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Antioxidant Properties of Tomato Lipids[1]

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THE OCCURRENCE of fat-soluble antioxidants in the non-saponifiable fraction of tomato and carrot extracts was reported by Bradway and Mattill (1). Solubility, vitamin E bioassay, and distillation studies which were reported indicated that these antioxidants were probably different from the tocopherols. However the isolation of vitamin E by Emerson *et al.* (2) and the demonstration of its antioxidant activity led Olcott (3) to suggest that some, if not all, of the antioxidant activity exhibited by the unsaponifiable portions of vegetable lipids can be attributed to the tocopherols. This view was confirmed in the case of carrot oil by Heftman (4). The purpose of the present investigation was to define more completely the antioxidant system of tomato lipids, which in initial work in this laboratory (10) showed considerable antioxidant activity.

Experimental

Determination of Antioxidant Activity. Stability tests were carried out at 100 ± 1°C. in a constant temperature oven equipped with a circulating fan. The fat substrate was bleached, deodorized lard,[2] 10-g. samples of which were placed in 9-cm. covered petri dishes for the individual tests. This lard [2] had a low peroxide value (1.0 millimole peroxide oxygen/kg.) and was essentially stripped of natural inhibitors, as evidenced by its lack of an induction period. The material to be tested was dissolved in a suitable solvent, and an aliquot of the solution was added to the substrate. Control lard samples were treated with solvent only. The solvent was removed by heating on a steam table. A peroxide value of 20, determined by a modification of the Wheeler method (5), was arbitrarily taken as the end of the induction period.

Preparation of Tomato Lipids. Field-ripened Baltimore tomatoes, which had been stored frozen at −18°C., were quartered, placed in enamelled trays, dried in an air oven at 100°C. for 24 hrs., and ground. Some 1,200 g. of the tomato powder were extracted in a four-liter Erlenmeyer flask with six successive one-liter portions and four 500-ml. portions of freshly distilled petroleum ether (30–60°C.). During each extraction the solvent was mixed with the tomato powder, the mixture was warmed on the steam bath with occasional rotation, and after several minutes the extract was decanted. The extracts were then combined, concentrated to one liter under reduced pressure, and filtered. Evaporation of an aliquot to dryness showed that 16.66 g., or 1.39%, were extracted from 1,200 g. of dried tomato powder. The antioxidant activity of various amounts of this crude tomato lipid preparation was tested at 100°C. in both fresh and oxidized lard.

Fractionation of the Tomato Lipids. To obtain a stock solution of tomato lipids for use in fractionation studies a second 1,175-g. portion of tomato powder was extracted in a four-liter Erlenmeyer flask with five consecutive 1,000-ml. portions and then five consecutive 500-ml. portions of purified petroleum ether (40–60°C.) in the manner described above. After the last decantation the insoluble tomato powder was placed in a large Buchner funnel and washed with several portions of warm petroleum ether until the filtrate became colorless. The extracts were then combined and concentrated to 2,000 ml. under reduced pressure. The 36.5 g. of tomato lipids obtained by this extraction represented 3.1% of the dried tomatoes.

The tomato lipids were then transferred from petroleum ether to absolute methanol. In this step 1,880 ml. of the crude tomato lipid solution (34.3 g.) which remained was evaporated to dryness under reduced pressure. The gummy residue was then extracted in a two-liter round bottom flask with two 500-ml. and six 250-ml. portions of absolute methanol. The extracts were combined, concentrated to two liters, and allowed to stand at 0°C. over night. The precipitate was removed by filtration and returned to the rest of the methanol-insoluble material. From 34.3 g. of crude tomato lipids 7.48 g., or 22.4%, were extracted by the absolute methanol. The stabilizing effects of these two fractions were tested (Table I).

The methanol-soluble fraction was subjected to phasic separation between methanol and petroleum ether. To this end 1,500 ml. of the methanol solution of tomato lipids (5.61 g.) were diluted to 92% methanol with water and exhaustively extracted in a separatory funnel with two 500-ml. and 18 250-ml. portions of purified petroleum ether (65–67°C.). The

TABLE I

The Stabilizing Effects of Methanol-Soluble and Insoluble Fractions of Tomato Lipids Added to Lard (100°C.)

