and heated under vacuum with a Glas-Col mantle until the contents of the flasks reached the desired temperatures (1.5-2 hr.). Rendering in a boiling water bath for 2 hr. was used as a control condition. All mixtures were filtered under nitrogen. Added stability was obtained by the use of high rendering temperatures (Table V), but increased color and odor of the lard were apparent.

Bleaching and Deodorizing. It was found that heated samples with strong odor and color between F. A. C. standards 2 and 3 were bland and light-colored (F. A. C. less than 1) after one hour's deodorization at 200°C. in contact with 1% bleaching clay, and subsequent filtration with the addition of 1% diatomaceous earth. Heat stabilized lard retained some degree of stabilization after this bleaching and deodorizing procedure (Table VI). Simultaneous heat treatment and deodorization gave a bland lard of fair stability but dark color.

Discussion

The foregoing results raise doubts as to the usefulness of some of the patented processes for stabilizing fats by heat treatments. The keeping quality of the most stable lard (heat treated with whey powder) after necessary bleaching and deodorizing, although considerably greater than that of the control material, was appreciably less than that obtained with the commercial antioxidants tested (cf. Tables IV and VI).

Although the direct application of heat treatment to lard stabilization appeared to be impractical, the

observations are of wider interest since edible fats are heated extensively with other materials during extraction or rendering and when used in the preparation of foods. The temperatures attained in such processes are generally not as high as the ones reached in these experiments, and the materials usually contain considerable moisture. However the tendency for the natural antioxidants in the heated fats to be destroyed may be offset to some extent by stabilization resulting from heating in contact with protein and (or) carbohydrate material. When heating conditions are severe this effect could assume greater importance, but it might be accompanied by undesirable changes in palatability and nutritive value.

Summary

The storage life of lard was increased by heating it under vacuum to 288-296°C. with 0.1 to 0.5% of various protein and carbohydrate materials (dried whey was the most effective substance used), but undesirable color and odor were produced in the lard by the process, and bleaching and deodorizing to a bland, light colored end-product resulted in a loss of most of the added stability.

Acknowledgments

TABLE VI

The Effect of Bleaching and Deodorizing on the Stability of Heat Treated Lard

Treatment	Storage life, days at 60°C.
No heat treatment, no deodorization	1
No heat treatment, deodorized one hour at 200°C	1
1% whey powder, heated to 296°C., no deodorization	80
1% whey powder, heated to 296°C., then simultaneously bleached and deodorized one hour at 200°C	8
0.5% whey powder, simultaneously heated and deodorized one hour at 225°C	1
0.5% whey powder, simultaneously heated and deodorized one hour at 250°C	6
0.5% whey powder, simultaneously heated and deodorized one hour at 296°C	11

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Evaluation of the Twitchell Isooleic Method: Comparison With the Infrared Trans-Isooleic Method[1]

FRANK L. JACKSON and JOSEPH E. CALLEN, Procter and Gamble Company, Ivorydale, Ohio

TWITCHELL (1), in 1921, proposed a lead salt-alcohol method for the separation of solid and liquid acids. His method was an important improvement over lead salt-ether method first suggested by Gusserow (2) in 1828 and subsequently examined by numerous other investigators. Twitchell clearly pointed out that the iodine value of the twice crystallized solid fatty acid (SFA) fraction represented "isooleic acids," a term which broadly includes all the unsaturated acids appearing in the solid fatty

acid fraction and which is most often applied to the unsaturated solid acids of hydrogenated fats (3). Cooperative investigation of the Twitchell method by a group of A.O.C.S. collaborators (4) led to its adoption as the A.O.C.S. official method (5) for determining solid and liquid unsaturated acids and incidentally isooleic acids.

The Twitchell method has been found satisfactory for determining saturated solid and total unsaturated fatty acids and partially satisfactory for determining the unsaturated solid acids in fats and oils. However in order to understand clearly the chemical nature

of the Twitchell unsaturated solid acids and the limitations of the Twitchell method, a critical evaluation using independent methods is indicated. The need for development of a dependable and completely understood method for isooleic acid determination had been pointed out by other investigators including Stillman and Andrews (6).

