

effects if the sliced fruit were dipped in a 0.1 to 0.2% solution of calcium chloride for 5 to 10 minutes. If the fruit had readings below 9 pounds, this treatment was progressively less effective and higher concentrations or longer periods of immersion adversely affected the flavor.

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## VEGETABLE OIL STABILIZATION

# Preparation and Evaluation of Two New Fat-Soluble Metal Inactivators

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Two new fat-soluble metal inactivators have been prepared and evaluated for stabilization of vegetable oils. One of the compounds, a mono-octadecyl ester of (carboxymethylmercapto)-succinic acid, is of the tridentate type, having two carboxy groups in either  $\alpha$  or  $\beta$  positions to the coordinating sulfur atom. The other, a mono-octadecyl ester of thiodisuccinic acid, belongs to the tetradentate class. Both compounds have the essential structure for effective chelation of metals. The tetradentate compound was not superior to the tridentate compound in stabilizing soybean oil against oxidative deterioration. The mono-octadecyl ester of (carboxymethylmercapto)-succinic acid represents a new type of ester of this acid. It did not impart an objectionable flavor to soybean oil and good solubility in oil was attained. The heat instability of the ester will limit its addition to the cooling phase of the deodorization. Good oxidative stabilities were obtained for the mono-octadecyl ester of thiodisuccinic acid in the usual concentration range employed for inhibitors. A significant improvement in the organoleptic flavor scores of the treated samples over that of the control was noted in both initial and aged samples. Again the disadvantage of heat instability limited the addition of the ester to the cooling phase of the deodorization.

SOYBEAN OIL is subject to deterioration by catalytic effects of pro-oxidant metals, such as iron and copper. A number of compounds, known as metal inactivators, have been and are being added to the edible oils to destroy the catalytic effects of these metals. At present the edible-oil industry lacks a metal-chelating agent which is nontoxic, does not impart undesirable odor, flavor, or color, and is heat-stable, readily available, and oil-soluble. The present investigation was undertaken in an effort to develop a fat-soluble metal inactivator more effective and satisfactory than any previously described.

Sulfur compounds of the tridentate class having two carboxy groups in either  $\alpha$  or  $\beta$  positions to the coordinating atom have been found very effective in stabilizing vegetable oils (7). Recently (carboxymethylmercapto)-succinic acid has been shown to possess high activity (7). This acid has two carboxy groups  $\alpha$  to the sulfur atom and one carboxy group in the  $\beta$  position. Actually, the molecule has one more carboxy group than is necessary for complexing the iron. It should be possible to prepare

an alkyl ester of this extra group with a long-chain fatty alcohol which might impart solubility in oil. However, it is important to esterify the proper carboxyl, so as not to lose the arrangement which permits the formation of chelate rings and the complexing of iron in an octahedral configuration.

Three types of esters of (carboxymethylmercapto)-succinic acid have been prepared by Mulvaney, Murphy, and Evans (5). Mattano and Hixon (3)

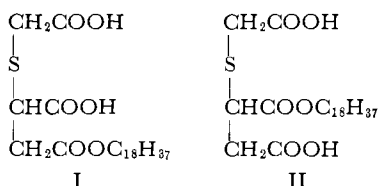
have been granted a patent on the rust-preventing action of these esters in turbine oils. These workers used either an ester of mercaptoacetic acid or a diester of maleic acid in their preparations. None of these combinations gives the best configuration for effective metal chelation. By employing mercaptoacetic acid and a monoester of maleic acid, a more desirable molecule is obtained, containing symmetrical carboxy groups in either  $\alpha$ - $\alpha$  or  $\alpha$ - $\beta$  positions to