Material tested [a]	Keeping time (hrs.)	Protective factor
Lard alone	1.0	1.0
Lard + 0.5% IF	1.5	1.5
Lard + 1.0% IF	1.5	1.5
Lard + 3.0% IF	3.5	3.5
Lard + 0.5% SF	9.0	9.0
Lard + 1.0% SF	18.0	18.0
Lard + 2.0% SF	49.0	49.0
Lard + 3.0% SF	178.0	178.0

[a] IF = insoluble fraction; SF = soluble fraction.

[1] Journal Paper No. 990 of the Purdue University Agricultural Experiment Station. The subject-matter of this paper was undertaken initially in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the armed forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsements of the sponsor.

[2] Kindly prepared and supplied by Swift and Company.

combined extracts were concentrated to a final volume of one liter. The petroleum ether fraction contained 4.96 g., or 88.4%, of the tomato lipids in the original absolute methanol solution. The antioxidant activity of each fraction, alone and combined, was compared with the activity of the original methanol solution (Table II).

TABLE II
The Stabilizing Effects of 92% Methanol and Petroleum Ether Phases Obtained from the Methanol-Soluble Fraction of Tomato Lipids Added to Lard (100°C.)

Material tested	Percentage added	Keeping time (hrs.)	Protective factor
1. Lard	....	1.0	1.0
2. Lard + original abs. MeOH soln	2%	49.0	49.0
3. Lard + 92% MeOH fraction	2%	2.0	2.0
4. Lard + petroleum ether fraction	2%	5.0	5.0
5. Lard + nos. 3 and 4 recombined	2%	51.0	51.0

Equivalent to 2% of the original abs. methanol soln.

Results and Discussion

Crude tomato lipids exhibited marked antioxidant activity in lard at 100°C. (Figure 1). The length of the induction period increased directly with concentration up to 3%, the highest level tested. Absence of a pro-oxidant action, even at high concentrations, was evidenced by the extremely slow accumulation of peroxides during the induction period. This is in contrast to the results obtained with alpha-tocopherol (Figure 2), in which a strong pro-oxidant action was observed with a much shorter keeping time. It was evident therefore that the active component was not solely tocopherol. When compared with NDGA, gossypol,[3] and alpha-tocopherol (Figure 3), the crude tomato lipids gave longer induction periods and showed no apparent optimum concentration.

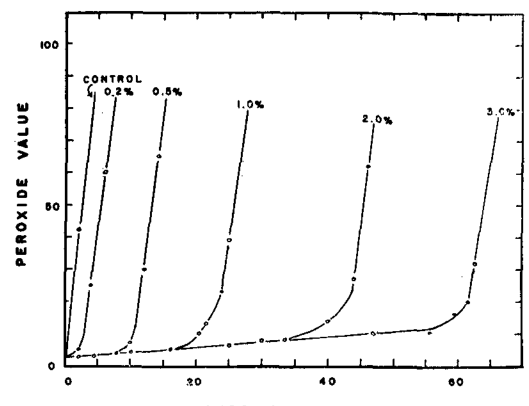

FIG. 1. The effect of various concentrations of a crude tomato lipid preparation on the stability of lard.

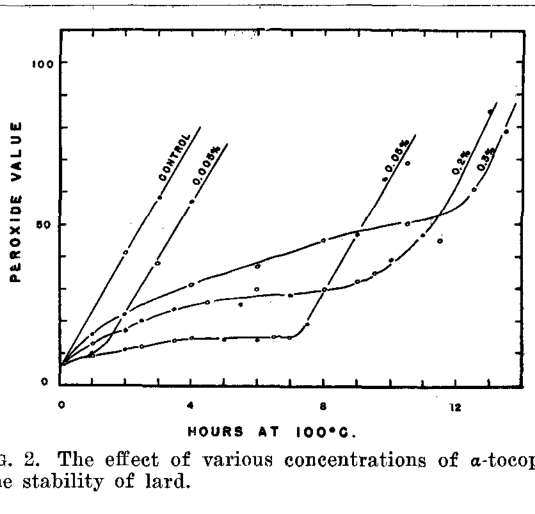

FIG. 2. The effect of various concentrations of α-tocopherol on the stability of lard.