The infrared method for trans-structure determination was developed largely in the past decade. Outside the fat and oil field various investigators (7, 8, 9, 10, 11, 12) have made use of the 10.36 μ trans-double bond absorption band for analyses. In the fat and oil field Rao and Daubert (13), Binkerd and Harwood (14), and Swern et al. (15, 16) have shown that trans-octadecenoic acids have strong absorption in the 10.36 μ region. In addition, Swern and co-workers set up a method for the quantitative determination of trans fatty material and compared briefly results of the Twitchell and infrared methods, with striking confirmation of the previously recognized failure of the Twichell method to detect all the isooleic acid present. There is still need of a more basic understanding of this deficiency in the Twitchell method, explanation of which is the primary aim of the present paper.

Unfortunately the term isooleic acid as used by various investigators has a somewhat obscure meaning and may or may not include all solid unsaturated acids. This, in some instances, may result in erucic acid being classified as isooleic acid. However in dealing with the bulk of oils commonly subjected to hydrogenation, namely, cottonseed and soybean oil, the term isooleic acids may be given a more specific meaning. In general the trans-octadecenoic acids, which have reported melting points of 44 to 63°C., are the truly solid unsaturated acids. It should be mentioned that elaidic acid, long recognized as the most prevalent isooleic acid, melts at 44°C. The cis-octadecenoic acids, on the other hand, are low melting (10-30°C.). With the exception of oleic acid they are thought to occur in very limited quantities in hydrogenated oils.

It is apparent then that "unsaturated solid acids" and trans-isooleic acids are synonymous whereas cis-isooleic acids are not classed as "unsaturated solid acids" in view of their low (10-30°C.) melting points.

Clearly then the superior isooleic method may be considered as the one giving the most accurate determination of the trans-isooleic acid content.

Experimental

Materials. The fatty materials used for standardization of the infrared method and for proof of its accuracy were carefully purified by accepted pro-

cedures. Analytical data for the purified fatty materials are given in Table I.

Lead Salt-Alcohol Isooleic Method. Official method Cd 6-38 of the American Oil Chemists' Society was used (5).

Infrared Spectrometric Trans-Isooleic Method. The infrared data were obtained, using a Perkin-Elmer Model 12-c spectrometer equipped with a sodium chloride prism and a lithium fluoride shutter. Determinations were made, using a fixed slit micrometer setting of .250 mm. and a 0.2 mm. sodium chloride cell. Fatty materials were examined as 10% solutions in carbon tetrachloride.

Though carbon tetrachloride has a weak band in the region of 10.36 μ and is not as satisfactory from a photometric standpoint as carbon disulfide, quite satisfactory results were obtained with this solvent using the "baseline" technique of Wright (17) and of Heigl, Bell, and White (18) for the measurement of optical densities. The disadvantages of the higher volatility, inflammability, and disagreeable odor of carbon disulfide were thus avoided. In addition, the "baseline" technique makes unnecessary a solvent absorption measurement for each determination.

An independent working curve was developed for each type of sample encountered, namely fatty acids, methyl esters, and triglycerides. Examination of "baseline" optical densities at 10.36 μ for pure oleic and stearic acids, revealed them to be small and equal for all practical purposes. Thus no correction was necessary for samples containing saturated materials.

Since the absorption coefficients of the pure trans-isomers for each of the three types of materials, as determined by the "baseline" technique, are not identical due to differences in background absorption, it follows that the triglycerides and methyl esters must be essentially free of fatty acid in order to obtain accurate results. An additional interference which has been encountered is absorption at 10.2 μ caused by conjugated diene. However in normal hydrogenated oils this interference is negligible.