Table I. Heat Stability of Mono-octadecyl Esters of (Carboxymethylmercapto)-succinic Acid and Thiodisuccinic Acid

| Ester, 0.01% Concn.              | Peroxide Values,<br>8 Hours A.O.M. | Oxidative<br>Index <sup>a</sup> | Heat Stability |
|----------------------------------|------------------------------------|---------------------------------|----------------|
| MECSA added before deodorization | 65                                 | 1.1                             | Unstable       |
| Control A                        | 72                                 |                                 |                |
| METSA added before deodorization | 30                                 | 1.2                             | Unstable       |
| Control B                        | 37                                 |                                 |                |
| MECSA added after deodorization  | 9.5                                | 5.7                             | Stable         |
| Control A                        | 54                                 |                                 |                |
| METSA added after deodorization  | 6.0                                | 5.5                             | Stable         |
| Control B                        | 33                                 |                                 |                |

<sup>a</sup> Ratio of peroxide value of control to peroxide value of oil-containing inactivator.

coordinating sulfur atom. Two possible products as a result of this addition are:



Product I is expected if polarizability effects are the determining factor. Addition of mercaptoacetic acid to a conjugate system is expected to take place as a 1-4 addition with rearrangements to give  $\beta$ -carboxymethylmercapto derivative of the ester (I).

Thiodisuccinic acid has been prepared recently and likewise found to be an effective metal inactivator (6). This acid is pentadentate and has two more carboxyl groups than are necessary for complexing iron. It should be possible to prepare an alkyl ester of one of these groups with a long-chain fatty alcohol in order to achieve fat-solubility. The approach was similar to that followed in the preparation of the mono-octadecyl ester of (carboxymethylmercapto)-succinic acid, except that mercapto-succinic acid replaced mercaptoacetic acid. Again there are two possible esters similar to I and II.

**Experimental** Octadecanol (270 grams, 1.0 mole) and maleic anhydride (98 grams, 1.0 mole) were heated on the steam bath for 4 hours. After cooling, the acid was neutralized with dry potassium carbonate and the product was dried in a vacuum oven at 100° C. The salt was then extracted in a glass extraction apparatus with hot absolute ethyl alcohol and subsequently the ethyl alcohol was distilled off. After drying in a vacuum oven at 80° C., there was obtained 382 grams (94% yield) of material melting at 140-142° C.

**Analysis.** Calculated for  $\text{C}_{22}\text{H}_{39}\text{O}_4\text{K}$ : K, 9.6. Found: K, 10.4.

**Mono-octadecyl Ester of (Carboxymethylmercapto)-succinic Acid [MECSA]**

Octadecyl potassium maleate (20.3 grams, 0.05 mole) and mercaptoacetic acid (4.6 grams, 0.05 mole) were heated under reflux with 100 ml. of ethyl alcohol for 5 hours. After standing at room temperature for several hours, a white solid separated and was collected on a sintered-glass funnel. After acidification with dilute hydrochloric acid and several washings with cold water, the product was dried in a vacuum desiccator over sodium hydroxide. The reaction gave an 89% yield of the mono-octadecyl ester melting at 88-89° C. and having a neutral equivalent of 238 (theory, 230).

**Analysis.** Calculated for  $\text{C}_{24}\text{H}_{44}\text{O}_6\text{S}$ : C, 62.6; H, 9.6; S, 6.9. Found: C, 63.1; H, 9.6; S, 6.0.

**Table II. Evaluation of Soybean Oil Treated with Mono-octadecyl Ester of (Carboxymethylmercapto)-succinic Acid**

| Ester Added   | Metal Added | Flavor Scores |                     | Oxidative Data                |       |
|---------------|-------------|---------------|---------------------|-------------------------------|-------|
|               |             | Initial       | After 4 days 60° C. | Peroxide value 8 hours A.O.M. | Index |
| 0.01% concn.  | 0.1 p.p.m.  |               |                     |                               |       |
| MECSA         | None        | 8.1           | 6.7                 | 5.2                           | 6.3   |
| None          | None        | 7.5           | 5.0                 | 33                            | ...   |
| MECSA         | Cu          | 7.3           | 5.5                 | 33                            | 5.5   |
| None          | Cu          | 3.0           | 2.3                 | 180                           | ...   |
| 0.005% concn. | 0.3 p.p.m.  |               |                     |                               |       |
| MECSA         | None        | 8.6           | 6.4                 | 3.0                           | 17    |
| None          | None        | 8.9           | 6.3                 | 51                            | ...   |
| MECSA         | Fe          | 5.4           | 4.6                 | 30                            | 3.8   |
| None          | Fe          | 5.3           | 3.3                 | 110                           | ...   |