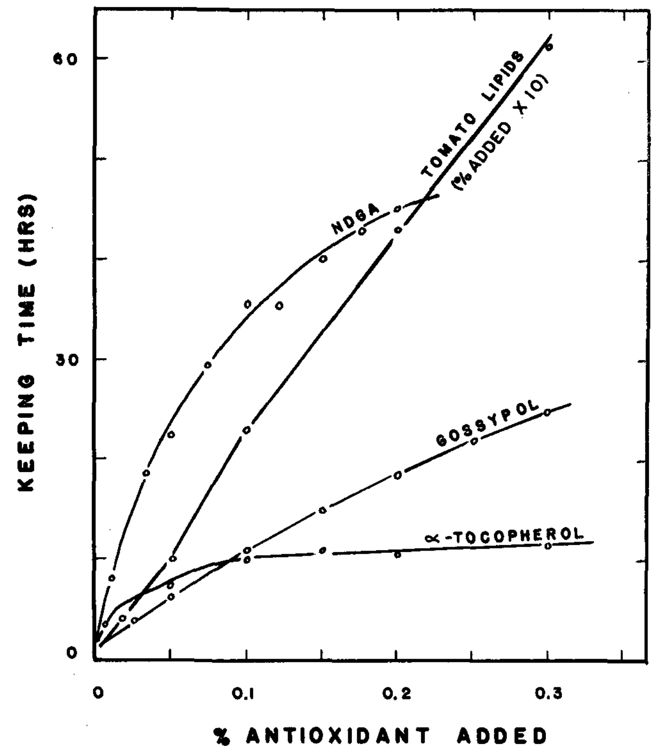

FIG. 3. A comparison of the antioxidant activities of crude tomato lipids, α-tocopherol, gossypol, and N.D.G.A. in lard at 100°C.

Much attention has been given in the past to the action of antioxidants in preventing peroxide development, and recently consideration has been given to the action of certain compounds in diminishing preformed peroxides. O'Leary (6) reported direct peroxide reduction by the use of di-thio fat antioxidants. Privett and Quackenbush (8, 9) found that both phosphoric acid and wheat germ phosphatides reduced the preformed peroxides of lard and formed a lipid-soluble complex.

The crude tomato lipids were tested for activity in reducing fat peroxides. When added at a 2% level to actively oxidizing lard of various peroxide values (Figure 4), the rapid reduction of an approximately constant fraction of the preformed fat peroxides was noted. It was also found that the length of the subsequent induction period varied inversely with the extent of peroxide reduction. The curves suggest that the peroxides of oxidizing lard contain an approximately constant percentage of a particular type of fat peroxide, which is immediately reduced by or combined with some component of the tomato lipid preparation. It is probable that the compounds in tomato lipids responsible for peroxide reduction are also connected with the prevention of peroxide accumulation and possibly with the lack of a pro-oxidant effect even at high extract levels. Schall and Quackenbush (12) reported that the pro-oxidant effect exhibited by high levels of pomiferin disappeared in the presence of a synergist.

[3] Kindly supplied by Procter and Gamble Company.

When larger amounts of tomato lipids were added to oxidizing lard (Figure 5), a rapid reduction of an almost constant fraction of the preformed fat peroxides was noted in the first hour. After this period of rapid reduction a period of relatively slow peroxide reduction was noted in the sample containing high concentrations of tomato lipids. This gradual

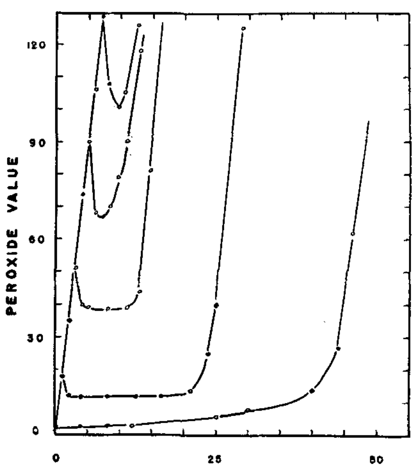

FIG. 4. The peroxide-reducing effect of tomato lipids added to oxidizing lard at a 2% level.

