Carbonyls in Oxidizing Fat. X. Quantitative Differences in Individual Aldehydes Isolated from Autoxidized Lard by Mild Methods of Extraction

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Abstract

Major aldehydes characteristic of the oxidation of the unsaturated fatty acids present in lard differed somewhat in proportions isolated by the mild methods of separation. This indicated an extra sensitivity of specific fatty acid primary oxidation products to conditions, and some deviation from the normal in the breakdown caused by the methods. Linolenate primary oxidation products showed the greatest sensitivity to separation conditions and the stage of oxidation. The mild methods differed in the amounts of individual aldehydes separated to a degree dependent on the stage of oxidation. These results favor the Girard T method for the isolation of free aldehydes from autoxidized lard.

Introduction

A COMPARISON OF QUANTITATIVE methods for isolation of aldehydes from autoxidized lard indicated an extreme sensitivity of monocarbonyl precursors to widely varied conditions of separation (9). Severity of conditions related to degree of acidity and heat affected the total monocarbonyl compounds and their class composition. The importance of isolation of free monocarbonyl compounds in flavor studies has been amply discussed (6,9,10,13). Quantitatively comparable mild methods (9), such as the pot still vacuum distillation of Lea and Swoboda (12), Girard T (8), and the Schwartz (15) procedures, have been proposed for the isolation of free aldehydes. These methods were found to differ at one or more of two apparent stages of autoxidation in total monocarbonyl compounds and proportions of classes (9).

This work was undertaken to determine the exact nature of the differences between the mild methods of isolation. Studies were confined to one lot of lard oxidized at 23C, and levels of autoxidation selected were those representative of early and advanced stages of autoxidation.

Experimental

Solvents and reagents were the same as described in previous publications (4,7–10). The lard used was rendered from a composite back fat sampling of six hogs. Rendering was performed a minimum length of time to a temperature of 105C. Storage of the lard was in vacuum-packed cans at −30F until used. Oxidation was carried out in thin layers at 23C under artificial light. This was conducted by spreading 500 g of lard in each of three 14½ in. × 8½ in. shallow Pyrex dishes. The contents of the dishes were mixed daily. Sampling was carried out by pooling equal amounts from each dish. Simultaneous determinations on duplicate 10.0 g samples were made at peroxide values of 29, 58, 119, and 256. Isolations of carbonyl 2,4-dinitrophenylhydrazones by vacuum distillation, Girard T, and Schwartz methods, separation of the monocarbonyl derivatives by column chromatography, resolutions into classes and individual compounds by paper chromatography and measurement of the various fractions by spectrophotometry at λ max were performed as described in earlier publications (1,2,4,5,8–10). The Pool and Klose method as modified by Keith and Day (11) was applied to all samples as a reference indicating the reservoir of monocarbonyl precursors present. Such isolates were carried to the class separation stage, and corrections were applied as recommended by Keith and Day (11). All results are calculated on the basis of μm per 10 g of oxidized lard.

Results and Discussion

Total Aldehydes and Their Class Composition

Results shown in Table I had essentially the same pattern observed in an earlier study of a number of oxidized lots of lard (9). This was characterized by close association between the vacuum distillation and Girard T values, with Schwartz values much higher, in the early oxidation stage (peroxide 29 and 58). Between peroxide values 58 and 119, positions were reversed with vacuum distillation and Schwartz values becoming similar and Girard T values much lower. The data clearly indicated that the differences were principally due to the magnitude of the alkanal classes. In the primary or early oxidation stage, the enal class was also higher in the aldehydes isolated by the Schwartz method. Volatile aldehydes at the four levels of oxidation were 7.8%, 5.6%, 9.0%, and 11.4%, respectively, of the known monocarbonyl com-

TABLE I
Class Separation of Monocarbonyl 2,4-Dinitrophenylhydrazones

	Vacuum distillation	Girard T	Schwartz	Pool and Klose
Peroxide 29				
Alkanal	0.332	0.401	0.902	3.707
Alk-2-enal	0.103	0.097	0.163	1.662
Alk-2,4-dienal	0.061	0.046	0.048	1.000
Total	0.496	0.544	1.113	6.369
Peroxide 58				
Alkanal	0.644	0.651	1.431	10.080
Alk-2-enal	0.183	0.164	0.358	3.757
Alk-2,4-dienal	0.081	0.046	0.163	2.417
Total	0.908	0.861	1.952	16.254
Peroxide 119				
Alkanal	1.488	1.063	1.517	13.984
Alk-2-enal	0.409	0.453	0.413	5.446
Alk-2,4-dienal	0.177	0.121	0.107	3.577
Total	2.074	1.637	2.037	23.007
Peroxide 256				
Alkanal	4.190	2.761	4.293	35.256
Alk-2-enal	1.098	1.033	1.004	7.068
Alk-2,4-dienal	0.343	0.247	0.244	7.277
Total	5.631	4.041	5.541	49.601

pounds isolated by the hydroperoxide destructive (6,10) Pool and Klose method. This summation does not include an unidentified, highly unsaturated monocarbonyl class previously observed (10) in substantial quantities.

