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The Flavor Problem of Soybean Oil. XII. Nitrogen Coordination Compounds Effective in Edible Oil Stabilization[1]

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DURING the past several years a study of the effects of metal contamination on the flavor problem of soybean oil has been undertaken at the Northern Regional Research Laboratory. Early work emphasized that certain compounds were stabilizers and their effects could be explained, at least in part, because they were metal deactivators. These studies enabled us to arrange compounds in order of their effectiveness and to formulate nitrogen-containing compounds which are effective metal deactivators. Herein we explain how the principles of coordination as applied to chelation complexes may predict the effectiveness of certain types of compounds as metal deactivators.

Nitrogen-Containing Metal Chelates

Coordination complexes were recognized over 100 years ago, but the first satisfactory theory to explain the nature of those complexes was presented by Werner in 1893. Werner's theory postulates the existence of two types of valence, ionic and nonionic, and indicates that the same radical or molecule can be held by either or both types of valence. For each central atom there is a fixed number of nonionic valences, and these nonionic valences are directed to definite positions in space. In 1923 Sidgwick suggested that the nonionic or secondary valence consists of an electron pair furnished by the coordinating atoms; that these electrons are shared between the coordinating and central atom; and that the central atom tends to build up sufficient external electrons to attain the configuration of the next higher inert gas. The coordinate bond may be considered as a special type of covalent bond. The difference is that both electrons of the electron pair are donated by the same atom in the coordinate bond. In many coordination complexes the distance between the coordinating atom and the central atom is as short or shorter than most carbon-carbon double bonds, and sometimes the distance is shorter than a triple bond (9, 22). The bond distance is directly related to the stability of the bond which explains the high stability of many coordination complexes.

Theoretically any molecule or atom having a free electron pair can act as a coordinating group. The most commonly found coordinating groups contain oxygen, nitrogen, sulfur, carbon, or halogens. Arsenic and phosphorus are also known to form coordination complexes. Coordination complexes can be of cyclic or linear structure. Chelates or the cyclic structures are of interest for metal deactivation in oil. The simplest complexes are formed through the coordination of a metal ion and neutral ligand, such as the cobalt or copper amines. Complex formation affects the properties of the central atom, and it loses its individual properties becoming a part of the whole complex. The charge on the complex is determined by both the coordinating group and the central atom. Extensive use of coordination complexes has been made in analytical chemistry because of the changes they produce in the properties of metal ions.

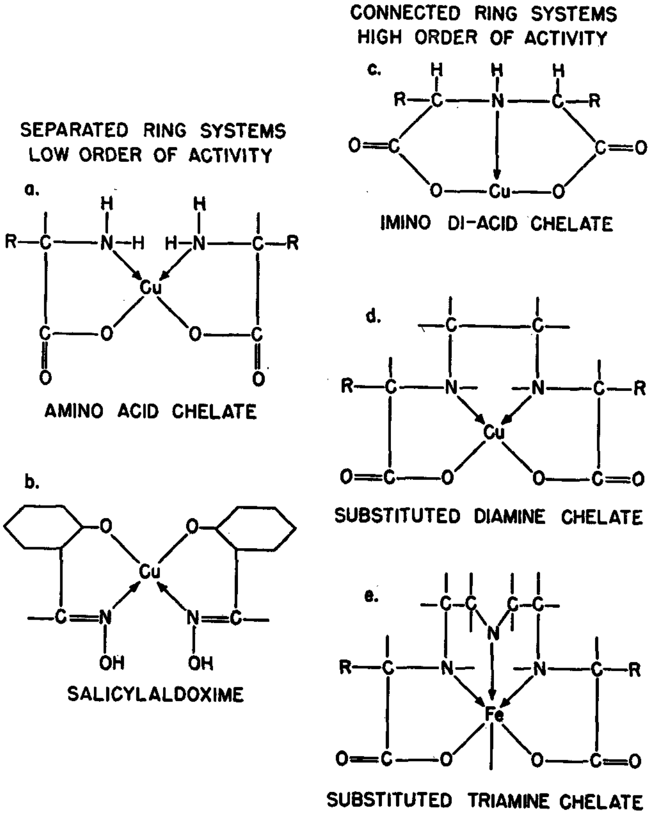

FIG. 1. Types of chelated ring systems.

