Carbonyls in Oxidizing Fat. VIII. Effect of the Pool and Klose Method on Monocarbonyl Precursors in Autoxidized Lard

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Abstract

Monocarbonyl compounds isolated by the Pool and Klose method greatly exceeded those obtained by a mild quantitative vacuum distillation method. As indicated by amounts of individual aldehydes, the Pool and Klose method of reaction with 2,4-dinitrophenylhydrazine on hydrated alumina partially decomposed hydroperoxides of unsaturated fatty acid glycerides and appeared to shift the normal balance of isomeric hydroperoxides. High proportions of unsaturated aldehydes were separated, and a new, highly unsaturated class in substantial amounts was present. Evidence, based on yield and type of aldehyde, suggests that in polyunsaturated fatty acids the hydroperoxide may preferentially form at the appropriate carbon furthest removed from the carboxyl group in the pentadiene system. This might partially explain the low proportions of alk-2,4-dienals in the free aldehydes of lard oxidized under mild conditions.

Introduction

THE ISOLATION AND MEASUREMENT of free carbonyl compounds in the study of flavor changes and deterioration in lipid-containing foods is important because of the major effect such compounds exert. Where the ubiquitous lipid oxidation has occurred, the potential carbonyl compounds measurable may be enormous in relation to the free carbonyl compounds present, and the system of precursors and capacity for change is complex and sensitive. An early consideration of this fact was given by Gaddis, Ellis and Currie (10) in an investigation of the distribution of volatile and nonvolatile carbonyl compounds in oxidized lard. Results indicated that a large part of the determinable carbonyls do not normally exist as such in the autoxidized fat. Since this work, new methods have been proposed for determining the free monocarbonyl compounds. In a comparison of several of the more promising methods, Gaddis et al. (12) noted an extreme sensitivity of monocarbonyl compound precursors to isolation conditions and a possible interaction due to stage of autoxidation. The methods are complex and time-consuming, and attention has been given to simple and rapid methods. For example, the Pool and Klose method (21) of reacting the oxidized fat with 2,4-dinitrophenylhydrazine on hydrated alumina, which has been modified by Keith and Day (14), for the determination of free monocarbonyl compounds. This method has been used by Day and Papaioannou (1) for the determination of free monocarbonyl compounds produced in irradiated milk fat. However, the early work on method comparison by Gaddis et al. (10) showed that the monocarbonyl compounds isolated by the Pool and Klose method from oxidized lard were higher than those isolated by the Lea mild vacuum distillation method (16). The conclusion drawn then was that the difference must represent free nonvolatile monocarbonyl compounds if hydroperoxides and other precursors were not decomposed on the hydrated alumina. It has since developed that all monocarbonyl compounds formed in normally oxidized fat are volatile. Examination of the data of Keith and Day (14) for oxidized lard shows what appears to be unusually high monocarbonyl values and large alk-2,4-dienal proportions for the degree of oxidation. Lea and Jackson (16) have recently demonstrated that the Keith and Day modification (14) is invalid due to a partial conversion of hydroperoxides to monocarbonyl compounds. The present investigation was undertaken to determine the nature of the differences previously observed between volatile monocarbonyl compounds and those isolated when the oxidized lard was reacted on hydrated alumina with 2,4-dinitrophenylhydrazine.

Experimental

Solvents, Reagents, and Materials

Solvents, such as carbon tetrachloride, benzene, and ethanol, were made carbonyl-free by treatment with 3 g trichloroacetic acid and 4–5 g 2,4-dinitrophenylhydrazine per liter, refluxing 1 hr, and distillation. Lard was rendered a minimum length of time to a maximum temperature of 105C and filtered through cheesecloth into cans which were vacuum-packed and stored at −30C until used. One batch of lard was used in this study. The grade of alumina used was that specified by Pool and Klose (21) and Keith and Day (14), and its 15% hydrated form was prepared as indicated by these investigators.

Methods

Vacuum distillations were carried out as described by Lea and Swoboda (17) except the distillation was continued for 50 min at the maximum temperature of 50C and ultimate pressure of 0.2 mm. Reaction of this distillate with 2,4-dinitrophenylhydrazine was conducted as described by Gaddis et al. (10). Similarly, monocarbonyl hydrazones were separated by chromatography on hydrated alumina (10). Reaction of the autoxidized lard on hydrated alumina was made according to the procedure outlined by Keith and Day (14). Monocarbonyl derivatives isolated by the alumina reaction method were separated from the fat by the Seasorb method of Schwartz, Haller, and Keeney (23) and further purified by the ion exchange resin method of Schwartz, Johnson, and Parks (22).

