The liquid and solid fatty acids were separately converted into methyl esters and systematically fractionated under vacuum. The percentages of the individual saturated acids in each ester fraction have been calculated according to the method of Baughman and Jamieson (5). The amounts of palmitic and C_{18} mono- and di-ethenoid acids and unsaponifiable in liquid esters are estimated on the basis of iodine values and saponification equivalents in conjunction with qualitative examination of each ester fraction. The results of the ester-fractionation are tabulated below.

Identification of Fatty Acids

Unsaturated acids. The oleic and linoleic acids were identified in the "liquid" ester fractions (L_1-L_5) in the form of their oxidation products (9:10 dihydroxy-stearic acid, m.p. 130°C.; 9:10:12:13 tetrahydroxy-stearic acid, m.p. 174°C.) obtained by the permanganate oxidation of the regenerated acids. Traces of palmitic acid (m.p. 59-61°) were isolated in the lowest boiling fractions (L_1-L_3) of liquid acid esters from light petroleum ether extracts of the oxidized product, followed by crystallization from aqueous alcohol. Further evidence of the presence of linoleic acid was obtained by the isolation of a tetrabromide m.p. 113-14° (which showed no depression on admixture with an authentic sample). No ether-insoluble hexabromide could be isolated, thus confirming the absence of linolenic acid in the liquid acids.

Saturated acids. The acids from each ester fraction were isolated and identified by their melting and mixed melting points.

Fractions

S_1-S_4: Palmitic acid, m.p. 59-61°; stearic acid, m.p. 69-71°.

S_5: Stearic acid, m.p. 68-70°; behenic acid, m.p. 78-80°.

S_6: Behenic acid, m.p. 78-80°, mean. mol. wt., 344.5 lignoceric acid, m.p. 78-80°, mean. mol. wt., 366.2.

The S.E. of the residue (S_6), which is in between that of methyl-behenate and methyl-lignocerate, clearly indicates their presence in this fraction.

TABLE III

Calculated Composition of Total Fatty Acids

	Solid	Liquid	Total	Excluding unsaponifiable
	%	%	%	%
	20.3	79.7	100.0	
Saturated acids				
Palmitic	10.24	2.43	12.67	12.74
Stearic	4.98	......	4.98	5.01
Behenic	3.62	......	3.62	3.64
Lignoceric	0.67	......	0.67	0.67
Unsaturated acids				
Oleic	0.79	22.71	23.50	23.63
Linoleic	......	54.01	54.01	54.31
Unsaponifiable	......	0.55	0.55	....

Examination of the Unsaponifiable Matter

The unsaponifiable matter obtained, prior to the liberation of the mixed fatty acids, when crystallized from absolute alcohol gave white crystalline needles m.p. 139-40°C. This appears to be sitosterol.

Summary

The fixed oil from the seeds of *Leucaena glauca*, Benth. (N. O. Leguminosae) has been studied for its component acids. The fatty acid composition, as determined by the ester-fractionation analysis, was found to be palmitic (12.74%), stearic (5.01%), behenic (3.64%), lignoceric (0.67%), oleic (23.63%), and linoleic (54.31%). The latter unsaturated acids are the major components.

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Metal Inactivation in Edible Oils by Carboxymethylmercapto Succinic Acid[1]

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INVESTIGATIONS with model compounds and the formulation of a theory to explain their activity in stabilizing soybean oil has led to the investigation of a new stabilizer possessing high activity. It is carboxymethylmercapto succinic acid and is relatively new to the chemical field.

Carboxymethylmercapto succinic acid, $HOOCCH_2$-$SCH(COOH)CH_2COOH$, has two carboxyl groups, alpha to the sulfur atom and one carboxyl group in the beta position. This arrangement of functional groups is ideal for the formation of chelate rings and the complexing of iron in an octahedral configuration (6, 7). Actually the molecule has one more carboxyl group than is necessary for complexing the iron, and it has been shown that this carboxyl group can be successfully esterified to achieve oil solubility and that the compound will still retain excellent metal-scavengering properties (15).