Sidgwick (24) has classified the chelate type of compounds according to the nature of the bonds that

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are present in the cyclic system. Chelate rings may be formed by a) two primary valences, by b) one primary and a secondary, or by c) two secondary valences. These rings result through the ability of the central atom to form both types of bonds. The stable chelates formed are either 5- or 6-membered rings. Although 4- and 7-membered rings are known, they are rare and usually unstable. Diehl (12) in his review and classification of the chelate rings has used the terminology of Morgan. The number of bonding links between the organic molecule and the metal ion determine the classification of the ligand as a unidentate, bidentate, tridentate, or polydentate compound.

The maximum number of chelate rings obtainable through coordination with the central atom is four for the divalent metals occurring in the periodic table between sodium and bromine. This only happens when the ligand itself is of a complex cyclic structure as in the porphyrins and phthalcyanines. The synthesis of such a completely closed ring system utilizing carboxylic acids is probably not possible because of the large space requirements of the carboxyl groups. A coordination number of 4 however can also be satisfied by a system of three consecutive rings, having one atom plus the central atom in common in each adjacent ring as illustrated in Figure 1 (d).

Amino acids form a series of metal complexes through the primary salt valence and the basic nitrogen coordination valence. Albert (1) states that copper has the greatest affinity for amino acids and magnesium the least. Metals in the transition series are the most easily coordinated because of the ease of fulfilling the valence shell. The order of the strength of bonds for the divalent metals is usually Mg < Mn < Fe < Cd < Zn < CO < Ni < Cu < Pd (18). Copper phthalocyanine, a coordination complex containing four inner rings with copper at the center, is one of the most stable organic compounds known. It can be heated to a dull red heat without decomposition; it is unaffected by molten potassium hydroxide, by boiling hydrochloric acid, or by solution in concentrated sulfuric acid. In an inert atmosphere or under low pressure it can be sublimed at about 580°C.

The relative coordination strengths of amines decrease in the following order: $NH_3 > RNH_2 > R_2NH > R_3N$ (2). Cyclic amines such as the pyridines give very stable complexes in aqueous systems. However the stability of a chelate must be defined with respect to a specific reaction or measurement. The effective complexing of iron or copper in edible oil requires that two or more chelate rings be around the metal ion and that the complexing agent be of the tridentate type. Cyclic nitrogen compounds of the tridentate type possessing the required structure are also very effective.

Copper and Iron Chelates

We have previously reported the stabilizing effects shown by iminodisuccinic acid (11). Since then a number of amino acid derivatives have been studied which should give the same type of metal chelates (20). The formulas in Figure 1 demonstrate how chelates are formed from any α,α'-iminodicarboxylic acid, which yields two 5-membered rings. An α,β-iminodiacid would yield a 5- and 6-membered ring, and a β,β'-diacid two 6-membered rings. Amino acids other than α or β do not form as stable chelate ring systems, nor are their complexes soluble in chloroform. The order of stability of these compounds, as will be shown in later tables, is greatest for the combination of two 5-membered rings and least for the combination of two 6-membered rings. The combination of one 5- and one 6-membered ring lies in between.

Nitrogen is a very efficient coordinating atom for copper, but the presence of both in the same system does not necessarily result in the formation of chelate rings. For example, when ammonia is added to a solution of copper glycinate, a freezing point depression occurs with each increment of ammonia added. However 4 equivalents of ammonia can be added to a copper sulfate solution without lowering the freezing point. Copper glycinate forms stable chelate rings, as shown in Figure 1 (a), whereas copper sulfate adds ammonia through coordination but does not form chelate rings.