Autoxidations of lard were carried out on thin layers at 23C exposed to artificial light. During this process the lard was mixed daily. Samples were taken for analyses after 14, 18, 21, 24, and 28 days' storage. All determinations on an oxidized sample were set up simultaneously. At the least, duplicate determinations, and usually more, were made for all monocarbonyl isolations. Vacuum distillations were made on 10.00 g of sample, and Pool and Klose isolations on from 4.00 to 1.00 g, depending on the degree of autoxidation. However, all results were calculated on the basis of 10.00 g. Absorbance values at λ max represent the amount of 2,4-dinitrophenylhydrazones in 100 ml of carbon tetrachloride. Suitable corrections for blanks were made. Isolated monocarbonyl derivatives were separated by paper chromatography into classes and individual compounds by previously reported methods and applications (2,3,9,11). The Pool and Klose method was reported by Keith and Day (14) to give incomplete recoveries of the classes

TABLE I

Differences in Classes of Monocarbonyls Isolated from Autoxidized Lard

Class	Peroxide	Vacuum distillation		Pool and Klose method	
		Absorbance values λ max	Class composition %	Absorbance[a] values λ max	Class composition %
Alkanal	15	0.066	62.2	0.263	48.4
Alk-2-enal		0.020	18.9	0.105	19.3
Alk-2,4-dienal		0.020	18.9	0.113	20.8
Unknown		....	......	0.063	11.6
Alkanal	40	0.132	60.0	1.017	43.8
Alk-2-enal		0.046	20.9	0.340	14.6
Alk-2,4-dienal		0.042	19.1	0.620	26.7
Unknown		....	......	0.345	14.9
Alkanal	78	0.223	65.1	1.604	45.1
Alk-2-enal		0.072	20.9	0.466	13.1
Alk-2,4-dienal		0.048	14.0	1.055	29.6
Unknown		....	......	0.433	12.2
Alkanal	159	0.484	69.7	3.300	46.3
Alk-2-enal		0.128	18.5	0.775	10.9
Alk-2,4-dienal		0.082	11.8	1.838	25.7
Unknown		....	......	1.218	17.1
Alkanal	252	0.776	71.7	4.499	47.4
Alk-2-enal		0.217	20.0	1.140	12.0
Alk-2,4-dienal		0.089	8.3	2.497	26.3
Unknown		....	......	1.353	14.3

[a] Corrected for deficient class recoveries.

of monocarbonyl compounds. Corrections as recommended by Keith and Day (4) allowing for recovery of 75% alkanals, 65% alk-2-enals, and 60% alk-2,4-dienals were made in the calculations. Peroxide values were determined on 0.20 g samples by the method of Kenaston et al. (15) and expressed as meq per 1,000 g.

Results and Discussion

Volatile and Pool and Klose Aldehydes

The total monocarbonyl compounds represented by absorbance at λ max values showed much larger quantities were isolated by the Pool and Klose method. Differences between the two methods increased rapidly with the progressive formation of hydroperoxides and leveled off when the volatile aldehyde absorbance values were about 15% of the Pool and Klose figures. The differences were actually greater since correction for the incomplete reaction of the Pool and Klose method had not been made at this point. This would appear to indicate a hydroperoxide decomposing effect of the hydrated alumina reaction method. Higher wavelengths of maximum absorption suggested that the Pool and Klose monocarbonyl compounds contained more unsaturated components.

Differences in Monocarbonyl Class Composition

Separation into classes by paper chromatography, as shown in data in Table I, revealed large differences in class components. The alkanal class was the major one, but in much higher proportions in the vacuum distillation method. The alk-2,4-dienals in the volatile aldehydes were the minor class and increased very slowly with autoxidation. However, this class was the second largest in the Pool and Klose aldehydes and responded quickly to oxidation. A substantial new class was present in the Pool and Klose aldehydes. This component separated cleanly below the alk-2,4-dienals, and was of similar magnitude to the alk-2-enals. The new class had maxima at 390–95 mμ in carbon tetrachloride and 495 mμ in alcoholic alkali. In the alkaline medium there was no secondary maximum; and the characteristic color did not fade during 30 min, indicating the fraction was composed of unsaturated aldehydes (13). This evidence suggests that the unknown might be alk-2,4,6-trienals. The new class is apparently volatile, since it could be obtained by steam distillation (100C) of oxidized lard after passage through hydrated alumina. This indicates it is probably formed by the action of the alumina on oxidation products. A somewhat similar type of compound has been previously observed in the steam distillates (100C) of autoxidized methyl linolenate and linseed oil (4,11).

