, were steeper and showed a trend similar to the peroxide value. A second pro-oxidant, chlorophyll (0.2%), showed polarographic effects similar to copper when added to lard and allowed to autoxidize at 100°C .

The pro-oxidant effects of high levels of α -tocopherol (0.25%, 0.50%, and 2.0%) were also observed polarographically (Figures 3 and 4). In Figure 3 curves

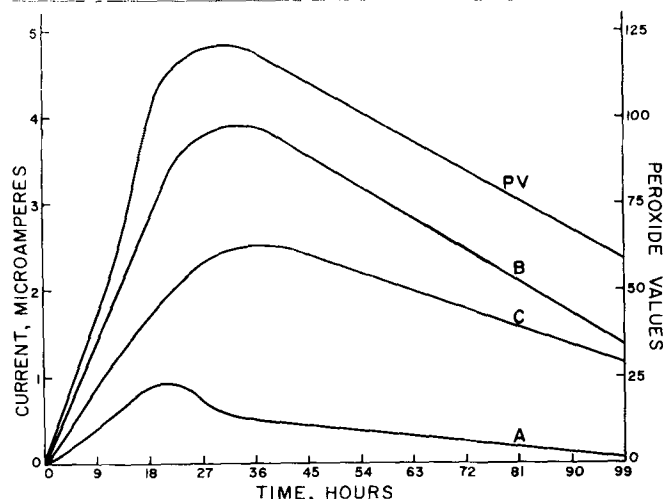


Fig. 2. Comparison of peroxide values with diffusion current for lard during autoxidation at 100°C .

A is ia at $E_{1/2} = -0.15$ v.; B is ia at $E_{1/2} = -0.68$ v.; C is ia at $E_{1/2} = -1.10$ v.; PV is peroxide value (m. mols. O_2/kg .)

are shown for the three concentration levels in two groups: the peroxide values and the Curve A ($E 1/2 = -0.15$). Tocopherol in concentrations from 0.25% to 2.0% did not affect the initial rate of peroxide formation. However, after 8 hrs., the effects of the tocopherol were noticeable. After about 20 hrs. a typical autocatalytic curve was obtained. The apparent effect of the higher concentration of tocopherol was to accelerate the rate of peroxide decomposition; this was observed previously by Privett and Quackenbush (8) in a different type of experiment. In a concentration of 0.25% tocopherol showed pro-oxidant action similar to that of copper (1.5 p.p.m.) and chlorophyll (0.2%). The polarographic Curve A also showed that reducible products accumulated more slowly and persisted longer with tocopherol increments.

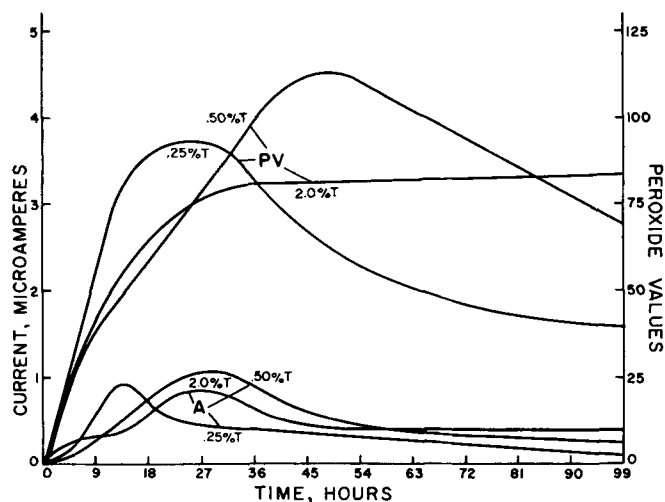


FIG. 3. Relationship of three concentrations of α -tocopherol (T) to peroxide values (PV) and diffusion current at $E 1/2 = -0.15$ v. (A) during autoxidation of lard at 100°C .

Curves B and C are shown in Figure 4. The B curve most nearly resembled the peroxide value curves for each of the various percentages of tocopherol. Curve C showed that higher concentrations of tocopherol delayed by at least 40 hrs. the development of a maximum concentration of the substance reducible at -1.10 volts.