The effectiveness of metal deactivators increases as the number of connecting rings in the system increases. If the rings are not connected, as in copper glycinate, no stabilizing effect can be measured. When the separate ring systems of the amino acid complexes are connected as in an iminodiacid or by an ethylene bridge, a great increase is observed in the stability of the complex. In glyceride oil systems salicylaldehyde oxime has shown no effect; however it would be predicted that the ethylene diamine disalicylaldehyde oxime would show activity. This compound has not been investigated; but we have shown that other compounds containing acidic phenolic hydrogens are effective in forming coordination complexes.

Many types of structures have been postulated (23) for the ethylene diamine tetraacetic acid complex; each structure varies somewhat because of the four carboxyl groups present. The metal complex formed however is a 1:1 mol ratio of ligand to metal. One of the structures postulated for this complex consists of three 5-membered rings containing one atom of copper.

Although there are sufficient ionic bonds to hold two copper ions to one of ethylene diamine tetraacetic acid, there are not enough coordinating bonds to saturate the coordination valence of the of the two copper ions. It would thus appear that, in the presence of excess ethylene diamine tetraacetic acid, the equilibrium is shifted to the more inert and stable form of the coordination complex rather than to the formation of an equivalent salt of 1 mol of acid to 2 mols of copper. The greater stability of the coordination complex is attributed to the formation of the covalent bonds.

Ethylene diamine tetraacetic acid is equally effective in oils against both iron and copper. In aqueous systems the iron complexes are very stable at high acidity, being more stable than the ferrocyanide or the thiocyanate, but are unstable at high alkalinity. However compounds forming extremely stable iron complexes, even at a pH of 12, recently have been reported (4). The structure of these iron complexes has not been released. It could be postulated for iron, as in the previous discussion on copper, that, by increasing the number of coordination atoms, a more stable complex would be obtained.

Experimental Results

We have found that free acids are essential for effective metal deactivation in edible oils. Esters and salts, except ammonium salts, are not active. Phenolic hydrogens are sufficiently active to show acidic properties, and metal deactivation is possible with this type of compound when a suitable coordinating group is also present.

The efficiency of a metal deactivator is established by the reduction in peroxide formation as determined by the active oxygen method and by organoleptic evaluation of the processed oil (21). The usual test conditions embody measurements on four samples: the control fat, the fat plus added metal, the fat plus added deactivator, and the control fat plus both the metal and deactivator. The usual procedure was to add both the metal and the deactivator at the beginning of the deodorization. For metal deactivators which were not heat-stable, the deactivators were added to the oil on the "cooling down side" of deodorization. The oil was cooled to temperatures below 100°C. before the heat-sensitive deactivator was added. When this processing technique was employed, it is referred to as "added on the downgrade."

TABLE I

Effect of Ethylene Diamine Tetraacetic Acid and Oxalohydroxamic Acid on the Oxidative Stability of Soybean Oil

Sample No.	Deactivator concentration, %		Iron p.p.m.[a]	Copper p.p.m.	Peroxide value[b] 8 hr.—100°C.
A	Control				28.0
A	E.D.T. acid[c]	0.01			2.0
A			0.3		56.0
A	E.D.T. acid	0.01	0.3		7.0
B	Control				55.0
B	E.D.T. acid	0.01			2.4
B				0.1	64.0
B	E.D.T. acid	0.01		0.1	2.4
C	Control				21.0
C	Oxalohydroxamic	0.01			4.7
C			0.3		76.0
C	Oxalohydroxamic	0.01	0.3		48.0
C				0.1	81.0
C	Oxalohydroxamic	0.01		0.1	8.6
C	Citric	0.01			2.7
A	Citric	0.01			8.4

[a] Parts per million.
[b] Peroxide value determined after 8 hours under A.O.M. conditions.
[c] E.D.T. acid—ethylene diamine tetraacetic acid.

Table I shows the improvement in oxidative stability for soybean oil deodorized in the presence of ethylene diamine tetraacetic acid and oxalohydroxamic acid. Ethylene diamine tetraacetic acid is shown to be very effective against both added copper and iron. The sodium salt is widely used as a sequestering agent, and a patent (3) has been granted for use of this material in edible oil refining. The question of chronic toxicity is unsettled. However a recent bulletin (19) on the pharmacological and physicological properties of ethylene diamine tetraacetic acid and its salts would indicate that these materials do not have a high order of toxicity.