TABLE II

Micromole Relationships Between Methods

Classes	Peroxide	Vacuum distillation	Pool and Klose	% Volatile[a]
		μmoles/10 g	μmoles/10 g	
Alkanal	15	0.320	1,281	25.0
Alk-2-enal		0.074	0.378	19.6
Alk-2,4-dienal		0.054	0.303	17.8
Total		0.448	1.962	22.8
Alkanal	40	0.644	4.963	13.0
Alk-2-enal		0.167	1.232	13.6
Alk-2,4-dienal		0.113	1.662	6.8
Total		0.924	7.857	11.8
Alkanal	78	1.088	7.824	13.9
Alk-2-enal		0.261	1.689	15.5
Alk-2,4-dienal		0.129	2.827	4.6
Total		1.478	12.340	12.0
Alkanal	159	2.361	16.097	14.7
Alk-2-enal		0.464	2.809	16.5
Alk-2,4-dienal		0.220	4.925	4.5
Total		3.045	23.831	12.9
Alkanal	252	3.785	21.945	17.3
Alk-2-enal		0.786	4.131	19.0
Alk-2,4-dienal		0.238	6.688	3.6
Total		4.809	32.764	14.7

[a] Vacuum distillation values expressed as per cent of Pool and Klose values.

In Table II, the data for the three known classes of monocarbonyl compounds have been converted to μmoles per 10 g. Vacuum distillation values expressed as per cent of Pool and Klose values differed with the monocarbonyl class and changed with the degree of autoxidation. These values for alkanals and alk-2-enals decreased rapidly in the primary oxidation stage, then leveled off, and with advanced autoxidation tended to increase. This is attributed to a change in the composition and nature of the primary autoxidation products and may be related to the two oxidation stages observed by Gaddis et al. (12). The per cent of the alk-2,4-dienals that were volatile decreased throughout the experiment. Isolation of large amounts of alk-2,4-dienals by the Pool and Klose

TABLE III

Individual Alkanals Found in Autoxidized Lard

	Peroxide	μmoles/10 g C-2	C-3	C-5	C-6	C-8	C-9	C-11
Vac. distn.	15	trace	0.087	(0.045)	0.057	0.026	0.072	(0.033)
Pool & Klose		0.091	0.516	....	0.364	0.109	0.201	....
Vac. distn.	40	0.049	0.089	(0.029)	0.237	0.076	0.127	(0.037)
Pool & Klose		0.127	0.721	....	3.003	0.676	0.436	....
Vac. distn.	78	0.035	0.121	(0.103)	0.505	0.113	0.178	(0.033)
Pool & Klose		0.179	1.391	....	4.617	1.065	0.572	....
Vac. distn.	159	0.106	0.405	....	1.350	0.326	0.174	....
Pool & Klose		0.289	2.809	....	10.305	1.685	1.009	....
Vac. distn.	252	0.087	0.291	....	2.357	0.643	0.291	(0.116)
Pool & Klose		0.424	3.329	....	14.213	2.928	1.051	....

TABLE IV

Individual Alk-2-enals Found in Autoxidized Lard

		μm/10 g								
	Peroxide	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12
Vac. distn.	15	Trace	Trace	Trace	0.012	0.020	0.016	0.015	0.011	Trace
Pool & Klose		Trace	Trace	0.034	0.068	0.058	0.109	0.054	0.055	
Vac. distn.	40	Trace	Trace	Trace	0.030	0.032	0.043	0.023	0.028	(0.011)
Pool & Klose		Trace	Trace	0.218	0.345	0.212	0.260	0.106	0.091	
Vac. distn.	78	Trace	Trace	Trace	0.056	0.048	0.069	0.048	0.040	
Pool & Klose		Trace	Trace	0.303	0.403	0.295	0.372	0.138	0.178	
Vac. distn.	159	Trace	Trace	Trace	0.092	0.133	0.112	0.073	0.054	
Pool & Klose		Trace	Trace	0.638	0.677	0.484	0.638	0.217	0.155	
Vac. distn.	252	Trace	Trace	0.152	0.108	0.227	0.129	0.085	0.085	
Pool & Klose		Trace	Trace	0.854	0.028	1.028	0.717	0.291	0.213	