Synergists of various types were compared, first without concurrent additions of antioxidants (Table 1). Ascorbic acid (0.05%) showed little or no effect upon the accumulation of reducible substances when compared to the control lard. Citric acid (0.05%) decreased both the time to reach the maximum of the reducible substances and the concentration of these substances at their maximum; the curves followed a trend roughly similar to that of the peroxide values. Phosphoric acid accelerated both the formation and subsequent breakdown of reducible substances; in-

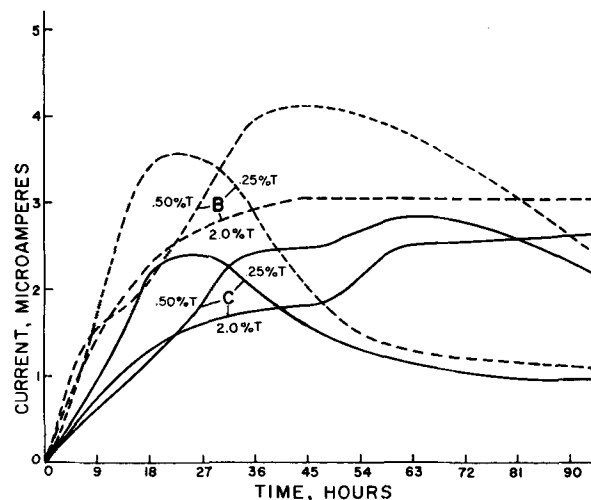


FIG. 4. Relationship of three concentrations of α -tocopherol (T) to diffusion current at $E 1/2 = -0.68$ v. (B) and $E 1/2 = -1.10$ v. (C) during autoxidation of lard at 100°C .

creasing concentration of phosphoric acid decreased the maximum accumulation. Neither ascorbic acid nor citric acid (0.05%) seemed to react with the peroxides formed in lard. However phosphoric acid formed a brown fat-soluble complex, perhaps polymeric in nature. The formation of this complex is thought to be responsible for diminished peroxide accumulation in autoxidized lard (8).

A typical antioxidant effect is shown with 0.011% catechol, tested in lard with and without synergist. Catechol alone delayed maximum accumulation of all of the reducible substances by about 6 hrs. (Figure 5). However, once the autoxidation started, the curves resembled closely those of the control lard (*cf.* Fig-

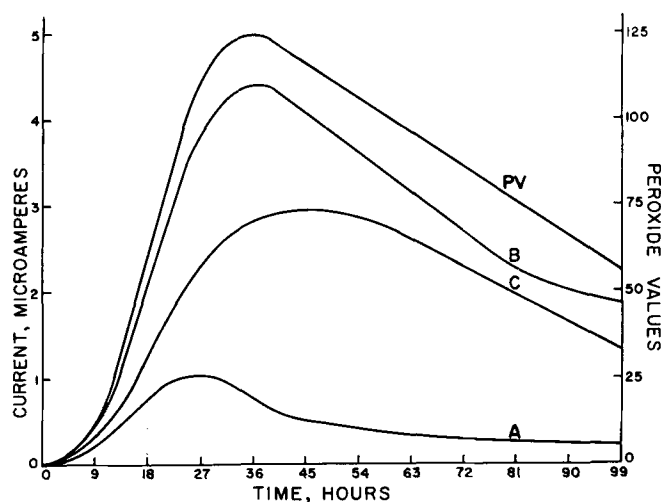


FIG. 5. The effect of catechol (0.011%) on the peroxide value and diffusion current curves A, B, and C during autoxidation at 100°C .

TABLE I
Effects of Synergists on Maximum Peroxidic Accumulation in Lard at 100°C .

Addition to lard	Polarographic						Titrimetric—peroxide value	
	Curve A (-0.15 v.) ^b		Curve B (-0.68 v.) ^b		Curve C (-1.10 v.) ^b		hrs.	m. mol./kg.
	hrs. ^a	ia	hrs.	ia	hrs.	ia		
None (control)	21	1.0	34	4.2	36	2.7	32	126
Ascorbic acid (0.05%)	21	0.7	34	4.2	36	3.0	33	120
Citric acid (0.05%)	14	0.6	23	2.4	19	2.1	29	105
Phosphoric acid (0.17%)	18	0.3	21	2.1	21	1.2	15	63
Phosphoric acid (0.34%)	7	0.4	14	2.0	7	1.6	7	52

^a Time in hours for maximum concentration to be reached. ^b Half-wave potential. ia—Diffusion current at maximum height.

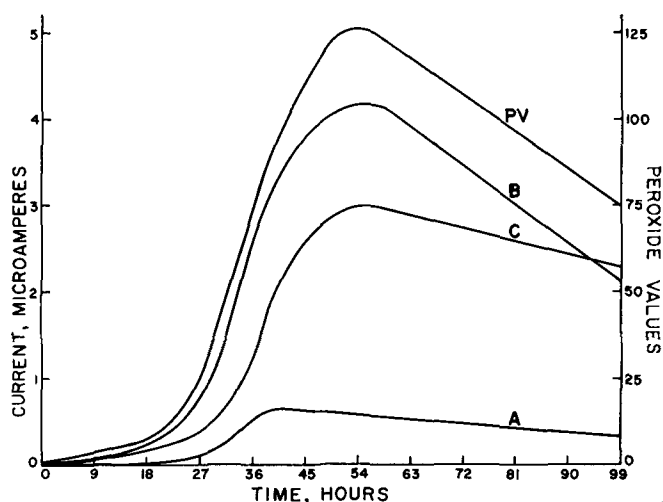


Fig. 6. The effect of catechol (0.011%) with citric acid (0.05%) on the peroxide value and diffusion current curves A, B, and C during autoxidation at 100°C.