Hydroxamic acids have been patented by Dietrich (14) as compounds capable of protecting organic materials from deterioration by inhibiting the catalytic effects of trace metals. Oxalohydroxamic acid is not specifically mentioned in the patent although other dihydroxamic acids as adipic and malonic acid derivatives are discussed. The statement is made that the dihydroxamic acids appear to be most efficient. From the discussion already presented, we believe that oxalohydroxamic acid will be the most effective derivative for glyceride oils. Oxidatively, oxalohydroxamic acid is not very effective in the presence of added iron, but it is quite effective against added copper (Table I). Soybean oil treated with this acid in the absence of added metals also shows a marked improvement in oxidative stability.

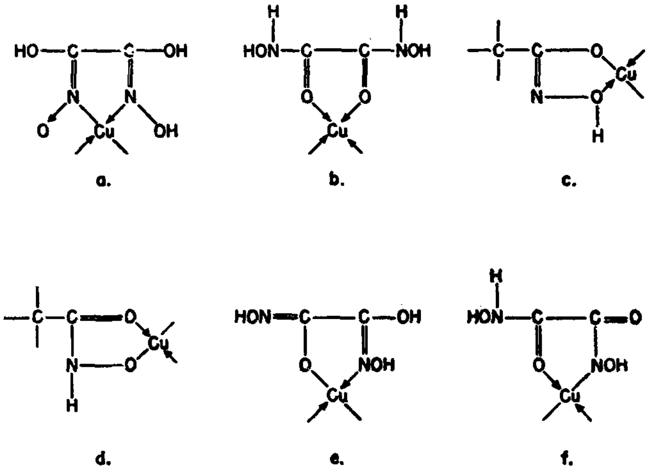


FIG. 2. Possible structures for the copper oxalohydroxmate complex.

TABLE II

Organoleptic Evaluation of Oxalohydroxamic Acid-Treated Soybean Oil

Oxalohydroxamic acid 0.01%	Iron 0.3 p.p.m.	Oxalohydroxamic plus iron	Control	Significant difference[a]
0 Time				
8.4 (0.3)[b]	4.8 (0.5)	6.3 (0.2)	8.1 (0.2)	1**2 * 3**4 2**4 † 1**3
After 4 days at 60°C.				
5.5 (1.7)	2.4 (1.9)			**
6.1 (1.7)		4.3 (4.7)		†
6.1 (1.6)			5.2 (1.5)	†
	2.9 (1.9)	4.6 (4.2)		**
	3.1 (1.8)		5.2 (1.5)	**
		4.0 (4.9)	4.8 (1.5)	†
A.O.M. conditions—8 hours				
Peroxide values				
4.4	76.0	48.0	21.0	

[a] † No significant difference; * Significant difference at 5% level; ** Highly significant difference at 1% level.
[b] Peroxide value at time of organoleptic evaluations shown in parenthesis.

Table II shows the organoleptic evaluation of oils treated with oxalohydroxamic acid. Taste data show that the acid itself does not adversely affect the flavor of the oil, but when used in the presence of added iron and copper, the scores of such oils are very low. The peroxides of oils that contain both the metal and hydroxamic acid and have been stored at 60°C. appear high. Oxygen-carrying metal chelates are known (8, 13), and if tests with oxalohydroxamic acid were repeated and similar results obtained, one might suspect that such a phenomenon has occurred with this complex.

It is possible to write several structures for copper oxalohydroxamate, depending upon whether nitrogen or oxygen serves as the coordinating atom and whether 5- or 6-membered rings are formed. The possibility of ionic resonance also exists and may be a contributing factor. Calvin (7) has investigated the stability of 21 copper chelates and has shown that the resonance effect is very important in determining the stability of these complexes.