method is a notable point of these results. An increase in alk-2,4-dienals is usually associated with heat treatment (12). Even steam distillation (100C) increases the amount of dienal isolated. Gaddis and associates (7,8,11,12) have observed that heating in air a short period of time at 165C produced a large increase in steam volatile dienals accompanied by little increase in the alkanals. Typically, in oxidized lard under the conditions used, there are very small proportions of volatile or free alk-2,4-dienals (12). This may be due to either greater stability of dienal precursors, the presence of relatively small amounts of such precursors, or secondary oxidation of free 2,4-dienals (24). The action of heat suggests an increase in the amount of the hydroperoxide isomer precursors of the dienals. Similarly the observed effect of hydrated alumina may indicate either the decomposition of a reservoir of fairly stable dienal precursors present or isomerization of hydroperoxides to dienal precursors followed by their scission.

Individual Aldehydes

Each aldehyde class was separated by paper chromatography into individual compounds, and the amounts present were measured. Data for the alkanals, enals, and dienals are shown in Tables II, IV, and V, respectively. The hydrated alumina affected all the alkanal precursors. The differences in composition between the volatile and Pool and Klose alkanals, which were particularly wide in the early oxidation stage, indicated that this was an abnormal effect. The action on oxidation products from linoleate (C_6 alkanal) and linolenate (C_3 alkanal) was the greatest. Similar results were evident in the case of the alk-2-enals, with differences in composition due to higher proportions of C_6 enal from linolenate and C_7, C_8, and C_9 enals from linoleate (11). The alk-2,4-dienals had the largest composition differences. The C_7 and C_{10} dienals were the major compounds in the volatile dienals. However, the C_9 dienal was the major compound in the Pool and Klose dienals, and the C_{10} dienal was the minor compound, an unusual occurrence. The C_7 and C_9 dienals have been shown to come from linolenate and the C_{10} dienals from linoleate. The C_9 dienal is usually a minor component of the class (11). Arachidonic acid can be a source of C_6 alkanal and C_{10} dienal (5,24); but the absence of other characteristic compounds, such as the C_7 alkanal, indicates that this may not be much of a factor here. The hydrated alumina apparently acted in some unusual way on the linoleate primary oxidation products. Although C_{10} dienal was a minor component, it nevertheless was increased as much as ten times as a result of the Pool and Kloss reaction conditions. This indicates the presence of either stable C_{10} dienal precursors that were decomposed by the hydrated alumina and/or formation of such precursors and their decomposition by its action. The amount of such precursors, however, must have been relatively small. Theoretically there are three isomeric hydroperoxides possible in autoxidized linoleate. These are the nonconjugated 11 and conjugated 9 and 13 dienoic isomers which would yield C_8 alk-2-enal, C_{10} alk-2,4-dienal, and C_6 alkanal, respectively. The conjugated hydroperoxides are reported (6) to be 90% of the total and consist of about equal amounts of the 9 and 13 isomers.

The unknown class was separated by paper chromatography into two individual compounds. Both of these compounds had maximum absorbance at 395 mμ in carbon tetrachloride. The major compound (75%) separated at the R_F value of C_9 dienal and the minor had the mobility of a C_{11} dienal. Identification and determination of the source of these compounds must await further investigation. A similar class has been isolated from oxidized methyl archidonate when subjected to the Pool and Klose column (5), as well as from methyl linolenate by steam distillation (11).

The Decomposition of Monocarbonyl Precursors by Heat

Since the action of hydrated alumina appeared to resemble in some respects the effect of heat on fat oxidation products, the two treatments were compared. A sample of lard separately autoxidized to a peroxide value of 22 was examined before and after heating at 165C under purified nitrogen for 15 min.

Heating as shown in Table VI increased the total μmole isolated by vacuum distillation approximately tenfold. The degree of increase was generally higher for the unsaturated aldehydes, but C_6 alkanal and C_{10} alk-2,4-dienal increased 12 and 15 times, respectively.