**Table III. Evaluation of Oils and Shortenings Treated with METSA and MECSA**

| Ester Added            | Metal Added | Flavor Scores |                     | Oxidative Data   |       |
|------------------------|-------------|---------------|---------------------|------------------|-------|
|                        |             | Initial       | After 4 days 60° C. | Peroxide value   | Index |
| 0.01% Concn.           |             |               |                     |                  |       |
| Soybean oil            | 0.1 p.p.m.  |               |                     | 8 hours A.O.M.   |       |
| METSA                  | None        | 7.7           | 7.4                 | 5.8              | 5.5   |
| None                   | None        | 5.6           | 5.7                 | 32               | ...   |
| METSA                  | Cu          | 7.6           | 6.8                 | 13               | 6.3   |
| None                   | Cu          | 3.8           | 3.0                 | 82               | ...   |
| Cottonseed oil         | 0.3 p.p.m.  |               |                     |                  |       |
| METSA                  | None        | 8.5           | 7.6                 | 10               | 1.4   |
| None                   | None        | 8.8           | 7.4                 | 14               | ...   |
| METSA                  | Fe          | 8.0           | 6.9                 | 11               | 4.1   |
| None                   | Fe          | 8.5           | 5.8                 | 45               | ...   |
| Soybean oil shortening | 0.2 p.p.m.  |               |                     | 30 hours A.O.M.  |       |
| MECSA                  | None        | 8.4           | 7.7                 | 2.4              | 1.8   |
| None                   | None        | 8.4           | 5.9                 | 4.2              | ...   |
| MECSA                  | Fe          | 8.2           | 7.6                 | 3.2              | 1.5   |
| None                   | Fe          | 7.4           | 6.2                 | 4.7              | ...   |
|                        |             |               |                     | 150 hours A.O.M. |       |
| METSA                  | None        | 8.5           | 7.9                 | 16               | 1.3   |
| None                   | None        | 8.5           | 7.4                 | 20               | ...   |
| METSA                  | Fe          | 8.3           | 8.0                 | 16               | 1.2   |
| None                   | Fe          | 8.1           | 6.7                 | 19               | ...   |
|                        | 0.1 p.p.m.  |               |                     |                  |       |
| MECSA                  | None        | 8.7           | 7.8                 | 15               | 1.2   |
| None                   | None        | 8.8           | 7.0                 | 18               | ...   |
| MECSA                  | Cu          | 8.1           | 6.2                 | 18               | 2.2   |
| None                   | Cu          | 7.6           | 5.6                 | 39               | ...   |
| METSA                  | None        | 8.7           | 8.4                 | 16               | 1.1   |
| None                   | None        | 8.1           | 7.5                 | 18               | ...   |
| METSA                  | Cu          | 8.5           | 7.7                 | 16               | 4.8   |
| None                   | Cu          | 7.1           | 5.5                 | 76               | ...   |

**Mono-octadecyl Ester of Thiodisuccinic Acid [METSA]**

Octadecyl potassium maleate (19.5 grams, 0.05 mole) and mercaptosuccinic acid (7.5 grams, 0.05 mole) were heated under reflux with 100 ml. of ethyl alcohol for 3 hours. The ethyl alcohol was then removed by distillation, leaving a white residue. The residue was acidified with 125 ml. of dilute hydrochloric acid and extracted with diethyl ether. The ether was removed by distillation, leaving a white waxy residue. This product was purified by recrystallization from ethyl alcohol. Yield, 11.2 grams; melting point 51-53°; neutral equivalent, 184.3 (theory, 172.6).