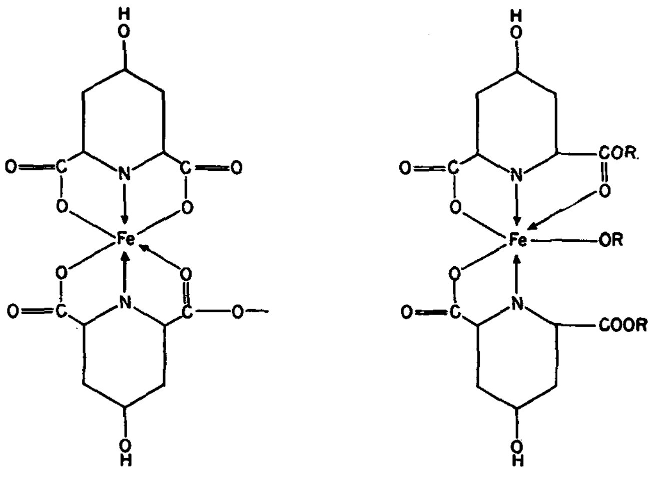
Among the possible structures for copper oxalohydroxamate are those shown in Figure 2. Formula a is commonly used to describe the structure of the dimethylglyoxime nickel complex. Tests with dimethylglyoxime in vegetable oils however were not successful. Thus it is believed that structure a is not predominant for copper oxalohydroxamate. Structure b represents the type of complex that might be expected from α-diketones, such as diacetyl, but since we have found no α-diketone to be effective, the importance of this structure is questioned. Experimental data with a monohydroxamic acid are not available at present. If the monohydroxamic acids are effective as claimed by Dietrich (14), then the structures represented in c and d may be of importance. On the other hand, ineffectiveness of the monohydroxamic acid together with the activity shown by the dihydroxamic acid would suggest that structures such as represented by e and f predominate.

Yale (25) reports that the oxime structure of $-\overset{|}{C}-NHOR$ and $-\overset{|}{C}{=}NOR$ do not give the ferric chloride test while structures of the type $-\overset{|}{C}-N(R)OH$ and $-C(R){=}NOH$ give the color test. This seems to indicate that for iron at least the free —NOH group is essential. The nitrogen atom in those compounds which no not give the ferric chloride test are available for coordination as Feigl (15) has shown. From this evidence it might be concluded that the oxalohydroxamic acid is probably not a nitrogen-coordinating complex but an oxygen type of chelate.

Other ring formations, particularly the 6-membered rings, are also possible, and their effect should not be overlooked. Factors other than those discussed here however might also account for the effectiveness of oxalohydroxamic acid. Burkin (5) discusses some of these factors in his review on the stabilities of complex compounds and the influence of both the ligand and the metal on the properties of the complex.

Chelidamic Acid

Chelidamic acid is a 4-hydroxypyridine 2-6 dicarboxylic acid. It offers the basic structure required for the formation of two chelation rings. Iron complexes of chelidamic acid are reported by Gorvin (16, 17), and a patent has been issued for the use of this complex as a therapeutic agent for the injection of iron. Of the 30 complexes tested, the iron triethanol amine chelidamic acid complex was the outstanding hemoglobin producer. Gorvin stated that crystalline dichelidamato ferric acid has been obtained. The octahedral structure requiring a coordination number of 6 has been assigned to the complex, in which an iron atom is associated with two tridentate acid molecules. The complex gives rise to two series of water-soluble salts, one containing a masked and the other a free carboxyl group. Absorption curves, acid dissociation constants, and the formulas shown in Figure 3 are given for the iron complexes.



CHELIDAMIC ACID CHELATES

FIG. 3. Chelidamic acid chelates.

TABLE III

Effect of Chelidamic Acid on the Oxidation of Soybean Oil

Sample No.	Chelidamic acid %	Iron p.p.m.	Copper p.p.m.	Peroxide value [a] 8 hr.—100°C.
D				39.0
D	0.01			5.5
D		0.3		91.0
D	0.01	0.3		21.0
D			0.1	104.0
D	0.01		0.1	21.0
Citric .01%				3.0
				A.O.M.
Hydrogenated				30 hr.—100°C.
E				44.0
E	0.01			13.0
E		0.3		72.0
E	0.01	0.3		21.0
Citric .01%				18.0

[a] Peroxide value determined after 8 hours A.O.M. conditions.