The preparation of carboxymethylmercapto succinic acid is described by Morgan and Friedmann (9) and by Larsson (4). This acid has recently been made available from production on a pilot-plant scale.[3] Es-

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[3] Evans Chemetics Inc., New York, New York. The name of this company is furnished for the reader's convenience and does not imply the Department's endorsement of its products over the products of another company.

ters of carboxymethylmercapto succinic acid have been prepared by Mulvaney, Murphy, and Evans (12). The monoester of the succinic carboxyls was prepared by a synthesis designed to achieve both metal-scavengering properties and oil solubility in our laboratory.

This publication presents the results obtained from a laboratory and pilot-plant investigation on the effectiveness of this acid in stabilizing liquid and hydrogenated soybean and cottonseed oils. Results also include a comparison in effectiveness with other metal deactivators now being used in the edible oil field.

Methods and Materials

The efficiency of a metal deactivator is measured by its effect in preventing the development of peroxides in the oil or shortening. Details of the methods employed in making these evaluations are presented in previous publications of this series (11, 14, 15). Organoleptic evaluations were made on most of the experimental runs because of the known odor and flavor problem which accompanies the use of many sulfur-containing compounds for edible purposes.

The liquid oils used in the laboratory tests were purchased as commercially refined but undeodorized oils. The pilot-plant tests were made on commercial crude soybean oils refined and deodorized in the pilot plant. The hydrogenated soybean oil was prepared and processed in our pilot plant from commercial crude oil. The margarine base oil was commercially prepared and processed from cottonseed oil.

Results and Discussion

The addition of the inactivator on the cooling side of deodorization (below 100°C.) resulted in a marked improvement in the stability of the oil. However the addition of 0.01% prior to deodorization showed absolutely no stabilizing effect. Table I shows the results from addition of the acid made on the warming up and on the cooling down side of deodorization. Carboxymethylmercapto succinic acid melts at 136° C. and is apparently stable at temperatures considerably higher than this. However it is completely inactivated when heated during a normal oil deodorization for 3 hours at 210°C. When suspended crystals of the acid are heated in an oil bath, bubbles are given off at temperatures near 195°C.: however in a sealed capillary, decomposition is not apparent at temperatures of 220°C. Sulfide and ethylenic linkages are isosteric and decarboxylation might be expected (2); also favorable conditions exist for the formation of anhydrides. Both reactions will hinder or destroy the compound's chelating ability.

TABLE I

Heat Instability of Carboxymethylmercapto Succinic Acid under Edible Oil Deodorizer Conditions

Samples concentration 0.01%	Peroxide values 8 hours A.O.M.	Index [a]
Added before deodorization	20	1.1
Control oil	23	
Added after deodorization	3.0	7
Control oil	21	
Added after deodorization	3.6	8.9
Control oil	32	
0.3 p.p.m. Fe added after	250	32
Fe and acid added after	7.8	

[a] Ratio of peroxide value of control divided by peroxide value of the oil containing inactivator.

Decomposition at deodorizer temperatures presents the problem of sulfurous odors and flavors being imparted to the oil. Shortenings containing 0.01% of carboxymethylmercapto succinic acid were heated for 5 minutes at 180° and at 200°C. before being submitted to the taste panel for flavor evaluation. The samples containing the inactivator scored higher than the control in both tests although a significant difference was not observed. The 180°-treated sample scored 4.3 against a control of 3.4, and at 200°C. the scores were 4.2 against 3.6. However, from a panel of 15, two people were able to pick out and identify a sulfurous or mercaptan odor in the treated samples of both tests. Another test which did not indicate a sulfur decomposition involved the evaluation of potato chips. The potato slices were soaked in an aqueous 0.10% solution of the mercapto acid for half an hour before frying. In this test no foreign odor or flavor was detected in the chips by the taste panel. Storage tests showed the potato chips treated in such a manner possessed improved odor and flavor stability.

Most tests were carried out at the 0.01% level because of the ease of comparison with other inactivators. Carboxymethylmercapto succinic acid however appears equally effective at concentration levels as low as 0.0025%. As is shown in Table II, concentrations as low as 0.001% show a beneficial effect although the maximum results are not obtainable at this level.