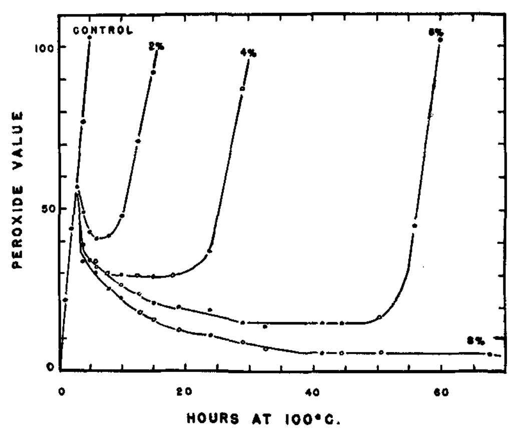

FIG. 5. The peroxide-reducing effect of increments of tomato lipids added to oxidizing lard.

decrease in peroxides may represent merely the thermal decomposition of the more stable peroxides as a result of prolonged heating at 100°C. However the higher levels of tomato lipids allowed the peroxides to fall to very low values and maintained this status for considerable periods.

The addition of alpha-tocopherol, gossypol, or NDGA to oxidizing lard (Figure 6) caused a temporary inhibition of peroxide formation but, in contrast to tomato lipids, showed no pronounced direct peroxide reducing action. The significance of this peroxide-reducing action is not yet clearly understood although the work of Privett and Quackenbush (8, 9) suggests that this effect might be the result of reactions between phosphoric acid or phosphatides with fatty peroxides to form complex polymeric substances.

The hexane-soluble tomato lipids prepared for fractionation studies exhibited an approximately linear relationship between the amount of lipids added and the keeping time (Figure 1). Extraction of the crude tomato lipids with absolute methanol concentrated the antioxidant approximately three-fold in the methanol-soluble fraction while the methanol-insoluble fraction exhibited only slight antioxidant activity (Table I). Dilution of the absolute methanol solution to 92%, followed by exhaustive extraction with petroleum ether, yielded two fractions: a) the petroleum ether fraction which exhibited some antioxidant activity and b) the 92% methanol fraction which exhibited negligible activity but showed marked synergistic activity when re-combined with the petroleum ether fraction (Table II).

That tocopherols might be responsible for the primary antioxidant activity of the tomato lipids was suggested by the solubility relation and the order of magnitude of the activity of the petroleum ether fraction (Table II). Analyses of the various tomato lipid fractions for tocopherols by the method of Parker and McFarlane (7) revealed that the original petroleum ether solution of crude tomato lipids contained 1.05% tocopherols calculated as *alpha*-tocopherol. Extraction of the gummy tomato lipids with absolute methanol removed 79% of the tocopherols. Dilution of the methanol solution to 92%, followed by exhaustive extraction with petroleum ether, concentrated 96.5% of the tocopherols in the petroleum ether fraction. This petroleum ether-soluble concentrate contained 4.1% of tocopherols.

For a further comparison with the activity of the petroleum ether soluble fraction, synthetic *alpha*-tocopherol (Merck) equivalent to the amount present in the petroleum ether aliquot (0.0073 g.) was combined with the same amount of the 92% methanol fraction used for the tests in Table II. The results (Table III) show that the petroleum ether fraction and an equivalent amount of synthetic *alpha*-tocopherol exhibited approximately the same antioxidant activity in lard at 100°C. when added alone or in

TABLE III

Synergistic Action of the 92% Methanol-Soluble Fraction of Tomato Lipids with *alpha*-Tocopherol in Lard (100°C.)

Material tested	Keeping time (hrs.)	Protective factor
Lard alone	2.0	1.0
Lard + 0.073% tocopherol	5.0	2.5
Lard + 0.073% tocopherol + 2% of 92% MeOH soluble fraction	90.0	45.0

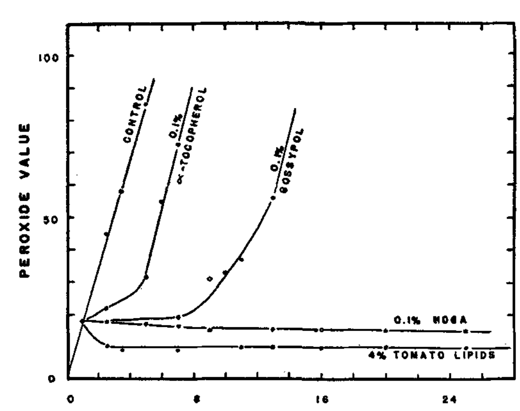

FIG. 6. A comparison of the peroxide-reducing effects of α-tocopherol, gossypol, N.D.G.A., and tomato lipids added to oxidizing lard.

combination with the 92% methanol (synergist) fraction. It is probable that the primary antioxidant activity of tomato lipids is caused chiefly by one or more of the tocopherols.