Table III shows the oxidative results obtained when soybean oil and hydrogenated soybean oil are treated with chelidamic acid in the presence of both copper and iron. This substance is equally effective against added iron and copper. When added to edible soybean oil, it is about as effective in inhibiting peroxide development as any nitrogen metal deactivator we have tested. Chelidamic acid is also very stable to heat. It melts with decomposition at 248°C., forming 4-hydroxypyridine. It is slightly soluble in water (637 parts of water), but we have obtained no data on its solubility in oil. When chelidamic acid is added on the downgrade of a deodorization, it is not as effective as when added at the beginning of a deodorization. This difference results probably because of the low solubility in oil and not because of heat activation.

Tables IV, V, and VI show the results of organoleptic evaluation of chelidamic acid in soybean oil, in

TABLE IV

Organoleptic Evaluation of Chelidamic Acid-Treated Soybean Oil

Chelidamic acid 0.01%	Copper 0.1 p.p.m.	Chelidamic plus copper	Control	Significant difference [a]
0 Time				
8.9 (0.3)	6.2 (0.3)	8.8 (0.3)	9.0 (0.3)	1**2**3 † 4 2**4 † 1 † 3
After 4 days at 60°C.				
7.1 (1.5)	2.8 (0.84)			**
7.2 (1.5)		5.8 (1.2)		*
7.3 (1.3)			6.0 (1.5)	*
	2.8 (0.86)	5.9 (1.3)		**
	2.6 (0.83)		6.4 (1.6)	**
		6.1 (1.3)	6.2 (1.7)	†
A.O.M. conditions—8 hours				
Peroxide values				
7.2	104.0	21.0	38.0	

[a] For explanation of symbols, see Table II.

TABLE V

Organoleptic Evaluation of Chelidamic Acid-Treated Hydrogenated Soybean Oil

Chelidamic acid 0.01%	Iron 0.3 p.p.m.	Chelidamic plus iron	Control	Significant difference [a]
0 Time				
9.3 (0.2)	5.4 (0.5)	8.6 (0.2)	5.4 (0.7)	1**2**3**4 2 † 4**1 * 3
After 7 days at 60°C.				
7.1 (1.0)	4.2 (6.1)			**
6.5 (1.1)		5.9 (0.80)		†
7.1 (1.0)			4.3 (8.1)	**
	4.0 (5.7)	6.6 (0.88)		**
	4.6 (3.9)		4.6 (7.2)	†
		6.5 (0.86)	3.8 (7.3)	**
A.O.M. conditions—30 hours				
Peroxide values				
13.0	63.0	16.0	44.0	

[a] For explanation of symbols, see Table II.

hydrogenated soybean oil, and in cottonseed oil, respectively. Marked improvements in flavor stability have been observed each experiment. In each evaluation the best oil contains chelidamic acid, and, in two tests, oil-containing iron plus chelidamic acid is better than the control. Chelidamic acid has shown a greater improvement in flavor scores of soybean oil containing added copper than those containing added iron. The flavor score of treated cottonseed oil also shows the improvement noted with soybean oil, and the inhibition of peroxide development is as great in cottonseed oil as it is in soybean oil. The pronounced improvement shown in the flavor scores, at zero time evaluation, of the hydrogenated oil is typical of a number of experiments. This oil was prepared and deodorized in the pilot plant and was not a high-scoring oil. It was redeodorized in the laboratory but still failed as an acceptable product. However the addition of chelidamic acid raised the quality of this shortening to one of the highest scoring products we have evaluated. The improvement is carried through even in the presence of added iron. Our experience to date would indicate that metal deactivators are just as important in shortenings as in liquid oils. It is difficult to explain the improvement in the 0-time scores of a twice-deodorized shortening by metal deactivation alone. A possibility exists that even under the best of deodorization conditions, some oxygen is present which can produce deterioration and result in the production of undesirable odors and flavors. When compounds present in concentrations of 1 p.p.m. or less are detectable through flavor, it is possible that the particular odoriferous materials are constantly being produced and removed during an ordinary deodorization.