Saturated Aldehydes Isolated

The individual alkanal compounds detected and measured in Table II. Most of the differences between the methods were due to size of the alkanal class, and there were some differences in the proportions of the individual aldehydes. This indicated a deviation from a normal breakdown by the more severe methods. The C_3 alkanal showed a tendency toward higher proportions in the alkanals isolated by the Schwartz method. This is the major aldehyde from linolenate (7). Extra sensitivity of the linolenate hydroperoxide to acid conditions might be indicated. The proportional differences in C_3 alkanal were greatest in the primary oxidation stage. Naturally present free and bound nonvolatile aldehydes were not of magnitude sufficient to be a factor in the differences observed. In the unoxidized lard, the Girard T method isolated 0.081 µm and the Schwartz method 0.157 µm of nonvolatile saturated aldehydes. Amounts of nonvolatile alkanal isolated by the Girard T and Schwartz methods in the primary stage of oxidation were similar to the control. These compounds were not detected in the advanced oxidation stages.

Alk-2-enal Compounds

As indicated in Table I, the Schwartz method had higher total enal values in the primary oxidation stage. Early in the oxidation, trace amounts of high molecular weight enals were detected in the Schwartz enals. These amounted to only 0.7% of the total aldehydes. Total amounts of alk-2-enals were similar in the advanced oxidation stages. Individual aldehyde profiles in Figure 1 for peroxide values 29, 58, and 119 differed considerably in the so-called primary oxidation stage. Schwartz C_4, C_5, C_6, C_7, C_8, and C_9 enals were higher; and the C_9 aldehyde was the major compound. The parent primary oxidation products giving rise to these compounds come from the polyunsaturated fatty acids (4,7). At peroxide 29, the major enals C_{10} and C_{11} isolated by the vacuum distillation and Girard T methods stemmed from oleate. As shown for peroxide 58, the vacuum distillation method responded more to increased autoxidation than the Girard T method with larger amounts of enals from polyunsaturated fatty acid oxidation products. This may indicate a greater breakdown of enal precursors by the vacuum distillation method than the Girard T. The changes in the major enal at different degrees of oxidation cannot be explained at this time.

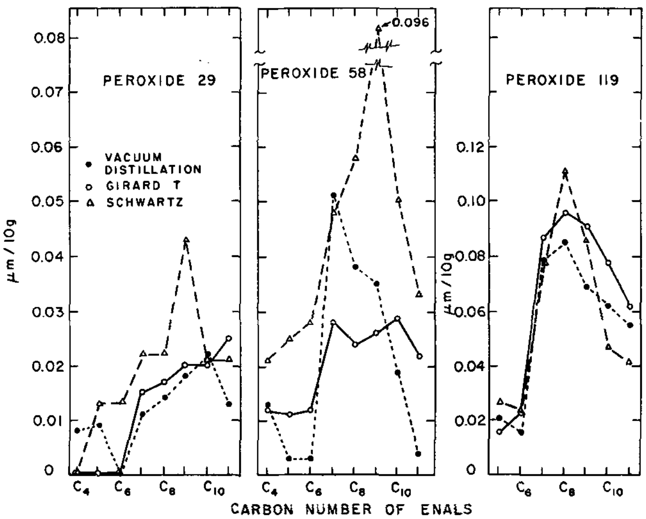

FIG. 1. Profiles of individual alk-2-enals.