To elucidate further the nature of the "synergist" present in tomato lipids, several acidic compounds were tested (Table IV). Of these, phosphoric acid was the only one which with alpha-tocopherol exhibited synergistic action of the same order of magnitude as the 92% methanol fraction of tomato lipids. The exact effective concentration of the phosphoric acid in the lard sample is not known because of its low solubility.

TABLE IV

A Comparison of the Synergistic Action of Phosphoric, Citric, Ascorbic, and Succinic Acids with *alpha*-Tocopherol in Lard (100°C.)

Material tested	Keeping time (hrs.)	Protective factor
Lard	1.0	1.0
Lard + 0.073% α-toc.	4.0	4.0
Lard + 0.073% α-toc. + 0.05% H_3PO_4	58.0	58.0
Lard + 0.073% α-toc. + 0.05% citric acid	26.0	26.0
Lard + 0.073% α-toc. + 0.05% ascorbic acid	26.0	26.0
Lard + 0.073% α-toc. + 0.05% succinic acid	9.0	9.0

The phosphorus content of the 92% methanol fraction was determined colorimetrically by the A.O.A.C. method (11) after digestion with concentrated sulfuric and nitric acids. The results of this analysis indicated that the 92% methanol fraction contained 2.4% phosphorus. This means that the 92% methanol fraction added to the lard in the tests in Tables II and III was equivalent to adding 0.00132 g. of phosphoric acid. The solubility of the tomato synergist fraction in both petroleum ether and lard indicates that the phosphorus is not present as free phosphoric acid but rather that it exists as an organic phosphorus compound. The peroxide-reducing properties of tomato lipids is similar to that reported for phosphoric acid and wheat germ phosphatides (8, 9) and may be caused by this phosphorus-containing fraction.

Summary

Highly active antioxidants for lard were extracted from dried tomato fruits with petroleum ether. When added to fresh lard, the tomato lipids protected against autoxidation at 100°C. for long periods with little accumulation of peroxides during the induction period. When added at a 2% level to actively oxidizing lard (peroxide values 20 to 130), the tomato lipids effected a rapid drop of approximately 25% in titrable peroxides. When added at a level of 8%, this immediate drop was followed by a second, more gradual, drop to a constant low value (*ca.* 5), which was maintained for a long period at 100°C.

The antioxidant activity of the tomato lipids was separated into two fractions, one consisting of a primary antioxidant and the other of a synergistic (potentiating) substance. The primary antioxidant activity was accountable in the tocopherol content of the extracts. The synergistic activity was qualitatively similar to phosphoric acid, and the synergistic fraction was found to contain 2.4% phosphorus. However solubility data indicated the phosphorus was present in an organic form, probably phosphatides.

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Recent Developments in Screw-Press Operations

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THIS PAPER is primarily a brief summary of developments related to cooking and continuous mechanical screw presses which have in recent years improved the efficiencies which can be expected from this method of processing. It is helpful and interesting in discussing these developments to trace the history of mechanical processing techniques since some of the basic fundamentals which were learned several decades ago are responsible for several of the more recent developments.

When pressing of cottonseed was first attempted, no cooking was done; then gradually it was discovered that a moderate cooking or tempering resulted in a higher degree of extraction. From this was evolved the idea of using a steam-jacketed conveyor to warm the material en route to the pressing machinery. Other types of horizontal cookers with paddles having steam-jacketed outer walls were also developed and were found to provide definite improvements over methods which used no cooking at all. All of these methods however gave way and were superseded by the individual batch-type of cooking kettle, which at the turn of the century was the most widely used type of cooking apparatus. The advantage of this type of cooking was that it provided absolute control over temperature, moisture, and length of time during which the material was processed. From this type of cooking arrangement evolved the stack cooker which super-imposed one kettle on another, providing greater capacity and greater control. This type of cooking arrangement was readily adapted to the continuous process by using automatic floating-type gates, which maintained the desired level of meats in each kettle continuously and thereby provided the exact control over the cooking process which is required for an efficient operation.

It is interesting to observe that when continuous mechanical presses were first introduced, processors labored through the same long cycle which they had experienced with hydraulic presses some years before with regard to cooking equipment. As with hydraulic presses, the first continuous mechanical presses were