TABLE VI

Organoleptic Evaluation of Chelidamic Acid-Treated Cottonseed Oil

Chelidamic acid 0.01%	Iron 0.3 p.p.m.	Chelidamic plus iron	Control	Significant difference [a]
0 Time				
8.6 (0.5)	6.2 (0.6)	8.8 (0.4)	8.5 (0.5)	1**2 † 3 † 4 2**4 † 1 † 3
After 4 days at 60°C.				
6.2 (2.7)	3.5 (5.0)			**
6.5 (2.2)		5.5 (3.4)		†
6.5 (2.3)			5.0 (6.0)	†
	3.5 (5.2)	5.8 (3.4)		*
	3.5 (5.1)		5.4 (5.2)	†
		5.4 (3.5)	3.5 (6.7)	**
A.O.M. conditions—8 hours				
Peroxide values				
15.0	72.0	24.0	39.0	

[a] For explanation of symbols, see Table II.

Iminodicarboxylic Acids

Iminodipropionic acid was prepared from the nitrile by the method of Chrodroff (10). A number of amino acid derivatives were prepared by the reaction of acrylontrile with individual amino acids (20). Hydrolysis of the product yielded an *α,β* type imino acid. Reaction of amino acids with chloroacetic acid yielded *α,α'*-imino acids, derivatives that should complex metals by means of 5-member chelation rings. These derivatives were effective as is shown by the oxidative data presented in Tables VII and VIII. A serious

TABLE VII

Effect of N-(β-Carboxy Methyl) *dl*-Leucine on the Oxidative Stability of Soybean Oil

Sample No.	Iron p.p.m.	Copper p.p.m.	Peroxide value [a] 8 hr.—100°C.
D, Control			38.0
D, Acid 0.01%			6.9
D, Control	0.3		96.0
D, Acid 0.01%	0.3		8.8
D, Control		0.1	104.0
D, Acid 0.01%		0.1	11.0
D, *Citric acid 0.01%*			3.0
Deactivator added on the cooling side of deodorization			
F, Control			34.0
F, Acid 0.01%			2.5
F, Control	0.3		72.0
F, Acid 0.01%	0.3		3.6

[a] Peroxide value determined after 8 hours A.O.M. conditions.

TABLE VIII

Effect of Amino Acid Derivatives on the Oxidative Stability of Soybean Oil

Deactivator, 0.01% concentration	Peroxide value [a] 8 hrs.—100°C.
α,α'-Imino (acetic acid) propionic acid	18.0
0.3 p.p.m. Iron	57.0
α,α'-Imino (acetic acid) propionic acid + 0.3 p.p.m. iron	19.0
Citric acid 0.01%	20.0
Control G	41.0
α,α'-Imino (acetic acid) propionic acid	2.9
0.3 p.p.m. Iron	50.0
α,α'-Imino (acetic acid) propionic acid + 0.3 p.p.m. iron	10.0
Control F	32.0
α,α'-Imino (acetic acid) propionic acid	17.0
α,β-Iminodipropionic acid	24.0
Control G	36.0
α,β-Imino (acetic acid) propionic acid hydrochloride	20.0
Control C	24.0
β,β'-Iminodipropionic acid	21.0
0.3 p.p.m. Iron	236.0
β,β'-Iminodipropionic acid + 0.3 p.p.m. iron	31.0
Control H	46.8
N-(β-carboxy ethyl) leucine	10.0
0.3 p.p.m. Iron	64.0
N-(β-carboxy ethyl) leucine + 0.3 p.p.m. iron	47.0
Control C	20.0
N-(β-carboxy ethyl) leucine	3.2
0.3 p.p.m. Iron	84.0
N-(β-carboxy ethyl) leucine + 0.3 p.p.m. iron	16.0
Control F	38.0
N-(β-carboxy ethyl) tyrosine	25.0
Control G	36.0
N-(β-carboxy ethyl) *dl*-aspartic acid	23.0
Control D	42.0
Effect on Cottonseed Oil	
α,α'-Imino (acetic acid) propionic acid	26.0
N-(Carboxy methyl) dl-leucine	27.0
N-(β-carboxy ethyl) *dl*-aspartic acid	22.0
β,β'-Iminodipropionic acid	39.0
Control	29.0
Chelidamic acid	15.0
0.3 p.p.m. Iron	72.0
Chelidamic acid + 0.3 *p.p.m. iron*	24.0
Control	39.0