TABLE II

The Concentration Effect of Carboxymethylmercapto Succinic Acid in Stabilizing Soybean Oil

Sample	Initial flavor score	Peroxide values 8 hours A.O.M.	Index
CMS acid [a] 0.1%	....	1.8	19
CMS acid 0.01	....	2.0	17
CMS acid 0.005	....	2.1	16
Control oil		34	
CMS acid 0.005%	8.4	2.0	28
CMS acid 0.0025	8.7	2.0	28
CMS acid 0.001	7.7	33	2
Control oil	8.1	56	

[a] CMS acid = carboxymethylmercapto succinic acid.

The effectiveness of this acid in improving the flavor and oxidative stability of soybean oil is shown in Table III. A significant improvement in the flavor scores of the treated samples over that of the control

TABLE III

Organoleptic Evaluation of Carboxymethylmercapto Succinic Acid Treated Soybean Oil in the Presence and Absence of Copper

CMS acid 0.01%	0.1 p.p.m. Cu.	CMS acid 0.1 p.p.m. Cu.	Soybean oil control	Significant difference [a]
Initial Flavor Score				
8.9(0.3)[b]	3.4(0.2)	8.7(0.3)	7.9(0.3)	1**2**3†4 2**4*1†3
Flavor Score After 4 Days' Storage at 60°C.				
7.7(0.9)	2.5(0.3)	7.5(0.8)	6.1(1.8)	1**2**3**4[c] 2**4*1†3
Peroxide Values A.O.M. Conditions 8 Hours				
1.8	172	2.2	45	

[a] † = No significance.
* = Significance at 5% level.
** = Significance at 1% level.

[b] Peroxide values.

[c] Aged flavor scores are averages obtained from the six paired tastings. Significance is calculated from scores of each paired tasting.

is shown in the initial evaluation and also for that of the aged samples. The improvement in flavor scores is however more pronounced with the metal-contaminated samples. The extremes shown by the flavor scores of 7.5 and 2.5 indicate a highly significant improvement in the quality of the aged samples. The effectiveness of this material is further exemplified by the superior score of the metal-contaminated but protected samples as contrasted with the flavor score of the control sample. The oxidative stabilities shown by the peroxide values at the bottom of the table confirms the flavor data. The control of the peroxide development to a level of only 2.2 milliequivalents of oxygen per kg. of oil compared to the copper control of 172 is remarkable, considering the ease with which soybean oil oxidizes. These values give an oxidative index of 80, which is one of the highest observed for any metal inactivator.

Similar results have been obtained for cottonseed oil, peanut oil, and lard although the same degree of effectiveness observed for soybean oil is not always attained for oils of less unsaturation. Some of these results are shown in Table IV, which also includes data obtained from pilot-plant tests on the effectiveness of this acid. The low score of the control in the first pilot-plant test indicates an oil of low quality which was significantly improved in both flavor and oxidative stability. The other test on a high quality oil shows no improvement in the flavor score, but a significant improvement is shown in the oxidative stability. The prevention of peroxide development in unstabilized prime steam lard is shown by the peroxide levels after 6 and 9 hours under A.O.M. conditions. Greater differences would be expected for lard in the presence of both added antioxidant and added metals (10).

TABLE IV

Organoleptic and Oxidative Evaluation of Carboxymethylmercapto Succinic Acid Stabilized Oils

Sample	Initial flavor score	Peroxide values 8 hours A.O.M.	Index
CMS acid 0.01%	....	13	2.8
Control oil D (CSO)	....	37	
0.3 p.p.m. iron	....	264	
CMS acid + Fe	....	18	15
CMS acid 0.01%	....	4	3.0
Control oil (peanut)	....	12	
CMS acid 0.01%	7.4	8	9.2
Control oil pilot plant (SBO)	4.1	74	
CMS acid 0.01%	8.1	4	11
Control oil pilot plant (SBO)	8.6	45	

Sample	Peroxide values 6 hours	Peroxide values 9 hours
Prime steam lard		
CMS 0.01%	3.5	7.0
Control	6.6	180
0.3 p.p.m. iron	15.0	220
CMS 0.01% + Fe	4.5	12