It is recognized that trans-9-trans-15-linoleic acid, if present, would cause an error in the estimation of trans-isooleic acid. It is assumed that such isolinoleic

TABLE I
Analytical Constants for Standardization Materials

Materials	Melting Point	Iodine Value [b]	Saponification Value [c]	Acid Value
Oleic Acid	......	89.3	198.3	......
Methyl Oleate	......	85.7	190.3	......
Triolein	5.1	85.2	......	0
Elaidic Acid	44.4	89.9 [a]	198.0	198.0
Methyl Elaidate	11.5	85.4 [a]	188.4	......
Trielaidin	42.2	85.1	......	0.1
Petroselinic Acid	30.1	88.0	198.8	196.5
Petroselaidic Acid	53.2	89.0 [a]	......	......
Methyl Petroselaidate	19.9	85.2 [a]	......	......
CSO Methyl Esters	......	114.2	194.3	.9

[a] These iodine values were obtained from hydrogen numbers.
[b] I. V. Theory: octadecenoic acid 89.9; methyl octadecenoate 85.6; trioctadecenoin 86.1.
[c] S. V. Theory: octadecenoic acid 198.6; methyl octadecenoate 189.3.

TABLE II
Infrared Trans-Isooleate Analyses of Synthetic Mixtures

Material	Trans-Material Added	Total Trans-Material Present	Infrared Trans-Isooleate	Infrared Error
	%	%	%	%
Methyl Elaidate	......	......	100	......
Methyl Petroselaidate	......	......	99	......
Elaidic Acid	......	......	100	......
Cottonseed Oil Me Esters	(IV=144)	......	.1	......
Hydrog. Cottonseed Oil FA's	(IV=75.7)	......	20.9	......
Hydrog. Soybean Oil FA's	(IV=86.7)	......	33.5	......
Me Elaidate/CSO Me Esters	5.0	5.1	5.0	−2.0
	15.0	15.1	14.8	−2.0
	25.0	25.1	24.6	−2.0
Me Petroselaidate/CSO Me Esters	3.9	4.0	3.8	−5.0
	7.9	8.0	7.8	−2.5
	14.7	14.8	14.8	0
	24.5	24.6	24.6	0
Elaidic Acid/Hydrog. CSO FA	8.8	27.8	27.8	0
	12.5	30.8	32.2	+4.5
	18.2	35.3	35.7	+1.1
	25.0	40.7	39.8	−2.2
	33.3	47.2	48.1	+1.9
Elaidic Acid/Hydrog. SBO FA	6.7	38.0	38.0	0
	14.3	43.0	41.9	−2.6
	27.7	51.9	51.6	− .6

acid arises from partial hydrogenation and isomerization of linolenic acid (19). In this case partially hydrogenated soybean and cottonseed oils (70-80 I.V.) would contain little if any isolinoleic acid in view of the low original linolenic content (6-8% and 0% respectively).

The accuracy of the infrared method for determining trans-isooleate material is indicated by the representative data in Table II. The usual variation in the infrared trans-isooleic results is about ± 1% of the actual isooleate material present.

Twitchell Isooleate Determinations

In evaluating Twitchell isooleic acid results, it is interesting to consider first the individual solubilities of various lead salts of fatty acids in alcohol. These data are shown in Table III. They are not conclusive but suggest that the Twitchell procedure should give fairly satisfactory results in the determination of trans-acids. As pointed out by previous investigators (16), petroselinic (Cis-6:7) acid could interfere, but this is not serious inasmuch as this acid would be found in very low concentration, if at all, in ordinary hydrogenated stocks which are normally subjected to Twitchell analyses.

TABLE III

Twitchell Solubilities of Isooleic and Saturated Acids

	M.P.	Solubility	Twitchell Recovery
	°C.	g./100 ml. EtOH	%
Stearic	69.6	.008	99
Palmitic	62.9	.020	97
Elaidic (9-trans)	44.5	.030	95
Petroselaidic (6-trans)	53.2	.027	96
Petroselinic (6-cis)	30.1	.222	73
Vaccenic (Synthetic 11-trans)	44.5	.034	95
"Vaccenic" from Tung Oil (trans-11 and -12)	39.0	.118	84
17-Octadecenoic	56.1	.050	93

Now that the Twitchell solubilities of individual isooleic acids are known, the next step is to determine the Twitchell recoveries of isooleic acids from simple mixtures. Perhaps the simplest mixture is the elaidic-oleic acid system. The data of Table IV support the conclusions drawn from Table III; namely, that the Twitchell solubility of elaidic acid is low. However it will be noted that the Twitchell SFA fractions are contaminated with about 5% of cis material.