The total μmoles isolated by the Pool and Klose method as the result of heating the oxidized fat was increased by only 50%, and there was a disappearance of the highly unsaturated unknown class. There was no change in the alkanal class, the enal class increased 77%, and the quantity of alk-2,4-dienals tripled, thereby accounting for most of the increase as the result of heating. C_6 alkanal decreased, C_{10} alk-2,4-dienal increased over 12 times, C_7 alk-2,4-dienal in-

TABLE V

Individual Alk-2,4-dienals Found in Auoxidized Lard

		μmoles/10 g		
	Peroxide	C-7	C-9	C-10
Vacuum distillation	15	0.009	0.009	0.036
Pool & Klose		0.082	0.155	0.066
Vacuum distillation	40	0.34	0.028	0.051
Pool & Klose		0.373	1.097	0.192
Vacuum distillation	78	0.048	0.032	0.049
Pool & Klose		0.677	1.755	0.395
Vacuum distillation	159	0.075	0.060	0.085
Pool & Klose		1.117	3.225	0.538
Vacuum distillation	252	0.098	0.068	0.072
Pool & Klose		1.738	4.180	0.770

TABLE VI
The Effect of Heating Autoxidized Lard at 165C
(15 Minutes; Peroxide Value 22)

	Absorbance at λ max or µmoles/10 g			
	Vacuum distillation		Pool and Klose	
	Unheated	Heated	Unheated	Heated
Classes (absorbance)				
Alkanal	0.066	0.483	0.605	0.633
Alk-2-enal	0.022	0.285	0.177	0.286
Alk-2,4-dienal	0.019	0.244	0.272	0.857
Unknown			0.132	
Classes (µm)				
Alkanal	0.322	2.356	2.953	3.089
Alk-2-enal	0.080	1.033	0.642	1.037
Alk-2,4-dienal	0.051	0.654	0.728	2.295
Unknown			?	
Total	0.453	4.043	4.323	6.421
Alkanal (µm)				
C-2	0.026		0.148	0.209
C-3	0.037	0.419	0.673	0.681
C-5	0.030			
C-6	0.104	1.258	1.655	1.498
C-8	0.027	0.295	0.209	0.367
C-9	0.073	0.384	0.268	0.334
C-11	0.025			
Alk-2-enal (µm)				
C-4	trace	trace	trace	trace
C-5	trace	trace	trace	0.114
C-6	trace	trace	0.126	0.092
C-7	0.011	0.290	0.152	0.286
C-8	0.017	0.196	0.108	0.133
C-9	0.028	0.270	0.144	0.210
C-10	0.013	0.125	0.056	0.092
C-11	0.011	0.152	0.056	0.110
Alk-2,4-dienal (µm)				
C-7	0.012	0.107	0.181	0.354
C-9	0.010	0.072	0.425	0.286
C-10	0.029	0.440	0.122	1.522
C-11		0.035		0.133

creased, and C_{11} alk-2,4-dienal made its appearance. Heating did not increase the C_9 alk-2,4-dienal, the major dienal isolated by the Pool and Klose method from the unheated oxidized fat. The major aldehydes isolated by the Pool and Klose method from the heated fat were equivalent amounts of C_6 alkanal and C_{10} alk-2,4-dienal.

The above data would appear to indicate and justify the following tentative conclusions, necessarily based on oxidized linoleate since the major aldehydes were characteristic of that compound. Hydrated alumina readily decomposed precursors of C_6 alkanal and C_{10} alk-2,4-dienal. These were mostly 13 and 9 conjugated linoleate hydroperoxides. Low proportions of C_{10} alk-2,4-dienal isolated from the unheated oxidized lard by both methods indicated the presence of little 9 conjugated linoleate hydroperoxide. The precursors of C_6 alkanal were present in large proportions since this aldehyde was the major one separated from the unheated oxidized fat by the Pool and Klose method. Heating the oxidized lard at 165C produced and partially decomposed large amounts of C_{10} alk-2,4-dienal precursors. This is shown by the presence of more free C_{10} alk-2,4-dienal in the heated oxidized fat, and the isolation of major amounts by the Pool and Klose method. Heating the oxidized lard produced a large amount of free C_6 alkanal, but less than that formed by action of the hydrated alumina. Amounts indicate that most of the C_6 alkanal isolated by the Pool and Klose method as a result of heating was free aldehyde. This means that heating produced a virtual disappearance of 13 conjugated linoleate hydroperoxide. It is not believed that such results would be obtained if the 13 and 9 conjugated linoleate hydroperoxy isomers were present in equal amounts as reported (6). The small proportions of dienals generally found have been accounted for on the basis of their secondary oxidation (19,20,24). The present results might be explained by an isomerization from 13 to 9 conjugated linoleate hydroperoxide by heat.