TABLE II

Individual 2,4-Dinitrophenylhydrazones Found

	Vacuum distillation		Girard T		Schwartz	
	µm	% Total[1]	µm	% Total[a]	µm	% Total[a]
Peroxide 29						
C-2	0.004	0.9	trace	......	trace	......
C-3	0.083	16.6	0.078	14.4	0.205	18.4
C-5	trace	......	....	......	....	......
C-6	0.119	23.9	0.165	30.5	0.323	29.0
C-8	0.047	9.4	0.061	11.1	0.129	11.6
C-9	0.061	12.3	0.040	7.3	0.091	8.2
C-11	0.019	3.9	....	......	....	......
C-16	....	......	0.038	6.9	0.084	7.6
C-18	....	......	0.019	3.5	0.070	6.3
Peroxide 58						
C-2	0.016	1.8	0.013	1.5	0.009	1.0
C-3	0.105	11.5	0.118	13.7	0.322	16.5
C-5	trace	......	....	......	....	......
C-6	0.317	34.9	0.331	38.4	0.660	33.8
C-8	0.095	10.4	0.068	7.9	0.175	9.0
C-9	0.091	10.0	0.048	5.6	0.111	5.7
C-11	0.020	2.2	....	......	....	......
C-16	....	......	0.047	5.5	0.086	4.4
C-18	....	......	0.026	3.0	0.069	3.6
Peroxide 119						
C-2	trace	......	trace	......	trace	......
C-3	0.206	9.9	0.153	9.4	0.320	15.7
C-5	....	......	....	......	....	......
C-6	0.917	44.2	0.620	37.9	0.765	37.6
C-8	0.170	8.2	0.208	12.7	0.267	13.1
C-9	0.195	9.4	0.082	5.0	0.165	18.1
C-11	....	......	....	......	....	......
C-16	....	......	....	......	....	......
C-18	....	......	....	......	....	......
Peroxide 256						
C-2	trace	......	0.229	5.7	0.118	2.1
C-3	0.451	8.0	0.295	7.3	0.492	8.9
C-5	....	......	....	......	....	......
C-6	2.633	46.8	1.762	43.6	2.429	43.8
C-8	0.639	11.4	0.475	11.8	0.772	13.9
C-9	0.467	8.3	trace	......	0.482	8.7
C-11	....	......	....	......	....	......
C-16	....	......	....	......	....	......
C-18	....	......	....	......	....	......

[a] Per cent of total aldehydes.

Alk-2,4-dienals

As indicated in Table I, these compounds were minor components throughout, and they responded very slowly to autoxidation. There were no consistent differences between the methods. There was a trend toward higher values for the vacuum distillation method which might be due to the slight heating (7). C_7, C_9, C_{10}, and C_{12} dienals were detected, among which the C_7 and C_{10} compounds were present in major quantities. There is evidence that under mild conditions of oxidation and treatment the hydroperoxide precursors of dienals are present in very low amounts (10). It is also possible that they are much more stable than the other hydroperoxide isomers.

Comparison of Major Individual Aldehydes Characteristic of Oxidized, Unsaturated Fatty Acids

The saturated aldehydes were the major compounds isolated, and these as a group showed the greatest differences between methods. It has been shown that under the conditions of oxidation used alkanals are the major monocarbonyl products of the oxidation of oleate, linoleate, linolenate (7), and arachidonate (4).

The major aldehydes found that are characteristic of the unsaturated fatty acids are shown in Table III. These data show variations of the trends previously reported (9) and also shown in the present study for total aldehydes. If these values are plotted

TABLE III
Major Monocarbonyl Products of Unsaturated Fatty Acids

	Oleate C_8 and C_9 alkanal	Linoleate arachidonate C_6 alkanal	Linolenate C_3 alkanal
Peroxide 29			
Vacuum distillation	0.108	0.119	0.083
Girard T	0.101	0.165	0.078
Schwartz	0.220	0.323	0.205
Peroxide 58			
Vacuum distillation	0.186	0.317	0.105
Girard T	0.116	0.331	0.118
Schwartz	0.286	0.660	0.322
Peroxide 119			
Vacuum distillation	0.365	0.917	0.206
Girard T	0.290	0.620	0.153
Schwartz	0.432	0.765	0.320
Peroxide 256			
Vacuum distillation	1.116	2.633	0.451
Girard T	0.475	1.762	0.295
Schwartz	1.254	2.429	0.492

against the degree of oxidation the C_3 alkanal shows the greatest differences in slope between all three methods. This may indicate that primary oxidation products from linolenate are the most sensitive to conditions of isolation. Plotted data of the Girard T method showed a large difference in slope from the other methods for all the major aldehydes. The stage of oxidation had a great influence on relationships between methods.

Choice of Methods

The comparisons made indicate that the Girard T method as developed by Gaddis et al. (8) extracts the lowest amounts of individual monocarbonyl compounds from autoxidizing lard. It would appear that it is superior in this respect to vacuum distillation and the Schwartz method. It has been indicated (9,12) that small amounts of carbonyl are formed during the vacuum distillation at 50C. The response of the vacuum distillation method to increased rate of oxidation and its close association with the Schwartz method at advanced stages (9) suggest a spontaneous breakdown of precursors under vacuum. Lea and Hobson-Frohock (14), in a recent publication, have shown that labile precursors of volatile carbonyls decomposed under vacuum even in the presence of an inhibitor at 20C. This investigation presents the Girard T method in a favorable light as a means of isolating free monocarbonyl compounds of autoxidized lard. However, for all kinds of fats and oils this may depend on the composition of the oxidized lipid. Recent work by Ellis et al. (4) comparing Girard T and Schwartz methods on oxidized methyl arachidonate indicated that both methods broke down precursors, but in a qualitatively different manner.

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