**Analysis.** Calculated for  $\text{C}_{26}\text{H}_{46}\text{O}_6\text{S}$ :

C, 60.2; H, 8.9; S, 6.1. Found: C, 59.4; H, 9.3; S, 5.9.

**Oxidative and Organoleptic Evaluations**

Evaluation of a metal inactivator in edible oils was accomplished by two procedures—oxidative and organoleptic. The first test was its performance in a screening test in which 0.01% concentration of the compound was added to soybean oil just prior to deodorization. Effectiveness of the compound was measured by peroxide development in the oil after 8 hours under active oxygen method (A.O.M.) conditions (2). An index of efficiency was determined by dividing the peroxide value of the control sample by the peroxide value of the treated

sample. Thus an index of 5 means that the compound was effective in holding the peroxide value to one fifth that of the control. After the activity was established, the effect of concentration was determined, in both the presence and absence of added iron and copper. Organoleptic evaluations were made by a taste-panel procedure described by Moser and others (4).

Oxidation rates of deodorized soybean oil were determined manometrically at 60° C. in both the presence and absence of metals and metal inactivators. Details of this procedure will be described in a separate publication.

Solubility of the monoesters was determined by stirring small amounts in refined soybean oil and recording temperature at which a clear solution was observed. Both esters were soluble to the extent of approximately 2% at 60° C.

### Results and Discussion

Table I shows the results from addition of the monoesters on the warming and the cooling phase of deodorization. The addition of the inactivator on the cooling phase of deodorization (below 100° C.) resulted in a marked improvement in the stability of the oil. However, the addition prior to deodorization showed no stabilizing effect.

The effectiveness of the mono-octadecyl ester of (carboxymethylmercapto)-succinic acid in stabilizing soybean oil is illustrated in Table II. Taste panel results did not show a significant improvement between the flavor scores of treated sample at 0.005% concentration when compared with the control sample. On storage, however, the treated sample with iron was found to have a higher flavor score than the untreated iron sample. Good oxidative indices were obtained in both the presence and absence of added iron. At a concentration of 0.01%, the taste panel found a significant difference between the flavor score of the treated and aged sample when compared with the aged control. In the initial flavor evaluation, the treated and copper-contaminated sample showed a highly significant improvement over the untreated copper sample.

Table III shows the evaluation of soybean oil, cottonseed oil, and soybean oil shorten-

ing treated with mono-octadecyl ester of thiodisuccinic acid and mono-octadecyl ester of (carboxymethylmercapto)-succinic acid. For soybean oil, a significant improvement was noted in flavor scores of treated samples over that of the control in evaluations of both the initial and aged samples. The improvement in flavor scores was much greater, as would be expected, for the metal-contaminated samples. With soybean oil shortening, the observed improvement in the initial flavor was not so pronounced as that of soybean oil. No improvement was found in the initial flavor of cottonseed oil, although an improvement is shown on storage for the metal-contaminated sample. This particular lot of hydrogenated soybean oil proved exceptionally stable and peroxide values are low even after aeration under active oxygen method conditions for as long as 150 hours. With less stable shortenings, greater improvements are observed in the stability, and greater differences are shown in the peroxide values of the treated and untreated control oils. The tetradentate compound, mono-octadecyl ester of thiodisuccinic acid, does not appear to be superior to the tridentate compound, mono-octadecyl ester of (carboxymethylmercapto)-succinic acid, in stabilizing soybean oil against oxidative deterioration.