The improvement in the initial flavor scores of hydrogenated oils treated with a metal deactivator is usually more pronounced than with liquid oils treated with an inactivator. In Table V are shown the organoleptic and the oxidative results for the evaluation of hydrogenated cottonseed oil. Similar results are shown in Table V for hydrogenated soybean oil. In both experiments the initial quality was significantly improved by deodorization in the presence of a metal inactivator. A considerable part of the improvement results from the removal of the typical hydrogenation odor and flavor found in all hardened oils. Repeated deodorization of a hardened oil will not raise the flavor score to as high a value as can be obtained through the use of a metal inactivator. Significant improvements in initial scores are shown for each of the six different metal inactivators shown in Tables VI and VII. Although the degree of improvement is perhaps more easily observed in the fresh sample, the results are statistically as good in showing improved flavor in the aged samples. Samples containing carboxymethylmercapto succinic acid score significantly better than

TABLE V

Organoleptic Evaluation of Hydrogenated Vegetable Oils Treated with Carboxymethylmercapto Succinic Acid in the Presence and Absence of Iron

CMS acid 0.01%	0.3 p.p.m. Fe	CMS acid + 0.3 p.p.m. Fe	Hydrogenated oil control	Significant difference[a]
		Cottonseed Initial Flavor Score		
9.3(0)[b]	6.9(0.3)	8.9(0)	7.2(0.2)	1**2**3**4 2†4**1†3
		Flavor Score after 7 Days' Storage at 60°C.		
7.3(1.0)	4.3(0.6)	6.4(1.6)	4.2(3.0)	1**2**3†4 2†4**1†3
		Peroxide Value A.O.M. Conditions 30 Hours		
14	280	17	37	
		Soybean Initial Flavor Score		
9.3(0.2)[b]	6.7(0.5)	8.8(0.2)	8.3(0.3)	1**2**3†4 2**4**1†3
		Flavor Score after 7 Days' Storage at 60°C.		
8.3(0.4)	4.2(1.3)	6.8(0.5)	4.6(2.2)	1**2**3**4 2†4**1*3
		Peroxide Values A.O.M. Conditions 30 Hours		
11	51	12	44	

[a, b] See Table III for explanation.

TABLE VI

Relative Effectiveness of Carboxymethylmercapto Succinic Acid in Stabilizing Hydrogenated Soybean Oil

Inactivator 0.01%	Initial flavor score	Aged flavor score 7 days	Peroxide values (A.O.M.-30 hrs.)	Index
CMS acid	9.3	8.3	11	3.6
Citric acid	8.8	6.7	18	2.2
Phytic acid	8.9	6.9	14	2.9
Chelidamic acid	9.3	6.9	13	3.1
Thiodiacetic acid	8.9	7.5	13	3.1
Phosphoric acid	9.0	5.8	14	2.9
Control av.	6.4	4.6	40	
CMS acid + 0.3 p.p.m. Fe	8.8	6.8	12	4.3
Citric acid 0.3 p.p.m. Fe	8.4	6.6	16	3.2
Phytic acid 0.3 p.p.m. Fe	8.8	6.6	17	3.0
Chelidamic acid 0.3 p.p.m. Fe	8.6	6.3	16	3.2
Thiodiacetic acid 0.3 p.p.m. Fe	8.7	7.2	17	3.0
Phosphoric acid 0.3 p.p.m. Fe	8.4	5.7	16	3.2
Control + 0.3 p.p.m. Fe (Av.)	5.7	4.3	51	

TABLE VII

Relative Effectiveness of Carboxymethylmercapto Succinic Acid in Stabilizing Cottonseed Margarine Oil

Inactivator 0.01%	Initial flavor score	Aged flavor score 7 days	Peroxide values (A.O.M.-30 hrs.)	Index
CMS acid	9.3	7.2	14	3.1
Citric acid	8.4	7.4	29	1.5
Phytic acid	8.1	5.7	13	3.4
Chelidamic acid	7.5	6.7	19	2.3
Control (av.)	7.1	3.9	44	
CMS acid + 0.3 p.p.m. Fe	8.9	6.4	17	16
Citric acid 0.3 p.p.m. Fe	8.6	6.6	24	11
Phytic acid 0.3 p.p.m. Fe	8.1	5.6	13	21
Chelidamic acid 0.3 p.p.m. Fe	8.6	6.3	18	15
Control + 0.3 p.p.m. Fe (Av.)	6.8	4.2	270	

the control, even in the presence of added metals, both initially and after 7 days' storage at 60°C.