The above assumptions are opposed by the following considerations. Precursors of C_{10} alk-2,4-dienal could come from some other mechanism. The data indicate greater stability of C_{10} alk-2,4-dienal precursors to heat. Equivalent amounts of C_6 alkanal and C_{10} alk-2,4-dienal were isolated under the most vigorous conditions, which might be construed to indicate equal amounts of their precursors. However, it is extremely likely that the Pool and Klose method does not completely decompose all hydroperoxides (6). Reaction of oxidized lard on celite containing 2,4-dinitrophenlhydrazone and 2 N HCl isolated much more monocarbonyl compounds (12) than the Pool and Klose method. These aldehydes had extremely low proportions of alk-2,4-dienals (12). Studies on the oxidation of methyl esters and glycerides of unsaturated fatty acids will be necessary to solve the problems indicated by this investigation.

The Pool and Klose method is not suitable for the separation of free aldehydes. Results with heated fat where considerable hydroperoxide breakdown had taken place indicate the method would be unsatisfactory for flavor analyses of cooked foods.

Decomposition of Hydroperoxides by Alumina

The Pool and Klose method is inefficient in the decomposition of hydroperoxides. Early workers used alumina to isolate and concentrate fat hydroperoxides (6). Poor recovery was obtained, and this depended on the particular fatty acid being studied, time of exposure, and grade of alumina. The oxidized fraction was preferentially adsorbed by the alumina, but the oxidation products were changed in some way by the process. A specially prepared sodium aluminum silicate gave some concentration of monohydroperoxides, but large proportions of oxygenated products other than hydroproxides were detected (6), indicating chemical changes. This investigation indicates a very considerable and abnormal breakdown of hydroperoxides by alumina of the grade and activity used.

REFERENCES

1. Day, E. A., and S. E. Papaioannou, J. Dairy Sci. *46*, 1201–1206 (1963).
2. Ellis, R., A. M. Gaddis and G. T. Currie, Anal. Chem. *30*, 475–479 (1958).
3. Ellis, R., and A. M. Gaddis, Ibid. *31*, 1997–2000 (1959).
4. Ellis, R., A. M. Gaddis and G. T. Currie, J. Food Sci. *26*, 131–138 (1961).
5. Ellis, R., A. M. Gaddis and G. T. Currie, J. Food Sci., in preparation.
6. Frankel, D. N., "Symposium on Foods; Lipids and Their Oxidation," ed. H. W. Schultz, E. A. Day and R. O. Sinnhuber, The Avi Publ. Co., Westport, Conn., 1962, p 51–78.
7. Gaddis, A. M., R. Ellis and G. T. Currie, Food Res. *24*, 283–297 (1959).
8. Gaddis, A. M., and R. Ellis Ibid. *24*, 392–400 (1959).
9. Gaddis, A. M., and R. Ellis, Anal. Chem. *31*, 870–875 (1959).
10. Gaddis, A. M., R. Ellis and G. T. Currie, Food Res. *25*, 495–506 (1960).
11. Gaddis, A. M., R. Ellis and G. T. Currie, JAOCS *38*, 371–375 (1961).
12. Gaddis, A. M., R. Ellis, J. Shamey and G. T. Currie, Ibid. *42*, 620–624 (1965).
13. Jones, L. A., J. C. Holmes and R. B. Seligman, Anal. Chem. *28*, 191–198 (1956).
14. Keith, R. W., and E. A. Day, JAOCS *40*, 121–124 (1963).
15. Kenaston, C. B., K. M. Wilbur, A. Ottolenghi and F. Bernheim, JAOCS *32*, 33–35 (1955).
16. Lea, C. H., and P. A. T. Swoboda, Chem. Ind. (London) *1958*, 974-975.
17. Lea, C. H., and P. A. T. Swoboda, J. Sci. Food Agr. *13*, 148–152 (1962).
18. Lea, C. H., and H. A. F. Jackson, Chem. Ind. (London) *1964*, 1429–1430.
19. Lillard, D. A., and E. A. Day, JAOCS *41*, 549–552 (1964).
20. Mookherjee, B. D., R. E. Deck and S. S. Chang, J. Agr. Food Chem. *13*, 131–134 (1965).
21. Pool, M. F., and A. A. Klose, JAOCS *28*, 215–218 (1951).
22. Schwartz, D. P., A. R. Johnson and O. W. Parks, Microchem. J. *6*, *37–44 (1962)*.
23. Schwartz, D. P., H. S. Haller and M. Keeney, Anal. Chem. *35*, 2191–2194 (1963).
24. Sulzbacher, W. L., A. M. Gaddis and R. Ellis, Proceedings of the 15th American Meat Institute Foundation Research Conference, Chicago, Ill., 1963, p 111–119.

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