In Table IV data of Swern et al. (16), as reported in a recent publication, are included. These investigators reported a decrease in Twitchell values with increase in selenium elaidinization time over one hour. They assumed this effect to be due to polymerization and/or hydrogenation during the selenium treatment.

The results of the present work show no evidence for any reaction, in the selenium treatment, except

cis-trans isomerization of the double bond. Distillation of the isomerized sample resulted in no change in the Twitchell or infrared isooleic values of the distillate, and there was no distillation residue. Furthermore iodine values of the original and selenium treated samples were essentially the same. It is evident then that selenium isomerization under carefully purified nitrogen does not result in polymerization or hydrogenation effects.

The next step in evaluation of the Twitchell isooleic method is to examine its behavior on known mixtures which are more complicated than the elaidic-oleic system just discussed. The mixtures to be considered are trans-ester-cottonseed ester, elaidic acid-hydrogenated cottonseed fatty acids, and elaidic acid-hydrogenated soybean fatty acids. The data, presented in Table V, show the Twitchell procedure to be subject to rather large loss errors, especially at low isooleic concentrations. It is noteworthy that elaidic acid added to hydrogenated oils is recovered in good yield. Figure 1, a graphical presentation of the Twitchell recovery of trans-esters added to unhydrogenated oils as recorded in Table V, indicates:

a) An initial solubility loss of lead isooleate, followed by a Twitchell recovery increasing in proportion to the amount of added trans ester.
b) A sharp initial increase in the percentage recovered of the total isooleic acids present, followed by a levelling off as the concentration of isooleic acids increase.

Figure 2, which shows the relationship between elaidic acid added to hydrogenated oils and Twitchell isooleic acid values, indicates:

a) Little or no initial lead elaidate loss through solubility in the alcohol solutions. This is reasonable in view of the large amount of isooleates already present in the hydrogenated basestocks.
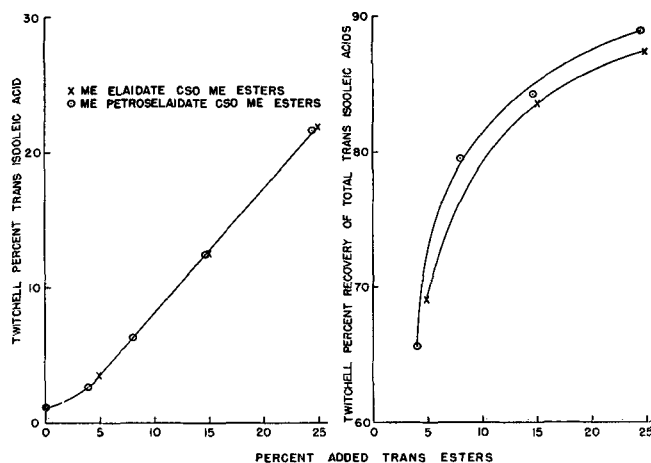

FIG. 1. Twitchell isooleic acid recoveries for trans-ester-cottonseed methyl ester mixtures.

TABLE IV

Twitchell and Infrared Analyses of Elaidic/Oleic Acid Mixtures

Material	Treatment	Iodine Value		Twitchell Isooleic	Infrared		Swern (16)	
		Original	After Treatment		Trans-Isooleic Total	Trans-Isooleic in Twitchell SFA Fraction	Twitchell Isooleic	Infrared Trans-Isooleic
				%	%	%	%	%
Oleic Acid	.3% Se; 1.5 hrs. at 220°C.	88.8	88.8	65.9	69.1	98.1	57.8 [a]	57.3 [a]
Oleic Acid	Same distilled	88.8	90.1	64.3	69.1	94.4	......	......
Oleic Acid	.3% Se; 3 hrs. at 220°C.	88.8	89.0	66.8	70.4	97.4	42.3	67.0
Oleic Acid	Same distilled	88.8	89.6	64.6	71.5	95.0	......	......