The oxidation curves in Figure 1 show that citric acid, mono-octadecyl ester of (carboxymethylmercapto)-succinic acid, and mono-octadecyl ester of thiodisuccinic acid retard the rate of oxidation and give considerably longer induction periods than that of the control sample. This effect is even more pronounced with added metals, as shown in Figure 2. In the absence of added metals, the induction period and the retarded rates of oxidation are about equal for mono-

octadecyl ester of thiodisuccinic acid, mono-octadecyl ester of (carboxymethylmercapto)-succinic acid, and citric acid, even though the molecular weights of the esters are roughly twice that of citric acid.

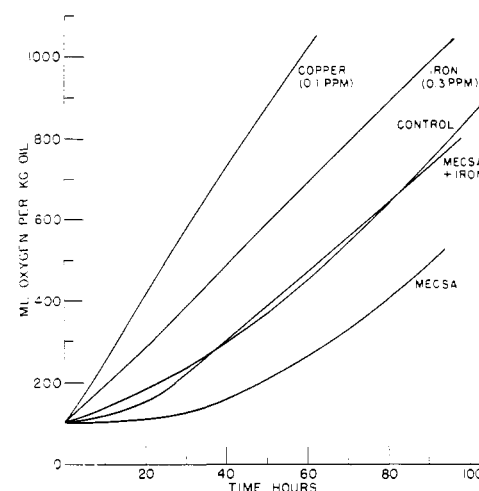
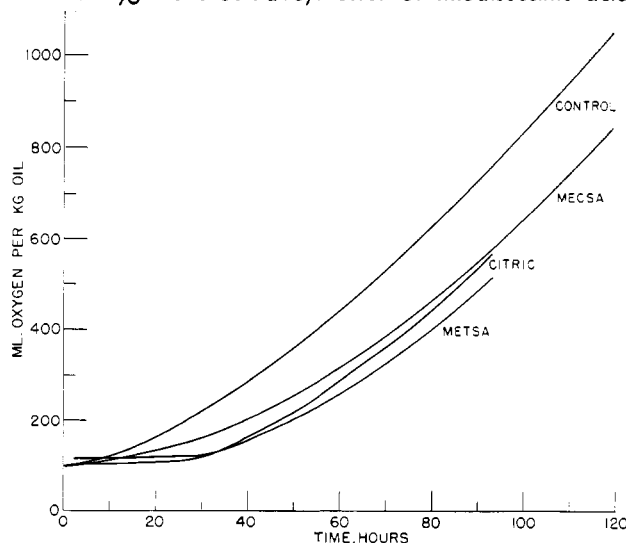


Figure 2. Oxidation rates of deodorized soybean oil at 60° C. in presence of 0.01% mono-octadecyl ester of (carboxymethylmercapto)-disuccinic acid, 0.1 p.p.m. copper, 0.3 p.p.m. iron, and 0.01% mono-octadecyl ester of (carboxymethylmercapto)-disuccinic acid plus 0.3 p.p.m. of iron

Figure 1. Oxidation rates of deodorized soybean oil at 60° C. in presence of 0.01% citric acid, 0.01% mono-octadecyl ester of (carboxymethylmercapto)-succinic acid, and 0.01% mono-octadecyl ester of thiodisuccinic acid



There is considerable disagreement in the fat field as to the suitability of various tests for evaluating antioxidants and metal inactivators. Although the A.O.-M. test is satisfactory when used as designed, it has not proved adequate for fats of high stability. Actual oxygen absorption tests are tedious and require more elaborate equipment, and the interpretation of the curve in terms of actual storage conditions is difficult. The induction period when a sharp break is obtained is probably the most meaningful of stability indices. However, with high stability fats such as vegetable oils and hydrogenated oils, sharp breaks may not occur in the rates of oxygen uptake. With citric acid, mono-octadecyl ester of thiodisuccinic acid, and mono-octadecyl ester of (carboxymethylmercapto)-succinic acid representative induction periods were obtained after 25 to 30 hours. The induction point of the control soybean oil was not sharp and in metal-contaminated oils the induction period is completely absent.