[a] Peroxide value determined after 8 hours A.O.M. conditions.

disadvantage of these complexes is their instability to heat and the development of a typical burnt-hair or protein odor. As shown in Table VII, greater activity is obtained if the material is added on the cooling side of deodorization. The flavor scores of these oils were always low, and the use of such compounds in edible fat products appears questionable. The improvement in oxidative stability utilizing amino acid derivatives, like N-(carboxy methyl) *dl*-leucine, is sufficiently good however to warrant their use in stabilizing non-edible fat products. The α-amino acids have not given effective stability to edible oils when tested in the manner employed in our laboratory.

Table VIII shows that the amino acid derivatives did not have as great a stabilizing effect in cottonseed oil as did a number of the other complexing agents, such as chelidamic and iminodisuccinic acids.

Summary

Metal deactivating agents containing nitrogen as the coordinating atom have been developed for use in edible oils. The most effective compounds were those containing two carboxyl groups, α,α' to the nitrogen. Those containing β,β' carboxyls were less effective, and the efficiency of α,β carboxyls was intermediate. The activity is explained on the basis of the formation of metal chelation rings—complexes believed to be typical Werner's coordination complexes. The nitrogen atom may be an amine or a cyclic nitrogen. Complex coordination compounds can also be formed from acidic nitrogen compounds, such as hydroxamic acids, when the proper structure for metal chelation exists.

Chelidamic acid has been found to be a very efficient metal deactivating agent for both copper and iron. Imino α or β dicarboxylic acids show varying degrees of effectiveness toward the complexing of iron and copper. The greater the number of 5-membered chelation rings that are possible around the metal atom, the greater is the observed stability.

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A Method for the Determination of Linoleic Acid and Conjugated Dienoic Acids in Materials Containing Eleostearic Acids[1]

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THE scope of the American Oil Chemists' Society Tentative Method for polyunsaturated acids, Cd 7-48, is limited to the analysis of ". . . animal and vegetable fats containing only small amounts of preconjugated material . . ." (1). For analyses such as the determination of linoleic acid in tung oil, it is apparent that the equations used must be corrected for the effect of the strong absorption of the naturally-occurring triene conjugated glyceride constituents (eleostearins) which interfere with measurements of absorption after the alkali isomerization.

Hilditch, Morton, and Riley (5) proposed methods for the analysis of fats containing various combinations of fatty acid constituents including oleic, linoleic, linolenic, and eleostearic acids, by first measuring the eleostearic acid content before isomerization. This value was "then to be taken into account in calculating the percentage of linolenic and linoleic acids from, respectively, $E^{1\%}_{1\,\mathrm{cm.}}$ at 268 mμ after alkali-glycol treatment at 170°C. for 15 min., and $E^{1\%}_{1\,\mathrm{cm.}}$ at 234 mμ after alkali-glycol treatment at 180°C. for 60 min." No details or equations for the proposed corrections were given. In a subsequent paper Hilditch and Riley (6) say that the method had been found to be unreliable. These workers describe a method they found satisfactory which involves separation of the mixed fatty acids by low temperature crystallization and subsequent analysis of each fraction. Low temperature crystallization separations are not considered suitable for routine analysis of a large number of samples.

The problem of deriving equations for calculations from spectrophotometric data, which would adequately correct for interfering trienoic absorption and permit direct determination after alkali isomerization, was reinvestigated. Samples of oils containing both linoleic and eleostearic acids, but no linolenic acids, were used. Hilditch and Riley (6) have shown that eleostearic and linolenic acids very probably never occur together in the same vegetable oil. The eleostearic acid isomers were determined by a previously described method (8), oleic acid was found by use of a recently described procedure using hydrogen-iodine values (10), and total saturated fatty acids were estimated by difference.

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