[a] Swern et al, used 1 hour heating with selenium.

TABLE V
Twitchell Isooleic Analyses of Synthetic Mixtures

Material	Trans-Material Added	Total Trans-Material Present	Twitchell		
			Trans-Isooleic Acid	Error	Recovery of added Trans-material
	%	%	%	%	%
Methyl-Elaidate	......	100[a]	......	......	......
Methyl Petros-elaidate	......	99[a]	......	......	......
Elaidic Acid	......	100[a]	......	......	......
Cottonseed Oil Me Esters	(IV=114)	.1[a]	1.2	+1200	......
Hydrog. Cottonseed Oil FA's	(IV=75.7)	20.9[a]	11.7	—44	......
Hydrog. Soybean Oil FA's	(IV=86.7)	33.5[a]	17.8	—47	......
Me Elaidate/ CSO Me Esters	5.0	5.1	3.5	—31	69
	15.0	15.1	12.4	—18	82
	25.0	25.1	21.9	—13	87
Me Petroselaidate/ CSO Me Esters	3.9	4.0	2.6	—34	66
	7.9	8.0	6.3	—20	80
	14.7	14.8	12.4	—16	84
	24.5	24.6	21.8	—11	89
Elaidic Acid/ Hydrog. CSO FA	8.8	27.8	17.9	—36	82
	12.5	30.8	21.7	—30	92
	18.2	35.3	26.3	—25	92
	25.0	40.7	32.1	—21	93
Elaidic Acid/ Hydrog. SBO FA	6.7	38.0	23.0	—40	96
	14.3	43.0	28.5	—34	92
	27.7	51.9	38.1	—27	91

[a] Determined by infrared analysis.

b) A nearly straight-line relationship between Twitchell per cent recovery of total isooleic acids present and elaidic acid added. This again indicates high recovery of the added elaidic acid.

Isooleic Analyses of Hydrogenated Oils. It has been shown that the infrared trans-isooleic method is accurate and is not influenced by the trans-double bond position. The Twitchell method seems to be fairly satisfactory for simple elaidic-oleic acid mixtures. It will also give acceptable results with simple trans esters and trans acids added to hydrogenated and unhydrogenated CSO and SBO oils. Up until now systems containing only one isooleic acid have
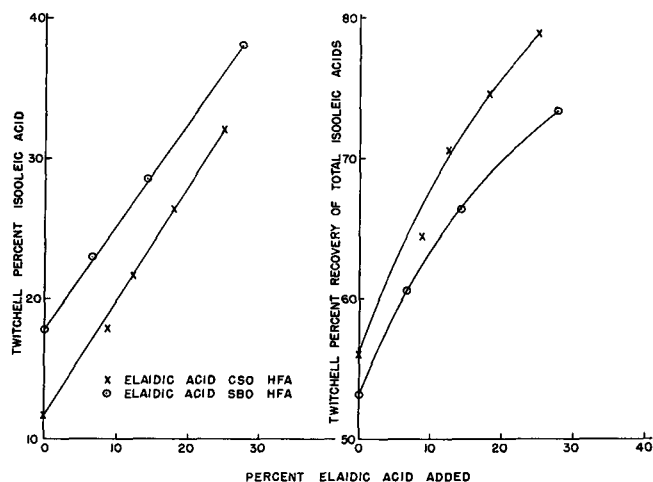

FIG. 2. Twitchell isooleic acid recoveries for elaidic acid-hydrogenated fatty acid mixtures.

been considered. Comparative analyses on hydrogenated oils which contain a probable minimum of six different trans-isooleic acids (Table VI) show the Twitchell method to be very inaccurate for such samples. It will be noted that for samples of a given oil hydrogenated under certain specified conditions, Twitchell errors are fairly constant at about —40%. However, with different oils using variable hydrogenation conditions, Twitchell errors vary from —27 to —60%.