ure 2). Citric acid (0.05%) with the catechol added 23 hrs. to the induction period of the lard (Figure 6) but did not otherwise change the order of formation and accumulation of reducible substances. The addition of 0.05% ascorbic acid to the catechol showed little effect other than to reduce slightly the total accumulation of reducible substances.

Phosphoric acid (0.17%) with the catechol showed an induction period of approximately 38 hrs. It also changed the order in which each curve reached a maximum. Curve C followed the trend of the peroxide value while both Curves A and B reached maximum values somewhat earlier (Figure 7). Previous work has shown that there is an increase in antioxidant activity when phosphoric acid is added to lard containing an antioxidant. This is probably caused partly by chelation of contaminant metal ions (1, 2) and partly by mutual sparing action between the partially oxidized form of the antioxidant and phosphoric acid (8).

Attempts to identify the individual peroxide structures represented by the three reduction waves led to only partial success. Measurements of half-wave potentials of a series of eight different hydroperoxides under our conditions had shown a range of values

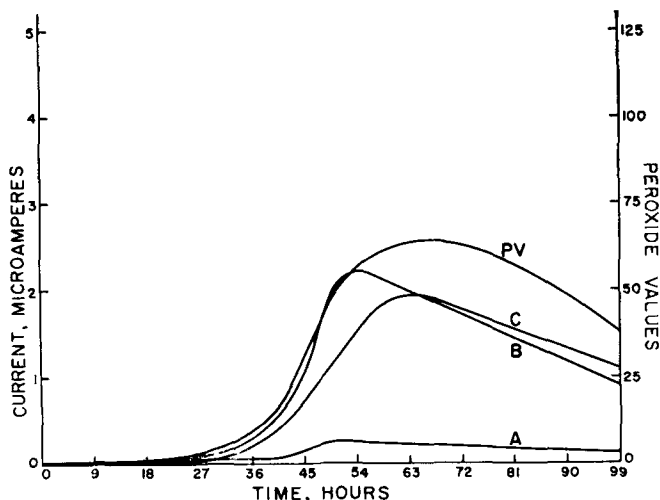


Fig. 7. The effect of catechol (0.011%) with phosphoric acid (0.17%) on the peroxide value and diffusion current curves A, B, and C during autoxidation at 100°C.

from -1.05 to -1.15 volts (6). Since the reduction waves represented by Curve C values (-1.10 to -1.15 volts) correspond well with this series, it is presumed that the reducible structure represented by Curve C is a hydroperoxide. Evidence for the types of peroxidic structure responsible for the other curves is less apparent. In our laboratory a number of peroxidic compounds have been shown to reduce polarographically at or near zero voltage (6). These include diacyl peroxides (lauroyl, acetyl) and bis (1-hydroxyheptyl) peroxide. The latter, which reduced at 0.00 voltage, was reported by Fritsch and Deatherage (3) to reduce at -0.3 volt. The A wave at -0.15 volt was observed with lard early in the autoxidation process, perhaps too early to be attributable to a product resulting from chain rupture and subsequent condensation. Accordingly, of the two types of peroxidic structure mentioned above, the diacyl would seem the more likely to be involved. However none of the types which we have studied corresponds closely with the A wave substance in all respects. At the present time no definite structure is suggested as responsible for Wave B. Some evidence indicates that this substance may be closely associated with the hydroperoxides. The late appearance of the D wave suggests a relation to secondary oxidation products.

Summary

In a polarographic study of lard during the early stages of autoxidation at 55° and 100°C. three reduction potentials were observed, and curves were plotted from the diffusion current values at the corresponding half-wave potential to show the changes in concentration of each of the reducible substances with time. All three curves followed the general pattern of the peroxide value, determined titrimetrically. However Curve A ($E_{1/2} = -0.15$ v.) reached a maximum much earlier than either Curve B ($E_{1/2} = -0.68$ v.) or Curve C ($E_{1/2} = -1.10$ v.). Pro-oxidants steepened the curves; antioxidants introduced a lag phase. Although the relative heights shifted somewhat, three curves persisted under all conditions. It is concluded that autoxidizing lard contains at least three types of primary "peroxidic" compounds and that these are not changed qualitatively by the presence of pro-oxidants and antioxidants.

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