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## CONSTITUENTS OF CORN

# Development of Starch and Phytoglycogen in Golden Sweet Corn

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Starch and phytoglycogen have been isolated from golden sweet corn at various stages of development. During ripening, the starch content increases from 8 to 15% (dry basis), with an apparent increase in the linear-chain characteristics of the starch. The phytoglycogen maintains the same molecular size during ripening, but increases from 7 to 30%. Occasional curdling of cream-style canned corn is attributed to coagulation of a heat-sensitive protein. In confirmation, the curd from coagulated canned corn analyzed 71% protein. This protein loses water solubility during field-drying of the corn, thereafter approaching the character of corn gluten. Ripe sweet corn offers a convenient source of pure and undegraded phytoglycogen, suitable for fundamental studies. The soluble protein is readily and completely removed by heat coagulation, and starch-free phytoglycogen has been prepared with a protein content as low as 0.04%. This avoids degradative treatment with trichloroacetic acid or hot alkali.

THE POLYSACCHARIDES OF SWEET CORN have been studied by several investigators in recent years. Morris and Morris (5) isolated a soluble polysaccharide which appeared to have properties similar to the animal glycogens of liver tissue and of various shellfish. Hassid and McCready (2) further characterized the product as glycogenlike in its chemical structure. Sumner and Somers (7) fractionated this soluble polysaccharide and suggested the name "phytoglycogen" to indicate its plant origin. Cameron (1) investigated the genetic control over the production of glycogen and starch type in several varieties of sweet corn.

Most of these studies were conducted on the dried corn and provide no evidence of the development of starch and soluble polysaccharide in the maturing corn. Some question may be raised regarding the purity and possible degradation of the phytoglycogen. For example, the phytoglycogen was separated from gelatinized or dissolved starch substance by fractional precipitation with glacial acetic acid, and there is no assurance that the glycogen is free from starchy carbohydrate. Protein was removed either by treatment with 10% trichloroacetic acid or by prolonged digestion in hot 60% caustic soda, with the possible hazards of hydrolytic or oxidative degradation.

It was thought that certain aspects of commercial corn canning might be rationalized by a better understanding of the nature and influence of the phytoglycogen and sweet corn starch. For example, the content of linear fraction in the natural sweet corn starch might be expected to influence the gelatinization and swelling properties of the starch granules and hence the cooking time and consistency of the canned corn. In the canning of cream-style corn, commercial food-grade corn or wheat starch is usually added to impart a smooth creamy consistency. In this type of product, sometimes an undesirable curdling occurs during pressure cooking, accompanied by syneresis of a thin liquid phase. The question has been raised whether this curdling is due to the sweet corn itself or to retrogradation of the added starch.

The purpose of the present investigation was threefold: to isolate and characterize the starch and phytoglycogen fractions, to trace the development of these substances during maturing of the corn, and to determine the cause of curdling during canning.

### Materials and Methods

Studies extended over 2 years. During the 1951 growing season, samples of green corn were furnished through the

courtesy of the California Packing Co., Rochelle, Ill. During 1952, samples were supplied by the Green Giant Corp., LeSueur, Minn. These sweet corn varieties are special strains developed by the individual canner, but they may be generally described as first-generation single-cross golden hybrids. The corn samples were successively picked at several stages of development ranging from completely immature (white kernels, small and incompletely developed) to overmature (full yellow kernels, somewhat "doughy" in consistency). The term "mature" as here used refers to that stage of ripening which is most suitable for canning purposes. Without husking, the ears were frozen in dry ice immediately after picking and maintained in a frozen state until processed within 2 or 3 days. The ears were thawed out at room temperature, husked, and wiped dry of surface moisture. Each sample consisted of 10 to 30 ears, visually selected for uniform representation of the desired stage of maturity. The total sample was weighed, the kernels were sliced off, the cobs were scraped, and the percentage of kernels was determined as a relative index of maturity. Dry substance on the kernels was likewise determined, though these values are necessarily influenced by the freezing and thawing of the corn.

The cut kernels were ground for 5