As the degree of hydrogenation of soybean oil is increased, metallic contamination shows less and less effect on the flavor score. A comparison of the flavor scores in Table V shows that they are similar in all respects. The addition of iron has catalyzed the production of peroxides to a greater extent at 100°C. in the hydrogenated cottonseed oil than in hydrogenated soybean oil. Iron probably catalyzes both the formation of fatty acid hydroperoxides and also the breakdown of these peroxides to aldehydes and other products. At 100°C. peroxide formation is catalyzed to a much greater degree than is the breakdown of the peroxide. At 60°C. the results would indicate that the rate of peroxide breakdown must be catalyzed to only a slight lesser extent than is their formation. The peroxide values at the time of tasting, shown in the parenthesis, are lower for the iron-containing samples than they are for the control. In the hydrogenated cottonseed oil the peroxides are at the lowest level in the iron-contaminated samples. Obviously peroxide values do not indicate the flavor quality of shortenings nearly as well as they do for liquid oils. The effect of temperature and metals on peroxide formation and decomposition in oils is a well-known phenomenon (1, 3, 5).

The relative effectiveness of carboxymethylmercapto succinic acid compared to other metal inactivators in stabilizing hydrogenated oils is shown in Tables VI and VII. These six materials are excellent trace metal inactivators for glyceride oils. In each of the comparisons the mercapto acid has been outstanding although the results are not significantly superior to the results of the other inactivators. The superior quality of the hydrogenated oils treated with a metal inactivator either in the presence or absence of added metals is clearly shown by these results.

Experiments on the hydrogenation of carboxymethylmercapto succinic acid-treated oils have shown that catalyst poisoning is observed. Hydrogenation of soybean oil under selective conditions required 40 minutes to reduce the iodine value of the control from 132 to 75, and 60 minutes were required for the sample containing 0.01% of the mercapto acid.

The presence of trace metals is known to cause oxidation, discoloration, and the impairment of flavor and nutritional value of many foods. Vitamin preparations, enzymes, special and dietetic foods, drugs, and similar products are all subject to deterioration through trace metal contamination. Food ingredients and additives of improved stability can be prepared through the use of metal-sequestering agents. Quality improvement results through the removal of iron and copper from such products as vinegar, gelatin, Irish moss, various stabilizing gums, flavors, and essential oils (16).

The discoloration of soaps from trace metals is prevented through the use of metal inactivators, and for many industrial uses a product of the highest activity is of prime importance. Mattano and Hixon (8) have been granted a patent on the rust-preventing action of esters of this mercapto acid in turbine oils.

The acute toxicity of this acid is reported by the manufacturer to be very low (13). The LD_{50} was determined on a single dosage test by intravenous injection into white mice. The LD_{50} for the sodium salt of carboxymethylmercapto succinic acid was reported as 1.32 grams per kg. body weight and a value of 0.225 gram per kg. was obtained for sodium citrate. No data are available on the chronic toxicity of this particular acid.

Summary

Carboxymethylmercapto succinic acid was one of the most effective metal inactivators examined for use in glyceride oils. The effectiveness is based on oxidative A.O.M. peroxide tests and on the organoleptic evaluation of various types of oils and shortenings. Indexes as high as 80-fold have been observed in preventing peroxide development. Extensive taste panel evaluations have shown significant improvements in the initial flavor and flavor stability of oils and shortenings treated with this mercapto acid. Indications are that the material has an extremely low order of toxicity. Acute tests have shown that the toxicity of the sodium salt is less than that of sodium citrate.

The thermal instability to withstand deodorization temperatures and the possibility of the development of a mercaptan odor and taste in oils subjected to a high temperature is a serious disadvantage. Under some conditions the low order of fat solubility and the poisoning of hydrogenation catalyst would be detrimental in some oil-processing operations.

Carboxymethylmercapto succinic acid and its derivatives may find use in salad oils, special hydrogenated oils for candy, icing, etc., vitamin preparations, drugs, and similar products where excellent trace metal-inactivating properties are required and where exposure to high temperatures is not encountered.

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