Reasons for Twitchell Error. It was indicated previously that all of the known trans-isooleic acids of any double bond position are "solid unsaturated acids" and should therefore be detected by the Twitchell method whereas cis-acids have low melting points and should not be included. Furthermore the Twitchell method gives fair accuracy with simple known trans-acid mixtures but fails with hydrogenated oils.

TABLE VI
Isooleic Analyses of Hydrogenated Stocks

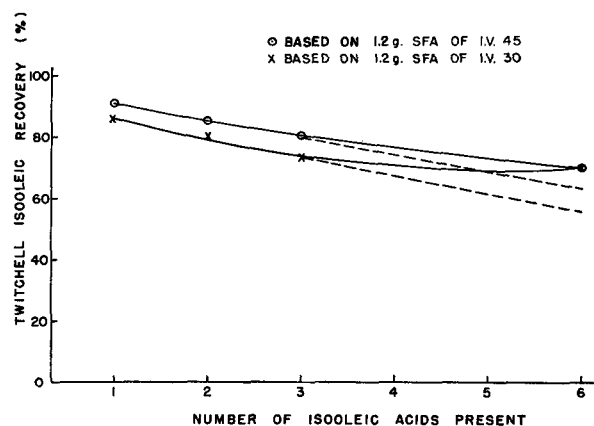| Material	I.V.	Isooleic		Twitchell Error
		Infrared	Twitchell	
Laboratory Hydrogenated Stocks (170°C.; .02% Ni)				
		%	%	%
Cottonseed Oil	75.7	20.9	11.7	—44
Cottonseed Oil	56.7	38.3	25.4	—34
Cottonseed Oil	56.5	36.1	21.9	—39
Cottonseed Oil	50.6	36.9	22.2	—40
Cottonseed Oil	46.5	34.2	18.5	—46
Cottonseed Oil	40.6	33.9	18.0	—47
Cottonseed Oil Me Esters	63.2	25.4	17.7	—30
Soybean Oil	76.1	24.4	15.5	—36
Olive Oil	41.3	28.3	18.2	—36
Olive Oil Me Esters	35.6	30.1	21.5	—29
Linseed Oil Me Esters	100.0	41.5	20.5	—51
Soybean Oils Hydrogenated at Various Temperatures and Pressures Using Numerous Catalysts				
Soybean Oil	86.7	33.5	17.8	—47
Soybean Oil	80.0	7.5	3.3	—56
Soybean Oil	77.2	38.5	28.1	—27
Soybean Oil	76.1	24.4	15.5	—36
Soybean Oil	73.9	16.8	8.0	—52
Soybean Oil	50.7	13.6	5.5	—60
Commercial Hydrogenated Stocks				
Shortening A	75.3	31.5	18.4	—42
Shortening A	75.2	31.5	18.2	—42
Shortening A	74.7	31.6	19.2	—39
Shortening B	71.1	29.1	17.1	—41
Shortening B	72.9	30.1	17.6	—42
Margarine Stock A	71.4	32.5	21.7	—33
Margarine Stock B	76.0	45.5	36.0	—20

FIG. 3. Twitchell isooleic recovery vs. number of isooleic acids present.

The explanation for the failure of the Twitchell isooleic acid method with hydrogenated oils lies in the increasing solubility of lead isooleate salts with increase in the number of individual isooleic acids present in the oil. Data supporting this point are found in Table VII and Figure 3. From the data presented, the following observations may be made:

TABLE VII

Twitchell Isooleic Determinations on Known Isooleate Mixtures

Fatty Acid Mixture	Lead Salt Solubility	Twitchell Recovery	
		Actual	Calculated (SFA wt. 1.2g.IV30)
	g./100 ml. *95% EtOH*	%	%
Elaidic	.030	95	87
Elaidic/Petroselaidic (1:1)	.047	93	81
Elaidic/vaccenic [a]/ Petroselaidic (1:1:1)	.109	85	65
Palmitic .5g Stearic .2g 9-trans- .5g 11-trans- .1g 12-trans- .2g Oleic 1.0g Linoleic 0.5g	.069 (SFA IV=44)	83	74
Isooleic Acids from Hydrogenated CSO [b]	.085	67	70
Isooleic Acids from Hydrogenated SBO [b]	.13	70	60

The lead salt-solubility data for CSO and SBO are low inasmuch as they were determined by a third lead salt crystallization of a regular Twitchell run. The more soluble lead isooleates were lost in the first two crystallizations.
 [a] Vaccenic acid from partially hydrogenated tung oil.
 [b] Hydrogenated CSO and SBO stocks are known to contain at least 6 trans-isooleic acids of different double bond position, based on unreported x-ray spectrophotometer studies of dihydroxy derivatives.

a) The solubility of lead isooleate salts increases in a more or less regular manner as the number of component isooleic acids increases.

b) The presence of "tung oil vaccenic acid," which is a mixture of mostly trans-11 and -12 acids, causes anticipated low recoveries.

c) The Twitchell recoveries with three and more individual isooleic acids approach the same order as those indicated by comparison of Twitchell and infrared isooleic analyses on hydrogenated stocks.

d) The broken lines indicate the anticipated Twitchell recoveries of six component mixtures.

Conclusion

The Twitchell and infrared isooleic methods have been compared. Assuming that all trans-isooleic acids are of equal significance as regards influence on shortening plasticity as "solid unsaturated acids," the infrared method is superior. This method has all the advantages of speed and accuracy of spectrophotometric methods. It handles simple and complex isooleate mixtures with equal ease in view of identical infrared absorption for trans-isomers of different double bond position.

The Twitchell method was shown, on close analysis, to have fair accuracy for simple trans-acid mixtures but give very low values (errors of 35 to 45%) with ordinary hydrogenated oils. The reason for the failure of the Twitchell method apparently lies in the increasing lead isooleate solubility with increase in the number of trans-isooleic acids.

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Phenolic Antioxidants for Carotene

E. M. BICKOFF, Western Regional Research Laboratory,[1] Albany, California

THE stability of carotene has been the subject of numerous investigations, most of which have been concerned with the rate of loss of carotene naturally present or added to food or feed ingredients. When antioxidants are incorporated into such systems, the interpretation of the results is often complicated by the possibility of synergistic action of the added antioxidants with those naturally present in the various materials comprising the provitamin A carrier (or by pro-oxidant materials which nullify the effect of the antioxidant). The results of studies, comparing antioxidant efficiency in natural and purified systems, indicate that the effectiveness of added antioxidants may be very different under various conditions of storage (1,2,5,9).

A wide variety of antioxidants is available for study, two of the most important groups being phenolic compounds and aromatic amines. The present investigation was primarily concerned with phenolic compounds and included aminophenols. A study of the effect of various substituting groups on the antioxidant efficiency of these compounds was undertaken

in order to obtain a better understanding of the relationship of antioxidant activity to structure. The results obtained were expected to be applicable to the stabilization of carotene in natural materials. For this work a simplified system comprising a solution of pure crystalline carotene in relatively inert solvent was employed. More than 100 phenolic compounds were studied.

Experimental

Commercial crystalline beta-carotene, repurified by precipitation from chloroform with methyl alcohol, was employed in this study. The substrate used for testing the antioxidants was a solution of this carotene in a highly refined medicinal mineral oil. The antioxidants in a suitable solvent, usually peroxide-free ether, were added to this solution and the solvent removed by bubbling a stream of nitrogen through the mixture. To facilitate the comparative evaluation of the antioxidants, the compounds were incorporated on an equivalent molecular basis rather than on a weight basis. Thus, in all cases, the test solution contained the added antioxidant in the proportion of one molecule of antioxidant to two molecules of carotene.

Stability Test. The details of the stability test have been published previously (11). Briefly, it consists